Clinicopathologic Correlation of Retinal Angiomatous Proliferation

Dinelli M. Monson, MD; Justine R. Smith, MBBS, PhD; Michael L. Klein, MD; David J. Wilson, MD

Objectives: To correlate clinical and histopathologic features of an eye with retinal angiomatous proliferation (RAP) secondary to age-related macular degeneration and to investigate the expression of von Willebrand factor (VWF) and vascular endothelial growth factor (VEGF) in this condition.

Methods: Histopathologic features from serial sections through the globe of an 87-year-old woman with RAP were studied and compared with fluorescein angiography and color fundus photographs obtained 4 months before death. Commercially available antibodies were used to detect expression of VWF and VEGF in tissue sections.

Results: The pathologic correlate of RAP was a circumscribed intraretinal angiomatous complex within the outer part of the neurosensory retina overlying a large pigment epithelial detachment. There were no breaks in the Bruch membrane. No choroidal neovascularization was present. Endothelial cells within the RAP lesion immunostained positively for VWF and VEGF. The Bruch membrane expressed VWF adjacent to the RAP.

Conclusions: Fundus examination and fluorescein angiography images of RAP in a patient with age-related macular degeneration correlated histopathologically with a neovascular intraretinal angiomatous complex, without the presence of sub-retinal pigment epithelial neovascularization. Immunostaining demonstrated that RAP expresses VWF and VEGF.

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RETINAL ANGIOMATOUS PROLIFERATION (RAP) is a term used to describe intraretinal neovascularization in patients with age-related macular degeneration (ARMD). This condition was first described by Hartnett et al1,2 in 1992 as deep retinal vascular anomalous complexes. Retinal angiomatous proliferation is a distinct form of neovascular ARMD, and the term RAP was coined by Yannuzzi et al3 in 2001.

An RAP is characterized by the early appearance of an intraretinal vascular complex, often associated with adjoining telangiectatic vessels. Retinal choroidal anastomosis is variable and is considered to be a late feature.3 In a recent prospective multicenter consecutive series of patients with newly diagnosed exudative ARMD, 15% of 205 eyes were diagnosed with RAP.4 There is a tendency toward bilateral involvement. In the series of 108 patients of Yannuzzi et al,3 there were 56 bilateral cases. Prior to the introduction of intravitreal antiangiogenic drug therapy, treatment outcomes were generally poor and most patients declined to a visual acuity of 20/200 or worse secondary to disciform scar formation.5

Prior to proposal of the concept of RAP, in 2000, Lafaut et al6 histologically examined neovascular membranes obtained during foveal translocation surgery from 6 patients who had angiographic features of RAP. In 2007, Shimada et al7 also examined neovascular membranes from 9 eyes with RAP, after excision of neovascularization. However, to our knowledge, there are no reports of histologic studies in whole eyes, and in the absence of a definitive histopathologic study, RAP has been defined primarily by its clinical features. In this study, we report the first clinicopathologic correlation of RAP in a whole eye.

An 87-year-old white woman complained of the recent onset of blurred vision in the right eye. The vision in the patient’s left eye had been poor due to long-standing exudative ARMD. The patient’s ocular history was also significant for glaucoma and cataract surgery in both eyes and left Nd:YAG capsulotomy for posterior capsular opacification.

At presentation, best-corrected visual acuity was 20/60 OD and 20/200 OS. Examination of the right fundus disclosed fluid in the macula accompanied by intraretinal hemorrhages located in the fo-
vea and just nasal. Large drusen and retinal pigment epithelial (RPE) changes were most prominent in the temporal macula (Figure 1). Funduscopic examination of the left eye revealed a large pigment epithelial detachment (PED) centered at the fovea with a few surrounding large drusen. Hyperpigmentation was present overlying the PED, and there was a focal, sharply demarcated area of pigmentary atrophy at the fovea (eFigure 1, www.archophthalmol.com).

Stereoscopic fluorescein angiography (FA) of the right eye in the early frames revealed telangiectatic vessels in the superficial retina at the nasal aspect of the fovea. There was focal hyperfluorescence and leakage deep to these vessels consistent with a neovascular lesion in communication with the retinal vasculature. Superior and nasal to the fovea were small areas of intraretinal blocked fluorescence corresponding to hemorrhage seen clinically (Figure 2A). In the later frames of the FA, there was further fluorescein leakage and late staining centered nasal to the fovea with small superficial cystic accumulations of dye (Figure 2B). Inferonasal to the fovea was an irregular circumscribed area of hyperfluorescence that did not leak and was consistent with a pigment epithelial window defect. Stereoscopic FA of the left eye revealed fluorescein pooling beneath a large PED involving the fovea with hyperfluorescence of the focal pigment epithelial atrophic defect at the fovea.

**METHODS**

**HISTOLOGIC PREPARATION OF THE GLOBE**

The eyes were enucleated post mortem and immediately fixed in 10% neutral buffered formalin. The globe was opened horizontally. Opening the globe revealed a posterior chamber lens implant and mild asteroid hyalosis, but the vitreous was otherwise normal. When compared with the final color photographs taken 4 months prior to death, there were no gross changes noted. The globe was dehydrated in graded ethanol solutions, cleared in xylene, and embedded in paraffin. The globe was sectioned serially at a thickness of 5 µm in a plane parallel to the pupil, optic nerve, and macula. Each section was numbered, from 1 to 674, and each number section represented a width of 5 µm. The sections were then stained with hematoxylin-eosin, and the histopathologic features were reviewed by light microscopy.

**IMMUNOHISTOCHEMICAL ANALYSIS**

Five-micron formalin-fixed, paraffin-embedded ocular sections were stained according to a previously published indirect immunohistochemical method that included antigen retrieval by boiling in citrate buffer. Primary polyclonal antibodies raised in rabbit against human von Willebrand factor (VWF) (catalog number A0082; DAKO, Glostrup, Denmark) and human vascular endothelial growth factor (VEGF) (catalog num-
Rabbit IgG (Vector Laboratories, Burlingame, California) was used as a negative control antibody. Sections including the RAP were stained to detect VWF and VEGF. Because of the limited number of ocular sections containing the lesion, an additional section cut remote from the lesion, but from the same eye, was stained with rabbit IgG in place of specific primary antibodies.

**RESULTS**

The histopathologic features of serial section 180 are illustrated in Figure 3A and B. An intraretinal vascular complex is present in the outer part of the neurosensory retina. This complex consists of a circumscribed mass of endothelial cells. This vascular core is surrounded by eosinophilic fibrous material. This vascular tissue is adjacent to the inner portion of the Bruch membrane. The RPE cells containing pigment epithelial granules are seen enveloping the lesion. This angiomatosus lesion is nasal to the fovea, and evidence of the lesion is present in section 170 through section 200. Intraretinal hemorrhages are present. In the parafoveal area there are multiple telangiectatic vessels extending into the outer neurosensory retina (Figure 2). The angiomatous complex, and associated telangiectatic vessels, corresponds to the FA image of a hyperfluorescent intraretinal vascular complex seen nasal to the fovea with associated telangiectatic vessels (Figure 2A). The intraretinal hemorrhages observed microscopically corresponded to the areas of intraretinal blocked fluorescence seen superior and nasal to the fovea.

Figure 3A and B also illustrates that the RAP lies over a broad, shallow PED. The Bruch membrane is separated along the length of this PED into its inner and outer components, as is often seen in PEDs.9,10 The RPE cells are adherent to the inner portion of the Bruch membrane. Retinal pigment epithelial cells containing pigment epithelial granules (arrows) are seen enveloping the lesion (hematoxylin-eosin, original magnification ×20 before reduction).

**Figure 3.** Serial section 180. A, Serial section 180 shows an intraretinal vascular complex present in the outer part of the neurosensory retina (arrow). This retinal angiomatous proliferation lesion lies over a large pigment epithelial detachment (asterisk). The Bruch membrane is separated along the length of this pigment epithelial detachment. There is no subretinal neovascularization. Intraretinal hemorrhages are adjacent to the vascular complex. Diffuse retinal thickening with cystic spaces is present with associated intraretinal exudates. Vitreous and choroid are indicated (hematoxylin-eosin, original magnification ×10 before reduction). B, The intraretinal vascular complex consists of a circumscribed mass of endothelial cells. The central area contains vascular spaces with a small number of red blood cells. This vascular core is surrounded by an eosinophilic fibrous material. This core appears adherent to the inner portion of the Bruch membrane. Retinal pigment epithelial cells containing pigment epithelial granules (arrows) are seen enveloping the lesion (hematoxylin-eosin, original magnification ×20 before reduction).

Vascular structures within the retina stain positively for VWF. Cells within the RAP also express VWF. The inner portion of the Bruch membrane (arrow) in this area is strongly VWF positive (anti-VWF antibody counterstained with fast red, original magnification ×400 before reduction).

**Figure 4.** Serial section 185 shows that vascular structures within the retina stain positively for von Willebrand factor (VWF). Cells within the retinal angiomatous proliferation also express VWF. The inner portion of the Bruch membrane (arrow) in this area is strongly VWF positive (anti-VWF antibody counterstained with fast red, original magnification ×400 before reduction).
the adjacent neurosensory retina (eFigure 3A). Expression of VEGF by the neurosensory retina has been previously reported.\textsuperscript{11-14} The RPE is strongly positive for VEGF, and the choroid exhibits little immunoreactivity (not shown). No positive staining in the retina is observed for the control section immunostained with rabbit IgG in place of the primary antibody (eFigure 3B).

Based on this case, we confirm that RAP is a distinct form of neovascular ARMD. In RAP, there is intraretinal neovascularization with angiomatic proliferation of capillaries within the retina. There was no subretinal neovascularization present in our case. The surgically excised lesions studied by Lafaut et al\textsuperscript{6} and Shimada et al\textsuperscript{25} were felt clinically to have some component of subretinal neovascularization or choroidal neovascularization. In 2001, it was hypothesized by Yannuzzi et al\textsuperscript{3} that RAP originated as a retinal proliferation that could progress posteriorly into the subretinal space. In 2003, Gass et al\textsuperscript{15} hypothesized that RAP originated on the choroidal side of the Bruch membrane with anterior progression. It seems plausible to us that neovascularization could develop on either side of the RPE, possibly based on perturbations of the balance of VEGF and pigment epithelium–derived factor in this microenvironment.\textsuperscript{19}

The Bruch membrane in the area of RAP stained strongly with VWF, though in other locations the Bruch membrane did not stain positively. von Willebrand factor is a large multimeric glycoprotein made by endothelial cells. Each monomer contains a number of domains, with known specific binding capabilities, though the physiologic significance of every domain is not known. Domain A3 is known to bind to collagen. von Willebrand factor binds specifically to several collagens including I, II, III, IV, V, and VI.\textsuperscript{17} Collagens I, III, IV, and V are known components of the Bruch membrane.\textsuperscript{18,19}

A small comparative cross-sectional study conducted by Lip et al\textsuperscript{20} demonstrated that VWF levels are elevated in patients with ARMD. In this study, VWF levels were compared in “dry” ARMD, “exudative” ARMD, and normal controls. The VWF levels were elevated in both ARMD groups, regardless of the presence of neovascular disease. Hence, Lip et al hypothesized that VWF may not just be an indicator of disease but may contribute to the pathogenesis of ARMD.

Inflammation may play a role in the pathogenesis of ARMD.\textsuperscript{21,22} Pendum et al\textsuperscript{23} hypothesized that VWF may contribute to inflammation and showed that neutrophils and monocytes may adhere directly to VWF. Though a causal relationship has not been identified between ARMD and VWF, the specific pattern of VWF staining in our case may support an association.

Although previous treatment methods for RAP, including laser photocoagulation and photodynamic therapy, had not been particularly effective in altering the course of the disease, the use of intravitreal bevaciuzumab has been recently investigated in a small cohort of patients, showing some benefit.\textsuperscript{24,25} Detection of VEGF in the RAP lesion from our patient suggests that treatments that block the actions of VEGF may have a role to play in the treatment of RAP. Interestingly, intravitreal injections of VEGF in the eyes of adult primates produce concentric layers of proliferating endothelial cells that are similar to the RAP lesion we have described.\textsuperscript{26}

In conclusion, fundus examination and FA images of a RAP lesion in a patient with ARMD correlate histopathologically to a neovascular intraretinal angiomatosus complex, without the presence of sub-RPE neovascularization. Immunostaining demonstrates that RAP expresses VWF and VEGF.

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Correspondence: David J. Wilson, MD, Casey Eye Institute, 3375 SW Terwilliger Blvd, Portland, OR 97239-4197 (wilsonda@ohsu.edu).

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REFERENCES


Ophthalmological Ephemera

In 1795, Dr Isaac Thompson concocted an eye water of zinc sulfate, saffron, camphor, and rose water. It was sold as late as 1939. This is 1 of a series of 32 medical trade cards advertising the product from 1875 through 1895.

Courtesy of: Daniel M. Albert, MD, MS.