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## Multimodal Imaging and Spatial Analysis of Ebola Virus Retinal Lesions and associated Dark Without Pressure in a Cohort of Fourteen Ebola Survivors

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### Abstract

**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 regarding potential pathogenesis.

**Design**—Consecutive, prospectively identified, cohort study

**Setting**—34 Military Hospital, Freetown, Sierra Leone

**Participants**—Fourteen EVD survivors of Sierra Leonean origin with identified Ebola virus retinal lesions. Mean age 37years (SD 8.8years) 43% Female.

**Exposures**—Ebola virus disease.

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**Main Outcomes and measures**—Multimodal imaging findings including ultra-widefield (UWF) scanning laser ophthalmoscopy, fundus autofluorescence, swept source optical coherence tomography (OCT), Humphrey visual field analysis, and spatial analysis.

**Results**—141 Ebola virus retinal lesions were observed in 22 of 27 eyes of fourteen survivors on UWF imaging. 41 lesions were accessible to OCT imaging. Retinal lesions are predominantly non-pigmented with a pale grey appearance. Peripapillary lesions exhibit variable curvatures in keeping with the retinal nerve fiber layer projections. All lesions respect the horizontal raphe and spare the fovea. OCT demonstrates a ‘V’ shaped hyperreflectivity of the outer nuclear layer overlying discontinuities of the ellipsoid zone and interdigitation zone in the smaller lesions. Larger lesions cause a collapse of the retinal layers and loss of retinal thickness. Lesion shapes are variable but sharp angulations are characteristic. Perilesional areas of dark-without-pressure (thinned ellipsoid zone hyporeflectivity) of variable extent, accompany 89% of lesions.

**Conclusions and relevance**—We demonstrate OCT evidence of localized pathological changes seen at the level of the photoreceptors in small lesions. The relevance of associated areas of dark-without-pressure remains undetermined.

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## Introduction

We previously conducted a case-control study that identified retinal characteristics specific to survivors of Ebola virus disease (EVD) in Sierra Leone utilizing ultra-widefield (UWF) retinal imaging. Of all retinal lesions characterized in the previous study, only one lesion of characteristic morphological appearance was exclusively identified in Ebola survivors by two masked graders.<sup>1</sup> On this basis, this lesion was deemed most likely to be secondary to Ebola virus infection. Identical lesions have been identified in other Ebola survivor cohort studies.<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

160 EVD survivors attended the ophthalmology clinic at 34 Military Hospital, Freetown between January 2016 and April 2017. Fourteen Ebola survivors met the eligibility criteria of at least one identified Ebola retinal lesions on UWF retinal imaging in keeping with the findings of our previous study.<sup>1</sup> All fourteen were recalled and attended the clinic for examination including OCT of accessible Ebola retinal lesions. Thirteen of these survivors were recruited to this study, one was excluded due to increased lens opacity preventing fundus imaging. One further eligible patient was identified in March 2017 and directly enrolled into the study. OCT lesion appearance was categorized, and lesion grading concordance was compared by two masked ophthalmologists. Informed consent was obtained from all participants. The study was approved by the Office of Sierra Leone Ethics and Scientific Review Committee on 31<sup>st</sup> January 2017 and followed the tenets of the Declaration of Helsinki. Examination protocol is summarized in eMethods 1.

## Results

Fourteen EVD survivors with 141 Ebola virus retinal lesions in twenty-two eyes (8 bilateral) were analyzed. OCT was obtained on 41 lesions from twenty eyes. Characteristics of all fourteen cases are summarized in eTable 1. Corresponding multimodal imaging and VFs are available in eFigures 2-23.

### Retinal Lesions

Ebola virus retinal lesions vary in size and shape although, distinctive linear borders with sharp angulations are characteristic (eFigure 11). Multimodal imaging features vary according to severity and extent of retinal structures involved. A lesion severity grading from 1 to 5 is outlined in eTable 2. Cohen's kappa statistic of intergrader agreement was 0.77. OCT of the smallest lesions demonstrates multiple vertical discontinuities of the ellipsoid zone (EZ) and interdigitation zone (IZ) with overlying 'V' shaped increased reflectance of the outer nuclear layer (ONL) (Figure 1 & eFigure 21F). Lesions appear light grey in color on fundus photography and are predominantly non-pigmented.

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

### Dark-without-pressure

Well-defined areas of dark-without-pressure (DWP) which correspond to a thinned, hyporeflective EZ and an absent IZ on OCT (Figure 2) were seen adjacent to 88.7% of Ebola retinal lesions in this case series. The extent of DWP was variable, ranging from a confined circumferential marginal zone (eFigures 2, 6, 8, 14, 19, and 20), to larger defined areas (Figure 2 (190 disc areas), eFigures 5 (40 disc areas), 7 (38 disc areas), and 15 (89 disc areas) and in some cases 360-degree pan-retinal involvement (406 disc areas) (eFigure 16). The extent of DWP in some eyes appears associated with the density of Ebola retinal lesions (Figure 2, and eFigure 15). In all cases, DWP appears to spare the macula. No associated vitreous inflammation or vitreous traction was visible on OCT.

120-point screen, 60-4 threshold tests or peripheral nasal 24-2 protocol (eFigure 10H) were unable to conclusively identify any definitive VF defect corresponding to areas of DWP. 24-2 HVF analysis of one survivor with right hemiparesis following acute infection demonstrated a right-sided homonymous hemianopia and left inferior quadrantanopia. (eFigure 19G and 20I).

## Spatial Analysis

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

## Discussion

We present a multi-modal imaging analysis of a series of 14 EVD survivors 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 the pathogenesis, OCT observations of small lesions revealed multifocal fine discontinuities of the EZ and IZ with overlying increased reflectivity of the ONL (Figure 1). These findings mirror the histological appearance of early herpes simplex virus (HSV) retinopathy observed in the contralateral retina via a retrograde axonal transmission following unilateral anterior chamber viral inoculation.<sup>4,5</sup> They have also been observed in the ipsilateral retina following unilateral anterior chamber viral inoculation<sup>5</sup>, although in all cases within this series, we did not identify signs of previous anterior chamber uveitis to suggest a direct anterior to posterior dissemination. The presence of peripapillary curvilinear lesions resembling the arcuate path of the ganglion cell axons, which respect the horizontal raphe (demonstrated both on imaging and VF analysis), provides further evidence that Ebola virus disease involves retinal ganglion cells and a lasting insult to their afferent photoreceptors.

Possible pathogenic mechanisms for the characteristic retinal lesions observed in ebola survivors 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>6</sup>

## Dark-without-pressure

Although not specific 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). Areas of DWP in this study correspond to a hyporeflective thinning of the second and loss of the third hyperreflective bands on OCT currently termed the 'ellipsoid zone' and 'interdigitation zone' respectively.<sup>7</sup> Although controversy continues over the precise anatomical correlates of these bands<sup>8,9</sup> recent cellular characterization using immunohistochemistry markers concurs that the second band is generated by the photoreceptor ellipsoids and 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 RPE.<sup>10</sup>

## Study Limitations

Due to the lack of histological evidence, pre-infection imaging or 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 here to a control group of retinal lesions secondary to other pathologies to confirm characteristics are unique to Ebola retinal lesions.

## Conclusion

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 which appear as a hyporeflectivity, thinned EZ in combination with an IZ absence on OCT. The importance of which remains to be determined. Follow up observations are ongoing. These findings suggest that ophthalmic evaluation of Ebola survivors would benefit from the inclusion of OCT analysis and visual field assessment in future outbreaks.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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## References

1. Steptoe PJ, Scott JT, Baxter JM, et al. Novel Retinal Lesion in Ebola Survivors, Sierra Leone, 2016. *Emerg Infect Dis*. 2017; 23(7):1102–1109. DOI: 10.3201/eid2307.161608 [PubMed: 28628441]
2. Shantha JG, Crozier I, Varkey JB, et al. Long-term Management of Panuveitis and Iris Heterochromia in an Ebola Survivor. *Ophthalmology*. 2016; 123(12):2626–2628.e2. DOI: 10.1016/j.ophtha.2016.07.013 [PubMed: 27594198]
3. Hereth-Hebert E, Bah MO, Etard JF, et al. Ocular Complications in Survivors of the Ebola Outbreak in Guinea. *Am J Ophthalmol*. 2017; 175:114–121. DOI: 10.1016/j.ajo.2016.12.005 [PubMed: 27998698]
4. Holland GN, Togni BI, Briones OC, Dawson CR. A microscopic study of herpes simplex virus retinopathy in mice. *Invest Ophthalmol Vis Sci*. 1987; 28(7):1181–1190. [PubMed: 3596994]

5. Vann VR, Atherton SS. Neural spread of herpes simplex virus after anterior chamber inoculation. *Invest Ophthalmol Vis Sci.* 1991; 32(9):2462–2472. [PubMed: 1714427]
6. Zeng X, Blancett CD, Koistinen KA, et al. Identification and pathological characterization of persistent asymptomatic Ebola virus infection in rhesus monkeys. *Nat Microbiol.* 2017; 2doi: 10.1038/nmicrobiol.2017.113
7. Staurengi G, Sadda S, Chakravarthy U, Spaide RF. International Nomenclature for Optical Coherence Tomography (IN•OCT) Panel. Proposed lexicon for anatomic landmarks in normal posterior segment spectral-domain optical coherence tomography: the IN•OCT consensus. *Ophthalmology.* 2014; 121(8):1572–1578. DOI: 10.1016/j.ophtha.2014.02.023 [PubMed: 24755005]
8. Spaide RF. Outer Retinal Bands. *Investig Ophthalmology Vis Sci.* 2015; 56(4):2505.doi: 10.1167/iops.15-16456
9. Jonnal RS, Kocaoglu OP, Zawadzki RJ, Lee S-H, Werner JS, Miller DT. Author Response: Outer Retinal Bands. *Investig Ophthalmology Vis Sci.* 2015; 56(4):2507.doi: 10.1167/iops.15-16756
10. Cuenca N, Ortuño-Lizarán I, Pinilla I. Cellular Characterization of Optical Coherence Tomography and Outer Retinal Bands Using Specific Immunohistochemistry Markers and Clinical Implications. *Ophthalmology.* 2017 Oct.doi: 10.1016/j.ophtha.2017.09.016

## Key Points

### Question

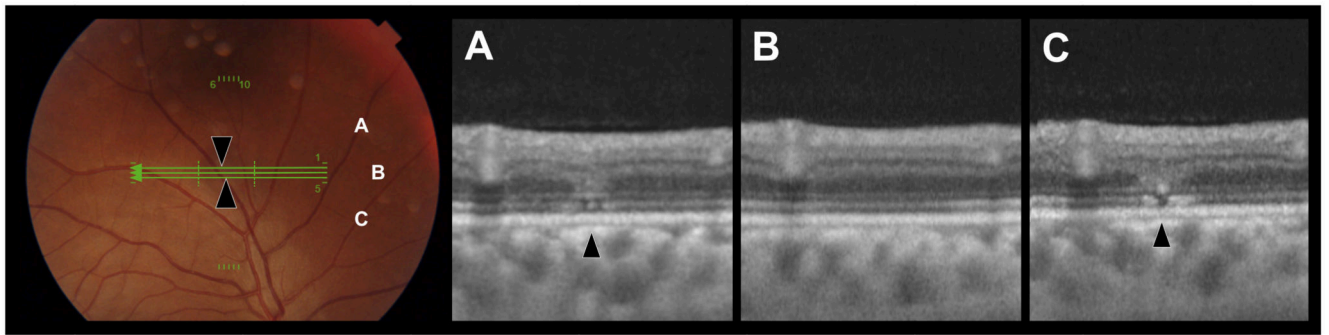
Can multimodal imaging of Ebola retinal lesions inform our understanding of their pathogenesis?

### Findings

In this prospective cohort of fourteen survivors, optical coherence tomography demonstrates 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 is detected in larger lesions. Corresponding visual field defects respect the horizontal raphe. Perilesional areas of dark-without-pressure (ellipsoid zone hyporeflexivity) accompany 89% of lesions.

### Meanings

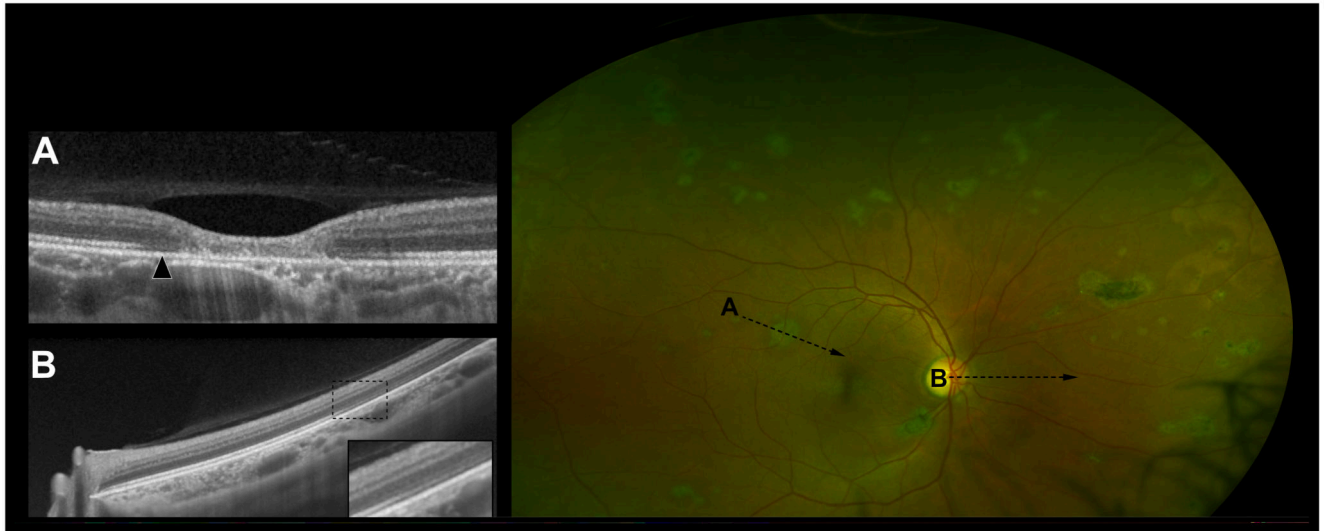
In these survivors, findings are consistent with a neuronal rather than vascular pathogenesis. The significance of dark-without-pressure is undetermined.



**Figure 1. Ebola Retinal Lesion.**

Left - Color fundus image. Green lines indicate OCT scan locations for corresponding OCTs A-C. Black arrows indicate lesion sites. A-C) OCTs through Ebola retinal lesions demonstrating the close proximity of multifocal discontinuities of the ellipsoid zone with an overlying 'V' shaped increased reflectance of the outer nuclear layer (of equal reflectance of the adjacent outer plexiform layer).





**Figure 2. Extensive Dark-Without-Pressure**

A) OCT through an Ebola retinal lesion. Arrow indicates an area of perilesional dark without pressure (DWP) corresponding to a thinned hyporeflective EZ and absent IZ. B) OCT demonstrating the transition between the normal retina and the circumferential extension of an area of DWP nasally. The enlarged box highlights the transitional zone. C) Right eye UWF image. Multiple Ebola retinal lesions and associated areas of DWP.