ORIGINAL CONTRIBUTION

Retinal Imaging by Laser Polarimetry and Optical Coherence Tomography Evidence of Axonal Degeneration in Multiple Sclerosis

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Background: Optical coherence tomography (OCT) and scanning laser polarimetry with variable corneal compensation (GDx) are similar yet provide information on different aspects of retinal nerve fiber layer (RNFL) structure (thickness values similar to histology for OCT vs birefringence of microtubules for GDx).

Objectives: To compare the ability of OCT and GDx to distinguish eyes of patients with multiple sclerosis (MS) from eyes of disease-free controls and thus identify RNFL abnormalities. We also sought to examine the capacity of these techniques to distinguish MS eyes from those without a history of optic neuritis and to correlate with visual function.

Design: Cross-sectional study.

Setting: Academic tertiary care MS center.

Participants: Eighty patients with MS (155 eyes) and 43 disease-free controls (85 eyes) underwent both OCT and GDx imaging using protocols that measure RNFL thickness.

Main Outcome Measures: Areas under the curve (AUC), adjusted for within-patient, intereye correlations, were used to compare the abilities of OCT and GDx temporal-superior-nasal-inferior-temporal average RNFL thicknesses to discriminate between MS and control eyes and to distinguish MS eyes with a history of optic neuritis. Visual function was evaluated using low-contrast letter acuity and high-contrast visual acuity.

Results: Average peripapillary RNFL thickness (360° around the optic disc) was reduced in patients with MS compared with controls for both methods. Age-adjusted AUC did not differ between OCT (0.80; 95% confidence interval [CI], 0.72-0.88) and GDx (0.78; 95% CI, 0.68-0.86; $P=.38$). Optical coherence tomography–measured RNFL thickness was somewhat better at distinguishing MS eyes with a history of optic neuritis from those without (OCT: AUC, 0.73; 95% CI, 0.64-0.82; GDx: AUC, 0.66; 95% CI, 0.57-0.66; $P=.17$). Linear correlations of RNFL thickness for OCT vs GDx were significant yet moderate ($r=0.67$, $P<.001$); RNFL thickness measures correlated moderately and significantly with low-contrast acuity (OCT: $r=0.54$, $P<.001$; GDx: $r=0.55$, $P<.001$) and correlated less with high-contrast visual acuity (OCT: $r=0.44$, $P<.001$; GDx: $r=0.32$, $P<.001$).

Conclusions: Scanning laser polarimetry with variable corneal compensation measurements of RNFL thickness corroborates OCT evidence of visual pathway axonal loss in MS and provides new insight into structural aspects of axonal loss that relate to RNFL birefringence (microtubule integrity). These results support validity for RNFL thickness as a marker for axonal degeneration and support use of these techniques in clinical trials that examine neuroprotective and other disease-modifying therapies.

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THE ANTERIOR VISUAL PATHways are a common site for axonal degeneration in multiple sclerosis (MS).[1] Even in the absence of a history of acute optic neuritis (ON), eyes of patients with MS have reduced numbers of retinal ganglion cell axons in pathologic studies.[1] Ocular imaging techniques, including optical coherence tomography (OCT) and scanning laser polarimetry with variable corneal compensation (GDx), have demonstrated retinal nerve fiber layer (RNFL) thinning in MS,[2-9] ON,[10-13] and other forms of optic neuropathy.[14-20]

Optical coherence tomography and GDx measures of RNFL thickness are reliable[21,22] and correlate well with histomorphometric findings in primate and human studies.[23-25] Retinal nerve fiber layer thinning by OCT is associated with visual dysfunction in MS and ON[2-4,7-13] and cor-

relates with brain atrophy and disease subtype.[5,6] These unique structure-function correlations make the anterior visual pathways an attractive model for studying neuroprotective therapies.[9] Used with increasing frequency in research studies, OCT and GDx provide noninvasive assessments of RNFL thickness, require only seconds to complete, and, because both are often available at academic centers, can be used in MS clinical trials to quantify axonal loss.

Despite these similarities, there are fundamental differences in the methodologies used by OCT and GDx to image the RNFL.[9,14-18] Optical coherence tomography uses interference patterns of backscattered near-infrared light, analogous to B-scan ultrasound, to determine RNFL thickness and yields measurements (in micrometers) that are within 5 to 6 μm of histologic parameters.[9,14,23,24] Scanning laser polarimetry quantifies shifts in polarization of near-infrared light (phase retardation) that are induced by RNFL birefringence, a tissue property that depends on the integrity of retinal ganglion cell axon microtubules and neurofilaments.[26,27] An estimate of RNFL thickness is then calculated using the phase retardation and birefringence.

Scanning laser polarimetry thus has the capacity not only to corroborate OCT findings of RNFL thinning, but may also provide insight into structural damage that may precede or occur in the absence of RNFL thinning by OCT.[27] A comparison of these techniques will be useful for validating the role of RNFL thickness as a marker for axonal loss in MS and will demonstrate how OCT and GDx may yield complementary information on RNFL abnormalities.

The purpose of this investigation was to compare the ability of GDx and OCT measures of RNFL thickness to discriminate eyes of patients with MS from those of disease-free controls and thus identify RNFL abnormalities in MS. We also sought to examine the capacity of these techniques to distinguish between MS eyes with and without a history of ON and to correlate with scores for low-contrast letter acuity, an emerging clinical measure that correlates with magnetic resonance imaging lesion burden and captured treatment effects in recent MS trials.[28]

METHODS

PATIENTS

Patients and healthy controls participated as part of an ongoing multicenter investigation of vision in MS. Analyses included individuals who had undergone both OCT and GDx in the same testing session and do not overlap with previously published reports.[3] Patients with comorbid ocular conditions not related to MS were excluded. A history (months to years before enrollment) of acute ON was determined by self-report and physician report and confirmed by medical record review. Eyes with ongoing ON or an episode within 3 months of testing were not included. Optic disc swelling was not noted among any participants.

Disease-free controls were recruited from staff and patients' families and had no history of ocular or neurologic disease. Control eyes were excluded if best-corrected high-contrast Snellen visual acuities were worse than 20/20. Protocols were approved by institutional review boards and participants provided written informed consent. The study was conducted in accordance with Health Insurance Portability and Accountability Act guidelines.

RETINAL IMAGING

Participants underwent measurement of RNFL thickness for both eyes using OCT (OCT-3, OCT 4.0 software; Carl Zeiss Meditec, Dublin, California) and GDx with variable corneal compensation (software version 5.5.1, Carl Zeiss Meditec). The fast RNFL thickness scan protocol was used for OCT (computes the average of 3 circumferential scans for 360° around the optic disc; 256 axial scans; diameter, 3.4 mm). Good-quality OCT scans were defined by a signal strength of 7 or greater (maximum, 10) and uniform brightness across the scan circumference. As in previous studies,[3] scanning was completed without the use of pharmacologic dilation if the pupils were large enough to permit imaging (generally ≥5 mm). Average RNFL thickness for 360° around the optic disc was recorded as the OCT summary measure.

Scanning laser polarimetry with variable corneal compensation was also performed to measure RNFL thickness. These scans were centered on the optic disc using a scan circle of 3.2 mm; the mean of 3 measurements was used. Adequate scan quality was defined as Q(GDx) values of 7 or greater. The temporal-superior-nasal-inferior-temporal average RNFL thickness was used as the summary parameter for GDx.

VISUAL FUNCTION TESTING

Low-contrast letter acuity testing was performed for each eye separately using retroilluminated low-contrast Sloan letter charts (1.25% contrast at 2 m; Precision Vision, LaSalle, Illinois).[28] High-contrast visual acuity was assessed using retroilluminated Early Treatment Diabetic Retinopathy Study charts at 3.2 m. The number of letters identified correctly (maximum of 70 per chart) were recorded for each eye for low- and high-contrast acuity.[28] Testing was performed by trained technicians experienced in examination of patients for research studies, and patients wore their habitual glasses or contact lenses for distance correction. Standardized protocols, including written scripts and instructions, were followed for testing.

STATISTICAL ANALYSIS

Analyses were performed using Stata, version 10.0 (Stata Corp, College Station, Texas), and SAS (SAS Institute, Cary, North Carolina). Both eyes of patients and controls were included when eligible; analyses were adjusted for potential correlations between eyes of the same participant. While ophthalmologic studies sometimes include only 1 eye per participant, methods used in this study maximize available data (in the case of MS, both eyes may be affected) while accounting for within-patient, intereye correlations.

The capacity of RNFL thickness by OCT and GDx to discriminate MS from control eyes was summarized by areas under the curves (AUCs). Similar analyses were performed for distinguishing eyes with a history of ON from those without. To accommodate the correlation between eyes of the same patient, bootstrap sampling was performed for AUC analyses by stratifying eyes on their disease state (MS vs control, ON vs non-ON) and drawing patients with replacement from each stratum. Confidence intervals for AUC were calculated based on the 2.5th percentile and 97.5th percentile from 2000 replications of bootstrap estimates.[29] Areas under the curve for OCT and GDx were compared using the bootstrap method to generate the variance and covariance of the estimates of the 2 correlated AUCs.[30]

Table 1. Characteristics of Eyes of Patients With MS and Disease-free Controls

Characteristic	Mean (SD)			
	All MS Eyes (n=155)[a]	MS Eyes Without ON (n=87)[b]	MS Eyes With ON (n=68)[c]	Disease-free Control Eyes (n=85)[d]
Age, y	42 (10)	42 (10)	43 (10)	34 (10)
Visual acuity, Snellen equivalent, median (range)	20/20 (<20/250 to 20/12.5)	20/20 (20/100 to 20/12.5)	20/25 (<20/250 to 20/12.5)	20/16 (20/20 to 20/12.5)
Low-contrast acuity, 1.25% level, letters	17 (12)	20 (11)	12 (12)	30 (6)
OCT average RNFL thickness, μm	89.6 (18.3)	95.6 (15.0)	81.8 (19.3)	104.6 (10.3)
GDx TSNIT RNFL thickness, μm	53.1 (8.9)	55.5 (7.6)	50.0 (9.5)	58.0 (5.6)

Abbreviations: GDx, scanning laser polarimetry with variable cornea compensation; MS, multiple sclerosis; OCT, optical coherence tomography; ON, optic neuritis; RNFL, retinal nerve fiber layer; TSNIT, temporal-superior-nasal-inferior-temporal.
[a] Eighty patients.
[b] Forty-eight patients.
[c] Forty-one patients. Eyes with a history of ON before study enrollment (patients with acute ON within 3 months of study enrollment were excluded).
[d] Forty-three patients.

Table 2. Comparison of AUC for RNFL Thickness by OCT and GDx

Eyes	Adjusted by Age	AUC (95% CI)[a]		*P* Value[b]
		Measured by OCT	Measured by GDx TSNIT	
MS vs control eyes	No	0.76 (0.68-0.84)	0.65 (0.56-0.74)	.03
	Yes	0.80 (0.72-0.88)	0.78 (0.69-0.86)	.38
MS eyes with ON vs without ON[c]	No	0.72 (0.62-0.80)	0.65 (0.55-0.75)	.21
	Yes	0.73 (0.64-0.82)	0.66 (0.59-0.78)	.17
MS eyes without ON vs control eyes	No	0.69 (0.59-0.79)	0.60 (0.50-0.70)	.09
	Yes	0.78 (0.69-0.86)	0.76 (0.66-0.85)	.46

Abbreviations: AUC, area under the curve; CI, confidence interval; GDx, scanning laser polarimetry with variable cornea compensation; MS, multiple sclerosis; OCT, optical coherence tomography; ON, optic neuritis; RNFL, retinal nerve fiber layer; TSNIT, temporal-superior-nasal-inferior-temporal.
[a] Based on the 2.5th percentile and 97.5th percentile from 2000 replications of bootstrap estimation of 95% CIs, adjusted for within-patient, intereye correlations.[30] Areas under the curve indicate the probability that a test will correctly rank any randomly selected pair of individuals as having a disease (in this case, MS or ON) or not. Areas under the curve range from 0.5 (ability to distinguish diseased from nondiseased, comparable with flipping a fair coin) and 1.0 (perfect ability to distinguish).
[b] Areas under the curve compared using method from Margolis et al.[30]
[c] Eyes with history of ON at least 3 months before study enrollment.

The relationship of GDx and OCT parameters with visual function in MS eyes was examined using Pearson linear correlation coefficients and generalized estimating equation techniques accounting for age and adjusting for within-patient, intereye correlations. Type 1 error for significance was set at $\alpha = 0.05$ for all analyses.

RESULTS

Clinical data for 80 patients with MS (155 eyes) and 43 disease-free controls (85 eyes) are summarized in **Table 1**. Characteristics were similar to the US MS population for sex (80% female) and age; most patients had relapsing-remitting MS (85%). Patients with MS were older than controls; analyses comparing eyes in these groups, therefore, included age adjustment. Retinal nerve fiber layer thickness was reduced in MS eyes compared with control eyes (Table 1). Consistent with reports for glaucoma and band atrophy, RNFL thickness values for GDx (polarimetric micrometers) were lower than those for OCT based on differences in imaging paradigms.[9]

Adjusting for age and within-patient, intereye correlations, the capacity to distinguish MS eyes from control eyes did not differ between OCT and GDx temporal-superior-nasal-inferior-temporal average RNFL thickness ($P = .38$) (**Table 2**). Optical coherence tomography and GDx were also similar in their capacities to discriminate eyes with a history of ON from those without. Linear correlations for OCT vs GDx RNFL thickness were moderate and significant for MS eyes both with and without ON (**Figure**).

Retinal nerve fiber layer thickness correlated moderately and to a significant degree with low-contrast letter acuity scores (OCT: $r = 0.54$, $P < .001$; GDx temporal-superior-nasal-inferior-temporal: $r = 0.55$, $P < .001$), indicating worse vision scores in the setting of RNFL thinning. Correlations with high-contrast visual acuity were lower (OCT: $r = 0.44$, $P < .001$; GDx temporal-superior-nasal-inferior-temporal: $r = 0.32$, $P < .001$). Adjustment for age and within-patient, intereye correlations confirmed associations between reduced visual function and RNFL thinning for both GDx and OCT ($P < .001$, generalized estimating equation models). In these models, 2-line (10-letter) differences in low-contrast acuity were associated, on average, with 8.1 μm differences in OCT (95% confidence interval, 5.9-10.2) and 4.0 μm differ-

ences in GDx RNFL thickness (95% confidence interval, 3.0-4.9). For low-contrast letter acuity, 2-line (10-letter) differences in score have been used in recent MS trials as a criterion for clinically meaningful change based on published reliability data.[31]

COMMENT

Results for GDx-measured RNFL thickness in this study provide evidence for anterior visual pathway axonal degeneration that reflects not only thinning of RNFL axons (measured by OCT) but also implicates disruption of birefringent axonal structures, such as microtubules (detected by GDx). Both GDx and OCT capture RNFL thinning in MS eyes and correlate well with visual function. Data from this study provide additional evidence that RNFL thickness is an important marker for axonal loss and suggest that these techniques will complement visual function assessments in clinical trials of MS and ON.

The recent development of candidate neuroprotective therapies for MS and other neurodegenerative diseases has brought to the forefront the potential role for the anterior visual pathways as a model for assessing clinical outcomes and axonal integrity.[9] While GDx and OCT use near-infrared light to produce measurements of RNFL thickness that are reliable,[21,22] noninvasive, and correlate with visual function,[2-4,7-13] differences in these techniques have provided a basis for comparative studies.[14-18] Optical coherence tomography yields measurements (in micrometers) that are similar to those of histologic sections.[9,14,23,24] Scanning laser polarimetry captures RNFL birefringence, which is largely dependent on the interaction of light with microtubules of ganglion cell axons[9,14,26,27]; GDx thus offers the ability to evaluate microtubule density changes, which have been demonstrated in animal models to be detectable by GDx, even in the absence of changes in RNFL thickness as measured by OCT.[27] These differing properties and measurements provided by OCT and GDx likely explain, at least in part, that correlations between OCT- and GDx-measured RNFL thickness in this and other studies are moderate in magnitude but not higher (r=0.57-0.69 in present study; r=0.63 in optic nerve band atrophy[18]; and r=0.71-0.85 in glaucoma[14,17]).

Because GDx not only estimates RNFL thickness but also evaluates an important aspect of axonal viability (microtubule integrity), this technology complements OCT in examining the RNFL in MS. Technical features of GDx that differ from OCT include its use of variable corneal compensation (measurement of corneal birefringence, measured first during the scan and subtracted from RNFL birefringence) and that patients undergoing GDx need to adequately fixate on a target (difficult with poor vision, primary gaze nystagmus) so that the scan can be obtained.[9] Whereas the technician performing OCT can visualize the optic disc to ensure proper scan placement, GDx does not allow for such visualization. On the other hand, elevations in RNFL thickness related to disc edema must be considered for OCT but are less problematic with GDx.[9] Retinal nerve fiber layer thickness measurements are proportional but differ in magnitude between GDx and OCT, with GDx values (in polarimetric micrometers) being approximately 0.55 times those of OCT (in micrometers) in the same eyes.[14-18]

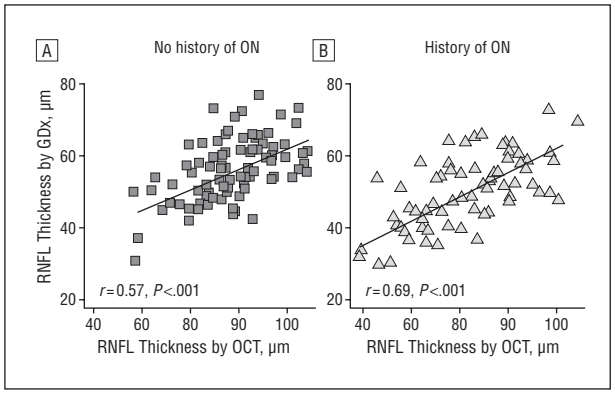

Figure. Scatterplot of optical coherence tomography (OCT) vs scanning laser polarimetry (GDx) temporal-superior-nasal-inferior-temporal average retinal nerve fiber layer (RNFL) thickness for eyes of patients with multiple sclerosis. A, Eyes of patients with multiple sclerosis and no history of optic neuritis (ON). B, Eyes of patients with multiple sclerosis and a history of ON at least 3 months before study enrollment. Linear correlation coefficients for OCT vs GDx measures were moderate and statistically significant. Lines indicate fitted values based on univariate regression analyses.

Measures of RNFL thickness for OCT in the present study were similar to those in previous investigations of MS and ON.[2-13] In 1 study of GDx,[8] 40% of MS eyes had an abnormal RNFL thickness but actual values were not presented. Areas under the curve were lower for our cohort compared with those in studies of glaucoma and band atrophy.[14-18] This is likely because, while glaucoma and band atrophy are defined by the presence of optic neuropathy, anterior visual pathway involvement and optic atrophy are not invariably present in MS and are not necessary for diagnosis. Correlations of GDx and OCT measurements with low-contrast letter acuity were similar (r=0.55 vs 0.54) but were relatively lower for high-contrast visual acuity vs GDx (r=0.32) and OCT (r=0.44). The relationship of low- vs high-contrast acuity measures with changes in RNFL thickness and birefringence is also under investigation in longitudinal studies. Importantly, data from our study demonstrate that RNFL thinning by both GDx and OCT are associated with reductions in low- and high-contrast acuity scores, supporting available evidence that axonal integrity in MS is likely an important contributor to afferent visual function.[2-13]

Additional studies of RNFL quadrant-specific analyses for GDx and OCT will provide insight into patterns of axonal loss in MS. Ongoing longitudinal studies will also determine the course and relationship among RNFL microtubule disruption (captured by GDx), visual dysfunction, and RNFL thinning by OCT. Our data support a role for ocular imaging techniques such as OCT and GDx in clinical trials of ON and MS that examine neuroprotective and other disease-modifying therapies.

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