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

This article contains online-only material. The following should appear online-only: Table 2.

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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 $P=0.019$,

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

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

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

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

Optical Coherence Tomography

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

lower dye pooling intensity.

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

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

Interestingly, 35 cystoid spaces had heterogeneity in the reflectivity levels with clear borders. We speculated that higher reflectivity might correspond to blood clots or hyaline deposits in pathohistologic findings,[34-35] whereas the areas with lower reflectivity might be filled with fluid. Among several possible components of hyaline deposits, most might be 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

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

We did not see fluorescein pooling in the subretinal fluids in eyes with SRD associated with DME, compared to the breakdown of outer BRB in central serous chorioretinopathy (CSC). Intriguingly, VMT was significantly associated with SRD in DME, which might explain this discrepancy. We further found that cystoid spaces had higher levels of reflectivity on OCT images than SRD. These data seem inconsistent with the negative correlation between fluorescein pooling and OCT reflectivity in the cystoid spaces. Additionally, VMT did not affect the qualitative characteristics in the contents of cystoid spaces, although their volume might be exacerbated. No studies have definitively reported the pathogenesis of SRD development or maintenance in DME, although the data in the current study suggested that the pathogenesis of SRD differs at least partly from that of the cystoid spaces. Recent studies showed that the continuity from the intraretinal cystoid spaces to subretinal fluids in SRD due to branch retinal vein occlusion[49] and the pathogenesis might be combined, after time passed by.

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

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

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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	*p*-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	*p*-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	*p*-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 (-)	*p*-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
hyperreflecitve 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 μm.

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 spases 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 μm.

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.

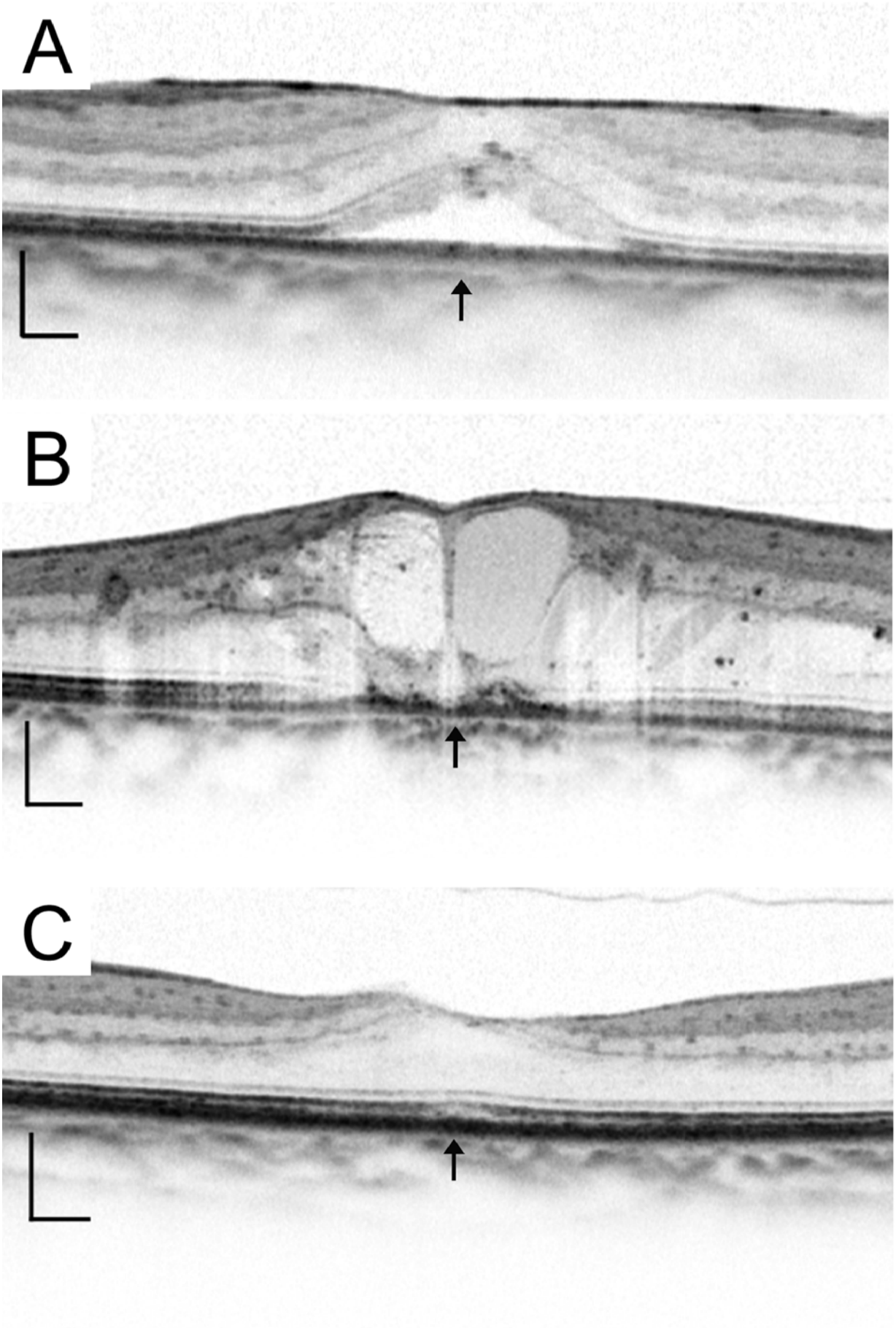


Figure 2.

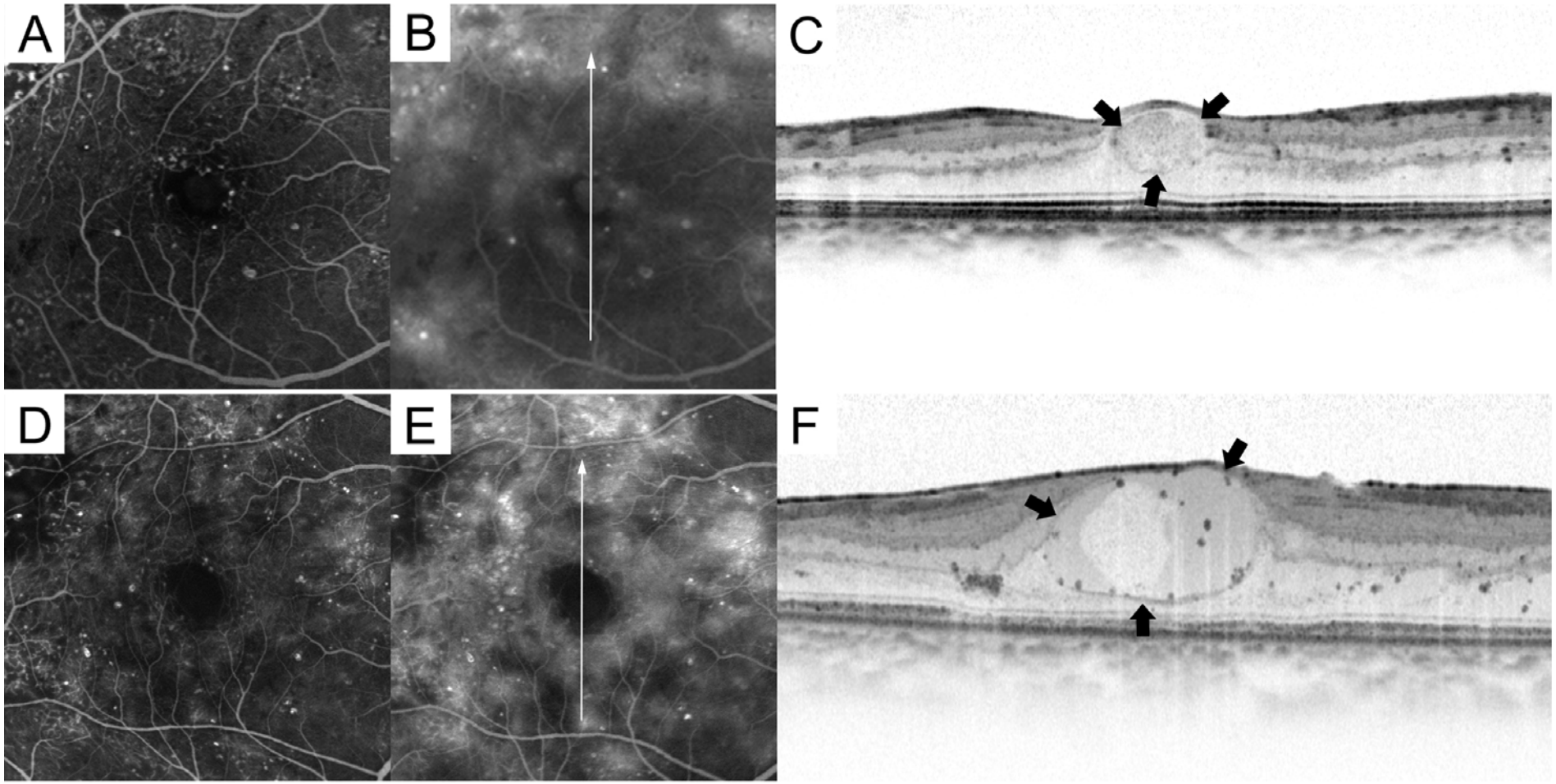


Figure 3.

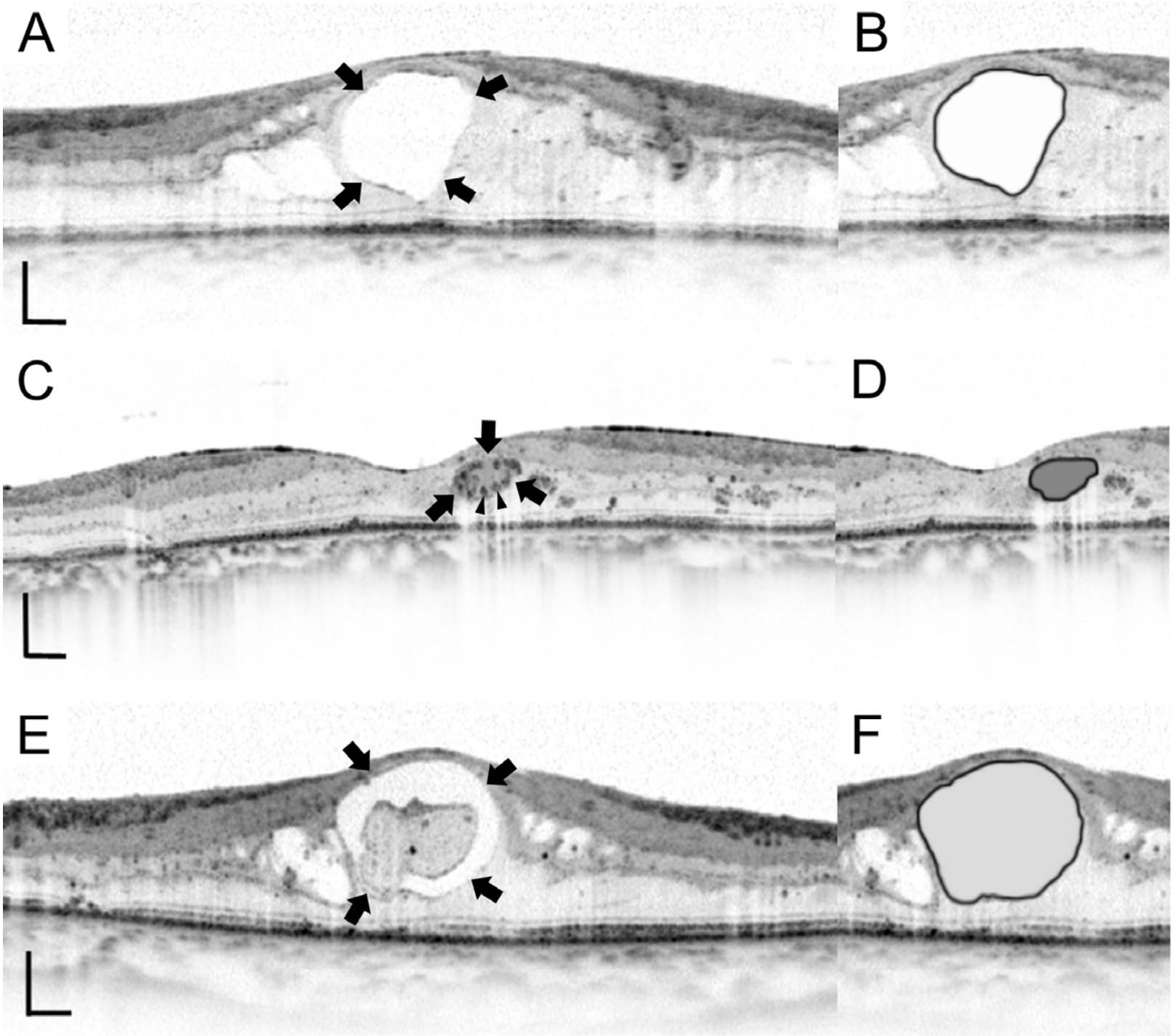


Figure 4.

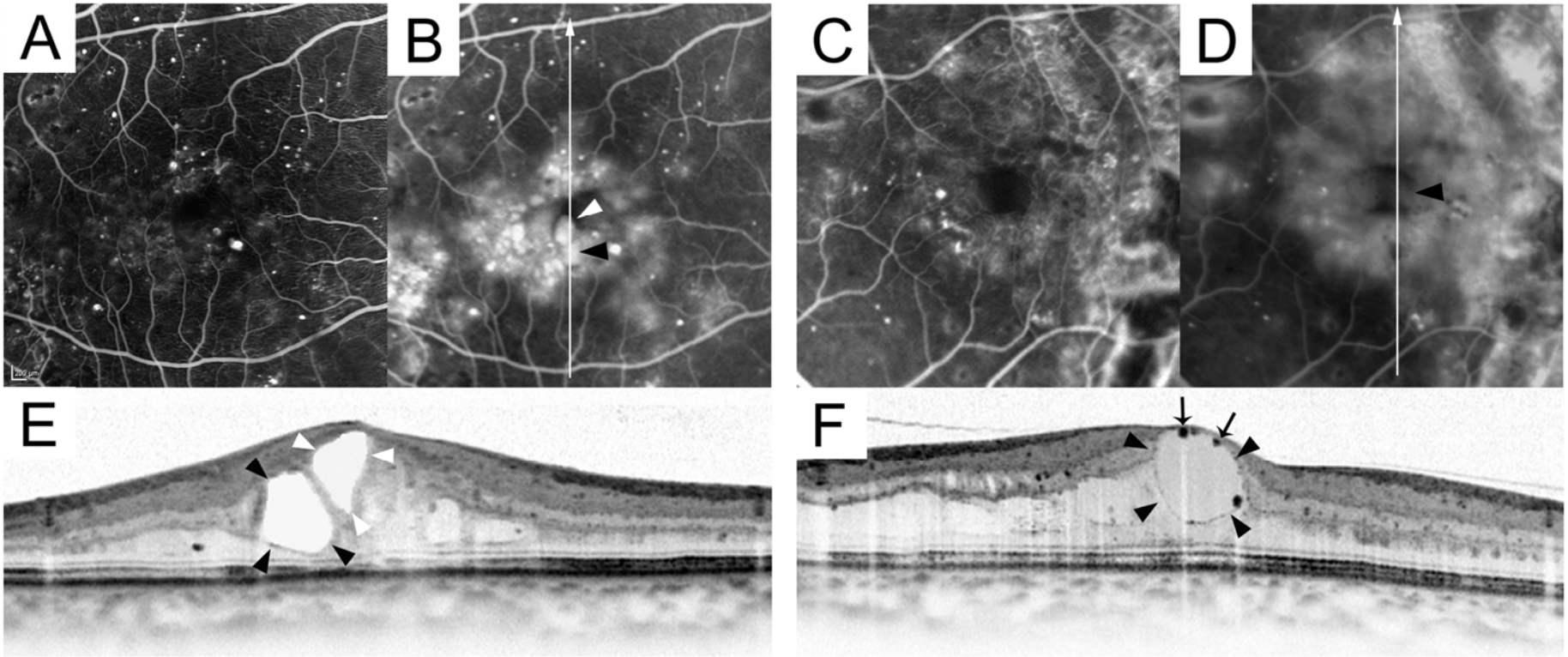
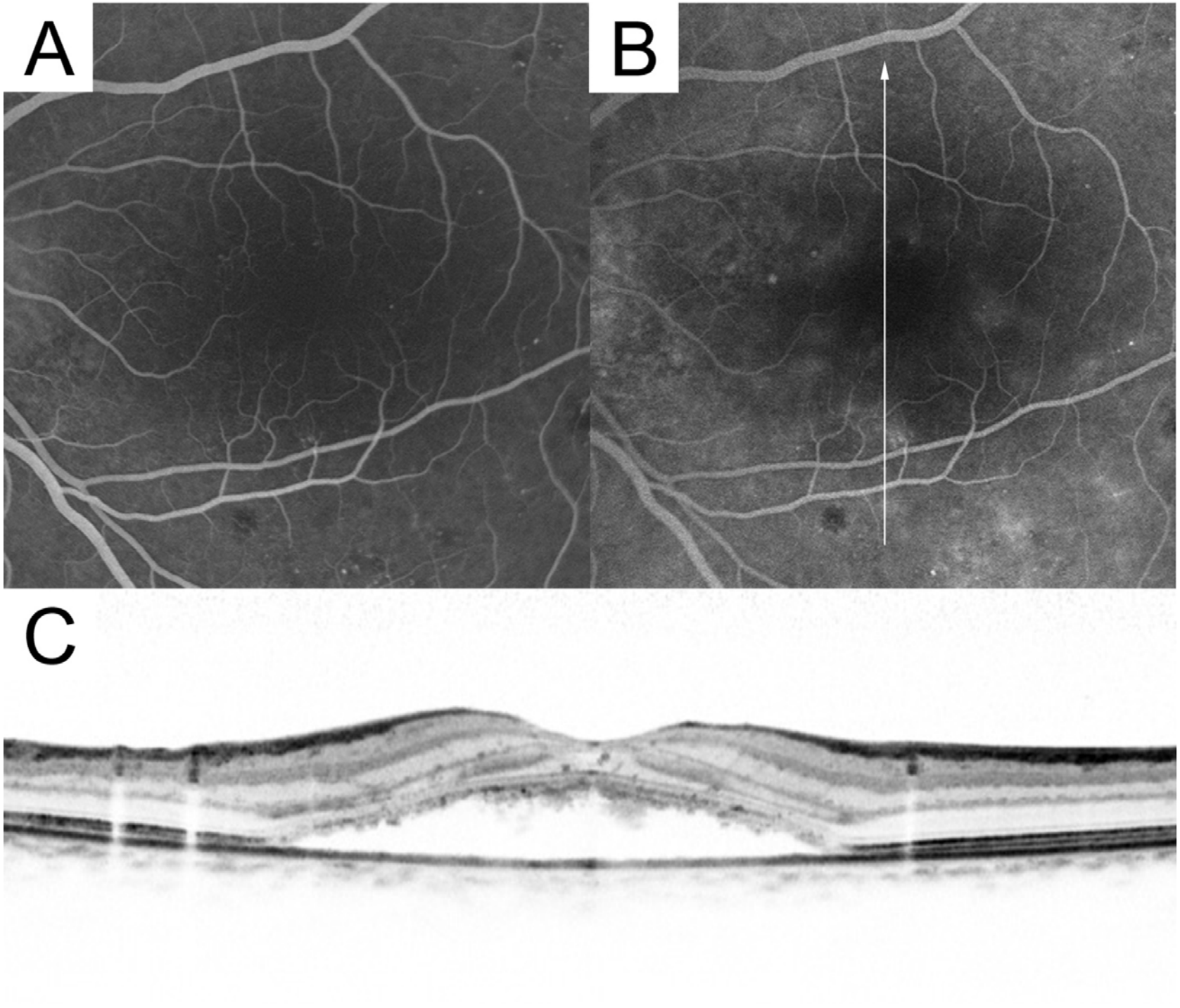


Figure 5.



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	*p*-value
fluorescein pooling		
marked	12.6±9.9	
modest	22.7±16.1	<0.001
hyperreflecitve foci		
present	24.3±12.6	
absent	14.2±15.1	<0.001
microaneurysms in fluorescein pooling		
present	22.8±11.9	
absent	17.0±15.7	0.001
VMT		
present	17.5±13.7	
absent	19.0±15.1	0.464

OCT = optical coherence tomography.
VMT = vitreomacular traction.