

# Multimodal Imaging and Spatial Analysis of Ebola Retinal Lesions in 14 Survivors of Ebola Virus Disease

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**IMPORTANCE** Differentiation between Ebola retinal lesions and other retinal pathologies in West Africa is important, and the pathogenesis of Ebola retinal disease remains poorly understood.

**OBJECTIVE** To describe the appearance of Ebola virus disease (EVD) retinal lesions using multimodal imaging to enable inferences on potential pathogenesis.

**DESIGN, SETTING, AND PARTICIPANTS** This prospective case series study was carried out at 34 Military Hospital in Freetown, Sierra Leone. Ophthalmological images were analyzed from 14 consecutively identified survivors of EVD of Sierra Leonean origin who had identified Ebola retinal lesions.

**MAIN OUTCOMES AND MEASURES** Multimodal imaging findings including ultra-widefield scanning laser ophthalmoscopy, fundus autofluorescence, swept-source optical coherence tomography (OCT), Humphrey visual field analysis, and spatial analysis.

**RESULTS** The 14 study participants had a mean (SD) age of 37.1 (8.8) years; 6 (43%) were women. A total of 141 Ebola retinal lesions were observed in 22 of 27 eyes (81%) of these 14 survivors on ultra-widefield imaging. Of these, 41 lesions (29.1%) were accessible to OCT imaging. Retinal lesions were predominantly nonpigmented with a pale-gray appearance. Peripapillary lesions exhibited variable curvatures in keeping with the retinal nerve fiber layer projections. All lesions respected the horizontal raphe and spared the fovea. The OCT imaging demonstrated a V-shaped hyperreflectivity of the outer nuclear layer overlying discontinuities of the ellipsoid zone and interdigitation zone in the smaller lesions. Larger lesions caused a collapse of the retinal layers and loss of retinal thickness. Lesion shapes were variable, but sharp angulations were characteristic. Perilesional areas of dark without pressure (thinned ellipsoid zone hyporeflectivity) accompanied 125 of the 141 lesions (88.7%) to varying extents.

**CONCLUSIONS AND RELEVANCE** We demonstrate OCT evidence of localized pathological changes at the level of the photoreceptors in small lesions among survivors of EVD with retinal lesions. The relevance of associated areas of dark without pressure remains undetermined.

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We previously conducted a case-control study that identified retinal characteristics specific to survivors of Ebola virus disease (EVD) in Sierra Leone using ultra-widefield (UWF) retinal imaging. Of all retinal lesions characterized in the previous study, only 1 lesion of characteristic morphological appearance was exclusively identified in Ebola survivors by 2 masked graders.<sup>1</sup> On this basis, this lesion was deemed most likely to be associated with Ebola virus infection. Identical lesions have been identified in other cohort studies of survivors of EVD.<sup>2,3</sup> This expanded analysis provides optical coherence tomography (OCT) interpretation and functional visual field (VF) analysis to provide further insights into the pathophysiology of Ebola retinal sequelae.

## Methods

A total of 160 survivors of EVD attended the ophthalmology clinic at 34 Military Hospital in Freetown, Sierra Leone, between January 2016 and April 2017. Of these, 14 survivors of EVD (8.8%) met the eligibility criterion derived from the findings of our previous study, which was having at least 1 Ebola retinal lesion identified on UWF retinal imaging.<sup>1</sup> All 14 patients were recalled to the clinic and attended examinations that included OCT of accessible Ebola retinal lesions. Thirteen of these 14 survivors (93%) were recruited to this study, and 1 (7%) was excluded because of increased lens opacity that prevented fundus imaging. One further eligible patient was identified in March 2017 and directly enrolled into the study.

Informed consent was obtained from all participants. The study was approved by the Office of Sierra Leone Ethics and Scientific Review Committee on January 31, 2017, and followed the tenets of the Declaration of Helsinki.<sup>4</sup>

The appearance of lesions on OCT was categorized, and lesion grading concordance was compared by 2 ophthalmologists (P.J.S. and N.A.V.B.) who were each masked to the grades of the other grader. The examination protocol is summarized in eMethods 1 and eFigure 1 in the [Supplement](#).

## Results

The 14 participants had a mean (SD) age of 37.1 (8.8) years. Six (43%) were women. The participants had a total of 27 eyes (1 patient had no view of the fundus available for retinal examination in 1 eye as a result of previous ocular trauma that had led to corneal and lens opacity followed by complicated surgery before Ebola infection). We analyzed 141 Ebola retinal lesions in 22 of the 27 eyes (81%; including 16 eyes of 8 patients with bilateral involvement and 6 eyes of 6 patients with unilateral involvement). Images were obtained by OCT of 41 of the 141 lesions (29.1%) in 20 of the 22 eyes (74% of the 27 total eyes). Characteristics of all 14 patients are summarized in eTable 1 in the [Supplement](#). Corresponding multimodal imaging and VF images are available in eFigures 2 through 23 in the [Supplement](#).

## Key Points

**Question** Can multimodal imaging of Ebola retinal lesions inform our understanding of their pathogenesis?

**Findings** In this case-series study of 14 survivors, optical coherence tomography demonstrated a V-shaped increased reflectivity of the outer nuclear layer overlying discontinuities of the ellipsoid zone and interdigitation zone in the smallest lesions. A collapse of the overlying retinal structures was detected in larger lesions, corresponding visual field defects respected the horizontal raphe, and perilesional areas of dark without pressure (ellipsoid zone hyporeflectivity) accompanied 89% of lesions.

**Meanings** These findings are consistent with a neuronal rather than vascular pathogenesis; the relevance of dark without pressure is undetermined.

## Retinal Lesions

Ebola retinal lesions varied in size and shape, but distinctive linear borders with sharp angulations were characteristic (eFigure 11 in the [Supplement](#)). Multimodal imaging features varied according to severity and extent of retinal structures involved. A lesion severity grading from 1 to 5 is outlined in eTable 2 in the [Supplement](#); results are shown in eTable 3 of the [Supplement](#). The Cohen  $\kappa$  statistic of intergrader agreement was 0.77 (eTable 4 in the [Supplement](#)).

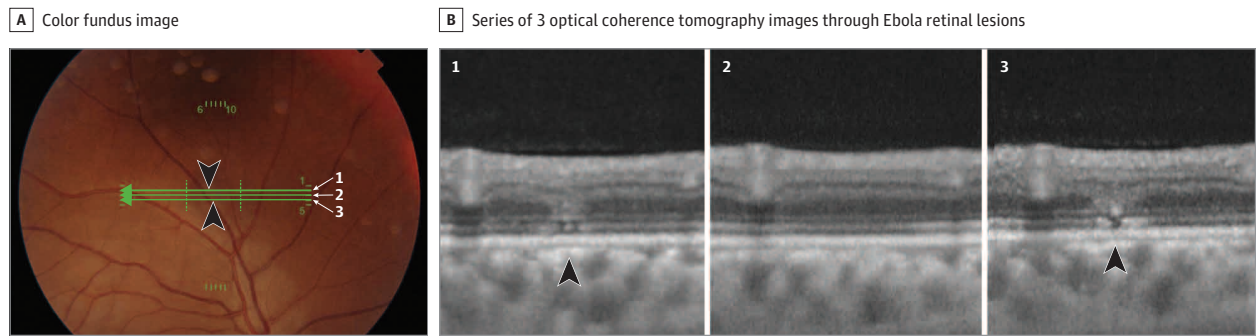
The OCT images of the smallest lesions demonstrated multiple vertical discontinuities of the ellipsoid zone (EZ) and interdigitation zone (IZ) with overlying V-shaped increased reflectance of the outer nuclear layer (Figure 1 and eFigure 21 in the [Supplement](#)). Lesions appeared light gray in color on fundus photography and were predominantly nonpigmented.

Peripapillary lesions demonstrated variable curvatures depending on the optic disc perimeter location and resembled the arcuate anatomical pathways of the retinal nerve fiber layer (ganglion cell axons) (eFigures 4, 10, 11, and 15 in the [Supplement](#)). Visual acuity and color vision were preserved in all cases in the absence of other pathology. Corresponding absolute VF defects that respected the anatomical horizontal raphe were observed on 24-2 Humphrey visual field analysis (eFigure 10 in the [Supplement](#)) and with a peripheral nasal 24-2 test protocol (eMethods 2 and eFigure 10 in the [Supplement](#)).

## Dark Without Pressure

Well-defined areas of dark without pressure (DWP) that corresponded on OCT imaging to a thinned, hyporeflective EZ and absent IZ (Figure 2) were seen adjacent to 125 of 141 Ebola retinal lesions (88.7%) in this series. The extent of DWP was variable, ranging from a confined circumferential marginal zone (eFigures 2, 6, 8, 14, 19, and 20 in the [Supplement](#)) to larger defined areas (eFigures 5, 7, and 15 in the [Supplement](#) and Figure 2) and in some cases 360° panretinal involvement (eFigure 16 in the [Supplement](#)). The extent of DWP in some eyes appeared to be associated with the density of Ebola retinal lesions (Figure 2 and eFigure 15 in the [Supplement](#)). In all cases, DWP appeared to spare the macula. No associated vitreous inflammation or vitreous traction were visible on OCT imaging.

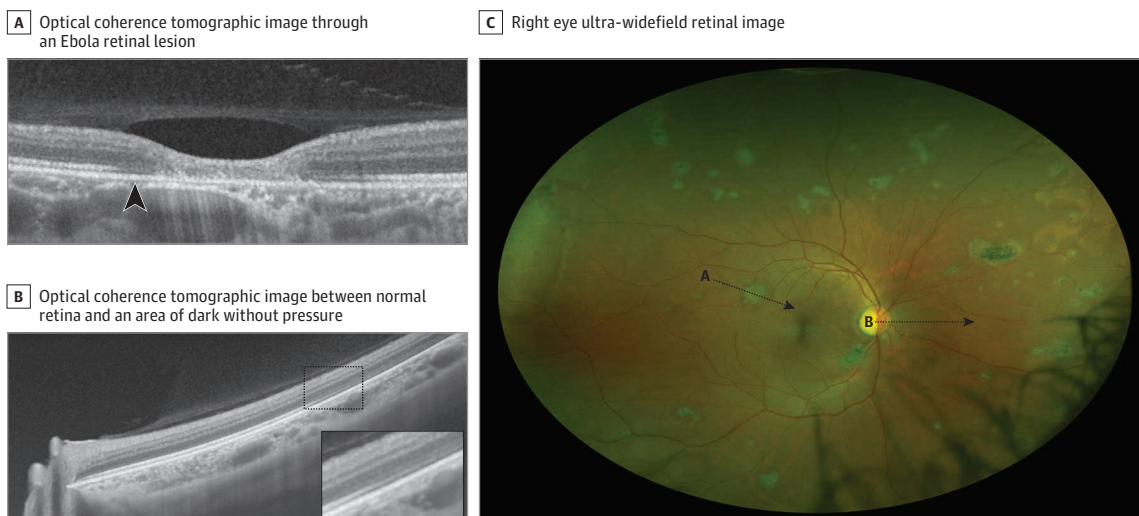
Figure 1. Ebola Retinal Lesions



A, In the color fundus image, green lines indicate optical coherence tomographic scan locations for corresponding optical coherence tomographic images; black arrowheads indicate lesion sites. B, Three optical coherence tomographic images through Ebola retinal lesions demonstrate the proximity of

multifocal discontinuities of the ellipsoid zone with an overlying V-shaped increased reflectance of the outer nuclear layer (equal to the reflectance of the adjacent outer plexiform layer). Black arrowheads indicate lesion sites.

Figure 2. Extensive Dark Without Pressure



A, An optical coherence tomographic image through an Ebola retinal lesion; arrowhead indicates an area of perilesional dark without pressure that corresponds to a thinned hyporeflective ellipsoid zone and absent interdigitation zone. B, An optical coherence tomographic image shows the transition between the normal retina and the circumferential extension of an area of dark without pressure near the nose. The enlarged inset highlights the

transitional zone. C, Multiple Ebola retinal lesions and associated areas of dark without pressure are visible in a right-eye ultra-widefield retinal image. The specific length and direction of the line labeled "A" precisely correlates to the length and direction of the cross-sectional optical coherence tomographic slice shown in image A, and the line labeled "B" correlates to the length and direction of image B.

The 120-point screen, 60-4 threshold tests, and peripheral nasal 24-2 protocol (eFigure 10 in the Supplement) were unable to conclusively identify any definitive VF defect corresponding to areas of DWP. The 24-2 Humphrey visual field analysis of 1 survivor with right hemiparesis after acute infection demonstrated a right-sided homonymous hemianopia and left inferior quadrantanopia (eFigures 19 and 20 in the Supplement).

### Spatial Analysis

Aligned and amalgamated retinal images with corresponding Ebola retinal lesion loci and longitudinal axis are shown in eFigure 24 in the Supplement. No overlapping axes or crossing of the horizontal raphe was observed.

### Discussion

We present a multimodal imaging analysis of a series of 14 survivors of EVD with Ebola retinal lesions, as characterized in our previous case-control study.<sup>1</sup> While OCT analysis of larger lesions involving all retinal layers provides little insight into pathogenesis, OCT observations of small lesions revealed multifocal fine discontinuities of the EZ and IZ with overlying increased reflectivity of the outer nuclear layer (Figure 1). These findings mirror the histological appearance of early herpes simplex virus retinopathy observed in the contralateral retina via a retrograde axonal transmission after unilateral anterior chamber viral inoculation.<sup>5,6</sup> They have also been observed in the

ipsilateral retina following unilateral anterior chamber viral inoculation,<sup>6</sup> although in all cases in this study, we did not identify signs of previous anterior chamber uveitis that would suggest a direct anterior to posterior dissemination. Peripapillary curvilinear lesions resembling the arcuate path of the ganglion cell axons were shown on both imaging and VF analysis to respect the horizontal raphe. Their presence provides further evidence that EVD involves retinal ganglion cells and creates a lasting insult to their afferent photoreceptors.

Possible pathogenic mechanisms for the characteristic retinal lesions observed in survivors of EVD could include retrograde neuronal transmission. Vascular ocular dissemination and involvement of the optic nerve leptomeninges has been demonstrated in a rhesus monkey model with acute fulminant Ebola infection.<sup>7</sup>

### Dark Without Pressure

Although nonspecific to Ebola retinal lesions, the frequency of circumferential marginal zones of DWP around Ebola retinal lesions strongly suggests an association. This is supported by the correlation between Ebola retinal lesion density and the extent of DWP in some eyes (eFigure 15 in the Supplement). Areas of DWP in this study correspond to a hyporeflexive thinning of the second hyperreflective band and loss of the third hyperreflective band on OCT imaging, currently termed the *ellipsoid zone* and *interdigitation zone*, respectively.<sup>8</sup> Although controversy continues over the precise anatomical correlates of these bands,<sup>9,10</sup> recent cellular

characterization using immunohistochemistry markers concurs that the second band is generated by the photoreceptor ellipsoids and is probably the result of the high number of mitochondria that they contain, while the third band corresponds to the cone phagosomes located in the top of the retinal pigment epithelium.<sup>11</sup>

### Limitations

Because of the lack of histological evidence, preinfection imaging, and retinal imaging during acute infection, an absolute temporal association with EVD and the Ebola retinal lesions and associated DWP has yet to be established. We have not compared the OCT findings presented in this study with a control group of patients with retinal lesions associated with other pathologies to confirm that these characteristics are unique to Ebola retinal lesions.

### Conclusions

In this study, we demonstrate pathological changes seen at the level of the photoreceptors on OCT in small lesions. We demonstrate associated areas of DWP that appear as a hyporeflexive, thinned EZ in combination with an absence of the IZ on OCT imaging. The importance of this finding remains to be determined, and follow-up observations are ongoing. These findings suggest that survivors of EVD in future outbreaks would benefit from ophthalmologic evaluation, including via OCT analysis and visual field assessment.

#### ARTICLE INFORMATION

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**Author Contributions:** Drs Steptoe and Semple had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Beare and Semple are co-last authors.

**Study concept and design:** Steptoe, Scott, Vandy, Sahr, Beare, Semple.

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#### Invited Commentary

## The Ophthalmic Sequelae of Ebola

Gary N. Holland, MD; Anne W. Rimoin, PhD, MPH

**In this issue** of *JAMA Ophthalmology*, Steptoe et al<sup>1</sup> describe in detail the characteristics of a specific retinal lesion type found in 14 survivors of Ebola virus disease, using various imaging techniques, including ultra-widefield scanning laser



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ophthalmoscopy, fundus autofluorescence, and swept-source optical coherence tomography. Structural analysis demonstrated disruption of the outer retina with perilesional ellipsoid zone hyporeflexivity. Spatial analysis suggested that lesions are located along axons in the nerve fiber layer. This article is a follow-up to their 2017 article,<sup>2</sup> in which they found several types of retinal lesion in survivors of Ebola virus disease. But among those lesion types, the particular type in this article<sup>1</sup> was found only in survivors compared with a large group of control participants.

Readers are undoubtedly familiar with the most recent epidemic of Ebola virus disease in west Africa, which lasted from 2013 to 2016 and was notable for the large number of people affected and for its high mortality rate. By the end of the epidemic in mid-2016, the World Health Organization had reported 28 616 Ebola virus disease cases in Guinea, Liberia, and Sierra Leone, with 11 310 deaths<sup>3</sup>; however, that leaves a large pool of survivors, many of whom have experienced ongoing, debilitating sequelae collectively termed *post-Ebola virus disease clinical syndrome*. It includes arthralgias, myalgias, weakness, anorexia, weight loss, abdominal pain, cardiac problems, orchitis, increased susceptibility to infections, neurological impairments including hearing loss and tinnitus, mental health problems, and eye problems with vision loss.

Ebola virus infection can be subclinical, and among those with Ebola virus disease, manifestations range from mild to severe. Close contacts may also have long-term health problems, suggesting that even those who are initially asymptomatic may experience the effects of Ebola virus infection. But the extent to which postinfection sequelae occur in individuals with unrecognized Ebola virus disease is unknown, to our

knowledge. Health care workers are also at risk. The magnitude of the outbreak in West Africa necessitates a better understanding of the long-term complications of Ebola virus disease, which will allow improved estimates of the future health needs of affected individuals.

Less well known are the long-term survivors of previous Ebola virus disease outbreaks. We are aware of 34 outbreaks of Ebola virus disease since 1976 (when the first human cases were reported), most of which have occurred in the Democratic Republic of Congo or other areas of Central Africa. Eye involvement has contributed to the long-term morbidity of these survivors, but the nature of the ophthalmic disorders is not well understood. There are a few known individuals who have survived for up to 40 years after being infected during past epidemics,<sup>4</sup> but to our knowledge, the longest reported follow-up period with a description of Ebola virus disease features is only 2 to 3 years.<sup>5</sup>

Shantha et al<sup>6</sup> have summarized a number of reports about the ophthalmic sequelae of Ebola virus disease in the recent and past outbreaks. The prevalence of uveitis has ranged from 18% to 34% of survivors; in their own series (from the recent outbreak in west Africa), more than one-third of those with uveitis were blind. In addition to cases of posterior uveitis, which can result in the retinal lesions described by Steptoe et al,<sup>1,2</sup> there were cases of isolated anterior uveitis and intermediate uveitis. Other ophthalmic problems included optic neuropathy and other neuroophthalmic problems.<sup>6,7</sup> Some affected eyes have progressed to phthisis.

In 1999, Kibadi et al<sup>8</sup> described late ocular complications in a small number of people who survived a 1995 outbreak of Ebola virus disease in the Democratic Republic of Congo, but they provided no detailed descriptions of ocular findings. For the most part, both early and late ophthalmic complications have been described only in general terms; specific characteristics of disease have not been included. Causes of vision loss have often not been reported for lack of detailed eye examinations.