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# Differential Optical Densities of Intraretinal Spaces

Daniel Barthelmes,<sup>1,2</sup> Florian K. P. Sutter,<sup>3</sup> and Mark C. Gillies<sup>4</sup>

**PURPOSE.** To test the hypothesis that hyporeflective spaces in the neuroretina found on optical coherence tomography (OCT) examination have different optical reflectivities according to whether they are associated with exudation or degeneration.

**METHODS.** Retrospective analysis of eyes with idiopathic perifoveal telangiectasia (IPT), diabetic macular edema (DME), idiopathic central serous chorioretinopathy (CSC), retinitis pigmentosa (RP), or cone dystrophy (CD) and eyes of healthy control subjects. OCT scans were performed. Raw scan data were exported and used to calculate light reflectivity profiles. Reflectivity data were acquired by projecting three rectangular boxes, each 50 pixels long and 5 pixels wide, into the intraretinal cystoid spaces, centrally onto unaffected peripheral RPE, and onto the prefoveal vitreous. Light reflectivity in the retinal pigment epithelium (RPE), vitreous, and intraretinal spaces for the different retinal conditions and control subjects were compared.

**RESULTS.** Reflectivities of the vitreous and the RPE were similar among the groups. Hyporeflective spaces in eyes with exudation (DME, RP, and CSC) had higher reflectivity compared with the mean reflectivity of the vitreous, whereas the cystoid spaces in the maculae of the eyes without exudation (CD and IPT) had a lower reflectivity than did the normal vitreous.

**CONCLUSIONS.** Analysis of the light reflectivity profiles may be a tool to determine whether the density of hyporeflective spaces in the macula is greater or less than that of the vitreous, and may be a way to differentiate degenerative from exudative macular disease. (*Invest Ophthalmol Vis Sci.* 2008; 49:3529–3534) DOI:10.1167/iovs.07-1320

Several retinal diseases, such as diabetic retinopathy, exudative age-related macular degeneration, or retinitis pigmentosa, exhibit intraretinal cystoid spaces that are related to exudation (seen in fluorescein angiography), whereas in other conditions, such as macular telangiectasia or cone dystrophy (CD), such spaces occur without retinal thickening and thus are presumably due to degenerative processes. These intraretinal spaces can be documented by optical coherence tomography (OCT).<sup>1–4</sup> To date, the true nature and composition of the content of these spaces is unknown. However, clinicians and

scientists may be interested in whether these spaces exhibit different optical properties in the noninvasive OCT, and having this information may help them to evaluate retinal diseases more thoroughly. For example, the presence of retinal cysts may be an indication for retreatment with ranibizumab for age-related exudative macular degeneration; however, if these cysts are degenerative rather than exudative, retreatment may best be withheld.

Macular telangiectasia is an uncommon condition characterized by dilatation and incompetence of the perifoveal retinal capillaries.<sup>5–7</sup> In contrast to type 1, or aneurysmal, telangiectasia, which is characterized by exudation and is usually unilateral, type 2, or idiopathic perifoveal telangiectasia (IPT), is generally found in both eyes and is characterized by progressive thinning of the neurosensory macula without exudation.<sup>5–7</sup>

Cone dystrophies (CDs) are rare hereditary retinal diseases that primarily affect the cones.<sup>8,9</sup> Slow decay of the cone photoreceptors leads to photophobia and decreased visual acuity; some patients eventually become legally blind. The retina may appear normal in clinical examination in the early stages of CD, whereas in later stages subtle atrophic changes with pigmentary reaction may be found. The Ganzfeld electroretinogram (ERG) shows decreased signal amplitudes for cone-driven signals, whereas the rod-driven signals remain within normal ranges.

Inner and outer retinal cystoid spaces are commonly found in IPT.<sup>10</sup> The nature of the inner cystoid spaces, which do not appear to be the result of exudation, since they are not associated with macular thickening, is entirely obscure. Until now, it was believed that these spaces were mainly found in IPT; however, we have found an identical appearance in an eye that subsequently developed typical features of CD.<sup>11</sup> We have also observed outer retinal, also apparently nonexudative, cystoid spaces in eyes with CD (Barthelmes D, Gillies MC, unpublished data, 2006).

It has been shown that there is little variation in the reflectivity of the retinal pigment epithelium and the vitreous between patients with inherited retinal diseases and those with normal eyes, while differences in the reflectivity of intraretinal layers can be used to distinguish different diseases when analyzed quantitatively.<sup>12</sup>

We used OCT to evaluate intraretinal spaces in eyes with IPT and CD and to compare them with those found in DME, CSC, and RP to test the hypothesis that hyporeflective spaces have different optical reflectivities according to whether they are associated with exudation or degeneration.

## METHODS

### Patient Selection

In this retrospective study, we examined eyes of patients with cone dystrophy (CD), idiopathic perifoveal telangiectasia (IPT), diabetic macular edema (DME), idiopathic central serous chorioretinopathy (CSC), or retinitis pigmentosa (RP). Three patients with CD were included; for the other diseases, six patients were included in each group, and one eye was examined in each patient. Eyes with type II IPT were chosen from 14 patients participating in a natural history study of that condition (see [www.mactelresearch.org/](http://www.mactelresearch.org/) The Macular Telangiectasia Project conducted by a consortium of scientists at centers in the

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United Kingdom and the United States). Diagnostic criteria for IPT were the presence of small telangiectatic vessels characteristically found inferotemporally within 1 disc diameter of the foveola; staining by fluorescein on angiography but without retinal thickening or exudation; loss of central macular transparency; and the potential presence of discrete superficial white crystals.

The diagnosis was confirmed in all cases by the Reading Centre at Moorfields Eye Hospital (London, UK). Eyes with CD and RP were chosen from patients referred to the Electrophysiology Laboratory at the Department of Ophthalmology of the University Hospital (Zurich, Switzerland). CD and RP were diagnosed based on best corrected visual acuity, slit lamp examination, Ganzfeld electroretinography (ERG), and funduscopy.<sup>13,14</sup> Patients with cystoid DME, secondary to diabetic retinopathy, and idiopathic CSC were chosen by sequential retrospective review of patient records, fluorescein angiography, and OCT findings from the retina clinics of the Sydney Eye Hospital. In addition, 20 eyes of 10 healthy control subjects were examined with OCT.

We identified six eyes with intraretinal spaces for each of four diagnoses—IPT, macular edema secondary to RP, DME, and idiopathic CSC—and three eyes with intraretinal space in the CD group. Based on the appearance of exudation in fluorescein angiography and its correlation with intraretinal spaces, these groups were chosen to represent exudative and degenerative, nonexudative maculopathies. Eyes for analysis were identified before performance of quantitative OCT analysis. Ethics committee supervision for this noninterventional retrospective analysis was not required. The study was performed in accordance with the tenets of the Declaration of Helsinki 1975 (1983 revision).

### Quantitative OCT Analysis

OCT scanning was performed (Stratus OCT, software ver. 4.01; Carl Zeiss Meditec AG, Oberkochen, Germany) with the built-in macular thickness scanning program, consisting of six radially arranged scan lines, each 6 mm long, 30° apart, and centered in the foveola. All OCT recordings were performed under the same conditions.

Only scans with excellent quality (quality factor, 9 or 10) were used. For quantitative analysis, unprocessed raw scan data were exported from the OCT machine. Interpreting the raw scan data as 32-bit gray-scale images resulted in 4096 levels of gray, ranging from 0 to 4095. Light reflection profiles (LRP) for each image were calculated for each OCT image (IGOR 5.05a; Wavemetrics, Inc., Lake Oswego, OR).<sup>12</sup> Reflectivity data were acquired by placing three rectangular boxes, each 50 pixels long and 5 pixels wide, into the cystoid spaces, centrally onto unaffected peripheral RPE, and in the prefoveal vitreous, by using the built-in image line profile procedure (Fig. 1). Instead of analyzing single data points, which can have rather high or low reflectivity values, we calculated the mean reflectivity of 250 data points from every area measured (RPE, vitreous, and cystoid space) in every eye. Details are given in Table 1. Because raw data were used, reflectivity was expressed in arbitrary units (AU) according to the reflectivity values instead of decibels.

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### Statistical Analysis

Statistical analysis was performed with commercial software (Statistica 6; StatSoft Inc., Tulsa, OK). One-way analysis of variance (ANOVA) was used to test for differences between the different groups, including post hoc testing. Statistical significance was prospectively defined as  $P < 0.05$ . Because of the low number of patients<sup>3</sup> with intraretinal spaces in the CD group, the data from this group were not included in the ANOVA. For evaluation, the data from the CD group were plotted against normal values.

## RESULTS

### Hyporeflective Spaces in Type II IPT and CD

Hyporeflective intraretinal spaces were found in 17 of 28 eyes with type II IPT and were similar to those previously described (Fig. 2).<sup>10,15</sup> We identified 42 patients with CD. For three of these patients, cystoid spaces were found in both eyes.

### Hyporeflective Spaces in DME and CSC

Figure 2 shows typical cross-sectional OCT studies through the central macula of eyes with RP, CSC, and DME.

### Quantitative OCT Analysis of Intraretinal Hyporeflective Spaces

Mean reflectivities of the RPE, vitreous, and intraretinal spaces for each eye and each condition are shown in Table 1. The mean optical reflectivities of the vitreous and the RPE were similar for all groups tested (Fig. 3). The mean optical reflectivity of hyporeflective spaces in eyes with intraretinal spaces due to exudation (DME, RP, and CSC) was higher than that of the vitreous, whereas that of the eyes with intraretinal spaces without exudation (CD and IPT) had a lower reflectivity than did the normal vitreous. The pooled data from IPT show a significantly lower reflectivity of the intraretinal spaces compared with the pooled reflectivity data from DME, CSC, and RP (Fig. 3).

This difference between the reflectivity of hyporeflective spaces in both groups persisted, even when the data from the group with CSC, which had the highest optical reflectivity, were removed (Fig. 3). In the group with exudative origin of the intraretinal spaces, each eye examined had a higher reflectivity within the intraretinal cavities compared with the reflectivity of the vitreous, whereas in the group without exudation, all intraretinal cavities had lower reflectivity than did the vitreous in each eye (Table 1).

An interesting finding was the presence of intraretinal spaces, not related to exudation, in patients with CD. A short description of each patient follows.

#### Case 1

A 53-year-old female presented with photophobia and visual acuity (VA) of 20/50 in both eyes. Clinical examination showed central atrophy and pigment changes. Ganzfeld ERG showed reduced cone-driven responses and normal rod-driven responses. OCT examination revealed significant central macular thinning and intraretinal spaces identical with those seen in type II IPT (Fig. 4).

#### Case 2

A 22-year-old female presented with VA of 20/200 in both eyes. A broadened foveal reflex with fine central pigment mottling but no other abnormalities was found on fundus examination.



**FIGURE 1.** Sites of analysis (*white bars*) of optical reflectivity of the vitreous (1), hyporeflective spaces (2) and the retinal pigment epithelium (3) in an OCT cross-sectional image from raw scan data.



TABLE 1. Reflectivity in Each Eye in Each Region

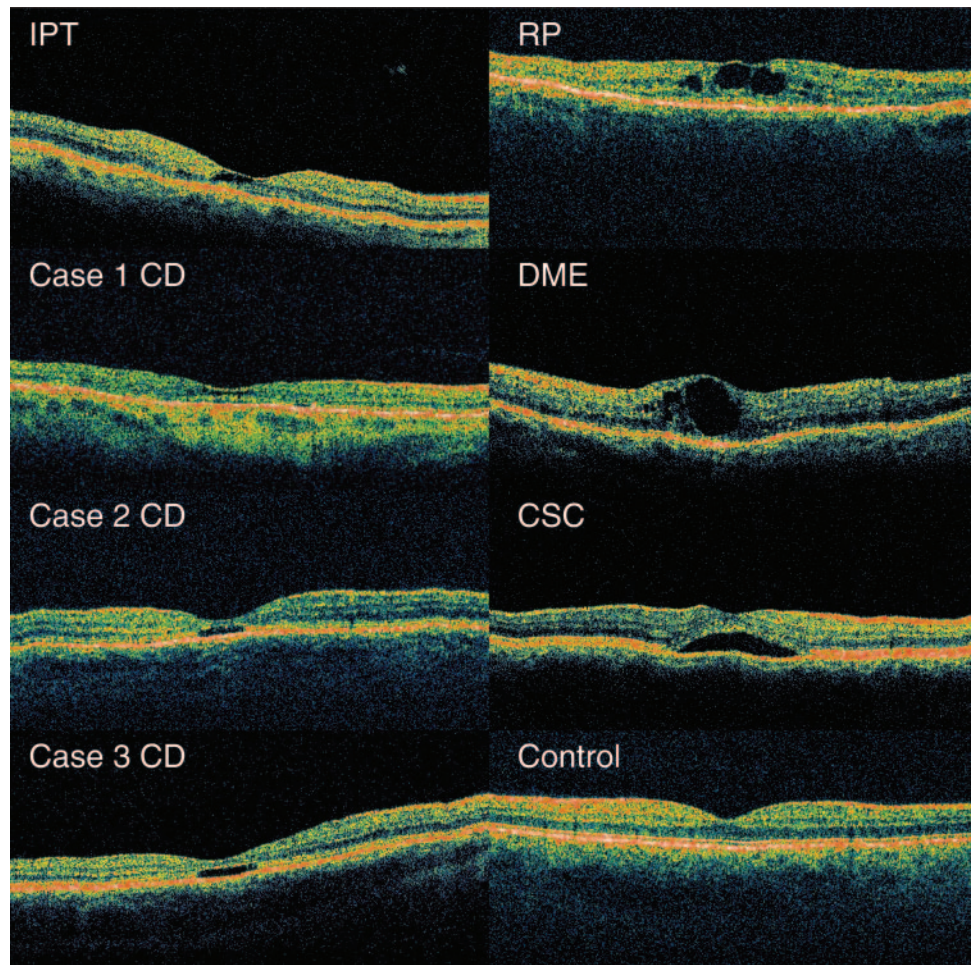
Group	Region Tested	Eye Tested					
		1	2	3	4	5	6
Normal	RPE	2215.7 ± 195.7	2211.6 ± 189.6	2153.24 ± 199.02	2194.83 ± 183.15	2281.87 ± 225.4	2166.44 ± 206.82
	Vitreous	966.86 ± 59.32	969.46 ± 66.08	964.46 ± 60.8	970.87 ± 62.25	969.85 ± 85.71	964.89 ± 64.44
	Cystoid space	NA	NA	NA	NA	NA	NA
CSC	RPE	2226.3 ± 213.7	2219.58 ± 177.83	2215.78 ± 208.4	2236.07 ± 212.05	2076.91 ± 235.05	2110.17 ± 219.88
	Vitreous	968.4 ± 66.98	962.3 ± 83.32	960.5 ± 41.64	965.06 ± 41.16	971.42 ± 46.27	974.22 ± 62.83
	Cystoid space	1009.79 ± 69.84	1016.38 ± 80.56	996.27 ± 52.33	997.8 ± 51.86	993.49 ± 48.04	1030.46 ± 89.15
DME	RPE	2097.32 ± 236.29	2047.41 ± 216.15	2131.45 ± 206.9	2221.72 ± 205.31	2239.83 ± 276.41	2356.29 ± 297.03
	Vitreous	965.95 ± 55.64	967.36 ± 50.95	964.94 ± 56.71	970.05 ± 64.63	968.91 ± 56.16	962.37 ± 57.69
	Cystoid space	968.18 ± 62.88	969.06 ± 58.66	970.6 ± 58.31	982.13 ± 59.98	989.75 ± 56.93	964.89 ± 56.64
IPT	RPE	2217.15 ± 163.55	2205.68 ± 167.96	2216.87 ± 215.69	2231.14 ± 238.78	2176.53 ± 244.95	2052.77 ± 199.01
	Vitreous	963.23 ± 49.3	952.69 ± 53.68	968.17 ± 78.01	987.82 ± 72.95	996.11 ± 75.15	979.28 ± 90.22
	Cystoid space	940.04 ± 60.97	952.49 ± 62.35	969.28 ± 57.67	936.05 ± 57.78	938.68 ± 48.73	961.99 ± 59.01
RP	RPE	2149.32 ± 245.88	2254.83 ± 219.34	2153.15 ± 231.33	2192.37 ± 210.23	2114.88 ± 226.17	2220.47 ± 212.48
	Vitreous	968.65 ± 49.76	965.36 ± 56.32	962.26 ± 55.72	962.39 ± 57.91	970.5 ± 52.63	971.65 ± 61.66
	Cystoid space	971.69 ± 54.78	967.18 ± 54.31	975.58 ± 57.47	981.27 ± 54.08	973.89 ± 54.5	979.84 ± 59.19
CD	RPE	2179.57 ± 162.57	2206.38 ± 235.82	2183.48 ± 287.08			
	Vitreous	967.38 ± 70.09	967.17 ± 81.42	969.05 ± 57.96			
	Cystoid space	940.43 ± 50.55	958.09 ± 79.95	961.43 ± 87.99			

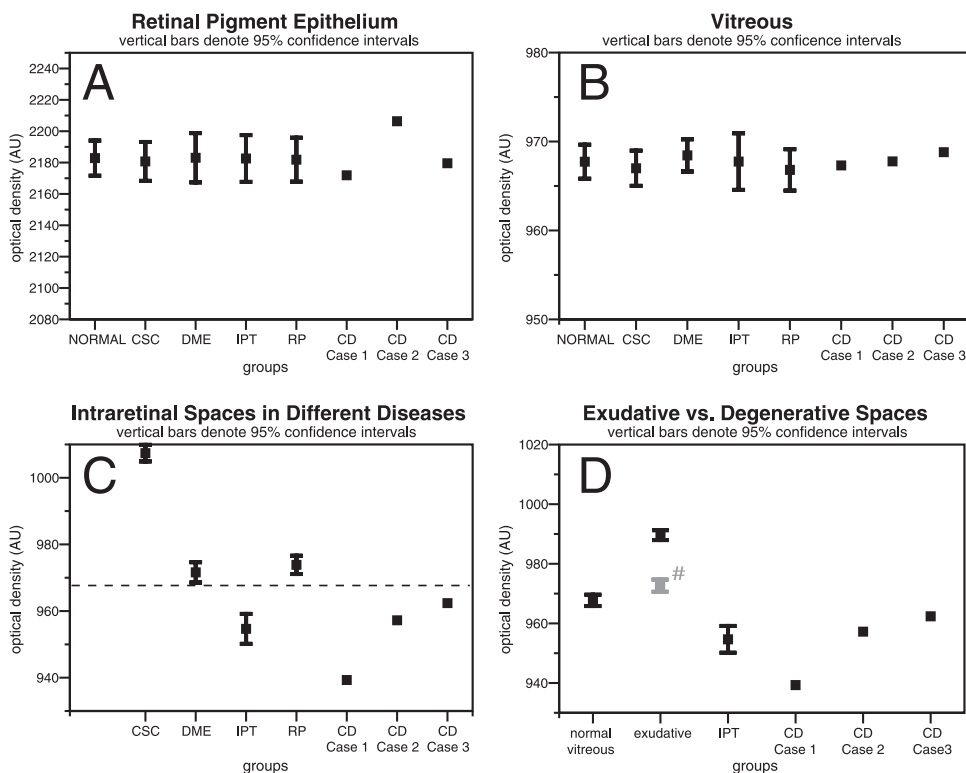
Reflectivity data are expressed as the mean AU ± SD.

Ganzfeld-ERG showed normal rod-driven responses. Cone-driven responses in single-flash stimulation were slightly sub-normal, but testing with 30-Hz flicker stimulation revealed a significant reduction of b-wave amplitude and a dramatic in-

crease in implicit time in both eyes. OCT revealed an outer retinal cystoid space in the central foveola. Fluorescein angiography did not show any leakage but identified a small window defect (Fig. 4).

FIGURE 2. Representative pseudocolor cross-sectional OCT images of the different maculopathies and a control subject are shown. The images were processed by OCT Stratus software (Carl Zeiss Meditec, AH, Oberkochen, Germany) and were exported as JPEG images. IPT represents a scan through an inner retinal cystoid space in an affected eye. Cases 1, 2, and 3 (CD) represent OCT scans through an inner cystoid space (case 1) and outer retinal cystoid spaces (cases 2 and 3) in patients with CD. A case of a macular edema secondary to RP is shown in the top right panel, and a DME in the panel immediately below. CSC represents a typical OCT scan through a focal neurosensory retinal detachment secondary to idiopathic CSC. Bottom right: a control subject's OCT scan.





**FIGURE 3.** (A) Mean optical reflectivities of the RPE in control subjects (normal) and the five disease groups. The mean results for the three CD cases are plotted. No significant differences are found. (B) Similar mean reflectivities of the vitreous of control subjects and patients. (C) Reflectivities of the different cystoid spaces. *Dashed line:* average reflectivity of the vitreous. The reflectivity of cystoid lesions in IPT was significantly less than the reflectivity of the cystoid spaces in eyes with exudative maculopathy. Also, the three values from the three patients with CD were below the average in normal vitreous. (D) Mean pooled reflectivity of cystoid spaces in IPT was significantly less than that of spaces in the exudative maculopathies (DME, CSC, RP; mean  $\pm$  SD,  $954.6 \pm 64.3$  vs.  $989.6 \pm 65.4$  AU  $P < 0.0001$ ). When the data from the CSC group were removed from exudative maculopathies, the comparison between nonexudative and exudative intraretinal cavities still showed a significantly higher reflectivity ( $954.6 \pm 64.3$  vs.  $972.8 \pm 57.7$  AU,  $\#P < 0.001$ ) for exudative cystoid spaces. The three CD patients showed much lower reflectivity than did patients with exudation-associated intraretinal spaces.

**Case 3**

A 21-year-old male presented with progressive loss of visual acuity (20/60 in both eyes). Some pigment epithelial changes were visible in the central macula in both eyes. Fluorescein angiography showed a window defect but no leakage, consistent with attenuation of the RPE.

OCT revealed outer retinal cystoid spaces in the central macula of both eyes. Ganzfeld ERG revealed normal rod-driven responses and significantly reduced cone-driven amplitudes in both single flash and 30-Hz flicker stimulation. Implicit times were abnormally increased under both conditions.

**DISCUSSION**

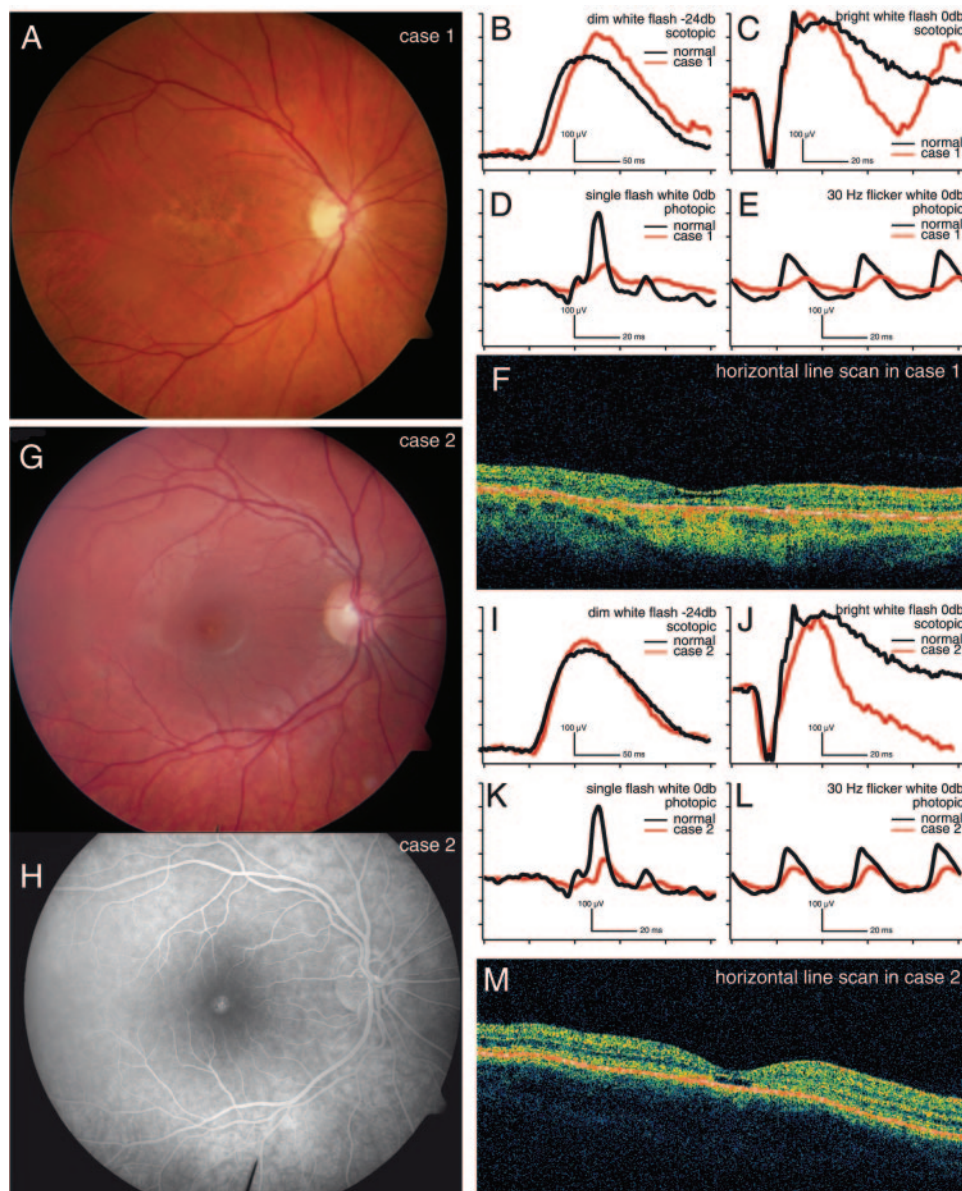
By quantitatively analyzing light reflectivity profiles from OCT studies, we have demonstrated that retinal hyporeflexive spaces in IPT and CD—conditions in which cysts occur without exudation—consistently have lower optical reflectivity than do spaces occurring in conditions associated with exudation. That exudative retinal cysts, for example in AMD, eventually subside in most eyes on treatment with anti-VEGF agents, whereas cysts associated with a nonexudative disease (IPT) are unaffected by such treatment, is further evidence of a difference between hyporeflexive spaces and is consistent with the

hypothesis that spaces without exudation have a degenerative etiology.<sup>16,17</sup>

The demonstration that intraretinal spaces of low reflectivity can have variations in appearance on OCT that are related to the underlying disease has the potential to improve clinical decision-making. For example, persistent intraretinal fluid, as evidenced by cystoid hyporeflexive intraretinal spaces, may be used as an indication to continue treatment with vascular endothelial growth factor inhibitors.<sup>1</sup> However, if these spaces result from nonexudative, or dry, degeneration, then the degeneration might be exacerbated by further treatment, and this treatment should be withheld.

The location of the cystoid spaces in these degenerative maculopathies may provide clues to their nature. Cystoid spaces in the outer retina are not unexpected in CD. Inner retinal spaces may also be anticipated in IPT, since the vascular changes that characterize the condition point to involvement of the inner retina as well. It is the presence of inner retinal cystoid spaces in eyes with CD that is surprising, since CD is understood to affect primarily the photoreceptors. The presence of inner retinal hyporeflexive spaces in eyes with CD suggests an influence of the outer retina on the inner retina, the nature of which is obscure. One possibility is that the death of photoreceptors results in retrograde degeneration of the inner retina, which eventually leads to formation of a cystoid space.





**FIGURE 4.** (A) Right fundus photograph from CD case 1, showing an atrophic central retina and the pigmentary changes in a 53-year-old patient. The OCT scan is shown (F). (B–E) ERG recordings with normal rod-driven responses and considerably decreased cone-driven responses. (G) A right eye fundus photograph from case 2 of CD. Compared with the older case 1, only slight pigment changes were found. (H) Respective fluorescein angiogram revealing a central window defect. (I–L) ERG recordings of normal rod-driven responses and significantly reduced cone-driven responses. (M) The corresponding OCT.

It has been proposed that the degeneration of the neural retina that characterizes IPT is primarily due to Müller cell damage.<sup>18,19</sup> Gass<sup>20</sup> drew attention to the “Müller cell cone,” a layer of Müller cells above the layer of Henle immediately beneath the inner limiting membrane (ILM) in the base of the foveal depression. Disease of the Müller cells may thus explain the “ILM drape” across cavities in the inner retina at the base of the foveal depression that are commonly found in eyes with IPT on OCT.<sup>10</sup>

Although the OCT reveals neither the origin nor the composition of the intraretinal spaces, the lower reflectivity of the spaces found in IPT and CD, compared with RP, CSC, and DME, may be due to the presence of albumin and acute-phase serum reactants in the latter, causing more backscattering. Macular edema secondary to diabetes and RP is recognized to be caused by a breakdown of the blood-retinal barrier, as is the subretinal fluid in CSC.<sup>21–24</sup> The lower reflectivity of spaces found in the putative degenerative maculopathies suggests that they have lower protein content, in which case they are probably not the result of breakdown of the blood-retinal barrier. It is more likely that the cysts represent areas of cell loss by apoptosis, which is not associated with inflammation or exu-

ation.<sup>18</sup> Therefore, we propose that cysts with an optical index greater than the vitreous have an exudative etiology, whereas those with reflectivity less than the vitreous are degenerative.

This study is limited by the relatively small number of participants. In particular, only two eyes of one participant with CD had inner retinal spaces identical with those seen in IPT. We have reported another case in which the development of inner retinal cystoid spaces typical of IPT was preceded by clinical and electrophysiological features of CD.<sup>11</sup> In the unlikely event that the association of inner cystoid spaces with CD was a coincidence, then conclusions drawn about the influence of the outer retina on the inner retina would be unfounded. However, the clear, quantifiable differentiation of intraretinal spaces on the basis of their optical reflectivity still stands.

Analyzing the light reflection profiles from OCT to determine whether the density of hyporeflexive spaces in the macula is greater or less than that of the vitreous may be a way to differentiate processes in the central macula. Further research is warranted to determine how this approach may be used to improve diagnosis and treatment of macular diseases.

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