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Comparison of Point Estimates and Average Thicknesses of Retinal Layers Measured Using Manual Optical Coherence Tomography Segmentation for Quantification of Retinal Neurodegeneration in Multiple Sclerosis

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Abstract

Purpose—The advent of macular optical coherence tomography (OCT) segmentation has enabled the *in vivo* quantitative assessment of retinal axonal and neuronal subpopulations. Recent studies employing OCT in multiple sclerosis (MS) have utilized various manual macular segmentation approaches to quantify retinal layer thicknesses. We investigated whether measurements of retinal layers solely at the points of maximal macular thickness (point estimates) within the central macular B-scan are representative of the corresponding average layer thicknesses for the ganglion cell + inner plexiform (GCIP) layers, inner nuclear layer (INL), outer plexiform layer (OPL) and outer nuclear layer (ONL) in MS and healthy controls. Additionally, we examined the correlation of manual segmentation-derived measures of composite layers with average thickness measures derived from automated 3-D segmentation of the macular cube.

Materials and Methods—Spectral-domain OCT central macular B-scans of 52 MS patients and 30 healthy controls (HCs) were manually segmented. Average layer thicknesses and layer thicknesses at the points of maximal macular thickness were calculated. Macular cube scans were also segmented utilizing a fully automated 3-D segmentation algorithm.

Results—GCIP, INL and OPL maximal thicknesses derived from point estimates correlated well with the average thicknesses of these layers within the central macular B-scan, whereas the ONL maximal thickness did not correlate as strongly. Manual segmentation-derived point estimates and average thickness measures of the GCIP correlated excellently with corresponding automated segmentation-derived measures. MS patients had significantly decreased GCIP maximal and average thicknesses relative to HCs. ONL average thickness was significantly decreased in MS compared to HCs, but this was not true of the ONL maximal thickness.

Conclusions—GCIP, INL and OPL maximal layer thicknesses may be used as surrogates to assess the gross structural integrity of these layers in MS, in a time-conservative fashion.

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Keywords

Multiple sclerosis; Optical coherence tomography; Retinal segmentation; Retinal pathology; Photoreceptor layer

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory demyelinating disorder of the central nervous system, with a predilection to affect the optic nerves both clinically and subclinically.^{1,2} Optic nerve demyelination results in retrograde axonal degeneration, leading to atrophy of the retinal nerve fiber layer, and culminating in ganglion cell death. This forms the pathophysiologic basis thought to primarily contribute to the retinal changes observed in MS, though studies have proposed that primary retinal mechanisms of disease may also play a role.^{3–5} The advent of macular optical coherence tomography (OCT) segmentation has allowed for the quantitative assessment of retinal axonal and neuronal subpopulations, enabling the *in vivo* examination and further characterization of the neuroretinal pathobiology of MS. Moreover, macular segmentation-derived measures have been proposed as potential outcome measures for clinical trials of putative neuroprotective and remyelinating agents.⁶

To date, automated OCT segmentation techniques employed in studies of MS eyes have been unable to delineate all visually identified borders, and mainly provide composite measurements of adjacent retinal layers.^{4,7} Advances in the field have led to the development of more sophisticated automated segmentation techniques,^{8,9} however these techniques are not widely commercially available. Thus, manual methods have been used extensively in research studies in order to isolate all visually discriminable retinal layers.^{10–13} Approaches utilizing manual segmentation in studies of MS eyes have included segmenting the central macular B-scan (traversing the fovea),¹¹ or segmenting multiple constituent B-scans of a macular cube scan.¹⁰ Recently, Albrecht et al. assessed retinal layer measurements solely at the points of maximal thickness within the central macular B-scan in a large cohort of MS patients¹², as well as in a cohort of patients with Parkinsonian syndromes.¹³ It is unclear though how these point estimates relate to the corresponding average thicknesses of these layers within the central B-scan.

The primary objectives of this study were (1) to examine in MS and healthy control (HC) eyes how measurements of individual retinal layers at the points of maximal thickness within the central macular B-scan relate to the corresponding average thicknesses of these layers within the central macular B-scan, and (2) to compare these measures between MS patients and HCs, in a previously described cohort.¹¹ As a secondary objective we examined the correlations of manual segmentation-derived measurements with those of automated 3-D segmentation of the macular cube (available to our group for composite layers).

MATERIALS AND METHODS

The study protocol was approved by the Institutional Review Board of Johns Hopkins University and written informed consent was obtained from all participants.

Imaging of the retina was performed with Spectralis OCT (Heidelberg Engineering, Germany) and macular scans were segmented utilizing fully manual segmentation (FMS), as previously described.¹¹ Briefly, segmentation of the central macular B-scan of the participants' right eyes was performed by a single segmenter, blinded to disease status (Figure 1). The following layers were defined: (1) ganglion cell layer + inner plexiform layer (GCIP; these two layers could not be reliably visually discriminated); (2) inner nuclear layer (INL); (3) outer plexiform layer (OPL); (4) outer nuclear layer (ONL). Additionally, the following composite layers were defined: (1) INL + OPL; (2) ONL+photoreceptor segments (PR). Average thicknesses of the segmented layers were calculated across a width of 5mm centered on the fovea. These measurements are hence referred to as GCIP_{avg}, INL_{avg}, OPL_{avg}, ONL_{avg}, INL+OPL_{avg}, and ONL+PR_{avg}.

Additionally, measurements of the GCIP, INL, OPL and INL+OPL were obtained at the points of maximal macular thickness temporally and nasally of the fovea (Figure 1), and averaged.¹² The ONL and ONL+PR were measured at their single thickest point at the fovea (Figure 1). These measurements are hence referred to as GCIP_{max}, INL_{max}, OPL_{max}, INL+OPL_{max}, ONL_{max}, and ONL+PR_{max}.

Macular cube scans were also acquired (on the same day) utilizing Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, CA, USA) and fully automated 3-D segmentation (FAS) of the macular cube was performed, as described in detail elsewhere.^{6,14} Briefly, the automated segmentation algorithm delineates the following composite layers: (1) GCIP; (2) INL+OPL; (3) ONL+PR. Average thicknesses were calculated in an annulus centered on the fovea, with an inner radius of 0.54 mm and an outer radius of 2.4 mm (this is the approach that has been utilized in previous studies^{4,6,11,14}). This approach provides measures derived from global macular sampling, in contrast to the thickness measurements derived from the manual segmentation approaches utilized in this study.

Statistical analyses were performed with Stata 11 (StataCorp, College Station, TX, USA). Layer thickness measures are presented as mean $\pm$ standard deviation. The Kolmogorov–Smirnov test was utilized to assess the normality of distributions (examined variables were normally distributed), and Pearson's correlation was used to assess the relationship between maximal and average layer thicknesses. Correlation coefficients ≥ 0.75 were classified as good to excellent, 0.50–0.75 were classified as moderate to good, and 0.25–0.50 were classified as fair.¹⁵ Comparison of segmentation measures between MS patients and HCs was performed with unequal variance two-tailed *t*-tests. Statistical significance was defined as $p < 0.05$.

RESULTS

Fifty-two MS subjects and 30 age and sex-matched HCs were included in the study. The demographics and clinical characteristics of this study population have been reported in detail elsewhere.¹¹

Correlation of Manual OCT Segmentation-Derived Maximal and Average Layer Thicknesses

GCIP_{max} demonstrated excellent correlation with GCIP_{avg} across the entire cohort of MS patients and HCs ($r = 0.94$) and in the MS cohort alone ($r = 0.93$). In the HCs alone, correlation for these measures was good ($r = 0.82$). Both INL_{max} and OPL_{max} correlated well with their corresponding average layer thicknesses across the entire cohort (INL: $r = 0.80$; OPL: $r = 0.79$) and in the MS cohort (INL: $r = 0.81$; OPL: $r = 0.84$). In the HCs, INL_{max} correlated well with INL_{avg} ($r = 0.78$), whereas correlation of the OPL_{max} and OPL_{avg} was moderate ($r = 0.58$). Finally, ONL_{max} demonstrated fair correlation with ONL_{avg} across the cohort ($r = 0.50$), as well as in the MS subjects ($r = 0.50$) and HCs ($r = 0.46$).

p values were < 0.01 for all of the above correlations. Scatter plots of the maximal and corresponding average layer thicknesses are illustrated in Figure 2.

Correlation of Manual and Automated OCT Segmentation-Derived Thicknesses of Composite Retinal Layers

GCIP_{avg} and GCIP_{max} demonstrated excellent correlations with corresponding Cirrus FAS-derived measurements, both across the cohort (GCIP_{avg}: $r = 0.92$; GCIP_{max}: $r = 0.90$) and in MS (GCIP_{avg}: $r = 0.92$; GCIP_{max}: $r = 0.88$), whereas in HCs GCIP measures correlated well, albeit less strongly (GCIP_{avg}: $r = 0.67$; GCIP_{max}: $r = 0.74$). INL+OPL_{avg} exhibited moderate correlations with Cirrus FAS-derived INL+OPL thickness across the cohort ($r = 0.62$), as well as in MS ($r = 0.67$) and HCs ($r = 0.56$), whereas the INL+OPL_{max} demonstrated weaker correlations (Across the cohort: $r = 0.47$; MS: $r = 0.44$; HCs: $r = 0.51$). Finally, ONL+PR_{avg} correlated well with Cirrus FAS-derived ONL+PR measurements (Across the cohort: $r = 0.78$; MS: $r = 0.77$; HCs: $r = 0.70$), whereas ONL+PR_{max} demonstrated much weaker correlations (Across the cohort: $r = 0.28$; MS: $r = 0.24$; HCs: $r = 0.28$).

p values were < 0.01 for the above correlations, except for the ONL+PR_{max} subgroup correlations, which were not significant (MS: $p = 0.10$; HCs: $p = 0.14$). Scatter plots of the manual and automated OCT segmentation-derived measures are illustrated in Figure 3.

Comparisons of OCT Segmentation Measures Between MS Subjects and HC

GCIP_{max} and average GCIP thicknesses were significantly reduced ($p < 0.0001$ for both) in MS eyes (GCIP_{max}: $81 \pm 10.8 \mu\text{m}$; GCIP: $59.3 \pm 8.1 \mu\text{m}$) relative to HCs (GCIP_{max}: $94.7 \pm 7.5 \mu\text{m}$; GCIP: $68.6 \pm 6.3 \mu\text{m}$). INL and OPL maximal and average thicknesses did not differ between MS (INL_{max}: $44.5 \pm 5.5 \mu\text{m}$; INL: $34 \pm 4.5 \mu\text{m}$; OPL_{max}: $23.9 \pm 5.4 \mu\text{m}$; OPL: $21.2 \pm 3.3 \mu\text{m}$) and HC eyes (INL_{max}: $45 \pm 4.6 \mu\text{m}$; INL: $33.8 \pm 3.5 \mu\text{m}$; OPL_{max}: $23.5 \pm 3.9 \mu\text{m}$; OPL: $20.7 \pm 2.3 \mu\text{m}$).

The ONL, as assessed by measuring the average thickness of this layer, was found to be significantly reduced ($p = 0.003$) in MS eyes ($75.2 \pm 8.7 \mu\text{m}$) in comparison to HC eyes ($80.7 \pm 7.0 \mu\text{m}$). This difference when utilizing the ONL_{max} measurement was not found to be significant (MS: $109.8 \pm 12.0 \mu\text{m}$; HCs: $114 \pm 14.2 \mu\text{m}$; $p = 0.18$).

The results of comparisons of automated 3-D segmentation-derived measures between MS subjects and HCs for this cohort have been reported in detail elsewhere.¹¹

Discussion

Our results demonstrate that the measurement of the GCIP at the points of maximal macular thickness ($GCIP_{max}$) correlates excellently in MS with the average thickness of this layer throughout the central B-scan of the macula, as well as with the automated segmentation-derived thickness over a large area of the macular cube. The INL_{max} and OPL_{max} measures also correlate well with their corresponding average thicknesses. This approach is significantly less time-intensive than segmentation of the retinal layer borders throughout the entire central macular B-scan. Thus, these measures may be considered to be representative of the average GCIP, INL and OPL thicknesses in MS and may be applied to rapidly estimate the gross structural integrity of these layers in large sample sizes in a time-conservative fashion.

On the contrary, ONL_{max} proved to be only fairly representative of the average ONL thickness in the central macular B-scan, and correlations of ONL_{max} with automated segmentation-derived average thicknesses were suboptimal. Additionally, this cohort has previously been demonstrated to exhibit significant ONL thinning, as assessed by measuring the average ONL thickness with both manual and automated segmentation techniques, in two spectral-domain OCT devices.¹¹ This difference was not statistically significant though when comparing the ONL_{max} measure between MS subjects and controls.

Studies employing electroretinography and/or OCT support the presence of functional and structural photoreceptor aberrations in MS.^{3–5,14} The mechanism of this pathology is unclear, although evidence points to a primary retinal process, since it appears that photoreceptor involvement occurs independently of inner retinal and optic nerve pathology.^{4,5,14}

Additionally, as expected, we observed significantly decreased GCIP thicknesses in the MS patients, but we were unable to detect alterations in the INL or OPL thicknesses derived from either method. Albrecht et al. recently reported decreased INL_{max} thicknesses in primary-progressive MS patients,¹² however we were unable to perform subgroup comparisons by MS subtype due to the small number of progressive patients in the study. Evaluation of a large cohort of primary-progressive MS patients is necessary to independently evaluate this finding.

In conclusion, we have demonstrated that the assessment of the GCIP, INL and OPL at the points of maximal macular thickness may be used as a less time-intensive approach to rapidly quantitatively assess the gross integrity of these layers in MS, although for the ONL this approach appears to be suboptimal. These results though do not mitigate the need for segmentation of the entire macular volume, an approach that is more informative concerning the integrity of the retinal layers throughout the entire macular cube, and allows the detection of localized aberrations of retinal layer thicknesses. Thus, the application in future studies of MS eyes of reproducible automated segmentation techniques isolating individual retinal layers will allow their quantitative assessment in a time-efficient fashion over a more extensive area of the macula, in larger study populations, and may bear important implications for further characterization of the neuroretinal pathobiology of MS.

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FIGURE 1.

Example of manual segmentation of a central macular B-scan. In addition to the average layer thicknesses, the point estimates $GCIP_{max}$, INL_{max} , OPL_{max} , and $INL+OPL_{max}$ were calculated by averaging the corresponding layer thicknesses measured at the nasal and temporal peaks of macular thickness (dashed lines). ONL_{max} and $ONL+PR_{max}$ were measured only at the central thickest point of the outer nuclear layer (solid line). GCIP, ganglion cell layer + inner plexiform layer; INL, inner nuclear layer; ONL, outer nuclear layer; OPL, outer plexiform layer.

FIGURE 2.

Scatter plots of maximal and corresponding average retinal layer thicknesses (central B-scan traversing the fovea). Fitted lines and correlation coefficients are for the entire cohort. Of note, the differences of the GCIP and ONL thicknesses between MS subjects and healthy controls may be visualized. GCIP, ganglion cell layer + inner plexiform layer; ONL, outer nuclear layer.

FIGURE 3.

Scatter plots of manual segmentation-derived measures (maximal and average retinal layer thicknesses of the central B-scan traversing the fovea) and Cirrus fully automated segmentation-derived average thicknesses. Fitted lines and correlation coefficients are for the entire cohort. Of note, the differences of the GCIP and ONL thicknesses between MS subjects and healthy controls may be visualized. GCIP, ganglion cell layer + inner plexiform layer; ONL, outer nuclear layer.