Correlation of Type 1 Neovascularization Associated With Acquired Vitelliform Lesion in the Setting of Age-Related Macular Degeneration

CHRISTINE A. CURCIO, CHANDRAKUMAR BALARATNASKINGAM, JEFFREY D. MESSINGER, LAWRENCE A. YANNUZZI, AND K. BAILEY FREUND

• PURPOSE: To correlate postmortem histology with previously recorded multimodal imaging from a patient with type 1 neovascularization (NV) associated with an acquired vitelliform lesion in the setting of age-related macular degeneration (AMD).
• DESIGN: Case study.
• METHODS: Multimodal imaging that was obtained antemortem was matched with ex vivo and high-resolution histologic images of the preserved donor macula. Anatomic correlates for multimodal imaging findings were then defined.
• RESULTS: Spectral-domain optical coherence tomography (OCT) revealed a split in the retinal pigment epithelium–Bruch membrane band. Type 1 NV in this case was composed of 6 layered components: (1) retinal pigment epithelium, (2) basal laminar deposits, (3) fibrovascular membrane, (4) fibrocellular scar, (5) hemorrhage, and (6) Bruch membrane. The anatomic correlates for the hyporeflective band on spectral-domain OCT included a thick basal laminar deposit. Not all structures could be readily separated on the basis of their reflectivity patterns.
• CONCLUSIONS: This is an important clinicopathologic correlation of NV secondary to AMD in the spectral-domain OCT era. Our findings of 6 layers include and extend the anatomic framework encapsulated by the double-layer and triple-layer signs. The resolution of current devices does not always permit distinction of the different layers of NV tissue. Thick basal laminar deposits may appear hyporeflective on spectral-domain OCT and may be confused with fluid from a neovascular process. It will be important to perform a larger clinicopathologic series to aid our anatomic interpretation of spectral-domain OCT images. (Am J Ophthalmol 2015;160(5):1024–1033. © 2015 by Elsevier Inc. All rights reserved.)

Choroidal neovascularization (NV) is a sight-threatening complication of age-related macular degeneration (AMD). The management of neovascular AMD has been greatly aided by spectral-domain optical coherence tomography (OCT), an imaging modality that utilizes the properties of light backscatter to provide fine detail about retinal and choroidal structures. Spectral-domain OCT permits anatomic localization of NV and also allows inference about the biological effects of vascular endothelial growth factor (VEGF) inhibitors on NV tissues localized to different compartments. In clinical practice, changes in spectral-domain OCT reflectivity patterns in the sub–retinal pigment epithelium (RPE), subretinal, and intraretinal compartments are used to judge response to anti-VEGF therapy and guide decisions concerning frequency of repeat therapy and interval review. Hemorrhage, exudates, and fibrovascular tissue can alter the reflectivity patterns of near-infrared light and thus confuse the interpretation of spectral-domain OCT images. Previous authors have described pathognomonic reflectivity patterns, such as the double-layer and triple-layer signs, to signify active angiogenesis in polypoidal choroidal vasculopathy (PCV) and AMD. However, these designations have yet to be validated histologically. Resolving the anatomic correlation for the reflectivity patterns seen on spectral-domain OCT is important, as it may aid our ability to assess neovascular disease activity in vivo. It may also provide new information that can be used to identify indolent NV structures that may have previously been unrecognized.

Vitelliform lesions may be seen in eyes with AMD and may also be encountered in Best macular dystrophy, cuticular drusen, vitreoretinal interface disorders, peripherin (retinal degeneration, slow) mutation-related...
macular dystrophies,\textsuperscript{12} and other degenerative maculopathies including adult-onset foveomacular vitelliform dystrophy.\textsuperscript{13} In this report we present a clinicopathologic correlation of type 1 (sub-RPE) NV\textsuperscript{3} in the setting of an acquired vitelliform lesion in an eye that demonstrated the clinical and pathologic characteristics of AMD. We correlate multimodal imaging results including fundus photography, indocyanine green angiography (ICGA), and spectral-domain OCT with histopathologic findings. Novel postmortem eye-tracking techniques are employed to ensure accurate alignment of in vivo and ex vivo B-scans and facilitate reliable comparisons between spectral-domain OCT and histology.\textsuperscript{14} The findings in this study are expected to aid in the clinical management of AMD.

Spectral-domain OCT images were obtained using the Spectralis HRA + OCT (Heidelberg Engineering, Heidelberg, Germany). Eye-tracking and image registration software was enabled during acquisition of spectral-domain OCT images. Color fundus photographs and red-free, fundus autofluorescence, and ICGA images were obtained with the Topcon TRC-50XF (Topcon America, Paramus, NJ). The time period between the most recent image by an imaging modality and the patient’s death was 13 months for color photography, 13 months for red-free photography, 13 months for fundus autofluorescence imaging, 8 months for near-infrared reflectance imaging, 8 months for spectral-domain OCT, and 28 months for ICGA.

**METHODS**

**THIS CASE STUDY COMPLIED WITH THE HEALTH INSURANCE Portability and Accountability Act of 1996 and followed the tenets of the Declaration of Helsinki. The institutional review board of the Manhattan Eye, Ear and Throat Hospital/ North Shore Long Island Jewish Hospital approved the retrospective review of clinical notes and imaging data. This board plus the Institutional Review Board at the University of Alabama at Birmingham approved the histopathology study.**

**CLINICAL CARE AND MULTIMODAL IMAGING:** Postmortem histopathologic examination was performed on the right eye of a long-term patient with AMD who had undergone serial examination and multimodal imaging as part of her clinical management. She had received care at Vitreous Retina Macula Consultants of New York for 11 years. Ocular morbidities other than AMD included pseudoexfoliation glaucoma with stable intraocular pressure control. The right eye also developed ciliary macular edema following cataract extraction and anterior chamber intraocular lens insertion 6 years prior to her death. Edema resolved following treatment with topical steroid and nonsteroidal anti-inflammatory therapy.

Serial multimodal imaging of her clinical course included fundus autofluorescence, spectral-domain OCT, high-resolution color fundus photography, red-free imaging, near-infrared reflectance imaging, and ICGA.

**HISTOPATHOLOGY:** Personnel of the Eye Bank for Sight Restoration (New York, NY) recovered eyes 8 hours and 55 minutes after donor death. Globes were opened with encircling cuts at the limbus and immersed in 10% neutral buffered formalin. Following overnight shipping to Birmingham, eyes were transferred to 2% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer and subject to multimodal ex vivo imaging as described.\textsuperscript{14,15} Eye-tracking software (Spectralis; Heidelberg Engineering, Heidelberg, Germany) was used to align in vivo and ex vivo B-scans from preserved globes. Then, 8-mm-diameter full-tissue-thickness punches centered on the fovea were postfixed in osmium tannic acid paraformaldehyde, embedded in epoxy resin, sectioned at 0.8 μm thickness, and stained with toluidine blue.\textsuperscript{16} This preparation makes lipid-containing material in basal linear deposits and soft drusen gray or brown and basal laminar deposits predominantly blue. Tissue was oriented during sectioning so that a line between the centers of the fovea and optic nerve head was always parallel to the edge of a diamond knife, aided by a fine line drawn across the resin block over these landmarks under a dissecting microscope. The distance between sections indicated by the controls of the ultramicrotome guided the correspondence to spectral-domain OCT scans. Histologic sections matched to the spectral-domain OCT scans were digitized in their entirety using image-stitching software (CellSens; Olympus, Center Valley, Pennsylvania, USA) and a 20× plan-apochromat objective. Correspondences between histology and spectral-domain OCT were verified by comparing images of entire sections to scans, with specific reference to the

**FIGURE 1.** Baseline examination findings of the case study and the temporal course of vitelliform collapse. Color photograph (Top left), fundus autofluorescence (Top right), and spectral-domain optical coherence tomography (OCT) images demonstrate an acquired vitelliform lesion in the central macula at baseline examination. Soft drusen are discernible (Top left, arrowheads). The acquired vitelliform lesion is characterized by yellow, subretinal, hyperautofluorescent material that is hyperreflective on spectral-domain OCT. The series of spectral-domain OCT scans represent changes to the acquired vitelliform lesion and outer retina over the final 43 months of management. Most of the acquired vitelliform lesion has reabsorbed by 24 months. Then, 2 hyperreflective bands correlating to the retinal pigment epithelial layer (red arrows) and tissue complex including Bruch membrane (yellow arrow) appear with an intervening hyporeflective space. Within the hyporeflective space are hyperreflective spots. There is little change in this configuration and reflectivity properties between 24 and 43 months (final visit).
contour of the inner retinal surface, contour of the inner nuclear layer,17 and the horizontal extent of basal laminar deposits and split RPE–Bruch membrane complex.18 Matching histologic sections and OCT scans at each level are presented in the Supplementary Figure (available at AJO.com). Selected areas were imaged using 60× oil immersion objectives (numerical aperture 1.40 and 1.42). Images were montaged, composited, and adjusted for exposure, contrast, and sharpness (Photoshop CS6; Adobe, San Jose, California, USA). Measurements of horizontal extents and thicknesses of histologic layers were made using the digital readout on the motorized microscope stage. Findings were compared to those from 52 similarly prepared advanced AMD eyes available at http://projectmacula, and to the descriptions of basal laminar deposits and RPE cells generated from these eyes.18 We previously reported clinicopathologic correlations of outer retinal tubulation and RPE degeneration in this patient.14,19

RESULTS

• CLINICAL COURSE AND MULTIMODAL IMAGING FINDINGS: Presenting visual acuity in the right eye was 20/50. Baseline retinal examination revealed an acquired vitelliform lesion in the central macula, soft drusen, and focal areas of pigmentary changes (Figure 1, Top left), as well as reticular pseudodrusen in the superior macula (Supplementary Figure, Left). There was no exudation, subretinal fluid, or hemorrhage at baseline examination.

Fundus autofluorescence imaging demonstrated hyperautofluorescence that correlated with the acquired vitelliform lesion surrounded by variable autofluorescence that correlated with the pigmentary changes (Figure 1, Top right). The acquired vitelliform lesion was characterized by subretinal hyperreflective material on spectral-domain OCT, and reabsorption of this material occurred over the course of 2 years (Figure 1). Following regression of the acquired vitelliform lesion, a hyporeflective band with punctate hyperreflective spots was observed to split the normally hyperreflective RPE–Bruch membrane complex. The RPE–Bruch membrane complex in this case had an irregular and sometimes interrupted hyperreflective band that correlated to the RPE (Figure 1, red arrows). Underlying this band was another band that correlated to a tissue complex (yellow arrows, Figure 1) notable for thin, closely spaced, and horizontally oriented hyperreflective lamellae. This distinctive signature was readily apparent on all spectral-domain OCT images from the point of acquired vitelliform lesion reabsorption to the final visit. We observed little change in the morphology, configuration, and reflectivity patterns of this finding between visits (Figure 1), with the exception of the RPE band becoming thinner and discontinuous. The underlying tissue complex became more visible, presumably owing to greater light penetration subsequent to RPE loss. In contrast, the complex was just barely visible prior to the acquired vitelliform lesion collapse at 24 months.

Visual acuity remained at 20/400 following reabsorption of the acquired vitelliform lesion. Clinically, a legacy of central chorioretinal atrophy and pigmentary changes were evident at the site of the prior acquired vitelliform lesion. ICGA performed 12 months before the final visit showed an indeterminate area of central staining (Figure 2), possibly consistent with type 1 NV. Intravitreal anti-VEGF injections were not administered during the course of this patient’s management. The death of the patient from gastric cancer occurred 8 months after her last clinic visit, where she was examined and also underwent spectral-domain OCT imaging.

• HISTOPATHOLOGY AND SPECTRAL-DOMAIN OPTICAL COHERENCE TOMOGRAPHY CORRELATION: Contours of the inner retinal surface, inner nuclear layer, and RPE–Bruch membrane morphology were well aligned in histologic sections and in spectral-domain OCT scans (Supplementary Figure). Histopathologic findings are detailed in Figures 2–4. Spectral-domain OCT scans and histologic sections covered the central 4.32 mm (superior to inferior diameter) of the macula and the histomorphometry is summarized in the Appendix.

Figure 2 (Top row) demonstrates central atrophy and a faint hyperfluorescent plaque by ICGA. A magnified spectral-domain OCT scan (Figure 2, Second row) shows hyperreflective RPE, the hyporeflective band, and the tissue complex including the Bruch membrane. Corresponding histology in Figure 2 (Third row and Fourth row) reveals multiple layers in the tissue complex consistent with type 1 NV. From inner to outer (Figure 2, Fourth row, left), dissociated RPE cells sit atop thick basal laminar deposits, which are internal to a fibrovascular scar. The scar ranged from 3 to 73 μm thick across the atrophic area (Appendix, available at AJO.com). Further external to the scar is hemorrhage that bows the Bruch membrane outward. Extravascular erythrocytes with minimal associated plasma are also visible within the scar (Figure 2, Fourth row, left). The Bruch membrane was calcified in patches, and choriocapillaris was atrophied, especially under the fibrovascular scar.

Corresponding to the hyporeflective band on spectral-domain OCT (Figure 2, Second row) was an elaborate basal laminar deposit (Figure 2, Third row and Fourth row). In the atrophic area basal laminar deposits sharply elevated the RPE (median thickness, 50 μm; Appendix), reaching a remarkable thickness of 90–120 μm in the fovea. This eye demonstrated early, late, and rivulet forms of basal laminar deposits (Figure 2, Fourth row, right).18,20 On its outer aspect, basal laminar deposits contained
FIGURE 2. Correlation between multimodal imaging and histopathology in type 1 neovascularization in the case study. (Top row, left) Color fundus photograph of the right eye at the final visit (8 months before death) demonstrates central chorioretinal atrophy. (Top row, right) Late-phase indocyanine green angiogram acquired 1 year before the final visit (20 months before death) reveals a faint central hyperfluorescent plaque possibly representing type 1 neovascularization. (Second row) Spectral-domain optical coherence tomography (OCT) image from the last visit acquired at the superior aspect of the central area of geographic atrophy. Red arrows indicate retinal pigment epithelium (RPE), which is a continuous epithelium on the left, and a series of dissociated cells elsewhere. Yellow arrows bracket a hyperreflective tissue complex that includes (from inner to outer) fibrovascular scar, hemorrhage, and Bruch membrane (BrM). Teal arrow indicates BrM without scar. (Third row) Histologic section that is matched to the OCT scan. Red-framed area is enlarged in the fourth row, left panel. Splitting of the Henle fiber layer (x) is a postmortem artifact. (Fourth row, left) Dissociated RPE cells are found atop a thick basal laminar deposit (BLamD). The BLamD is internal to a fibrovascular scar (fv s). External to the scar is hemorrhage (*), which bows outward Bruch’s membrane (bracketing white arrowheads) and covers a druse (d). Green frame is enlarged in fourth row, right panel. Fourth row, right: BLamD has 3 sublayers: rivulet (R), late (L), and early (E). Basal mounds contain calcific nodules (black arrowhead). ONL = outer nuclear layer.
aggregations of granules shed from RPE and basal mounds (focal areas of soft druse material) (Figure 2, Fourth row, right).

Figure 3 demonstrates details of extracellular lesions, type 1 NV with associated exudation, and neurodegeneration that collectively establish this case as AMD. Figure 3 (Top panel) shows basal linear deposit and subretinal drusenoid deposit. In contrast to the extensive basal laminar deposits and fibrovascular scar (Appendix), active NV was confined to the superior-nasal quadrant. Figure 3 (Third row, left) shows sub-RPE NV with endothelium, pericytes, intravascular blood cells with plasma, minimal extracellular matrix, and overlying intraretinal exudation. Hemorrhage was found in subscar, intrascar, subretinal, and intraretinal locations throughout the atrophic area (Figure 3, Second row, left). In the center of the atrophic area and within the scar were ovoid RPE mixed with erythrocytes (Figure 3, Third row, right). Of note was a marked depopulation of the outer nuclear layer and corresponding swelling of Müller cell processes (Figure 3, Second row, left). Within the outer nuclear layer were pockets of fluid that contained lipid (Figure 3, Second row, right).

FIGURE 3. Histopathology of neovascular age-related macular degeneration in the setting of a collapsed vitelliform lesion in the case study. Sub-micrometer epoxy resin and toluidine blue stain sections demonstrate regions of exudation, neovascularization, retinal pigment epithelium (RPE) atrophy, and neurodegeneration within the specimen. (Top row) Basal linear deposit (white arrowheads) and subretinal drusenoid deposit (SDD) are both extracellular in position. (Second row, left) Hemorrhages in the outer nuclear layer (ONL), Henle fiber layer, and subretinal space are seen. Hemorrhage is also found between basal laminar deposits and Bruch membrane (bracketing black arrowheads at left) and within the fibrovascular scar (bracketing black arrowheads at right). The ONL contains few photoreceptor nuclei and many swollen Müller cell processes. (Second row, right) Gray-staining fluid-containing cells in the ONL are identified at sites of exudation. (Third row, left) Type 1 neovascularization (green arrowheads) has endothelium and pericytes. The poor preservation of overlying photoreceptors is artifact. (Third row, right) Sloughed RPE (yellow arrowhead) and hemorrhage (teal arrowhead). RPE cells are entombed in the fibrocellular scar. INL = inner nuclear layer. External limiting membrane is indicated by red arrows in all panels.

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DISCUSSION

ADULT-ONSET FOVEOMACULAR VITELLIFORM DYSTROPHY can manifest vitelliform lesions in later life.\(^\text{21}\) However, the diagnosis of AMD was used in this case because of the occurrence of soft drusen and RPE abnormalities, which typify AMD.\(^\text{22}\) Reticular pseudodrusen were also present in this eye. Although this feature is not currently used in classifying AMD, increasing evidence suggests that reticular pseudodrusen are important in the pathogenesis of AMD.\(^\text{23}\) Histologic analysis of this case also supported the diagnosis of AMD, namely the identification of extensive basal linear and basal laminar deposits.\(^\text{24}\) In contrast, for another condition associated with vitelliform lesions, pattern dystrophy due to mutations of peripherin (retinal degeneration, slow), histopathology has demonstrated minimal basal laminar deposit without either basal linear deposits or drusen.\(^\text{25}\)

Our study joins a select group of reports that correlated clinical imaging and histopathology of intact eyes with neovascular AMD (Table). Our report is notable for correlating NV histology to multimodal imaging (including spectral-domain OCT) and for using histology that is both panoramic and sufficiently high-resolution to comprehensively disclose delicate sub-RPE tissue. Previous correlations between histology and angiography\(^\text{26–31}\) employed routine paraffin histology (5- to 7-μm-thick-sections; approximately 600–800 sections per eye) to create 2-dimensional en face maps. These maps were used to demonstrate NV membranes in subretinal and sub-RPE compartments, breaks in the Bruch membrane, and vessels penetrating the choroid in a format that recapitulated en face ophthalmoscopy and angiography.\(^\text{26–34}\) Paraffin sections are suboptimal for basal laminar and basal linear deposits, however. We used sub-micrometer-thick epoxy sections that provided spatial resolution similar to low-magnification electron microscopy.\(^\text{24}\) Compositional information was attainable via a lipid-preserving postfixation technique and a polychromatic stain. Stepped histologic sections were matched within an estimated 50–100 μm to spectral-domain OCT scans (Supplementary Figure). This was enabled by a novel application of eye-tracking software to register the vascular landmarks in clinical and ex vivo scanning laser ophthalmoscopy images of the same eye, thus ensuring precise histology-OCT correlation.\(^\text{14,15}\)

Although the findings of NV were indeterminate on ICGA, type 1 NV with hemorrhage was identified on

<table>
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<tr>
<th>Reference</th>
<th>Year</th>
<th>Eyes</th>
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<th>Last Clinical Examination Before Death</th>
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<tr>
<td>31</td>
<td>1976</td>
<td>OS</td>
<td>FA, 29 months</td>
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<td>1976</td>
<td>OD</td>
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<td>OD</td>
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<td>3 weeks</td>
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<td>27</td>
<td>1994</td>
<td>OU</td>
<td>FA/ICGA, 15 months</td>
<td>3 months</td>
</tr>
<tr>
<td>34</td>
<td>2000</td>
<td>OS</td>
<td>FA/ICGA, 4.5 months</td>
<td>4.5 months</td>
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<tr>
<td>26</td>
<td>2006</td>
<td>OS</td>
<td>FA, 6 months</td>
<td>6 months</td>
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<tr>
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<td>OD</td>
<td>FA, 21 months</td>
<td>21 months</td>
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<tr>
<td>26</td>
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<td>Current</td>
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<td>FA/ICGA, 28 months</td>
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FA = fluorescein angiography; ICGA = indocyanine green angiography.

FIGURE 4. Six histologic layers in type 1 neovascularization identified in the case study. A histologic specimen is matched to the inset represented on the spectral-domain optical coherence tomography scan. At the thickest point of the hemorrhage, all 6 layers are apparent: retinal pigment epithelial layer (RPE), basal laminar deposit (BLamD), fibrovascular (fv) and fibrocellular (fc) membranes, hemorrhage (H), and Bruch membrane (BrM).
postmortem histopathology. This finding is consistent with our previous report showing that type 1 NV is typically ill-defined on ICGA. In the present report, the discrepancy between clinical and histopathologic findings may have been owing to evolution of type 1 NV lesion since the last clinic visit. However, we believe that this is an unlikely reason because there was strong correlation in the morphology of the sub-RPE and outer retinal compartments between postmortem histology and spectral-domain OCT images obtained at the final visit. Klein and Wilson proposed that fluorescence signals from small-caliber vessels within neovascular tissue may be masked by overlying RPE and dense fibrovascular tissue. In our case, vessels with neovascular tissue were small and restricted to 1 quadrant of the atrophic area, possibly contributing to difficulty in clinical identification of NV. An important insight as to why type 1 NV was not identified on multimodal imaging was the presence of thick (≈30–40 μm) and elaborate basal laminar deposits throughout the atrophic area. We propose that basal laminar deposits may have a masking effect similar to a fibrovascular scar on multimodal imaging. Basal laminar deposits represent staged thickening of the RPE basement membrane that is common in aged eyes and especially abundant in AMD eyes. Its constituents are primarily extracellular matrix proteins and lipoprotein particles en route to the choriocapillaris. In our specimen, basal laminar deposits reached a maximum thickness of 120 μm and were comparable to the thickest basal laminar deposit measurement reported in advanced AMD. The mean values in our specimen (Appendix) were also greater than basal laminar deposit measurements previously reported in neovascular AMD. Very thick basal laminar deposits are characteristic of several adult-onset inherited maculopathies that also feature NV.

Histologic findings in our study affirm and extend previous observations (Table) concerning the cellular composition of type 1 NV tissue. We identified 6 layers in our case: (1) RPE, (2) basal laminar deposits, (3) fibrovascular membrane, (4) fibrocellular scar, (5) potential space where hemorrhage and serous fluid can accumulate, and (6) Bruch membrane. Importantly, our study confirms that vessels in type 1 NV complexes occur on the outer surface of basal laminar deposits and shows that hemorrhage can occur on the inner surface of the Bruch membrane under a fibrovascular scar. By providing definitive histologic evidence that a separation between basal laminar deposits and the inner collagenous layer of the Bruch membrane can fill with numerous distinct sublayers, our data strengthen the concept raised by previous authors that this is a natural cleavage plane.

Our data provide anatomic knowledge that can refine the interpretation of spectral-domain OCT images in the setting of AMD. The double-layer and triple-layer signs are imaging terms used to denote the presence of type 1 NV in PCV and AMD. The double-layer sign of Sato is characterized by 2 hyperreflective bands (RPE and Bruch membrane) with an intervening hyporeflective space proposed to represent fluid accumulation from NV. In PCV this space has also been interpreted as penetrating vessels and the inner reflective band as RPE attached to Bruch membrane separated from choroid; this idea is currently unsupported by the limited histology available. The triple-layer sign includes (from the RPE band outward) large, irregular, dilated structures consistent with vessels; a horizontally multilamellar component consistent with fibrovascular tissue; and a hyporeflective prechoroidal cleft between the fibrous component and hyporeflective Bruch membrane. The triple-layer appearance is thought to result from a combination of vascular exudation and fibrovascular contraction; basal laminar deposits were not included among the layers. Although in vivo spectral-domain OCT findings in our patient superficially resembled the double-layer sign, histopathologic correlation indicated that it also contained layers of the triple-layer sign, that is, lamellae attributable to fibrovascular scar and the cleft. A cleft that was clearly identified on histology was not evident on spectral-domain OCT, possibly because internal reflectivity of the hemorrhage reduced overall contrast relative to surrounding tissues. An important finding in our study is that basal laminar deposits can appear hyporeflective on spectral-domain OCT in some cases. Because thicker basal laminar deposits can displace the RPE and lead to a hyporeflective band on the outer surface of the RPE, it can be misinterpreted as fluid from NV on spectral-domain OCT. Clinicians should therefore interpret this imaging finding in the context of the disease.

Our study also demonstrates that indolent NV tissue may be present within an area of geographic atrophy without clear-cut clinical or multimodal imaging evidence of NV. The relevance of this finding to a decision whether anti-VEGF therapy should be initiated to manage these lesions is beyond the scope of this report. The results of our clinicopathologic correlations support the anatomic framework encapsulated in part by the double- and triple-layer signs. However, it also demonstrates that not all NV cases have pathognomonic spectral-domain OCT appearances and that current imaging devices lack sufficient resolution to separate the 6 known histologically possible elements of NV tissue. These findings underscore the importance of newer imaging modalities, including OCT angiography, that use dynamic parameters, such as flow, to detect NV.

Type 1 NV is a common subtype of NV in AMD. This report directly correlates histopathology to multimodal imaging in neovascular AMD. Our results highlight the advantages and limitations of spectral-domain OCT in evaluating the anatomy of type 1 NV. It will be important to perform a larger series of similar correlations and also to expand the study to include other NV subtypes. Knowledge from these investigations will be invaluable in defining multimodal imaging indices that can be reliably used to guide AMD management.
REFERENCES


26. Grossniklaus HE, Wilson DJ, Bressler SB, et al. Clinicopathologic studies of eyes that were obtained postmortem from four patients who were enrolled in the submacular surgery


Christine A. Curcio, PhD is professor of ophthalmology at the University of Alabama School of Medicine. She investigates retinal aging and age-related macular degeneration through clinical image validation, cell biology, lipoprotein biology, neurodegeneration, and transcriptomics in >110 peer review articles. She is a member of the Investigative Ophthalmology & Visual Science editorial board and an NIH study section. She was awarded the 2014 Ludwig von Sallmann Prize for lifetime contribution to vision research.
SUPPLEMENTARY FIGURE. Correlation between spectral-domain optical coherence tomography and histology findings for type 1 neovascularization in the setting of a collapsed acquired vitelliform lesion in the case study. Clinical imaging was obtained in April 2013, 8 months before death. Infrared reflectance scanning laser ophthalmoscopy (Left panel) shows green lines at the 19 levels at which B-scans were obtained (Middle panel). Histology images (Right panel) were scanned from toluidine blue–stained sub-micrometer sections of epoxy-embedded tissue that had been postfixed with osmium tannic acid paraphenylenediamine. Sections were matched to the in vivo scans using ex vivo scanning laser ophthalmoscopy, eye-tracking software (Spectralis, Heidelberg Engineering), and the section counter on an ultramicrotome. The fovea was present at levels 9–10. An area of central atrophy was present at levels 3–13. Level 11 was shown in Figure 2 of the main article; levels 6, 12, and 13 were shown in Figure 3; and level 12 was shown in Figure 4. Histology of outer retinal tubulation at levels 5–6 and RPE morphologies at level 8 have been published separately.\textsuperscript{10,15} The splitting of outer retina in levels 12–19 and the undulation of inner retinal layers in most levels is artifact due to the postmortem interval to fixation (8:55 h), which resulted in edema and autolysis.
### APPENDIX. Histomorphometric Measurements of Basal Laminar Deposit, Fibrovascular Scar, and Hemorrhage

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<th>BLamD</th>
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<th>Scar</th>
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BLamD = basal laminar deposit; NA = not applicable.