Published in final edited form as:

Retina. 2017 August ; 37(8): 1620–1624. doi:10.1097/IAE.0000000000001672.

Clinicopathologic Correlation of Retinal Angiomatous Proliferation Treated with Ranibizumab

Alison H. Skalet, MD, PhD, Audra K. Miller, MD, Michael L. Klein, MD, Andreas K. Lauer, MD, David J. Wilson, MD

Department of Ophthalmology, Casey Eye Institute, Oregon Health and Science University, Portland, Oregon

Abstract

Purpose: To describe histopathologic features of an eye with retinal angiomatous proliferation (RAP) secondary to age-related macular degeneration (ARMD) treated with serial ranibizumab injections and to correlate these findings to spectral domain optical coherence tomography (SD-OCT).

Methods: Histopathologic features from serial sections through the globe of a 93-year-old man with ARMD were studied and compared to SD-OCT images obtained 7 weeks before his death.

Results: The pathologic correlate of ranibizumab-treated RAP was a circumscribed, branching paucicellular vascular complex extending from the inner plexiform layer to Bruch's membrane. The histopathologic findings corresponded to an area of hyper-reflectivity on SD-OCT imaging, substantiating the reported tomographic appearance of RAP lesions. A frank anastomosis with choroidal or retinal vasculature was not seen in this treated RAP lesion. There was a lack of retinal pigment epithelium (RPE) underlying the lesion in an area of RPE detachment. The elastic portion of Bruch's membrane appeared intact. Treatment with ranibizumab over an extended period of time may have been associated with a loss of cellularity of the RAP lesion.

Conclusion: Fundus examination, fluorescein angiography, and SD-OCT images of RAP in a patient with ARMD extensively treated with ranibizumab correlated histopathologically with a paucicellular intraretinal vascular complex.

Summary:

The histopathologic features of an eye with retinal angiomatous proliferation (RAP) treated with serial ranibizumab injections were correlated with the findings on spectral domain optical coherence tomography (SD-OCT). The histopathologic findings corresponded to an area of hyper-reflectivity on SD-OCT imaging, substantiating the reported tomographic appearance of RAP lesions.

Corresponding Author: Alison H. Skalet, 3375 S.W. Terwilliger Blvd., Portland, OR 97239-4197, Tel: (503) 494-7881, Fax: (503) 494-7233, skalet@ohsu.edu.

Author Contributions:

Study Design: AHS, MLK, AJL, DJW

Data Analysis: AHS, AKM, MLK, AJL, DJW

Manuscript: AHS, AKM, MLK, AJL, DJW

Presented at the American Association of Ophthalmic Oncology and Pathology Annual Meeting, Orlando, FL, October 21, 2011.

Keywords

Age-related macular degeneration; Anti-VEGF; Choroidal Neovascularization; Retinal Neovascularization; Clinicopathologic Correlation; Ranibizumab; Retinal Angiomatous Proliferation

Introduction

Retinal angiomatous proliferation (RAP) is a form of intraretinal neovascularization in patients with age-related macular degeneration (ARMD) that is distinct from the occult and classic choroidal neovascularization (CNV) seen in ARMD¹⁻⁴. In RAP lesions there is active proliferation of telangiectatic neovascular vessels within the retina. Formation of retinal-choroidal anastomoses is seen, but thought to be a late feature.

There have been numerous descriptions of the tomographic features of RAP lesions using spectral domain optical coherence tomography (SD-OCT)⁵⁻¹². These studies have described the intraretinal neovascularization of RAP as a highly reflective lesion in the neurosensory retina. A consistent tomographic feature in eyes with RAP has been absence of retinal pigment epithelium beneath the RAP lesion. To date, no histopathologic study has been conducted to confirm the tomographic appearance of RAP lesions.

Here we present the histopathology of a case in which a RAP lesion was identified in a post-mortem eye that had been imaged with SD-OCT seven weeks prior to the patient's death. We provide histopathologic evidence supporting the presumed OCT appearance of RAP lesions. In addition, we describe the first histopathologic examination of a RAP lesion treated with serial ranibizumab.

Case Report

A 93-year-old man with longstanding ARMD presented for routine evaluation for ranibizumab intravitreal injection. He had previously received treatment for RAP in the left eye with intravitreal triamcinolone on three occasions followed by 25 serial injections of ranibizumab over a period of 50 months (November 2006 through June 2011). His ocular history was also significant for pseudophakia and primary open angle glaucoma in both eyes. At the time of the examination, the patient's visual acuity measured 20/400 in the right eye due to a longstanding disciform macular scar. The visual acuity measured 20/40 in the left eye in which a RAP lesion had been previously identified by fluorescein angiography (Figure 1). The left eye was examined with SD-OCT and found to have a small amount of residual fluid and was treated with an additional ranibizumab injection. The patient died 7 weeks after, subsequent to a hip fracture.

Methods

The patient was evaluated at a tertiary care referral center where he underwent clinical evaluation that included a complete eye examination and SD-OCT (Spectralis® Heidelberg Engineering Inc, Heidelberg, Germany) on the date of his last examination. His most recent

color fundus photographs and fluorescein angiography were performed 6 years prior to this last evaluation (Figure 1). The eyes were obtained post-mortem and were fixed in 10% neutral buffered formalin. They were then dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Serial 5-micrometer sections were prepared from the left globe in a plane parallel to the pupil, the optic nerve, and the macula. Sections were then stained with hematoxylin and eosin and evaluated with light microscopy.

Results

The histopathologic features of a section through the central retina at a location 370 microns superior to the fovea are illustrated in Figure 2A. There is a vascular complex located in the outer retina abutting the inner portion of Bruch's membrane. The location of this lesion corresponds to the RAP lesion on fluorescein angiogram. There is generalized loss of the photoreceptor outer segments, outer nuclear layer and some of the outer plexiform layer. The retinal pigment epithelium (RPE) monolayer is disrupted underlying the RAP lesion; however, the inner portion of Bruch's membrane is intact and there is a continuous layer of basophilic basal laminar deposit present. There is artifactitious separation of the inner portion of Bruch's membrane and the basal laminar deposits from the underlying elastic portion of Bruch's membrane. There is a thin band of fibrous tissue within the pigment epithelial detachment. It is likely that this tissue represents regressed type 1 choroidal neovascularization, although no vascular elements could be identified. A section through another area of the RAP lesion located 30 microns inferior to the first area (Figure 2B), shows the RAP lesion has branched. There are only sparse cells present within the structure of the complex. Moving further inferiorly to an area 250 microns superior to the fovea (Figure 2C), the RAP lesion is located more superficially in the retina, now abutting the inner nuclear layer. As the lesion terminates, 200 microns superior to the fovea, it is located in the inner plexiform layer (Figure 2D). Examination of each 5 micron section failed to reveal a definite anastomosis with a retinal vessel.

SD-OCT images obtained 7 weeks ante-mortem through the location of the RAP lesion (Figure 3A) revealed a highly-reflective lesion located in the outer neurosensory retina. This lesion was consistently seen in all ante-mortem spectral domain OCTs through this area, up to 2 years prior to death (Figure 3B). This feature was also noted in OCT obtained at 1 year and 5 months prior to the patient's death.

Discussion:

Intravitreal ranibizumab injections have been found to be effective in improving visual acuity and reducing OCT macular thickness in patients with RAP lesions^{1,5,11,13}. Based upon this case, we confirm that RAP lesions appear as highly-reflective lesions on OCT. In addition, we show that treatment with serial ranibizumab was associated with a RAP lesion with decreased cellularity as compared with previously published lesions^{14,15}. In our patient, treatment with ranibizumab failed to eliminate the RAP lesion completely. Given the thick walled fibrotic appearance of the regressed lesion, it seems unlikely that the lesion could completely disappear. A published report of tomographic evidence of a RAP lesion disappearing after bevacizumab may reflect an earlier stage and less developed RAP lesion,

or possibly a small difference in segmentation on subsequent scans.⁶ Another consideration is that our patient was treated with anti-VEGF therapy alone; so it is unclear whether or not combination of photodynamic therapy with ranibizumab would have led to a difference in the histologic structure of the RAP lesion^{16,17}.

The origin of retinal neovascularization in RAP lesions remains a subject of debate. In 2001, Yannuzzi and colleagues suggested that RAP develops first in the retina, then may progress to involve the choroid through retinal-choroidal anastomoses.³ In 2003, Gass et al suggested an alternative hypothesis that RAP begins first with occult type 1 neovascularization.¹⁸ Yannuzzi and Freund later revised their classification to include this possibility^{1,2}. OCT studies have supported both theories^{6,7}. Unlike our prior reported cases,^{14,15} this case had a thin band of fibrous tissue underlying the RAP lesion deep to the RPE which may represent a regressed occult CNV membrane. However, there were no anastomoses seen between this tissue and the RAP lesion on serial histopathologic sections. Both the choroid and the retinal circulation remain possible sources for the origin of RAP lesions, but the location of the RAP lesions overlying an intact inner portion of Bruch's membrane favors an origin from the retinal circulation. In this case, as in our previous cases, fluorescein angiography demonstrated there was a pigment epithelial detachment in association with the RAP lesion. This PED had resolved during the course of therapy with ranibizumab. The contact of the RAP lesion with the inner portion of Bruch's membrane, an area devoid of RPE, suggests that leakage of fluid from the vascular complex could contribute to the formation of a pigment epithelial detachment as was present in the angiogram of this patient several years previously.

Financial disclosure:

Supported by a Lloyd Research Endowment Faculty Grant (AHS); Core grant P30 EY010572 from the National Institutes of Health (Bethesda, MD) and by an unrestricted departmental grant from Research to Prevent Blindness (New York, NY).

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Figure 1.

Color Fundus Photograph and Fluorescein Angiography performed 6 years prior to the patient's death. Early hyperfluorescence with late leakage is seen in the superior macula consistent with the angiographic appearance of RAP. The location of the RAP lesion is indicated with an arrow in the earliest frame of the fluorescein angiogram. In the temporal macula there is an area of transmitted hyperfluorescence, consistent with pigment epithelial atrophy. In the late frames of the angiogram there is a broad area of hyperfluorescence consistent with a pigment epithelial detachment.

Figure 2. Histopathology of retinal angiomatous proliferation (RAP) at 370 (A), 340 (B), 250 (C) and 200 (D) microns superior to the fovea. The RAP lesion (arrow) appears as a hypocellular, thick walled vascular complex abutting the inner portion of Bruch's membrane, which has artifactuously separated from the outer Bruch's membrane. As the lesion is followed inferiorly, moving closer to the fovea, the RAP lesion branches (B, arrows) and is found in the more inner layers of the retina; finally terminating in the inner plexiform layer (D). There is a third layer of fibrous tissue immediately below the RPE, but no neovascular tissue was identified. (hematoxylin-eosin, original magnification x10 before reduction).

Figure 3.

Appearance of RAP lesion on SD-OCT examination 7 weeks ante-mortem (A) and 2 years ante-mortem (B) as well as histopathology (C). The tomographic appearance of the lesion is a highly-reflective lesion located in the outer neurosensory retina (arrows). The corresponding histopathologic appearance of the RAP lesion (C, arrow) is that of a vascular complex in the outer neurosensory retina.