Optical Coherence Tomographic Imaging of Sub−Retinal Pigment Epithelium Lipid

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Objective: To describe an optical coherence tomographic finding of layered hyperreflective bands beneath the retinal pigment epithelium (RPE), the so-called onion sign believed to represent lipid within a vascularized pigment epithelial detachment.

Methods: This retrospective observational case series involved reviewing clinical histories of patients with the onion sign. Imaging studies analyzed included spectral-domain optical coherence tomography, color and red-free photographs, near infrared reflectance, fundus autofluorescence, and blue-light fundus autofluorescence.

Results: A total of 22 eyes of 20 patients with sub-RPE hyperreflective bands were identified. There were 15 women and 5 men with a mean patient age of 76 years (range, 60-92 years). Snellen best-corrected visual acuities ranged from 20/25 to counting fingers, with a median of 20/80. Two patients had bilateral involvement, and 3 of 17 eyes had multifocal onion signs in the same eye. All eyes had neovascular age-related macular degeneration, with type 1 (sub-RPE) neovascularization. In all patients, the onion sign correlated with areas of yellow-gray exudates seen clinically that appeared bright on red-free and near infrared reflectance imaging. No specific fundus autofluorescence or blue-light fundus autofluorescence pattern was identified.

Conclusions: The onion sign refers to layered hyperreflective bands in the sub-RPE space usually associated with chronic exudation from type 1 neovascularization in patients with age-related macular degeneration. With an associated bright near infrared reflectance, these bands may correspond to lipid, collagen, or fibrin. Because the onion sign colocalizes to areas of exudation that are known to consist of lipoprotein, we propose that this finding may represent layers of precipitated lipid in the sub-RPE space. To our knowledge, this is the first report of lipid detected in the sub-RPE space on clinical examination.

Spectral-domain (SD) optical coherence tomography (OCT) has enhanced our ability to image the subretinal pigment epithelium (RPE) space in retinal disorders associated with pigment epithelial detachments (PEDs).1−5 With serial SD-OCT imaging, greater insight into the pathogenesis and natural history of PEDs may be gained and may supplement older modalities such as fluorescein angiography (FA), indocyanine green angiography (ICGA), and histopathology specimens.5−11 Using SD-OCT, we identified a unique finding associated with PEDs in patients with neovascular age-related macular degeneration (AMD)—the so-called onion sign. Reminiscent of the layers of an onion, this finding appears as layers of hyperreflective bands between the RPE and Bruch membrane within vascularized PEDs. Coscas12 previously reported this OCT finding as bands of fibrovascular tissue. However, we propose that these hyperreflective bands likely represent sub-RPE lipid trapped within fibrovascular tissue that originates from type 1 neovascular exudation. Although intraretinal and subretinal lipid are commonly present in a multitude of retinal diseases including diabetic retinopathy, retinal vein occlusions, hypertensive retinopathy, Coats disease, macular telangiectasia type 1, radiation retinopathy, and Bartonella neuroretinitis, we are not aware of other conditions that commonly produce sub-RPE lipid.13−19

METHODS

We retrospectively reviewed clinical and imaging data from all eyes noted to have the
onion sign on SD-OCT performed as part of routine follow-up in patients with neovascular AMD. Patients were seen at the Vitreous Retina Macula Consultants of New York, the Jules Stein Eye Institute, the Centro Brasileiro de Ciencias Visuais, and the University and Polytechnic Hospital. The Western Institutional Review Board approved this retrospective review, and patients provided written informed consent for study participation.

High-resolution digital color photographs, red-free (RF) photographs, and FA were obtained in each patient. Near infrared reflectance (NIR) using a light stimulus of 815 nm, blue-light autofluorescence (bFAF) using a light stimulus of 488 nm, fundus autofluorescence (FAF) using an excitation light band-width from 535 to 585 nm, and SD-OCT examinations were obtained with the Spectralis HRA + OCT (Heidelberg Engineering). For each scan, qualitative data including location, shape, relative orientation of the hyperreflective bands, and the presence of posterior optical shadowing were recorded.

RESULTS

Twenty-two eyes of 20 consecutive patients were identified as having sub-RPE hyperreflective bands, the onion sign. Two patients had bilateral involvement and 3 of 22 eyes had multifocal involvement in the same eye (Figure 1). There were 15 women and 5 men, with a mean age of 76 years (range, 60-92 years). Snellen best-corrected visual acuity at the most recent visit ranged from 20/25 to counting fingers, with a median of 20/80. Review of medical histories revealed 13 patients (65%) with hypertension, 5 (20%) with hypercholesterolemia, and 5 (20%) with type 2 diabetes mellitus.

All eyes had been diagnosed as having neovascular AMD. Of the 22 eyes, 11 were receiving injections of intravitreal ranibizumab, 5 of intravitreal bevacizumab, 2 of both intravitreal ranibizumab and bevacizumab (sequentially), and 4 were being observed without treatment. The length of treatment with intravitreal anti-vascular endothelial growth factor (anti-VEGF) therapy prior to detection of the onion sign ranged from 3 months to 54 months. One eye had been treated for 3 months, 1 for 6 months, 2 for 12 months, 1 for 18 months, and 14 for more than 24 months. Six eyes had evidence of chorioretinal anastomosis, 1 had a RPE tear, 1 had reticular macular disease (subretinal drusenoid deposits), and 1 had angiographic evidence of polypoidal choroidal vasculopathy.

On SD-OCT, the layered hyperreflective bands were found to localize to beneath the hyperreflective RPE band and above the hyperreflective band believed to repre-
sent the Bruch membrane (Figure 2). The onion sign was not detected in the neurosensory retina, subretinal space, or beneath the Bruch membrane in the choroid or sclera. The horizontal length of the bands appeared to correlate with the size of the overlying PED. The shape of the bands varied from straight to curvilinear. Eyes with larger PEDs had straighter bands, while eyes with flatter PEDs had more curvilinear bands. The hyperreflective bands did not produce posterior optical shadowing of the underlying tissues as is seen with calcification. The total number of bands per eye was difficult to estimate because SD-OCT spatial resolution was insufficient to distinguish bands when stacked closely together.

The sub-RPE hyperreflective bands seen on SD-OCT correlated with deep yellow-gray deposits on clinical examination and color photographs (Figure 3). On clinical examination and color photography alone, it was difficult to appreciate the sub-RPE location of these deposits. Within these yellow-gray deposits, there were glistening patches that appeared to represent crystalline material. In several eyes, we were able to document the development of an onion sign in fundusoscopic locations where prior color photography had documented an absence of this finding.

When available, we reviewed RF, NIR, FAF, and bFAF images. In all eyes, the onion sign correlated with areas of hyper-reflectance on RF and NIR images. When both imaging modalities were available, the area of hyper-reflectance was larger in the NIR images than in the RF images. In 6 of 11 eyes in which bFAF images were available, the onion sign was associated with areas of mild hypo-autofluorescence. In the remaining 5 eyes, there were no discernable findings correlating with these lesions (Figure 4).

A 64-year-old woman had bilateral neovascular AMD. Medical history was significant for hypercholesterolemia treated with atorvastatin and osteopenia treated with oral alendronate. On presentation, best-corrected visual acuity was 20/25 OD and 20/20 OS.

At the initiation of monthly intravitreal anti-VEGF therapy, clinical examination and color photographs of the right eye showed paravascular pigmentary changes and drusen but no evidence of yellow-gray deposits or refractile material in the temporal macula (Figure 5). Despite monthly treatment with intravitreal ranibizumab, 0.5 mg/0.05 mL, every 3 to 5 weeks for 3 years, this eye had SD-OCT evidence of continued growth of type 1 neovascularization with persistent fluid noted at each monthly examination. One year later, a new PED was noted in the inferotemporal macula that flattened following a RPE tear. During the next 4 months, a well-defined area of yellow-gray deposits with foci of brighter refractive material was noted to develop temporal to the RPE tear.

Serial eye-tracked SD-OCT B-scan images through this area documented the changes resulting in the appearance of the yellow-gray lesions. A predominantly serous PED noted 24 months after initiating intravitreal anti-VEGF therapy developed, increasing internal reflectivity during the ensuing 4 months. This increase in reflectivity initially appeared to represent increasing vascularity, or type 1 neovascularization, between the RPE and the Bruch membrane, but later it appeared as intense, layered linear bands believed to represent lipid-rich material. Simultaneous SD-OCT and NIR imaging localized the clinically apparent yellow-gray material to the sub-RPE space above the Bruch membrane.

The onion sign is a novel SD-OCT finding characterized by the presence of hyperreflective bands detected within vascularized PEDs. We believe this finding may represent lipid-rich material due to chronic leakage from type 1 neovascularization, most commonly occurring in eyes with AMD. To our knowledge, sub-RPE lipid has not been previously described as a clinical or OCT finding. As there are no published clinicopathologic reports describing this finding, speculation about the etiology is based on insights provided by multimodal imaging techniques.

Clinically, the onion sign correlates with matte yellow-gray deposits with interspersed refractile areas localizing to type 1 neovascular tissue. Punctate yellow-white crystalline deposits have been described in eyes with neovascular AMD. On OCT, these deposits appear as hyperreflective foci and localize more superficially within the outer nuclear and outer plexiform layers of the neurosensory retina. Lima and colleagues proposed that these deposits could represent lipid or degenerated Müller cells.
In eyes with diabetic macular edema, similar exudative deposits can be seen as hyperreflective foci on OCT in all retinal layers. When confluent, these foci can form plaques in the outer plexiform layer on OCT that correlate to areas of hard exudates seen clinically. These hyperreflective foci and plaques are thought to represent lipid-rich deposits.\textsuperscript{13,19,21-23}

While eyes with retinal vascular disease develop intraretinal lipid, the eyes in our study appeared to accumulate sub-RPE lipid within vascularized PEDs (Figure 4). This localization of our findings to the sub-RPE space is unique. Just as a breakdown of the inner blood-retina barrier results in exudation anterior to the external limiting membrane in diabetes, exudation through the fenestrated endothelium of a type 1 neovascular membrane could result in buildup of lipoprotein-rich deposits bound anteriorly by the RPE. Perhaps precipitation or crystallization of the lipid components of these deposits appears as hyperreflective bands on OCT. Phases of intermittent exudation could result in layers of parallel hyperreflective bands similar to strata of deposited sedimentary rock seen on a mountainside. We have observed similar bands above the RPE in patients with diabetic macular edema with hard exudates.

In addition to deposition of lipid, other explanations of these bands must be considered. Drusen regression can result in calcification that may produce a crystalline appearance with deposits just above the Bruch membrane.\textsuperscript{24-27} However, in the onion sign, bands are found at many levels between the Bruch membrane and the RPE.

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**Figure 3.** Left eye of an 81-year-old woman with neovascular age-related macular degeneration receiving monthly alternating injections of ranibizumab and off-label bevacizumab for 3 years. A, Color photograph shows a central vascularized pigment epithelial detachment with yellow-gray material at its margins. This material was not present at the initiation of anti–vascular endothelial growth factor therapy 2 years earlier. Laminar venous and recirculation phase fluorescein angiography show an occult leakage pattern consistent with type 1 neovascularization (B and C). D, Red-free imaging shows areas of brightness corresponding to the yellow-gray material present in the color photograph (A). Early and late phase indocyanine green angiography shows an early focal hotspot with late washout consistent with polypoidal choroidal vasculopathy (PCV) (E and F). The PCV lesion is found within a larger late staining plaque consistent with type 1 neovascularization. Spectral-domain optical coherence tomographic B-scan lines show sub–retinal pigment epithelium hyperreflective bands (G and H) that corresponded to the yellow-gray areas in the color photograph (A) and areas of increased brightness on red-free image (D and inset).
This dissimilarity, along with the absence of posterior optical shadowing typically caused by calcification on OCT, makes the possibility of a calcified drusen less likely.

Other imaging modalities may be useful in identifying the onion sign. Both RF and NIR imaging highlight areas with an onion sign. When both of these modalities are available, the sign appeared larger and brighter on NIR when compared with RF images. Melanin, fibrin, and collagen are bright on NIR images and should be included in a list of possible explanations of the onion sign.28 We did not observe any bFAF and FAF associated with the onion sign in the sub-RPE space.

While there is no histopathologic evidence that melanin accumulates within type 1 neovascular tissue, fibrin and collagen are possible contributors to the onion sign. Electron micrographic evaluation of surgically excised choroidal neovascular (CNV) membranes revealed that 25-nm collagen and fibrin bands were among the most commonly seen extracellular components, found in 84% and 79% of specimens, respectively.29 In 12% of specimens, there was evidence of uncommon components including calcification and curvilinear filaments. Lafaut and colleagues30 analyzed 35 surgically removed CNV membranes and attempted to assess the distribution of fibrin deposition in these specimens. They did not observe fibrin in curvilinear sub-RPE bands as seen on OCT in our patients.

Although fibrin is very common in excised CNV specimens, the NIR images of our patients showed fibrin may not be a major component of the onion sign. Typically, fibrin in occult CNV lesions is seen as punctate areas of increased NIR reflectance interspersed within an area of decreased NIR reflectance.28,31 By contrast, the NIR images of all of our patients showed confluent areas of increased NIR reflectance. These areas of increased NIR reflectance are peculiar because PEDs, within which the onion signs are found, are usually associated with decreased central NIR reflectance with a brighter halo.28 While fibrin is not seen on FA, it typically stains with ICGA. In eyes in which FA and ICGA were available, there was no finding consistent with the more intense hyperfluorescent staining of fibrin, only the late faint hyperfluorescence of type 1 neovascularization commonly referred to as a plaque (Figure 5).

Collagen is another possible component of the onion sign, as it is a common component in sub-RPE deposits.24,25 Knupp and colleagues22 investigated collagen in the Bruch membrane and beneath the RPE. They showed that collagen, particularly collagen VI, can aggregate into bands that form basal laminar deposits. However, collagen is inconsistent with the yellow-gray refractile deposits seen on color fundus photographs.
Figure 5. The right eye of a 64-year-old woman with neovascular age-related macular degeneration. A. Color photograph at the onset of intravitreal ranibizumab injections revealed parafocal drusen. B. One year later, there was a vascularized pigment epithelial detachment (PED) detected in the inferotemporal macula. The arrowhead shows its location in the color photographs and in the corresponding spectral-domain optical coherence tomographic scans (SD-OCT) (D). C. Three years after presentation, color photographs revealed pigment hyperplasia in the central macula. The inferotemporal PED had flattened following a retinal pigment epithelial (RPE) tear, leaving an area of subretinal fibrosis. Temporal to the resolved PED, a well-defined area of yellow-gray deposit with foci of brighter refractile material is shown (C, green arrow). D. The serial eye-track SD-OCTs show evolution of a second PED correlating with an accumulation of yellowish exudates (D, week 6, green arrow). The progression appears to be a gradual enlargement and vascularization of an initially serous PED with the development of sub-RPE hyperreflective bands, the onion sign.

Based on the clinical progression of our patients, we hypothesize that the onion sign resulted from layers of deposited lipid within type 1 neovascular tissue. Clinically, these patients, all with neovascular AMD, had areas where chronic exudation preceded the development of these hyperreflective bands on OCT. Histopathology of hard exudates has revealed the presence of lipoprotein material and lipid-laden macrophages. These lipid exudates can appear as foci of extreme NIR hyperreflectance in neovascular AMD, as seen in our patients. Multiple physical forms of sub-RPE lipid-rich deposits have been observed by apolipoprotein immunofluorescence. However, to our knowledge, the presence of cholesterol crystals or stacked linear bands has never been reported.

In summary, we report a novel SD-OCT finding we call the onion sign appearing as hyperreflective bands within vascularized PEDs detected in the setting of ongoing exudation in eyes with neovascular AMD. These bands appear to be related to lipoprotein exudation and likely represent layers of lipid-rich material trapped within type 1 neovascular tissue. Based on NIR findings, collagen and/or fibrin may also contribute to this finding. To our knowledge, this is the first report of sub-RPE lipid that can be detected on OCT examination.

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