

Relationship between Fluorescein Pooling and Optical Coherence Tomographic

Reflectivity of Cystoid Spaces in Diabetic Macular Edema

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Running head: Fluorescein Pooling and OCT Reflectivity in Diabetic CME

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Abstract

Objective: To study the characteristics of the reflectivity of the cystoid spaces and serous retinal detachment (SRD) on spectral-domain optical coherence tomography (SD-OCT) and the correlation with fluorescein findings in diabetic macular edema (DME).

Design: Retrospective, observational, cross-sectional study.

Participants: Consecutive 134 eyes of 114 patients with clinically significant macular edema for whom SD-OCT and fluorescein angiography (FA) were performed on the same day.

Methods: FA using Heidelberg Retina Angiograph 2 and OCT images using Spectralis OCT were obtained. The reflectivity of the cystoid spaces and SRD on the OCT images was evaluated qualitatively and quantitatively and compared to the fluorescein pooling intensity on FA images.

Main Outcome Measures: The relationship between fluorescein pooling and the reflectivity characteristics of the cystoid spaces on SD-OCT images.

Results: One hundred forty-one cystoid spaces in 101 eyes were delineated on OCT images, and 138 (97.9%) spaces had fluorescein pooling. Fifty-five (39.9%) cystoid spaces with marked fluorescein pooling intensity had lower reflectivity on OCT images than those with modest pooling (12.1 ± 10.4 vs. 22.0 ± 15.4 , $P < 0.001$). The heterogeneity of the reflectivity of the cystoid spaces on the OCT images was associated significantly ($P < 0.001$) with modest fluorescein pooling. The hyperreflective foci in the cystoid spaces were correlated significantly with modest fluorescein pooling and higher or heterogeneous reflectivity on OCT images ($P < 0.001$, $P < 0.001$, and $P = 0.005$, respectively). In addition, the cystoid spaces with microaneurysms had higher or heterogeneous reflectivity on OCT images more frequently than those without microaneurysms ($P < 0.001$ and $P = 0.019$,

1 respectively). The reflectivity levels in the SRD were significantly ($P=0.005$) lower than in
2 the cystoid spaces, and only one (3.3%) eye had heterogeneous reflectivity on OCT images.

3 **Conclusions:** The results provided a novel interpretation of fluorescein pooling and
4 OCT characteristics of cystoid spaces and SRD in DME and suggested several mechanisms
5 by which the blood-retinal barrier is disrupted and concomitant edematous changes
6 develop.

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INTRODUCTION

Diabetic macular edema (DME) and angiogenic complications are major causes of severe visual loss in diabetic retinopathy (DR).¹⁻² Breakdown of the blood-retinal barrier (BRB) especially causes accumulation of intracellular or extracellular fluids and exacerbation of neuroglial dysfunction and concomitant visual disruption.³⁻⁴ Although several therapeutic strategies have been reported, all are limited and many patients with DME still have poor visual prognoses.⁵⁻¹⁰ This suggests several patterns of pathogenesis in DME, which remain to be elucidated.

The diagnosis of DME initially was based primarily on the results of slit-lamp biomicroscopy and fundus stereophotography. Another modality, fluorescein angiography (FA), delineates vascular lesions with higher contrast and various patterns of hyperfluorescence in the retinal parenchyma, i.e., focal or diffuse leakage and pooling with petalloid or honeycomb-like patterns.¹¹⁻¹² The extravasation of fluorescein dye suggests a vascular pathophysiology and thickening of the retinal parenchyma. The major component of the inner BRB is believed to be retinal vascular endothelial cells with a highly integrated intercellular junctional complex, efflux transporters of wastes or toxic materials, and limited transcytosis, which are modulated by perivascular cells, pericytes, or glial cells.¹³⁻¹⁷ Diabetes exacerbates the breakdown of the BRB depending on several mechanisms: disrupted tight junctions, increased transcellular transports, and endothelial cell death, which are mediated via growth factors, cytokines, and several biochemical pathways.^{3-4, 18-20} Despite advances in basic research in vascular pathophysiology, the cellular or molecular mechanisms that determine the clinical fluorescein findings in DME remain unclear.

Optical coherence tomography (OCT), which enables the capture of in vivo sectional

images of the retinal pathology, has accelerated clinical research in DME.²¹⁻²⁴ Time domain-OCT delineates various morphologies in DME: serous retinal detachment (SRD), cystoid macular edema (CME), and sponge-like retinal swelling.²¹ The objective quantification using OCT identified a modest correlation between macular thickness and visual acuity (VA) in DME.²² Recent technologic advances have resulted in spectral domain (SD)-OCT, which improved the delineation of smaller structures with much higher resolution. Fine lesions, i.e., hyperreflective foci, have been newly delineated on SD-OCT images, and the detailed structural characteristics in microaneurysms have been reported.²⁵⁻²⁷ Another advantage of SD-OCT with averaging processes is reduced speckle noise, which guarantees more accurate definition of reflectivity levels of the individual retinal layers and lesions. SD-OCT has been used to evaluate the external limiting membrane in DME and segmentation of the individual retinal layers.²⁸⁻²⁹

We evaluated qualitatively and quantitatively the reflectivity levels of the cystoid spaces and SRD on SD-OCT images and their relationship to hyperfluorescence in the images. We found that the fluorescein pooling intensity was related to the OCT reflectivity and its heterogeneity in the cystoid spaces in DME.

Methods

Patients

We retrospectively reviewed 134 eyes of 114 patients (age range, 33-89 years; mean, 65.0 ± 10.8 years; 13 eyes with mild nonproliferative diabetic retinopathy [NPDR], 73 moderate NPDR, and 48 severe NPDR) who visited the Department of Ophthalmology in Kyoto University Hospital from May 2009 to February 2011. The inclusion criteria were eyes with clinically significant macular edema and those for whom FA and OCT images of

1 sufficient quality were obtained. Eyes with PDR were excluded, because intravitreal
2 hyperfluorescence from neovascular tissue makes it difficult to evaluate the exact levels of
3 intraretinal dye pooling. Since postoperative inflammation might modulate vascular
4 permeability, eyes in which cataract surgery were performed within 6 months and
5 vitrectomized eyes were also omitted. All research and measurements adhered to the tenets
6 of the Declaration of Helsinki. The ethics committee of our institution approved the study
7 protocol. Each patient provided informed consent after receiving a detailed explanation of
8 the nature and possible consequences of the study procedures.

9 **Optical Coherence Tomography**

10 After measuring the best-corrected VA (BCVA) and performing fundus biomicroscopy,
11 retinal sectional images of the macula were acquired and evaluated using SD-OCT
12 (Spectralis OCT, Heidelberg Engineering, Heidelberg, Germany) with infrared images.
13 Cross-hair scans (30 degrees) centered on the presumed fovea were obtained, and the
14 vertical images were used for further investigation. We then evaluated the contents of each
15 cystoid space and SRD within 1 mm of the center of the presumed fovea. The averaging
16 processes in SD-OCT reduce speckle noise and improve delineation of the pathological
17 structures, which encouraged us to evaluate the levels of reflectivity in each space
18 qualitatively and quantitatively. The reflectivity in some spaces has heterogeneity either
19 with or without a clear border, and others showed homogeneous reflectivity. Qualitative
20 characteristics were estimated by two independent graders, and a third higher-level grader
21 reviewed the images at the disagreement. To quantify the reflectivity levels, the margin of
22 each space was manually traced using the images with inverted grayscale. The average
23 reflectivity in the area encircled was measured using image processing software (Photoshop,
24 Adobe Systems, San Jose, CA). We used the reflectivity levels of the vitreous cavity and

nerve fiber layer (NFL) as the standard in each image. After measuring the averaged reflectivity in each area as shown in the cystoid spaces, we defined the level in the vitreous as 0 and in the NFL as 100. The reflectivity values of the cystoid spaces or SRDs were calculated as an arbitrary unit (AU) according to the formula:

$$\text{relative reflectivity (A.U.)} = \frac{\text{reflectivity (cystoid space)} - \text{reflectivity (vitreous)}}{\text{reflectivity (NFL)} - \text{reflectivity (vitreous)}} \times 100$$

The values were well agreed between two independent graders (intraclass correlation coefficient [ICC] = 0.992), and the average was applied to further analysis.

For an objective confirmation, we quantified OCT reflectivity of cystoid spaces automatically. We modified the method for automated detection of the margin of each cystoid space which was described previously.³⁰ To be brief, after gray-scale TIFF images were imported into ImageJ (NIH, Bethesda, MD), an ImageJ plugin, E-Snake, was applied for the edge detection of cystoid spaces. We targeted each cystoid space using eight control points inside itself. The exact contour was determined by the optimization algorithm of the snake, followed by the calculation of the signal intensity in the encircled area of each cystoid space.

After the exclusion of two cystoid spaces in which the contour cannot be automatically decided, the reflectivity levels by this protocol were significantly correlated to those determined manually (ICC=0.992).

Fluorescein Angiography

Early- and late-phase FA images (6-10 minutes after intravenous injection) were obtained using Heidelberg Retinal Angiography 2 (HRA2, Heidelberg Engineering) as reported previously.²⁴ We evaluated the pattern of hyperfluorescence on a vertical line that dissected

the presumed foveal center (within 1 mm centered on the presumed fovea) as focal, diffuse, or pooled (petalloid or honeycomb-like) as reported previously.¹¹⁻¹² The fluorescein pooling intensity then was divided into two levels. Briefly, after tracing the margin of each area of pooled dye, the fluorescein intensity was averaged using image processing software (Photoshop). Marked pooling indicated a higher level of fluorescein intensity than that of nearby venules, and modest pooling indicated a lower or the same level. Microaneurysms in fluorescein pooling were defined as those in the cystoid spaces.

In eyes with a SRD, we evaluated the presence of subretinal fluorescein pooling, which was defined as hyperfluorescence with a clear margin in the area corresponding to the SRD on the OCT images. Briefly, we first determined the presence of SRD at the presumed fovea and traced the subretinal spaces to the periphery until the margin of the SRD was identified. We then determined whether subretinal pooling of fluorescein dye was present. The margin of the pooled dye in the subretinal spaces should be very clear and round compared to the various patterns of hyperfluorescence in the retinal parenchyma.

Statistical Analysis

The results are expressed as the mean $\pm$ standard deviation. The Student's *t*-test was used to compare the quantitative data populations with normal distributions and equal variance. The data were analyzed using the Mann-Whitney *U*-test for populations with non-normal distributions or unequal variance. Significant differences in the sampling distribution were determined by Fisher's exact test. $P < 0.05$ was considered statistically significant.

Results

The disrupted BRB in DR leads to several types of structural changes and accumulation of extracellular fluids.²¹ SD-OCT showed foveal cystoid spaces in 101 eyes and SRD in 30

eyes (Fig 1). We further investigated the patterns of the extracellular fluids in FA and SD-OCT in each lesion in DME.

Association between Fluorescein Pooling and Optical Coherence Tomographic

Reflectivity of the Cystoid Spaces

We first evaluated the fluorescein and OCT findings in 101 eyes with foveal cystoid spaces and found that 138 of 141 cystoid spaces delineated on OCT images had pooling of fluorescein. However, three cystoid spaces were not hyperfluorescent, and two were accompanied with epiretinal membrane (ERM) (Fig 2). The discrepancy in these cases suggests the limitation of FA for evaluating the cystoid spaces and mechanisms other than vascular hyperpermeability in the pathogenesis of the cystoid spaces.

Recent studies have reported that the morphologic patterns of hyperfluorescence are associated with the locations of the cystoid spaces in DME, which prompted us to investigate the relationship between fluorescein intensity and the OCT characteristics of 138 foveal cystoid spaces with fluorescein pooling.¹¹⁻¹² The average reflectivity in the cystoid spaces with marked fluorescein pooling was significantly lower than that with modest pooling ($P<0.001$) (Figs 3, 4; Table 1, 2, available at <http://aaajournal.org>). Interestingly, 35 cystoid spaces showed heterogeneity in the reflectivity levels (Fig 3), which was significantly associated with modest fluorescein pooling ($P<0.001$) (Table 1).

Hyperreflective Foci in the Cystoid Spaces

The accumulation of hyperreflective foci in the subretinal spaces indicates a poor prognosis in DME,²⁶ which encouraged us to investigate the characteristics of the hyperreflective foci in the foveal cystoid spaces. Curiously, their presence was associated significantly with modest fluorescein pooling ($P<0.001$; Table 3). It seems inconsistent with the general belief that severe disruption of the BRB increases proteins, lipids, or lipid-laden macrophages and

concomitant deposition of hard exudates in the retinal parenchyma. The cystoid spaces with hyperreflective foci had significantly higher levels of OCT reflectivity than those without foci ($P<0.001$; Table 3). There also was a significant association between the hyperreflective foci and heterogeneity of the reflectivity in the cystoid spaces ($P=0.005$; Table 3). These data suggested that higher reflectivity might represent condensed proteins or lipids, resulting in increased hyperreflective foci.

Reflectivity in the Cystoid Spaces with Microaneurysms

Microaneurysms are accompanied by vascular hyperpermeability and concomitant edema in the retinal parenchyma in DR. The association between microaneurysms and foveal cystoid spaces was reported recently, suggesting the contribution of microaneurysms to the pathogenesis of the cystoid spaces.²⁴ When we investigated the OCT reflectivity in the cystoid spaces with microaneurysms, we found that these spaces had higher and heterogeneous reflectivity on OCT images more frequently than those without microaneurysms ($P<0.001$ and $P=0.019$, respectively) (Table 4). This suggested that the disrupted BRB in microaneurysms often allowed the proteins and lipids to move into the cystoid spaces, at least in part. However, cystoid spaces with microaneurysms were not associated with fluorescein pooling intensity ($P=0.348$) (Table 4).

No Subretinal Fluorescein Pooling in Serous Retinal Detachments

We investigated the characteristics of the FA and OCT findings in 30 eyes with SRD. Compared to the intraretinal hyperfluorescence in the parafoveal area,²⁴ we did not find fluorescein pooling in the subretinal spaces (Fig 5). A further evaluation of the OCT images showed that the reflectivity levels in the SRD were significantly lower than in the cystoid spaces (12.3 ± 15.2 vs. 18.6 ± 15.2 , $P=0.005$). The heterogeneity in OCT reflectivity was delineated in only one eye with SRD (3.3%) compared to that in the cystoid spaces. The

differences in fluorescence and reflectivity on the OCT images suggested that the pathologic mechanisms in SRD differ from those in the cystoid spaces.

Vitreomacular traction

Vitreomacular traction (VMT) has been reported as another mechanism exacerbating DME,^{9, 31-33} which prompted us to evaluate its association with fluorescein intensity and OCT reflectivity in cystoid spaces. Spectralis OCT delineated VMT including epiretinal membrane (ERM) in 19 (18.8 %) of 101 eyes with foveal cystoid spaces, and either qualitative or quantitative parameters in the contents of cystoid spaces were not associated with VMT (Table 5). Intriguingly, 16 (53.5 %) of 30 eyes with SRD were accompanied with VMT, whereas OCT delineated VMT in only 30 (28.8 %) of 104 eyes without SRD ($P=0.017$). It suggests that tractional forces by posterior hyaloid might have an influence on the development or maintenance of SRD, at least in part.

Discussion

The current study showed for the first time the different levels of OCT reflectivity in the cystoid spaces in DME, suggesting the diversity of the contents. Increased reflectivity on the OCT images might be similar to flare in the intraocular humor, which can be seen by light reflection. In other words, the reflectivity could suggest the presence of concentrated proteins and lipids in the cystoid spaces. Pathohistology showed that hyaline deposits are often present in the cystoid spaces due to retinal vascular diseases,³⁴⁻³⁵ and the deposits might also have higher reflectivity on OCT images.

Barthelmes et al demonstrated the different levels of OCT reflectivity in cystoid spaces in different diseases, and speculated that the levels might be related to the pathogenesis of intraretinal spaces; exudation or degeneration.³⁶ The heterogeneity in OCT

reflectivity was often delineated in cystoid spaces associated with retinal vascular diseases, whereas neuroglial degeneration in X-linked retinoschisis or high myopia results in lower and homogeneous reflectivity in intraretinal spaces.³⁷⁻³⁸ Considering the significant association between fluorescein intensity and OCT reflectivity in this current study, the diversity of OCT reflectivity levels in DME should depend on the different mechanisms in BRB breakdown, at least in part.

The physiologic BRB depends on a highly integrated intercellular junction complex, decreased transcellular transport, and active efflux of smaller molecules in the vascular endothelial cells, which are supported by perivascular components including the basement membrane, pericytes, and glial cells.¹³⁻¹⁷ These systems might be disrupted during the different stages of DR. Several pathological or biologic studies have reported that endothelial cell death, induced by inflammatory stimulation and biochemical pathways, increase the flux through the BRB in diabetes.^{19, 39-40} Tight junctions were disrupted in diabetic rats or because of treatment with cytokines or growth factors, which results in increased paracellular flux and concomitant vascular hyperpermeability,^{18, 41} and transcellular transport also was stimulated by growth factors, mediated via pinocytosis and vesiculovacuolar organelle.²⁰

Endothelial cell death might be compatible with the current data that the cystoid spaces with higher reflectivity have lower fluorescein pooling intensity.¹⁹ We speculated that this kind of break in the retinal vasculature may allow the blood constituents to diffuse freely from or to the cystoid spaces, resulting in almost the same oncotic and hydrostatic pressure in the cystoid spaces as that in nearby capillaries. In other words, there are no active forces in transport into the cystoid spaces that depend on pressure differences. The amount of fluorescein moving into the cystoid spaces therefore decreased, with resultant

1 lower dye pooling intensity.

2 Partial disruption of the BRB might explain the findings that the cystoid spaces with
3 lower reflectivity have higher intensity fluorescein pooling. Transgenic mice deleting
4 claudin-5, an endothelial tight junction protein, had vascular hyperpermeability in smaller
5 but not larger molecules.⁴² Several cellular and molecular mechanisms, result in the
6 breakdown of tight junctions, which might permit smaller but not larger molecules to
7 migrate through the capillary walls into the cystoid spaces.^{18, 41-45} In such situations,
8 Starling's equation, which describes the balance between hydrostatic and oncotic pressures,
9 might be applied.⁴⁶ Albumin, a main serum protein, could not move into the cystoid spaces,
10 resulting in a difference in oncotic pressure between the cystoid spaces and the nearby
11 capillaries. To neutralize it, the hydrostatic pressure in the capillaries should be higher than
12 that in the cystoid spaces, which promotes movement of water and smaller molecules
13 including fluorescein (molecular weight, approximately 330) into the cystoid spaces and
14 increases the fluorescein pooling intensity.

15 Another explanation for the negative correlation between fluorescein intensity and
16 reflectivity on OCT might be blockage or scattering of fluorescent light by the concentrated
17 proteins. Based on this hypothesis, the negative correlation should be mathematically
18 accurate, although we found some cystoid spaces without this association, suggesting that
19 this explanation is not reasonable.

20 Interestingly, 35 cystoid spaces had heterogeneity in the reflectivity levels with clear
21 borders. We speculated that higher reflectivity might correspond to blood clots or hyaline
22 deposits in pathohistologic findings,³⁴⁻³⁵ whereas the areas with lower reflectivity might be
23 filled with fluid. Among several possible components of hyaline deposits, most might be
24 extravasated serum proteins or fibrins. We showed that heterogeneous reflectivity was

positively associated with modest fluorescein pooling. One explanation might be the limited volume of the fluid phase, and another might be that the cystoid spaces are accompanied by severe disruption of the BRB and concomitant movement of serum proteins and lipids, as discussed previously. This theory might be supported by the positive association between the heterogeneous reflectivity and focal fluorescein leakage from microaneurysms, in which the vascular walls are sometimes ruptured.²⁷

Hyperreflective foci, which may be precursors of hard exudates, were associated significantly with heterogeneity or higher levels of reflectivity in the cystoid spaces on the OCT images. In other words, the foci were observed in the cystoid spaces with severe disruption of the BRB. Although the origin of the hyperreflective foci remains controversial,²⁵⁻²⁶ condensed proteins or lipids in the cystoid spaces might deposit as hyperreflective foci, resulting in emergence of hard exudates. Another possibility is that lipid-laden macrophages might take up the concentrated proteins or lipids in the cystoid spaces. A severe break in the vascular walls might allow macrophage migration into the cystoid spaces, regardless of whether the BRB breakdown is or is not induced by inflammatory cells.^{19, 39-40} In addition, considering the different appearances of the hyaline-like deposits and the hyperreflective foci on the OCT images, the foci may be lipid-laden macrophages rather than protein or lipid deposition. Since the Early Treatment Diabetic Retinopathy Study showed that the foveal hard exudates predict poor visual prognosis,⁴⁷⁻⁴⁸ clinicians should evaluate the OCT reflectivity in the foveal cystoid spaces, which also might exacerbate the subfoveal hard exudates.

Recent publications have reported an association between microaneurysms and cystoid spaces in DME.^{24, 27} Cystoid spaces with microaneurysms had higher and heterogeneous reflectivity, suggesting that the BRB in the microaneurysms often was

1 damaged severely and allowed serum proteins and lipids to move into the cystoid spaces.
2 SD-OCT showed the structural diversity in the vascular walls of the microaneurysms, and
3 the absence or rupture of the thick walls often was associated with the heterogeneous
4 reflectivity in the nearby cystoid spaces. In addition, circinate hard exudates often were
5 seen around the microaneurysms in DR. We then speculated that microaneurysms, other
6 vascular lesions, or mixed mechanisms contribute to the pathogenesis of the cystoid spaces
7 with fluorescein pooling. A longitudinal study after direct coagulation of the
8 microaneurysms might clarify this.

9 We did not see fluorescein pooling in the subretinal fluids in eyes with SRD
10 associated with DME, compared to the breakdown of outer BRB in central serous
11 chorioretinopathy (CSC). Intriguingly, VMT was significantly associated with SRD in
12 DME, which might explain this discrepancy. We further found that cystoid spaces had
13 higher levels of reflectivity on OCT images than SRD. These data seem inconsistent with
14 the negative correlation between fluorescein pooling and OCT reflectivity in the cystoid
15 spaces. Additionally, VMT did not affect the qualitative characteristics in the contents of
16 cystoid spaces, although their volume might be exacerbated. No studies have definitively
17 reported the pathogenesis of SRD development or maintenance in DME, although the data
18 in the current study suggested that the pathogenesis of SRD differs at least partly from that
19 of the cystoid spaces. Recent studies showed that the continuity from the intraretinal
20 cystoid spaces to subretinal fluids in SRD due to branch retinal vein occlusion⁴⁹ and the
21 pathogenesis might be combined, after time passed by.

22 In the current study, we showed for the first time a negative correlation between
23 fluorescein pooling intensity and OCT reflectivity of the cystoid spaces in DME,
24 suggesting various patterns of BRB disruption. Recent advances in medicine and biology

1 have enabled application of several therapeutic strategies, although any treatment has only
2 partial effects on DME. Identification of several patterns of disruption of the BRB would
3 lead to development of customized therapies for DME.

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References

1. Klein R, Klein BE, Moss SE, Cruickshanks KJ. The Wisconsin Epidemiologic Study of Diabetic Retinopathy. XV. The long-term incidence of macular edema. *Ophthalmology* 1995;102:7-16.
2. Aiello LP, Avery RL, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. *N Engl J Med* 1994;331:1480-7.
3. Gardner TW, Antonetti DA, Barber AJ, et al. Diabetic retinopathy: more than meets the eye. *Surv Ophthalmol* 2002;47(suppl):S253-62.
4. Antonetti DA, Barber AJ, Bronson SK, et al, JDRF Diabetic Retinopathy Center Group. Diabetic retinopathy: seeing beyond glucose-induced microvascular disease. *Diabetes* 2006;55:2401-11.
5. Mohamed Q, Gillies MC, Wong TY. Management of diabetic retinopathy: a systematic review. *JAMA* 2007;298:902-16.
6. Early Treatment Diabetic Retinopathy Study Research Group. Photocoagulation for diabetic macular edema: Early Treatment Diabetic Retinopathy Study report number 1. *Arch Ophthalmol* 1985;103:1796-806.
7. Haritoglou C, Kook D, Neubauer A, et al. Intravitreal bevacizumab (Avastin) therapy for persistent diffuse diabetic macular edema. *Retina* 2006;26:999-1005.

- 1 8. Jonas JB, Sofker A. Intraocular injection of crystalline
2 cortisone as adjunctive treatment of diabetic macular edema.
3 Am J Ophthalmol 2001;132:425-7.
- 4 9. Lewis H, Abrams GW, Blumenkranz MS, Campo RV. Vitrectomy
5 for diabetic macular traction and edema associated with
6 posterior hyaloidal traction. Ophthalmology 1992;99:753-9.
- 7 10. Aiello LP. The potential role of PKC beta in diabetic
8 retinopathy and macular edema. Surv Ophthalmol
9 2002;47(suppl):S263-9.
- 10 11. Otani T, Kishi S. Correlation between optical coherence
11 tomography and fluorescein angiography findings in diabetic
12 macular edema. Ophthalmology 2007;114:104-7.
- 13 12. Bolz M, Ritter M, Schneider M, et al. A systematic
14 correlation of angiography and high-resolution optical
15 coherence tomography in diabetic macular edema. Ophthalmology
16 2009;116:66-72.
- 17 13. Shakib M, Cunha-Vaz JG. Studies on the permeability of
18 the blood-retinal barrier. IV. Junctional complexes of the
19 retinal vessels and their role in the permeability of the
20 blood-retinal barrier. Exp Eye Res 1966;5:229-34.
- 21 14. Schinkel AH, Smit JJ, van Tellingen O, et al. Disruption
22 of the mouse *mdrla* P-glycoprotein gene leads to a deficiency
23 in the blood-brain barrier and to increased sensitivity to drugs.
24 Cell 1994;77:491-502.

15. Coomber BL, Stewart PA. Three-dimensional reconstruction of vesicles in endothelium of blood-brain barrier versus highly permeable microvessels. *Anat Rec* 1986;215:256-61.
16. Cunha-Vaz J. The blood-ocular barriers. *Surv Ophthalmol* 1979;23:279-96.
17. Bernstein MH, Hollenberg MJ. Fine structure of the choriocappillaris and retinal capillaries. *Invest Ophthalmol* 1965;4:1016-25.
18. Antonetti DA, Barber AJ, Khin S, et al, Penn State Retina Research Group. Vascular permeability in experimental diabetes is associated with reduced endothelial occludin content: vascular endothelial growth factor decreases occludin in retinal endothelial cells. *Diabetes* 1998;47:1953-9.
19. Joussen AM, Poulaki V, Mitsiades N, et al. Suppression of Fas-FasL-induced endothelial cell apoptosis prevents diabetic blood-retinal barrier breakdown in a model of streptozotocin-induced diabetes. *FASEB J* 2003;17:76-8.
20. Hofman P, Blaauwgeers HG, Tolentino MJ, et al. VEGF-A induced hyperpermeability of blood-retinal barrier endothelium in vivo is predominantly associated with pinocytotic vesicular transport and not with formation of fenestrations. *Curr Eye Res* 2000;21:637-45.
21. Otani T, Kishi S, Maruyama Y. Patterns of diabetic macular edema with optical coherence tomography. *Am J Ophthalmol*

1999;127:688-93.

22. Diabetic Retinopathy Clinical Research Network.

Relationship between optical coherence tomography-measured central retinal thickness and visual acuity in diabetic macular edema. *Ophthalmology* 2007;114:525-36.

23. Sakamoto A, Nishijima K, Kita M, et al. Association between foveal photoreceptor status and visual acuity after resolution of diabetic macular edema by pars plana vitrectomy. *Graefes Arch Clin Exp Ophthalmol* 2009;247:1325-30.

24. Murakami T, Nishijima K, Sakamoto A, et al. Foveal cystoid spaces are associated with enlarged foveal avascular zone and microaneurysms in diabetic macular edema. *Ophthalmology* 2010;118:359-67.

25. Bolz M, Schmidt-Erfurth U, Deak G, et al, Diabetic Retinopathy Research Group Vienna. Optical coherence tomographic hyperreflective foci: a morphologic sign of lipid extravasation in diabetic macular edema. *Ophthalmology* 2009;116:914-20.

26. Ota M, Nishijima K, Sakamoto A, et al. Optical coherence tomographic evaluation of foveal hard exudates in patients with diabetic maculopathy accompanying macular detachment. *Ophthalmology* 2010;117:1996-2002.

27. Horii T, Murakami T, Nishijima K, et al. Optical coherence tomographic characteristics of microaneurysms in diabetic

retinopathy. Am J Ophthalmol 2010;150:840-8.

28. Murakami T, Nishijima K, Sakamoto A, et al. Association of pathomorphology, photoreceptor status, and retinal thickness with visual acuity in diabetic retinopathy. Am J Ophthalmol 2011;151:310-7.

29. Tan O, Chopra V, Lu AT, et al. Detection of macular ganglion cell loss in glaucoma by Fourier-domain optical coherence tomography. Ophthalmology 2009;116:2305-14.

30. Fernandez DC. Delineating fluid-filled region boundaries in optical coherence tomography images of the retina. IEEE Trans Med Imaging 2005;24:929-45.

31. Kaiser PK, Riemann CD, Sears JE, Lewis H. Macular traction detachment and diabetic macular edema associated with posterior hyaloidal traction. Am J Ophthalmol 2001;131:44-9.

32. Diabetic Retinopathy Clinical Research Network Writing Committee. Vitrectomy outcomes in eyes with diabetic macular edema and vitreomacular traction. Ophthalmology 2010;117:1087-93.

33. Bhagat N, Grigorian RA, Tutela A, Zarbin MA. Diabetic macular edema: pathogenesis and treatment. Surv Ophthalmol 2009;54:1-32.

34. Tso MO. Pathology of cystoid macular edema. Ophthalmology 1982;89:902-15.

35. Wolter JR. The histopathology of cystoid macular edema.

- 1 Albrecht Von Graefes Arch Klin Exp Ophthalmol 1981;216:85-101.
- 2 36. Barthelmes D, Sutter FK, Gillies MC. Differential optical
- 3 densities of intraretinal spaces. Invest Ophthalmol Vis Sci
- 4 2008;49:3529-34.
- 5 37. Gregori NZ, Berrocal AM, Gregori G, et al. Macular
- 6 spectral-domain optical coherence tomography in patients with
- 7 X linked retinoschisis. Br J Ophthalmol 2009;93:373-8.
- 8 38. Benhamou N, Massin P, Haouchine B, et al. Macular
- 9 retinoschisis in highly myopic eyes. Am J Ophthalmol
- 10 2002;133:794-800.
- 11 39. Kowluru RA. Effect of advanced glycation end products on
- 12 accelerated apoptosis of retinal capillary cells under in vitro
- 13 conditions. Life Sci 2005;76:1051-60.
- 14 40. Nishikawa T, Edelstein D, Du XL, et al. Normalizing
- 15 mitochondrial superoxide production blocks three pathways of
- 16 hyperglycaemic damage. Nature 2000;404:787-90.
- 17 41. Murakami T, Felinski EA, Antonetti DA. Occludin
- 18 phosphorylation and ubiquitination regulate tight junction
- 19 trafficking and vascular endothelial growth factor-induced
- 20 permeability. J Biol Chem 2009;284:21036-46.
- 21 42. Nitta T, Hata M, Gotoh S, et al. Size-selective loosening
- 22 of the blood-brain barrier in claudin-5-deficient mice. J Cell
- 23 Biol 2003;161:653-60.
- 24 43. Hammes HP, Lin J, Wagner P, et al. Angiopoietin-2 causes

pericyte dropout in the normal retina: evidence for involvement in diabetic retinopathy. Diabetes 2004;53:1104-10.

44. Armulik A, Genove G, Mae M, et al. Pericytes regulate the blood-brain barrier. Nature 2010;468:557-61.

45. Furuse M, Tsukita S. Claudins in occluding junctions of humans and flies. Trends Cell Biol 2006;16:181-8.

46. Salathe EP, Venkataraman R. Interaction of fluid movement and particle diffusion across capillary walls. J Biomech Eng 1982;104:57-62.

47. Chew EY, Klein ML, Ferris FL III, et al, ETDRS Research Group. Association of elevated serum lipid levels with retinal hard exudate in diabetic retinopathy: Early Treatment Diabetic Retinopathy Study (ETDRS) report 22. Arch Ophthalmol 1996;114:1079-84.

48. Fong DS, Segal PP, Myers F, et al, Early Treatment Diabetic Retinopathy Study Research Group. Subretinal fibrosis in diabetic macular edema: ETDRS report 23. Arch Ophthalmol 1997;115:873-7.

49. Tsujikawa A, Sakamoto A, Ota M, et al. Serous retinal detachment associated with retinal vein occlusion. Am J Ophthalmol 2010;149:291-301.

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TABLES

Table 1. Correlation between fluorescein pooling and optical coherence tomographic characteristics of cystoid spaces in diabetic macular edema

	fluorescein pooling marked	fluorescein pooling modest	<i>p</i> -value
reflectivity levels in cystoid spaces on OCT images	12.1±10.4	22.0±15.4	<0.001
reflectivity patterns in cystoid spaces			
homogeneous	52	51	
heterogeneous	3	32	<0.001

OCT = optical coherence tomography.

Table 3. Hyperreflective foci in cystoid spaces in diabetic macular edema

	hyperreflective foci in cystoid spaces present	hyperreflective foci in cystoid spaces absent	<i>p</i> -value
fluorescein pooling			
marked	13	41	
modest	48	36	<0.001
reflectivity levels in cystoid spaces on OCT images	23.8±11.3	13.6±15.2	<0.001
reflectivity patterns in cystoids spaces			
homogeneous	38	65	
heterogeneous	23	12	0.005

OCT = optical coherence tomography.

Table 4. Association between microaneurysms and optical coherence tomographic reflectivity in diabetic macular edema

	microaneurysms in fluorescein pooling present	microaneurysms in fluorescein pooling absent	<i>p</i> -value
fluorescein pooling			
marked	14	40	
modest	29	55	0.348
reflectivity levels in cystoid spaces on OCT images	23.4±12.8	16.3±15.7	<0.001
reflectivity patterns in cystoids spaces			
homogeneous	26	77	
heterogeneous	17	18	0.019

OCT = optical coherence tomography.

Table 5. Fluorescein pooling or optical coherence tomographic characteristics in cystoid spaces with vitreomacular traction in diabetic macular edema

	VMT (+)	VMT (-)	<i>p</i> -value
fluorescein pooling			
marked	11	43	
modest	11	73	0.341
reflectivity levels in cystoid spaces on OCT images	14.9±9.8	18.7±15.2	0.301
reflectivity patterns in cystoid spaces			
homogeneous	17	86	
heterogeneous	5	30	1.000
hyperreflective foci in cystoid spaces			
present	8	53	
absent	14	63	0.488
microaneurysms in fluorescein pooling			
present	5	38	
absent	17	78	0.455

VMT = vitreomacular traction.

OCT = optical coherence tomography.

FIGURE LEGENDS

Figure 1. Three patterns of foveal pathomorphologies in diabetic macular edema.

Spectralis optical coherence tomography delineated serous retinal detachment (A), cystoid macular edema (B), and retinal swelling (C) at the presumed foveal center. Arrow: presumed foveal center. Scale bar = 200 μ m.

Figure 2. Fluorescein pooling and cystoid spaces delineated by optical coherence tomography.

Fluorescein angiography in the late phase (B) showed fluorescein pooling at the fovea, compared to the early phase (A). (C) A cystoid space was delineated on the optical coherence tomography (OCT) image, in the area corresponding to the pooling. (F) In several eyes, cystoid spaces on OCT image were not accompanied with hyperfluorescence in both the early (D) and late (E) phases. OCT images were dissecting along the white arrows in the late phases. Black arrow: cystoid spaces.

Figure 3. Various patterns of optical coherence tomographic reflectivity in cystoid spaces.

(A) A cystoid space had the homogeneous and lower reflectivity on optical coherence tomography image. (C) A cystoid space with homogeneous and higher reflectivity was accompanied with hyperreflective foci. (E) A cystoid space showed the heterogeneity in reflectivity. Cystoid spaces were traced and encircled (black curved line), followed by the quantification of the average reflectivity, as shown in the Methods section. The relative reflectivity in each cystoid space was -1.3 (B), 108.9 (D), or 23.8 (F). Arrows: cystoid spaces. Arrowheads: hyperreflective foci. Scale bar = 200 μ m.

Figure 4. Negative correlation between fluorescein intensity in pooling and optical coherence tomographic reflectivity in cystoid spaces.

Fluorescein angiography in the late phase (B) showed marked intensity of fluorescein pooling (arrowheads), compared to the early phase (A), and corresponding cystoid spaces (arrowheads) had lower reflectivity on optical coherence tomography (OCT) image (E). (D) Fluorescein intensity in pooling was modest in the late phase (arrowheads), whereas dye pooling was absent in the early phase (C). The corresponding cystoid space (arrowheads) showed higher reflectivity with hyperreflective foci (black arrows) on OCT image (F). OCT images were dissecting along the white arrows in the late phases.

Figure 5. No fluorescein pooling in serous retinal detachment.

Fluorescein angiography in the early (A) or late phase (B) did not show fluorescein pooling in the area corresponding to serous retinal detachment delineated on optical coherence tomography image (C).

Figure 1.

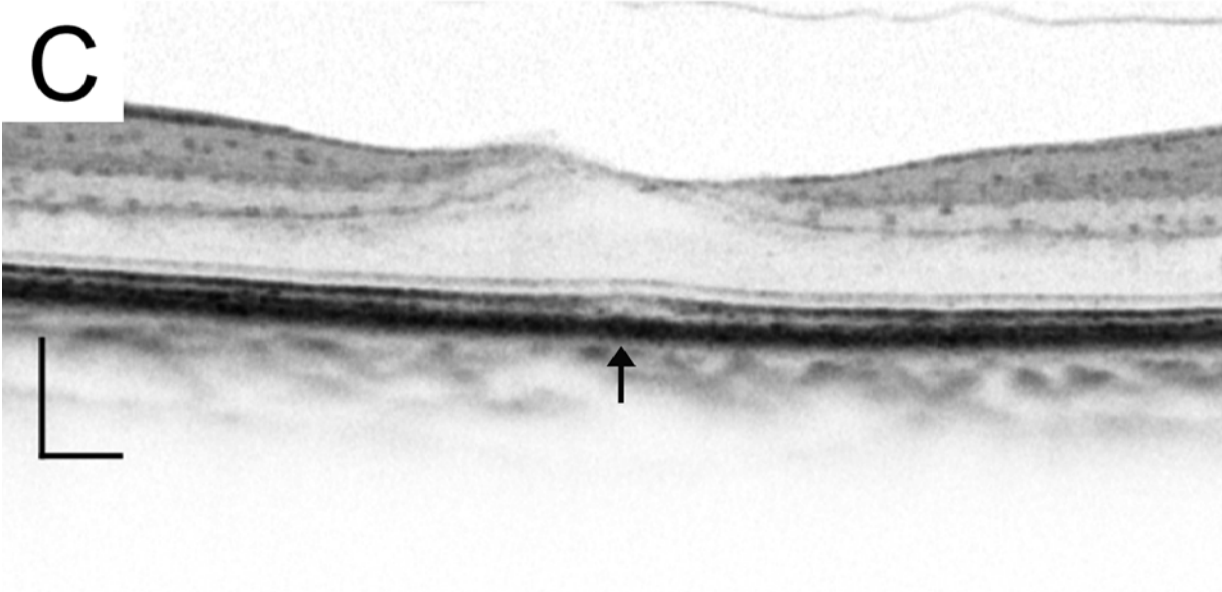
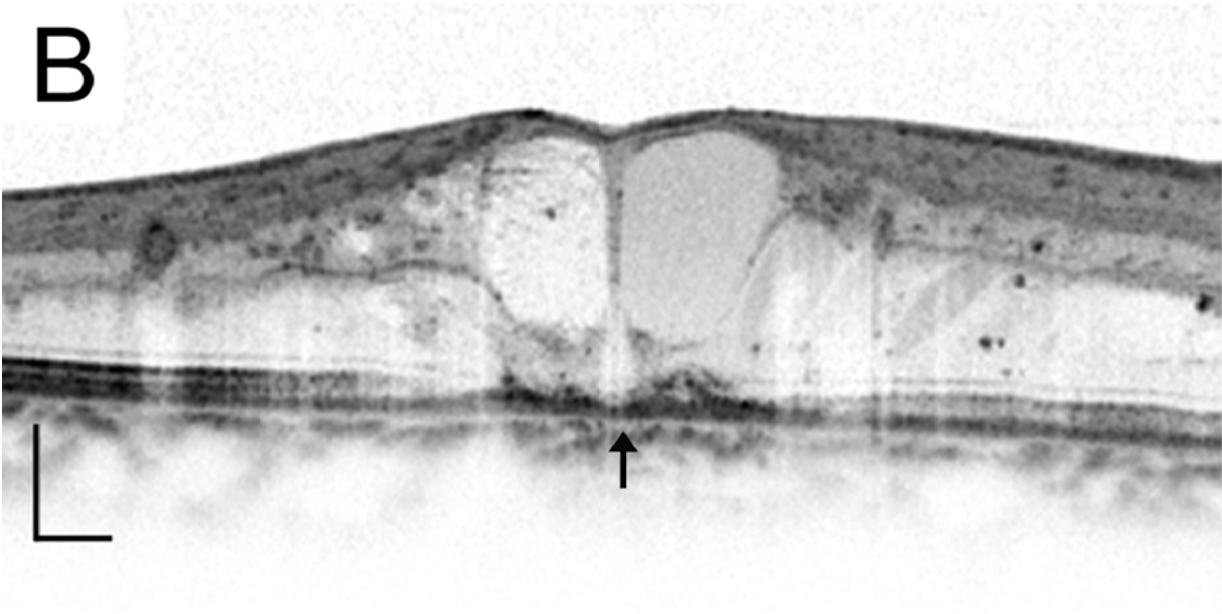
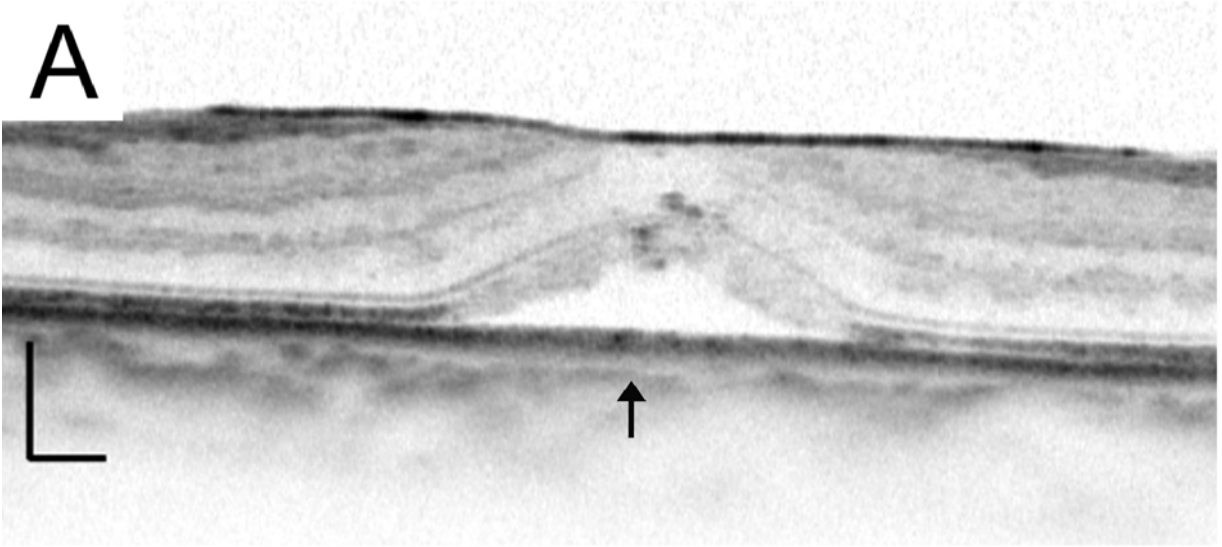


Figure 2.

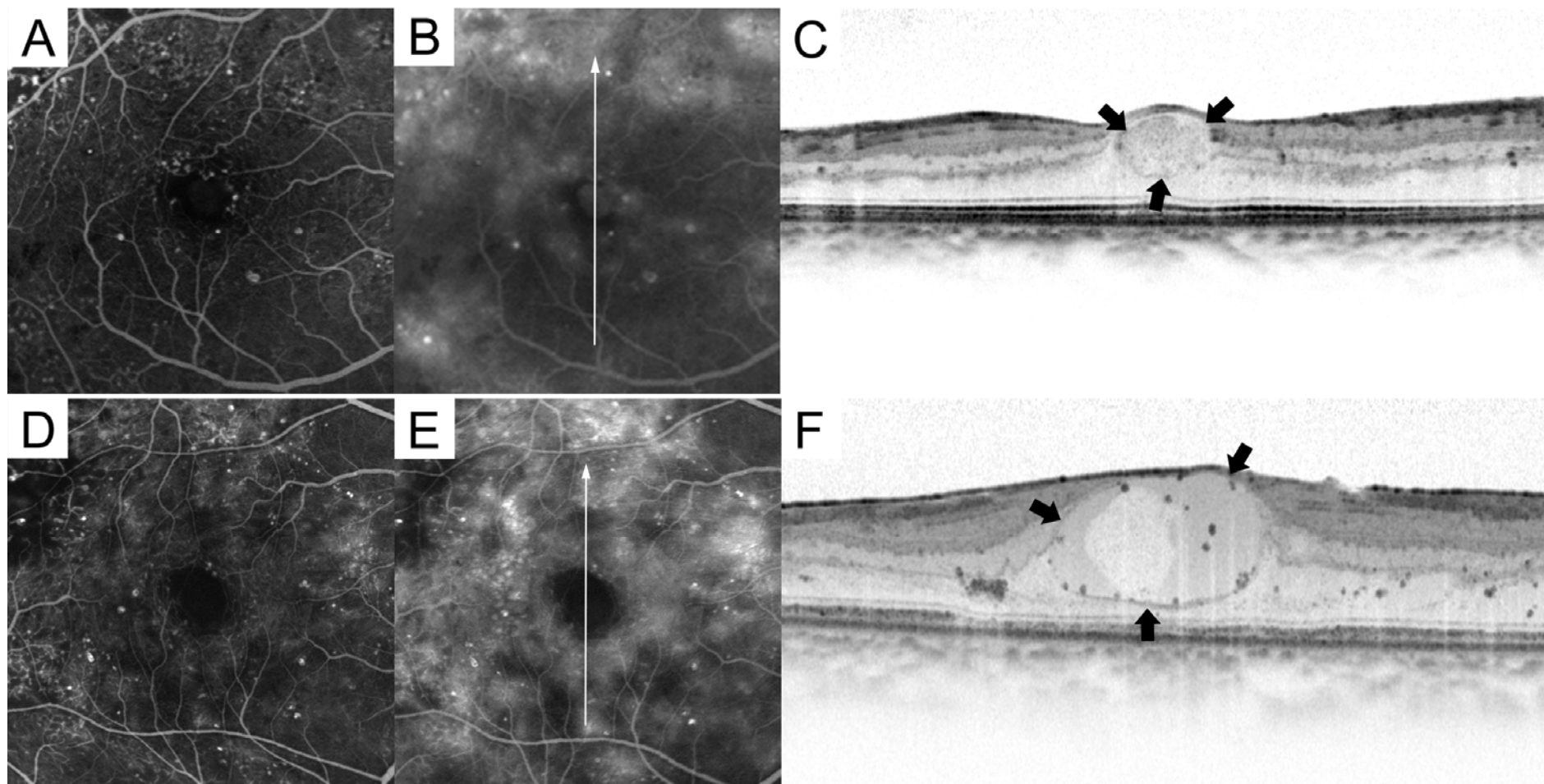


Figure 3.

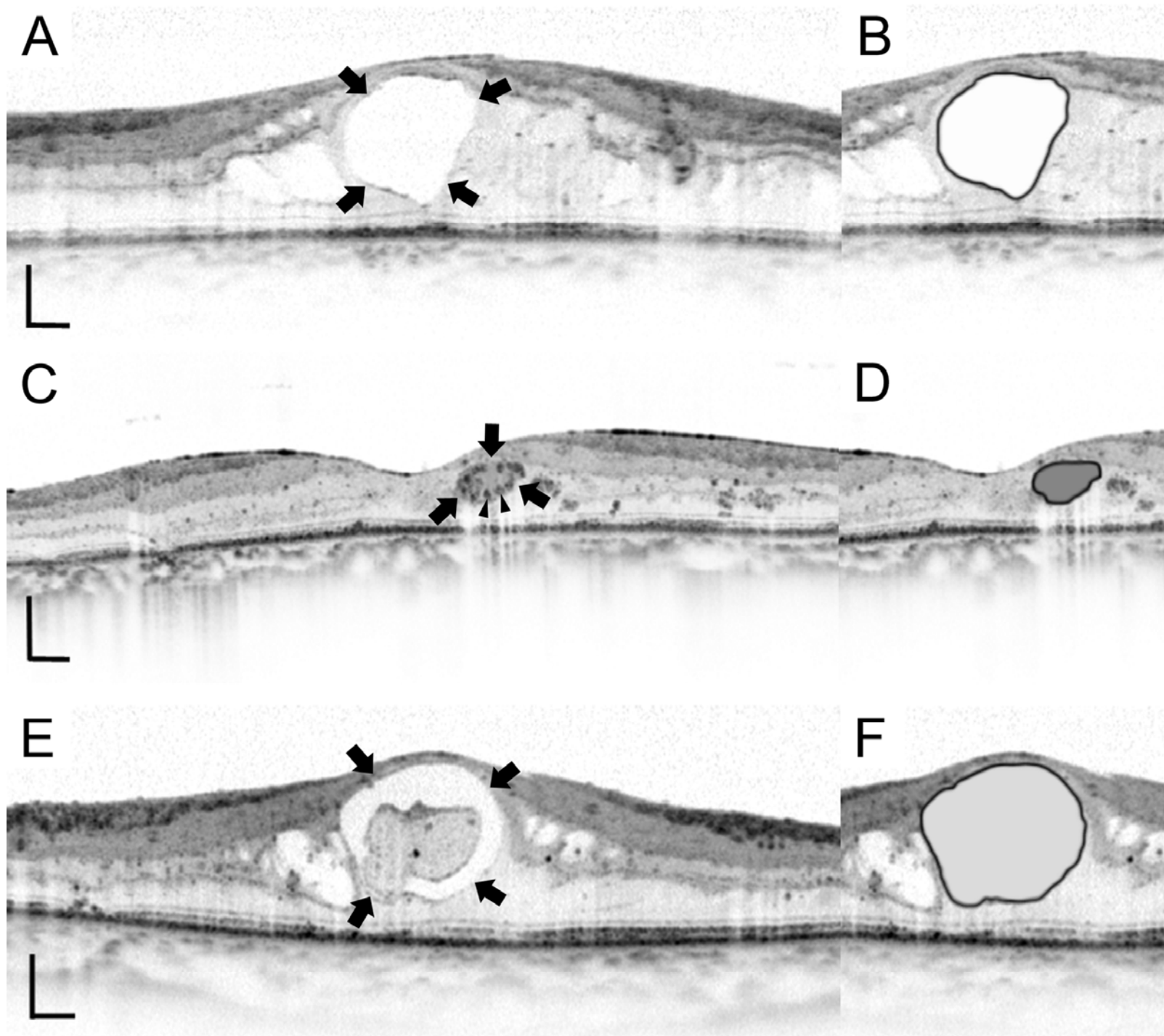


Figure 4.

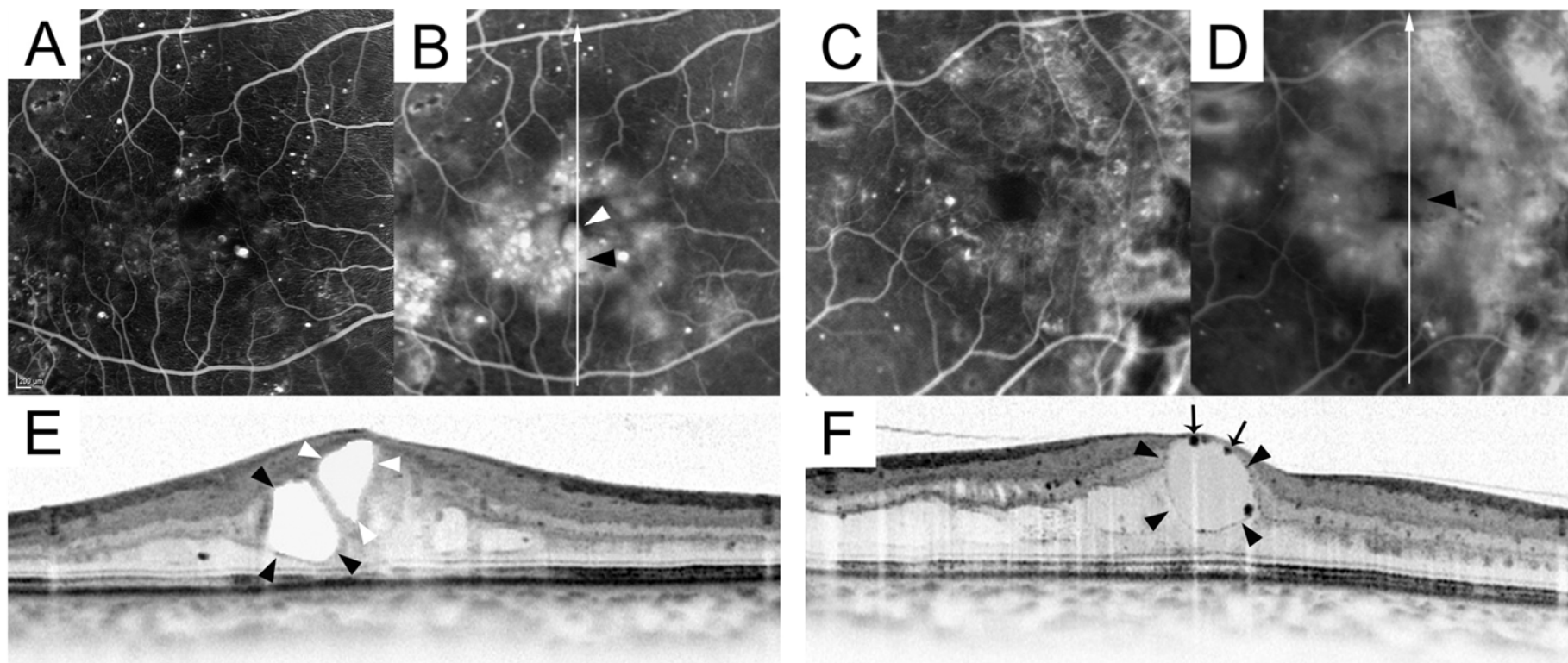
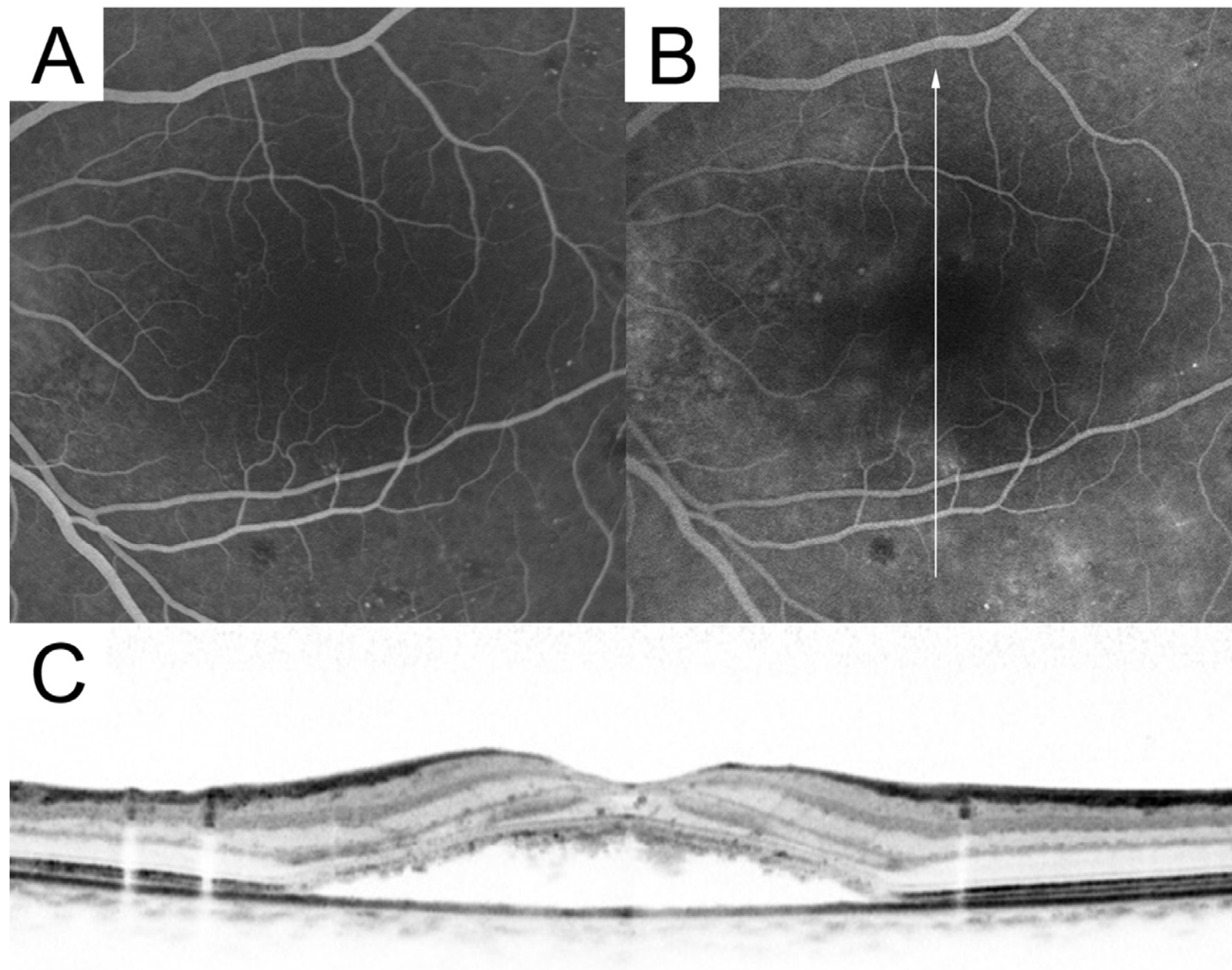


Figure 5.



Supplemental Data

Table 2. Automatically calculated reflectivity levels in 139 cystoid spaces on optical coherence tomography images in diabetic macular edema

	reflectivity levels in cystoid spaces on OCT images	<i>p</i> -value
fluorescein pooling		
marked	12.6±9.9	<0.001
modest	22.7±16.1	
hyperreflective foci		
present	24.3±12.6	<0.001
absent	14.2±15.1	
microaneurysms in fluorescein pooling		
present	22.8±11.9	0.001
absent	17.0±15.7	
VMT		
present	17.5±13.7	0.464
absent	19.0±15.1	

OCT = optical coherence tomography.

VMT = vitreomacular traction.