Appearance of Regressing Drusen on Optical Coherence Tomography in Age-related Macular Degeneration

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Objective: To describe and interpret a multilaminar sub-retinal pigment epithelium (RPE) intense hyperreflectivity observed in vivo in eyes clinically diagnosed with regressing drusen.

Design: Observational case series.

Participants: Twenty-three consecutive patients clinically diagnosed with regressing calcific drusen due to nonneovascular age-related macular degeneration (AMD).

Methods: Patients were submitted to confocal scanning laser ophthalmoscopy (cSLO) fundus imaging and “eye-tracked” spectral-domain optical coherence tomography (SD-OCT).

Main Outcome Measures: Localization and possible origin and composition of the multilaminar sub-RPE hyperreflectivity.

Results: Thirty eyes of 23 consecutive patients (8 male and 15 female; mean age, 82.7 ± 10.1 years) showing on SD-OCT an intense multilaminar sub-RPE hyperreflectivity, which matched with regressing calcific drusen as visualized by cSLO infrared (IR) and MultiColor (Heidelberg Engineering, Heidelberg, Germany) images, were included in this study. The multilaminar hyperreflectivity was found to localize to beneath the RPE and above the outer Bruch’s membrane (oBM) layer. A mean of 1.2 multilaminar sub-RPE hyperreflectivities per SD-OCT scan were identified by 2 readers. The SD-OCT analysis allowed the 2 readers to describe 3 different types of sub-RPE hyperreflectivity.

Type 1: laminar/multilaminar hyperreflectivity (found in 24 scans of 12 eyes) was characterized by an intense signal originating from what we interpreted as the inner Bruch’s membrane (iBM) layer.

Type 2: multilaminar hyperreflectivity (found in 130 scans of 27 eyes) was characterized by an intense signal originating from the oBM layer.

Type 3: multilaminar fragmented hyperreflectivity (found in 22 scans of 11 eyes) was characterized by an intense signal originating from what we interpreted as both the iBM and the oBM, showing different degrees of fragmentation.

Conclusions: We describe a novel SD-OCT finding appearing as multilaminar sub-RPE intense hyperreflectivity observed in vivo in eyes with regressing drusen. This multilaminar sub-RPE hyperreflectivity could be interpreted as layers of lipid mineralization (membranous debris also called “lipoprotein-derived debris” developing calcification), internal and external to the basement membrane, with different degrees of fragmentation. Ophthalmology 2014;121:173-179 © 2014 by the American Academy of Ophthalmology.

Deposition of extracellular material between the basal lamina of the retinal pigment epithelium (RPE) and the inner collagenous layer of Bruch’s membrane is generally referred to as “drusen.”1 Drusen usually appear after the age of 50 years and are considered to be the main risk factors of age-related macular degeneration (AMD).2 Large AMD drusen are yellow-white, mound-like RPE elevations typically 63 to ≥1000 μm in diameter.3 Histopathologic investigations showed that large drusen are mounds external to the RPE basement membrane containing membranous debris (also called “lipoprotein-derived debris”), a neutral lipid-rich material.3 Basal linear deposits and large drusen should be regarded as the same lesion.4-6 A basal linear deposit consists of a thin, relatively extensive layer of membranous profiles.7 This is in contradistinction to a basal laminar deposit, a diffusely deposited material that lies internal to the RPE basement membrane, and is composed of fibrils, amorphous material, and a banded component that is reminiscent of long-spacing collagen.8 Drusen growth is characterized by the continued deposition (overproduction/underclearance) of drusen-associated constituents (membranous debris) developing calcification, internal and external to the basement membrane, with different degrees of fragmentation. Ophthalmology 2014;121:173-179 © 2014 by the American Academy of Ophthalmology.
photoceptor disk shedding, and assembly of an apolipoprotein B (apoB)-lipoprotein by RPE.

In drusen “maturation,” several layers of coiled membranes lay between the RPE and its basement membrane (membranous debris crossing the basal laminar deposit in strands or mounds). These membranous debris extend inward between adjacent RPE cells. A thin stratus of membranes also lay external to the basement membrane. Regressing drusen show evidence of not only reduced formation of membranous debris but also its elimination by macrophages. Material that is not removed develops calcification. A better understanding of the physiopathologic features of drusen is required to develop rational and effective AMD therapies. Drusen size, type, and composition reveal both past formative processes and future progression of disease. Because of the paucity of histopathologic information about eyes previously imaged in the clinic, we could refer to contemporary multimodal, high-resolution in vivo imaging to interpret differences in drusen appearance.

Spectralis high-resolution spectral-domain (SD) optical coherence tomography (OCT) (Spectralis SD-OCT; Heidelberg Engineering, Heidelberg, Germany) is a high-speed OCT system (up to 40 000 axials scans per second) using SD/Fourier domain detection, with an optical axial image resolution of 7 μm (digital axial image resolution of 3.5 μm). In Spectralis SD-OCT, confocal scanning laser ophthalmoscopy (cSLO) with real-time eye tracking technology allows one to track the eye and guide OCT to the selected location, thus giving a real-time reference for the automatic real-time averaging can be used with both IR and MultiColor to minimize noise. The Spectralis high-resolution SD-OCT system provides in vivo details of the anatomy of the retina that nearly resembles histologic specimens (optical axial image resolution of 7 μm). A new approach to improve depth imaging by OCT, termed “enhanced depth imaging” OCT, has been shown to reliably image the choroid. Enhanced depth imaging OCT uses a Spectralis SD-OCT positioned closer to the eye than usual such that a stable inverted image is produced. The net effect of this practice is that the sensitivity of the imaging in deeper layers of tissue is increased.

The SD-OCT minimum acquisition protocol included 19 horizontal lines, each composed of 9 averaged OCT B-scans (1024 A-scans per line) at 240-μm intervals and covering a 6 x 6-mm area; in a subset of eyes, further high-resolution 9-mm single B-scans (each composed of up to 100 averaged enhanced depth imaging OCT B-scans) were guided from the regressing drusen as visualized on IR and MultiColor images (yellow-gray deposits with interspersed refractile areas). The SD-OCT images consecutively collected were proportionally magnified for better visualization of sub-RPE changes and viewed with the contained Heidelberg Eye Explorer software (version 1.7.0.0, Heidelberg Engineering).

Qualitative description was independently performed by 2 senior retinal physicians (readers: G.Q. and E.H.S.) on the high-quality, confidently tracked (IR and MultiColor images) SD-OCT scans. Disagreement between readers regarding the detection of features was resolved by open adjudication. To describe the SD-OCT images, according to Spaide and Curcio, the following correspondence has been applied to the outer retinal layers: The innermost band reflects the external limiting membrane; a second band corresponds to the photoreceptors’ inner segment ellipsoid portion/outer segment (OS) interface, also known as the “ellipsoid zone”; a third band represents the RPE/OS junction, also known as “interdigitation zone”; and the most external band corresponds to the RPE/Bruch’s membrane complex. To interpret the multilaminar sub-RPE hyperreflectivity, according to Bloom and Singal, the hyperreflective band underlying areas of RPE elevation was defined as the outer Bruch’s membrane (oBM) layer.

When available, previous SD-OCT examinations (matching areas of regressing drusen) were investigated in regard to sub-RPE reflectivity and time to development of multilaminar hyperreflectivity. Spectralis SD-OCT allows confidence in detecting and assessing small changes over time by using cSLO technology to track the eye and guide OCT to the selected location. By using a selected prior reference scan, the Spectralis SD-OCT aligns the reference fundus image with the live patient fundus image at the selected location.
follow-up. The eye tracker recognizes the retina and then directs the SD-OCT scan to the same location. This eliminates the potential bias of subjective placement of the scan by the operator.

The statistical analysis included descriptive statistics for demographic data and a qualitative description of the findings. Data were analyzed with the SPSS software version 20.0 for Mac (IBM/SPSS, Inc., Chicago, IL).

Results

A total of 30 eyes of 23 consecutive patients (8 male and 15 female; mean age, 82.7±10.1 years; range, 71–98 years) showing on SD-OCT an intense multilaminar sub-RPE hyperreflectivity that matched with regressing calcific drusen as visualized by IR and MultiColor images (yellow-gray deposits with interspersed refractile areas) were included in this study. Seven patients had bilateral lesions, and 15 of 30 eyes had multifocal lesions in the same eye. Best-corrected visual acuity ranged from 20/25 to 20/160.

Overall, 142 of 626 SD-OCT scans showed at least 1 multilaminar sub-RPE hyperreflectivity and thus were selected for analysis. On SD-OCT, the multilaminar hyperreflectivity was found localize beneath the RPE and above the oBM layer but was never detected in the neurosensory retina, in the subretinal space, or beneath the Bruch’s membrane (in the choroid or sclera). A mean of 1.2 multilaminar sub-RPE hyperreflectivities per SD-OCT scan were identified by the 2 readers (k statistics = 0.88; P < 0.001). The hyperreflective laminae varied from straight to slightly curvilinear. Despite the calcific appearance of regressing drusen on both fundus examination and IR/MultiColor images, not all the multilaminar hyperreflectivities produced a posterior optical shadowing of the underlying tissues. The SD-OCT analysis allowed the 2 readers to describe 3 different types of

Figure 1. Three different types of sub-retinal pigment epithelium (RPE) hyperreflectivity. A, Type 1 multilaminar hyperreflectivity (arrows) originating from what we interpreted as the inner Bruch’s membrane (iBM) layer. B, Type 2 multilaminar hyperreflectivity (arrows) originating from the outer Bruch’s membrane (oBM) layer. C, Type 3 multilaminar fragmented hyperreflectivity (arrows) originating from what we interpreted as both the iBM and the oBM, showing different degrees of fragmentation.

Figure 2. Combined MultiColor (Heidelberg Engineering, Heidelberg, Germany) image and spectral-domain optical coherence tomography (SD-OCT) scan of type 1 multilaminar hyperreflectivity in a patient with nonexudative age-related macular degeneration. MultiColor image (left) shows a regressing calcific drusen (arrowhead), and tracked SD-OCT (right) shows an intense laminar hyperreflective signal (arrows) beneath the retinal pigment epithelium (RPE) layer, apparently originating from the inner Bruch’s membrane (iBM) layer (note the underlying outer Bruch’s membrane [oBM] layer).
Figure 3. Combined MultiColor image and spectral-domain optical coherence tomography (SD-OCT) scan of type 1 multilaminar hyperreflectivity in a patient with nonexudative age-related macular degeneration. MultiColor image (left) shows a regressing calcific drusen (arrowhead), and tracked SD-OCT image (right) shows an intense multilaminar hyperreflective signal (arrows) beneath the retinal pigment epithelium (RPE) layer, apparently originating from the inner Bruch’s membrane (iBM) layer (note the underlying outer Bruch’s membrane [oBM] layer).

Figure 4. Combined infrared (IR) image and spectral-domain optical coherence tomography (SD-OCT) scan of type 1 multilaminar hyperreflectivity in a patient with nonexudative age-related macular degeneration. MultiColor image (left) shows a regressing calcific drusen (arrowhead), and tracked SD-OCT (right) shows an intense multilaminar hyperreflective signal (arrows) beneath the retinal pigment epithelium (RPE) layer, apparently originating from the inner Bruch’s membrane (iBM) layer (note the underlying outer Bruch’s membrane [oBM] layer).
sub-RPE hyperreflectivities (Fig 1). “Type 1” laminar/multilaminar hyper-reflectivity (Figs 2–4; Fig 5, available at http://aaojournal.org) was found in 24 scans of 12 eyes (κ statistics = 0.89; P < 0.001), characterized by an intense signal originating from what we interpreted as the inner Bruch’s membrane (iBM) layer. “Type 2” multilaminar hyperreflectivity (Fig 6; Figs 7–10, available at http://aaojournal.org) was found in 130 scans of 27 eyes (κ statistics = 0.91; P < 0.001), characterized by an intense signal originating from the oBM layer. “Type 3” multilaminar fragmented hyper-reflectivity (Fig 10, available at http://aaojournal.org; and Fig 11) was found in 22 scans of 11 eyes (κ statistics = 0.85; P < 0.01), characterized by an intense signal originating from what we interpreted as both the iBM and the oBM, showing different degrees of fragmentation.

In 12 eyes of 12 patients for whom previous SD-OCT examinations (matching areas of regressing drusen) were available, multilaminar hyperreflectivities developed after a mean of 15.1±10.1 months (Figs 8 and 9, available at http://aaojournal.org).

**Discussion**

In this study, we describe a multilaminar sub-RPE intense hyperreflectivity observed in vivo by SD-OCT in eyes clinically diagnosed with regressing drusen (documented by retrospective evaluation of progression) due to nonneovascular AMD. The SD-OCT analysis allowed us to identify 3 different types of sub-RPE hyperreflectivities: “type 1” laminar/multilaminar hyperreflectivity, characterized by an intense signal originating from what we interpreted as the iBM layer; “type 2” multilaminar hyperreflectivity, characterized by an intense signal originating from the oBM layer; and “type 3” multilaminar fragmented hyper-reflectivity, characterized by an intense signal originating from what we interpreted as both the iBM and the oBM, showing different degrees of fragmentation.

In all scans (n = 142) showing at least 1 of the 3 different types of intense multilaminar sub-RPE hyperreflectivity, the tracked SD-OCT matched with regressing calcific drusen as visualized by cSLO IR and MultiColor images (yellow-gray deposits with interspersed refractile areas). Because we have no clinicopathologic correlations for these findings, speculation about the actual localization and possible origin/composition is based on insights provided by multimodal imaging (combined cSLO IR/MultiColor images and SD-OCT).

Large drusen in eyes with AMD consist of sub-RPE mounds of cholesterol-enriched (~40% of drusen content) membranous debris (a lipid-rich material). Membranous debris is more highly unesterified cholesterol-enriched than expected if this material is composed of OS or RPE membranes only, suggesting not only a plasma source but also synthesis and assembly of an apoB-lipoprotein by RPE for cholesterol in the sub-RPE space. This mechanism is similar to what has been proposed for the origin of atherosclerotic liposomes: cholesterol excreted from intimal cells, apoB-lipoproteins of plasma origin modified in the extracellular space, or partly hydrolyzed chylomicron remnant particles. In drusen “maturation,” several layers of coiled membranous debris accumulate between the RPE and the Bruch’s membrane (internal and external to the basement membrane). Close to areas of atrophy, the mounds of membranous debris appear fused: The membranes pass through the basement membrane to separate it from the inner collagenous layer. It is noteworthy that in drusen regression there is evidence not only of reduced formation of membranous debris but also of its removal by macrophage. Similar to what occurs in atherosclerosis (similar composition/progression and reflectivity), material not removed develops calcification.

The stratified multilaminar sub-RPE intense hyperreflectivity in the current study described by SD-OCT in eyes clinically diagnosed with regressing drusen could be interpreted as the in vivo visualization of calcific (not removed) membranous debris. Layers of lipid mineralization (in part similar to atherosclerotic changes) may develop internal and external to the basement membrane in the form of type 1 and type 2 multilaminar hyperreflectivity (originating from the iBM and oBM SD-OCT layers), respectively. Type 3 multilaminar fragmented hyperreflectivities (originating from both the iBM and the oBM SD-OCT layers) may correspond to fractures in the Bruch’s membrane, which develop after calcification and could lead to neovascularization.

To the best of our knowledge, this is the first study to describe a stratified multilaminar sub-RPE intense hyperreflectivity in eyes with regressing drusen. Mukkamala et al described an OCT finding of layered hyperreflective bands beneath the RPE in eyes with vascularized pigment epithelial detachment that the authors called the “onion sign.” The onion sign was usually associated with chronic exudation from type 1 neovascularization in patients with AMD. Because the onion sign colocalized to areas of exudation that are known to consist of lipoprotein, the authors proposed that it may represent layers of precipitated lipid in the sub-RPE space; such precipitated (possibly with calcification) lipid also may be found in the neurosensory (intracystic) retina (Fig 12, available at http://aaojournal.org). In our study, multilaminar sub-RPE intense hyperreflectivity appeared fairly similar to the onion sign. This favors the idea that SD-OCT may allow visualization of precipitated lipid in the sub-RPE space, not only in neovascular AMD but also in dry AMD. In the current study, all types of SD-OCT multilaminar sub-RPE hyperreflectivities colocalized to IR/MultiColor refractile lesions (regressing drusen), which histologically are largely composed of lipid (i.e., membranous debris, also called lipoprotein-derived debris) developing calcification (precipitated lipid mineralization due to reduced removal by macrophage).

Another possible explanation for the multilaminar sub-RPE hyperreflectivity in our series might be the presence of subclinical focal neovascularization (not distinguishable by fluorescein angiography and indocyanine green angiography) localized within regressing drusen. Our findings share similarities with the recently reported layers of tissue within vascularized pigment epithelium detachment (just beneath the RPE layer), which Spaide suggested to represent sub-RPE neovessels. This may also explain the iBM/oBM disjunction found in our series.

**Study Limitation**

The main limitation of this study is the lack of corroborative data by histologic studies or other in vivo high-resolution

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Querques et al. *Sub-RPE Hyperreflectivity in Regressing Drusen*
imaging that confirms or contradicts these findings. Therefore, this interpretation needs to be confirmed by future research.

In conclusion, we describe a novel SD-OCT finding appearing as multilaminar sub-RPE intense hyperreflectivity observed in vivo in eyes with regressing drusen. This multilaminar sub-RPE hyperreflectivity could be interpreted as layers of lipid mineralization (membranous debris, also called lipoprotein-derived debris, developing calcification), internal and external to the basement membrane, with different degrees of fragmentation. To our knowledge, this is the first study of sub-RPE lipid mineralization in nonneovascular AMD that can be detected on SD-OCT examination.

Figure 6. MultiColor image and combined infrared (IR) image and spectral-domain optical coherence tomography (SD-OCT) scan of type 2 multilaminar hyperreflectivity in a patient with nonexudative age-related macular degeneration. MultiColor image (left) shows a regressing calcific drusen (arrowhead), and IR-tracked SD-OCT image (right) shows an intense multilaminar hyperreflective signal (arrows) beneath the retinal pigment epithelium (RPE) layer, apparently originating from the outer Bruch’s membrane (oBM) layer, and reaching the inner Bruch’s membrane (iBM).

Figure 11. Combined MultiColor image and spectral-domain optical coherence tomography (SD-OCT) scan of type 3 multilaminar hyperreflectivity in a patient with nonexudative age-related macular degeneration. MultiColor image (left) shows a regressing calcific drusen (arrowhead), and tracked SD-OCT image (right) shows a multilaminar fragmented hyperreflectivity, apparently originating from both the inner Bruch’s membrane (iBM) and the outer Bruch’s membrane (oBM) layers.
References


Footnotes and Financial Disclosures

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