Article

Confocal Adaptive Optics Imaging of Peripapillary Nerve Fiber Bundles: Implications for Glaucomatous Damage Seen on Circumpapillary OCT Scans

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Received: 30 December 2014

Accepted: 18 February 2015

Published: 17 April 2015

Keywords: glaucoma; adaptive optics; optical coherence tomography; optic disc; retinal nerve fiber layer

Citation: Hood DC, Chen MF, Lee D, et al. Confocal adaptive optics imaging of peripapillary nerve fiber bundles: implications for glaucomatous damage seen on circumpapillary OCT scans. *Trans Vis Sci Tech.* 2015;4(2):12, <http://tvstjournal.org/doi/full/10.1167/tvst.4.2.12>, doi:10.1167/tvst.4.2.12

Purpose: To improve our understanding of glaucomatous damage as seen on circumpapillary disc scans obtained with frequency-domain optical coherence tomography (fdOCT), fdOCT scans were compared to images of the peripapillary retinal nerve fiber (RNF) bundles obtained with an adaptive optics-scanning light ophthalmoscope (AO-SLO).

Methods: The AO-SLO images and fdOCT scans were obtained on 6 eyes of 6 patients with deep arcuate defects (5 points ≤ -15 db) on 10-2 visual fields. The AO-SLO images were montaged and aligned with the fdOCT images to compare the RNF bundles seen with AO-SLO to the RNF layer thickness measured with fdOCT.

Results: All 6 eyes had an abnormally thin (1% confidence limit) RNF layer (RNFL) on fdOCT and abnormal (hyporeflective) regions of RNF bundles on AO-SLO in corresponding regions. However, regions of abnormal, but equal, RNFL thickness on fdOCT scans varied in appearance on AO-SLO images. These regions could be largely devoid of RNF bundles (5 eyes), have abnormal-appearing bundles of lower contrast (6 eyes), or have isolated areas with a few relatively normal-appearing bundles (2 eyes). There also were local variations in reflectivity of the fdOCT RNFL that corresponded to the variations in AO-SLO RNF bundle appearance.

Conclusions: Relatively similar 10-2 defects with similar fdOCT RNFL thickness profiles can have very different degrees of RNF bundle damage as seen on fdOCT and AO-SLO.

Translational Relevance: While the results point to limitations of fdOCT RNFL thickness as typically analyzed, they also illustrate the potential for improving fdOCT by attending to variations in local intensity.

Introduction

Frequency domain optical coherence tomography (fdOCT) is useful in detecting glaucomatous damage. The most commonly used OCT measure of such damage is the thickness of the retinal nerve fiber (RNF) layer (RNFL) around a circle centered on the disc. This circumpapillary measure of RNFL thickness can be obtained directly from a circular scan around the disc or derived from a volumetric scan of

the disc. In either case, summary statistics of circumpapillary RNFL thickness, such as the overall average or the average within quadrants of the disc, have proven to have good sensitivity and specificity in detecting glaucomatous damage (see recent review¹).

The ability of the OCT to detect glaucomatous damage still can be improved with two techniques. First, a direct topographical comparison of the abnormal locations on the visual field (VF) to the abnormal locations on the fdOCT allows subtle damage to be confirmed.² Second, we have proposed

that the actual circumpapillary images should be scrutinized with the care a radiologist would view a computerized axial tomography (CAT) or magnetic resonance imaging (MRI) scan.^{2,3} Although the fdOCT scans have the same complexities as these scans, and even better spatial resolution, glaucoma specialists typically rely on summary statistics. The actual scan rarely is examined and, in any case, the image typically displayed in commercial reports is too small for evaluation.

If the circumpapillary OCT image is large enough, one can see important details. For example, segmentation algorithm failures, which yield misleading indicators of the RNFL thickness, can be detected.² In addition, we reported that small, hyporeflective regions (“holes”) can be seen in the RNFL of glaucoma suspects and patients.⁴ These holes are due to RNF dropout and can be seen in glaucoma suspects; that is, patients with normal-appearing VFs.

Here we explore confocal adaptive optics scanning light ophthalmoscopy (AO-SLO) imaging as a way to further improve our interpretation of circumpapillary fdOCT scans. With AO-SLO, the RNF bundles can be imaged^{5–9} and changes due to glaucoma visualized.^{6,7,9} These changes include those difficult or impossible to see with currently available commercial fdOCT.⁹

In the present study, we compared AO-SLO images of RNFL bundles near the disc to the circumpapillary fdOCT scans of patients with arcuate-like defects in the central $\pm 10^\circ$ of vision.

Methods

Subjects

The AO-SLO images were obtained near the optic disc on 6 eyes of 6 patients with open-angle glaucoma. These patients were selected prospectively for testing based upon the presence of a deep arcuate defect on the total deviation plot of the 10-2 VF (Humphrey VF Analyzer; Carl Zeiss Meditec, Inc., Dublin, CA). In particular, there were at least 5 test points with total deviation values ≤ -15 dB. Five of the 6 patients were part of a study comparing vertical AO-SLO images to OCT and 10-2 VFs⁹ and were selected from the 7 eyes in that study because they already had AO images near the disc or were willing to return for AO imaging; the sixth eye was excluded from that study based upon the poor quality of the macular AO images. Inclusion criteria included a best-corrected visual acuity better than 20/40, clear media, refractive

error within ± 4.0 D, and pupil dilation ≥ 6 mm. The study was approved by the Columbia University and New York Eye and Ear Infirmary of Mount Sinai Institutional Review Board and adheres to the tenets set forth in the Declaration of Helsinki, and the Health Insurance Portability and Accountability Act. Written informed consent was obtained from all subjects.

AO-SLO Imaging

After the pupil dilation, images were obtained with a custom-built, confocal AO-SLO system described previously.¹⁰ Confocal reflectance image sequences of 150 frames were obtained of RNF bundles near the temporal half of the optic disc margin. A montage of the AO-SLO images was created by aligning them on a fundus photograph using blood vessels as landmarks.

Circumpapillary fdOCT Scan

Circumpapillary circle scans were obtained for all eyes using fdOCT (3D-OCT 2000; Topcon Corp., Paramus, NJ). **Figure 1B** shows the scan for the left eye of patient P1 obtained along the circumpapillary path, 3.4 mm in diameter, shown in green in **Figure 1A**. The circumpapillary RNFL thickness plot (**Fig. 1D**) was obtained from the machine after manually correcting the borders of the RNFL (**Fig. 1C**) where needed. The RNFL thickness was plotted with the temporal (T) quadrant of the disc in the center of the scan image and the nasal (N) quadrant at the ends.² **Figure 1D** shows this NSTIN plot for patient P1, with RNFL thickness plotted as the distance from the center (0°) of the temporal quadrant. This places the disc region associated with the axon bundles from the macula in the center of the plot (**Fig. 1C**).

Results

All 6 eyes had upper VF/inferior disc damage, and one, P1, had lower VF/superior disc damage as well. All 7 hemifields had deep defects on the 10-2 VFs within the central $\pm 8^\circ$ (the macula) and a corresponding RNFL thinning consistent with an anatomical model^{11,12} of the macula. To illustrate this point, the 10-2 VF total deviation plots for 3 of the eyes are shown in **Figure 2A–C**, along with their RNFL thickness plots in **Figure 2D**. The red circles have a radius of 8° . According to the model, on average the central $\pm 8^\circ$ of the VF is associated with the portion of the optic disc indicated by the red line with arrows in

Figure 1. (A) Fundus photograph showing the direction of the scan images in (B) and (C). (B) Circumpapillary circle scan from nasal (N) to superior (S) to temporal (T) to inferior (I) to N regions. (C) The same NSTIN scan with segmentation lines indicating the edges of the RNFL layer. (D) A NSTIN RNFL thickness plot with the region associated with the macula (central $\pm 8^\circ$) of VF.

Figure 2D. Portions of the abnormally thin region of the RNFL (white arrows in **Fig. 2D**) fall between the red vertical lines, the region of the disc associated with the central $\pm 8^\circ$. Further, the thinning in the inferior hemiretina included the macular vulnerability zone (MVZ) (between dashed blue lines in **Fig. 2D**); this is the macular portion of the disc that is particularly susceptible to glaucomatous damage.^{11,12}

AO-SLO Images

The key features of the peripapillary AO-SLO images of the 6 eyes can be seen in **Figures 3–5**, which show the images for 3 of the eyes. First, 5 of the 6 eyes had reasonably large regions without clearly detectable RNF bundles as indicated by the solid red arrows in **Figures 3A** and **4A**. These regions can be contrasted with regions (green arrows) at approximately the same location in the upper half of the disc. The sixth eye also had a region of missing RNF bundles, but this region was only a bundle or two

wide (dashed red arrow in **Fig. 5A** and inset). Second, lower contrast and/or less densely packed RNF bundles were present in all 6 eyes, especially near the borders between regions with healthy and missing bundles (orange arrows in **Figs. 3A, 4A, 5A**). Third, in 2 eyes RNF bundles were seen between regions with barely detectable, or undetectable, RNF bundles. Patient P1 is an example of the latter; the yellow arrow in **Figure 3A** points to the isolated region of a few RNF bundles. Patient P4, discussed below, is the second example.

Comparison of AO and OCT Images

Figures 3–5 illustrate our approach to comparing the AO images to fdOCT scans. The large green circle in **Figures 3A–5A** is the location of the OCT circumpapillary scan; the black portion is the temporal half of the disc. The fdOCT scan is shown in **Figures 3D–5D**, where the black horizontal line with arrows indicates the temporal half ($\pm 90^\circ$) of the circle scan. This temporal portion is reproduced in **Figures 3B–5B**, where it is oriented vertically and presented without (**Figs. 3B–5B**) and with (**Figs. 3C–5C**) the RNFL boundaries marked. The purple lines indicate corresponding blood vessels, seen as shadows on the OCT scan. These landmarks allow us to locate corresponding points on the AO-SLO image and fdOCT scan.

First, consider the relatively large regions with missing RNF bundles, which were seen on the AO-SLO images of 5 of the eyes. These regions without RNF bundles were associated with abnormally thin RNFLs on the fdOCT scan. This can be seen in **Figures 3** and **4** where the red arrows indicate a region on the AO-SLO images (**Figs. 3A, 4A**) without RNF bundles and a region of the OCT scan with a thin RNFL (**Figs. 3B, 4B**). These thinned regions fall within the abnormal range of the OCT RNFL layer thickness plot (red vertical arrows in **Figs. 4D, 4E**). Interestingly, the OCT RNFL thickness in these regions of missing RNF bundles was not zero, but was approximately 20 μm thick. Additionally, these residual RNFL regions appeared hyporeflective in places as compared to regions of RNFL with normal thickness. This can be seen in **Figure 6**, which shows portions of the scans within the red rectangles in **Figures 3** and **4**. Notice the hyporeflective regions, as seen for example at the points indicated by the white and gray arrows in the upper panels of **Figures 6A–D**.

Second, consider the regions with clearly abnormal (hyporeflective)–appearing bundles. Patient P3 has a relatively large region of bundles of reduced contrast

Figure 2. (A) The total deviation plots of the 10-2 VF for 3 patients with the central $\pm 8^\circ$ shown (red circle). (B) The NSTIN RNFL thickness plots for the same 3 eyes.

throughout the affected region (orange arrow in Fig. 5A). This region is associated with the abnormal thinning of the RNFL as shown by the vertical orange line in Figures 5D and 5E. As mentioned above, in the other 5 eyes, abnormal-appearing bundles were typically seen in regions between the healthy and missing bundles (orange arrows in Figs. 3–5). As might be expected, on the OCT RNFL thickness plot,

they fell near the border between normal and abnormal RNF bundles.

Third, there were two eyes with isolated bundles, which looked better than the adjacent regions. In the case of P1 (yellow arrows in Fig. 3), the RNFL thickness for the region with the isolated bundles was approximately the same as neighboring regions (Fig. 3E). This is easier to see in the enlarged image in Figure 6A, where the yellow arrows mark the location

Figure 3. (A) Montage of the peripapillary AO-SLO images superimposed upon the fundus photograph of P1. The *green circle* indicates the location of the OCT circumferential scans and the *black semicircle* the temporal half of this scan. (B, C) The temporal portion of the OCT circumferential scan is shown without (B) and with (C) the RNFL borders marked. The *purple lines* extending to (A) indicate corresponding blood vessels. (D, E) The circumferential scan (D) and NSTIN RNF layer thickness plot (E) for this eye. In all panels, the arrows point to locations with RNF bundles on the AO-SLO images that were normal in appearance (*green*), missing (*red*), appeared abnormal in contrast (*orange*), or appeared relatively normal (*yellow*), but were sandwiched between regions without bundles. Scale bar: in (A) is 100 μm .

on the OCT corresponding to the isolated bundles. Notice that the region associated with the preserved bundles appeared more intense (dense) than its neighbors (yellow and gray arrows in Fig. 6A). The other eye, P4, had a region (see Fig. 6E) that appeared on AO-SLO (left) to be a mixture of missing bundles (red arrow) and relatively preserved bundles (yellow). The right panels in Figure 6E show the corresponding locations on the OCT.

Discussion

Circumpapillary RNF thickness is the most common OCT measure used in the clinic to detect glaucomatous damage. We compared the AO-SLO images and OCT scans of the disc in patients with relatively similar deep, central arcuate defects to better understand this OCT measure. The circumpapillary regions of these eyes had abnormally thin

Figure 4. Same as [Figure 3](#) for patient P2. Scale bar: in (A) is 100 μm .

RNFLs of approximately the same thickness on OCT scans, but they had very different appearances on AO-SLO images. These abnormally thin regions can be largely devoid of RNF bundles, have isolated areas of reasonably normal bundles, or have abnormally-appearing bundles of lower contrast. These findings raise two important questions.

First, what is included in the OCT measure of RNFL thickness when there is no sign of RNF bundles on AO-SLO images? One possibility is that the residual OCT thickness is due to segmentation errors, even though a manual correction was used. In particular, the segmentation may include less intense structures (e.g., retinal ganglion cell [RGC] + inner plexiform layer [IPL]), mistakenly included within the

RNFL. In fact, in some cases (gray arrows in [Figs. 6A–6D](#)), the segmentation appears to include a small part of the RGC+IPL layer. However, these deviations are relatively minor and overall the manually corrected segmentation accurately represents the RNFL, which can be less intense (white arrows in [Figs. 6A–6D](#)). That is, there appears to be an irreducible minimal thickness of approximately 20 μm , which typically is less intense than the neighboring thicker RNF regions. While this irreducible minimum includes the inner limiting membrane, its contribution should be approximately 2 μm . In some cases, not shown here, an epiretinal membrane (ERM) can add to the thickness. In any case, we have not accounted for almost 20 μm . Glial cells

Figure 5. Same as Figure 3 for patient P3. Scale bars: in (A) are 100 μm .

probably contribute, perhaps including the end feet of the Müller cells. Finally, axons that are no longer functional may remain. Such axons may contribute to thickness measures, but not to intensity due to axonal cytoskeletal abnormalities.^{13,14}

Second, with AO-SLO we see details that the current analyses of circumpapillary OCT scans miss. Can some of these details be seen on OCT scans? For example, in 2 eyes, there was a clear indication of RNF bundles surrounded by regions apparently devoid of RNF bundles. A close examination of the OCT scans revealed a corresponding region of near normal intensity in the corresponding circumpapillary region. This is illustrated for P2 by the yellow arrows

in Figures 3A, 3B, and 6A and was true as well in the second eye (Fig. 6E). Although these local differences in intensity are not easy to identify with confidence on OCT scans, the fact that they can be seen with current commercial OCT suggests that new OCT technology and/or analysis should allow us to identify these local variations in damage.

Conclusions and Clinical Implications

The RNF bundles can vary in appearance in RNFL regions of equal thickness as measured with conventional analysis of OCT. In these regions, the bundles may be missing, appear abnormal, or even contain a few bundles that are relatively normal in

Figure 6. (A–D) Portions of the OCT scans from Figures 3–5 (with red rectangles) illustrating regions with preserved bundles (yellow arrows), hyporeflexive regions (white arrows) and regions where the segmentation may have included a small portion of the RGC+IPL region (gray arrows). (E) The AO-SLO images (left) and OCT scans for the second eye with local preserved bundles.

appearance. Further, while these results point to some limitations of RNFL thickness as typically analyzed with fdOCT, they also illustrate the potential to further improve the use of this valuable clinical tool by close examination of the OCT scan. With

improvements in OCT resolution and contrast, these regions should be even easier to see. However, these clinically relevant details will only be revealed if we look carefully at the scan images, as opposed to trusting RNFL thickness measures generated by

commercial algorithms. In general a close examination of OCT images allows the detection of local glaucomatous damage missed on commercial reports.²

Acknowledgments

The authors thank Ali Raza for his assistance at various stages of this project, Jason Nunez for his assistance in the preparation of this manuscript, and Alexander Gan, Alexander Pinhas, Moataz Razeen, Nishit Shah, and Daiyan Xin for their assistance in scheduling and imaging the patients.

Supported by National Institute of Health Grant EY-02115 (DCH) and grants from Research to Prevent Blindness (AD), Glaucoma Research Foundation (AD), the Marrus Family Foundation (RBR), Bendheim-Lowenstein Family Foundation (RBR), Chairman's Research Fund of the New York Eye and Ear Infirmary (RBR), and Jane Banks Research Fund of the New York Glaucoma Research Institute (RR).

Disclosure: **D.C. Hood**, Topcon, Inc. (F, C); **M.F. Chen**, None; **D. Lee**, None; **B. Epstein**, None; **P. Alhadeff**, None; **R.B. Rosen**, None; **R. Ritch**, None; **A. Dubra**, P, Cannon USA (F); **T.Y. Chui**, None.

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