Relation of Visual Function to Retinal Nerve Fiber Layer Thickness in Multiple Sclerosis

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Purpose: To examine the relation of visual function to retinal nerve fiber layer (RNFL) thickness as a structural biomarker for axonal loss in multiple sclerosis (MS), and to compare RNFL thickness among MS eyes with a history of acute optic neuritis (MS ON eyes), MS eyes without an optic neuritis history (MS non-ON eyes), and disease-free control eyes.

Design: Cross-sectional study.

Participants: Patients with MS (n = 90; 180 eyes) and disease-free controls (n = 36; 72 eyes).

Methods: Retinal never fiber layer thickness was measured using optical coherence tomography (OCT; fast RNFL thickness software protocol). Vision testing was performed for each eye and binocularly before OCT scanning using measures previously shown to capture dysfunction in MS patients: (1) low-contrast letter acuity (Sloan charts, 2.5% and 1.25% contrast levels at 2 m) and (2) contrast sensitivity (Pelli–Robson chart at 1 m). Visual acuity (retroilluminated Early Treatment Diabetic Retinopathy charts at 3.2 m) was also measured, and protocol refractions were performed.

Main Outcome Measures: Retinal nerve fiber layer thickness measured by OCT, and visual function test results.

Results: Although median Snellen acuity equivalents were better than 20/20 in both groups, RNFL thickness was reduced significantly among eyes of MS patients (92 μm) versus controls (105 μm) ($P<0.001$) and particularly was reduced in MS ON eyes (85 μm; $P<0.001$; accounting for age and adjusting for within-patient intereye correlations). Lower visual function scores were associated with reduced average overall RNFL thickness in MS eyes; for every 1-line decrease in low-contrast letter acuity or contrast sensitivity score, the mean RNFL thickness decreased by 4 μm.

Conclusions: Scores for low-contrast letter acuity and contrast sensitivity correlate well with RNFL thickness as a structural biomarker, supporting validity for these visual function tests as secondary clinical outcome measures for MS trials. These results also suggest a role for ocular imaging techniques such as OCT in trials that examine neuroprotective and other disease-modifying therapies. Although eyes with a history of acute optic neuritis demonstrate the greatest reductions in RNFL thickness, MS non-ON eyes have less RNFL thickness than controls, suggesting the occurrence of chronic axonal loss separate from acute attacks in MS patients.
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Visual dysfunction is a leading cause of disability in multiple sclerosis (MS).[1,2] As many as 50% of patients with MS experience visual loss as a presenting symptom, and 80% develop some degree of visual impairment during the course of their disease.[1,3,4] Visual symptoms in MS may be present even among patients with normal Snellen acuities and in those with no history of acute optic neuritis.[5–10]

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Despite the importance of vision to disability and quality of life in MS, the quantitative assessment of visual function in clinical trials traditionally has been limited to nonstandardized tests of Snellen acuity, a method that does not capture visual loss in most MS patients. The extent to which vision may be affected by standard and novel disease-modifying therapies for MS is not yet known, and even the newest clinical outcome measure, the MS Functional Composite (MSFC), lacks a component for visual assessment.[11–14]

Recent cross-sectional and longitudinal studies have demonstrated that low-contrast letter acuity (Sloan charts) and contrast sensitivity (Pelli–Robson charts) have the greatest capacity to capture visual dysfunction in MS patients.[15,16] In addition, Sloan and Pelli–Robson chart tests are clinically practical, demonstrate high degrees of interrater reliability,[10,17] and correlate with visual evoked potential testing in MS patients.[18,19] Sloan charts have been incorporated into several recent MS clinical trials,[15,16] and Pelli–Robson testing was used as a primary outcome in the Optic Neuritis Treatment Trial.[20–24] Testing for each of these measures may be performed binocularly to capture overall function with both eyes open,[25–27] or with each eye separately to reflect individual optic nerve function.

Correlation with biological markers of disease is one of the most important considerations in the assessment of validity for clinical outcome measures. Traditionally in MS, standard brain magnetic resonance imaging (MRI) techniques have provided information regarding disease burden, with emphasis on inflammation and demyelination. However, the capacity for MRI techniques to quantify precisely axonal and neuronal loss within the brain has been limited to research methods such as diffusion tensor imaging and magnetic resonance spectroscopy. Furthermore, MRI provides essentially no information regarding chronic disease in the anterior visual pathways. Although optic neuritis and acute demyelination are important contributors to visual dysfunction in MS, irreversible axonal and neuronal degeneration also represent final common pathways to permanent visual loss.[28]

Optical coherence tomography (OCT) is a noninvasive high-resolution technique that uses near infrared light to measure the thickness of ocular structures, particularly the retinal nerve fiber layer (RNFL).[29] Optical coherence tomography has been used successfully to capture retinal ganglion cell axon loss in early glaucoma and in other forms of anterior visual pathway disease, including traumatic optic neuropathy, chiasmal lesions, and acute optic neuritis.[30–35] In patients with glaucoma and visual field (VF) abnormalities, RNFL thickness has been shown to correlate significantly with automated perimetry results.[30,36–40] Optical coherence tomography is a highly reliable technique for measuring RNFL thickness. For example, one recent study demonstrated high levels of reproducibility for the third generation of commercial OCT (OCT-3, Carl Zeiss Meditec, Inc., Dublin, CA) in eyes of normal subjects.[41] Intraclass correlation coefficients calculated for RNFL thickness both before and after pharmacologic pupillary dilation demonstrated high degrees of test–retest and interobserver reliability (intraclass correlation coefficients, 0.79–0.83). Intravisit and intervisit standard deviations (SDs) were <3 μm.

Unlike MRI measures of brain or optic nerve atrophy, OCT provides a unique opportunity to measure a structure within the central nervous system that consists of isolated axons (because axons within the RNFL are not myelinated). Accessibility of the retina for imaging and the capacity to correlate directly RNFL thickness with visual function make OCT a strong candidate biomarker for clinical trials of MS and optic neuritis. Although pilot studies have demonstrated reductions in overall average RNFL thickness in MS and in acute optic neuritis,[34,35] the relation of RNFL thickness to visual function in heterogeneous MS cohorts has not been established.

The purpose of our investigations was to examine the relation of visual function to RNFL thickness as a structural biomarker for axonal loss in MS. We also sought to compare RNFL thicknesses among MS eyes with a history of acute optic neuritis (MS ON eyes), MS eyes without an optic neuritis history (MS non-ON eyes), and eyes of disease-free controls. Because the MS disease process affects multiple regions of the central nervous system, we explored the relation of RNFL thickness to measures of overall neurologic impairment.

Materials and Methods

Subjects

Patients and disease-free control subjects in the MS Vision Prospective Cohort Study,[15] an ongoing investigation of visual outcome measures, were invited to participate. Multiple sclerosis was diagnosed by standard clinical and neuroimaging criteria.[42] Disease duration, disease-specific therapies (e.g., immunomodulatory agents) and their duration, and MS disease phenotype (relapsing–remitting, secondary progressive, primary progressive) were ascertained for each MS patient. Patients with comorbid ocular conditions not related to MS (ascertained by a detailed history and examination) were excluded. A history of $\geq$1 episodes of acute optic neuritis was determined for eyes of MS patients by self-report and physician report and confirmed by medical record review. Patients experiencing an acute attack of optic neuritis and those whose most recent attack had occurred less than 1 month prior were not included in these analyses. Optic disc swelling was not noted among any study participants.

Disease-free control participants were recruited from among staff and family members of patients and had no history of ocular or neurologic disease. Patients and controls with refractive error in the absence of other ocular comorbidities were invited to participate to best capture the ocular status of patients who may participate in MS trials. Although no absolute criteria for refractive error were used for participation, one patient with MS was excluded on the basis of severe congenital myopia (<-15.00 spherical equivalent [SE]). Multiple sclerosis patients were excluded if Snellen visual acuity (VA) equivalents were worse than 20/200 in both eyes, because this would preclude testing of low-contrast letter acuity; control eyes were required to have acuities of 20/20 or better. Institutional review board approval was obtained. All participants provided written informed consent, and the study was conducted in accord with regulations of the Health Insurance Portability and Accountability Act.

Visual Function Testing

Participants underwent testing using the following: (1) low-contrast letter acuity (low-contrast Sloan letter charts, which involve identification of gray letters of progressively smaller size on a white/retroilluminated background at 2 m; 1.25% and 2.5% contrast levels; Precision Vision, LaSalle, IL),[15,16,43] (2) contrast sensitivity (Pelli–Robson charts, which capture the minimum contrast level at which patients can perceive letters of a single large size at 1 m; Lombart Instrument Co., Norfolk, VA),[20,44] and (3) high-contrast VA (Early Treatment Diabetic Retinopathy Study [ETDRS] charts at 3.2 m; Lighthouse Low-Vision Products, Long Island City, NY). Sloan charts have a standardized format based on that of the ETDRS VA charts (5 letters per line).[45,46] Each Sloan chart corresponds to a different contrast level, and charts are scored based on the number of letters identified correctly. This format may allow Sloan charts to capture losses of contrast at small letter sizes that have been reported in MS and other neurologic disorders.[47]

Pelli–Robson contrast sensitivity charts consist of 16 groups of 3 uppercase letters (triplets, or lines). Letters on this chart are of a single large size (~20/680 Snellen equivalent).[44] Unlike the Sloan charts, which measure threshold acuity at different levels of contrast, the Pelli–Robson chart provides a measure of contrast sensitivity at a single letter size. All testing was performed for each eye separately as well as binocularly; binocular testing was included to provide a summary measure of overall visual functioning with both eyes open.[25]

Monocular and binocular summary scores for visual function tests were calculated as follows: (1) Sloan charts and ETDRS VA, number of letters identified correctly (maximum, 70) and number of lines correct (letters correct/5), and (2) Pelli–Robson charts, log contrast sensitivity (maximum log score, 2.25 [48 letters]) and number of lines correct (letters correct/3). Snellen equivalents were also recorded for ETDRS VA measurements.

Before vision testing, participants underwent detailed refractions to minimize potential bias between patients and controls with respect to correction of refractive error. Refractions were performed for each eye at 3.2 m (ETDRS chart R) and adjusted for the different distances used for other vision tests. Testing was performed by trained technicians experienced in examination of patients for research studies. Although it was not feasible for the examining technicians to be masked to MS versus control group status, strict standardized protocols, including written scripts and instructions for testing, were followed.

Optical Coherence Tomography

Optical coherence tomography was performed for both eyes of each participant using OCT-3 with OCT 4.0 software (Carl Zeiss Meditec). Using low-coherence interferometry, OCT generates cross-sectional tomograms of the retina with an axial resolution of ≤ 10 μm.[29] The fast RNFL thickness scan protocol was used (computes the average of 3 circumferential scans 360° around the optic disc, 256 axial scans, 3.4-μm diameter). Optical coherence tomography scanning was performed by trained technicians after visual function testing. Scans were performed without flash photography to optimize patient comfort. If the participant's pupils were large enough to permit adequate OCT imaging (5-mm diameter), scanning was completed without the use of mydriatic eyedrops. Dilation has been shown to have little impact on OCT values and reproducibility, and may not be consistently feasible in the MS clinical trial setting.[41] Pupils were dilated with 1% tropicamide if adequate scans could not otherwise be obtained. Good scans were defined according to specifications in the OCT-3 users' manual: signal strength of ≥ 7 (maximum, 10) and uniform brightness across the scan circumference. In this cohort, all scans met this requirement, and the median signal strength was 10 (range, 7–10). Internal fixation was used for all OCT scans, and a patch was placed over the nontested eye to improve fixation.

Average overall RNFL thickness (averaged for peripapillary retina 360° around the optic disc) and thickness values for each of 4 quadrants (temporal, superior, nasal, inferior) were recorded from the OCT printouts for MS and disease-free control eyes.

Neurological Assessment

The Expanded Disability Status Scale (EDSS) and MSFC, measures used in MS clinical trials, were performed for MS patients to characterize degrees of neurological impairment.[12,48] The MSFC includes quantitative tests of leg function/ambulation (Timed 25-Foot Walk [T25FW]), arm function (9-Hole Peg Test [9HPT]), and cognition (Paced Auditory Serial Addition Test with a 3-second interstimulus interval [PASAT3]). The MSFC component and composite Z scores represent the number of SDs from a disease-free control group mean score.[15] Composite Z scores are calculated as follows: MSFC Z score = (ZT25FW+Z9HPT+ZPASAT3)/3.0.

Statistical Methods

All data analyses were performed using Stata statistical software (version 8.0, StataCorp, College Station, TX). Generalized estimating equation (GEE) models were used for primary analyses that examined the relation of visual function to RNFL thickness. Generalized estimating equation models are generalized linear models that allow for specification of within-group correlations when examining the capacity of one or several independent variables to predict a dependent variable. In this investigation, GEE models were used to determine how well visual function scores predicted average overall RNFL thickness, accounting simultaneously for age. Because both eyes of each MS patient and control were included in this study, and eyes of the same patient would be expected to have some degree of intercorrelation with respect to visual function and RNFL thickness, GEE models allowed us to adjust for these within-patient intereye correlations.

Generalized estimating equation models were also used to compare patient (MS eyes, MS ON eyes, MS non-ON eyes) and disease-free control groups with respect to RNFL thickness values (average overall and 4 quadrants) and to examine the relation of neurologic status to RNFL thickness. Indicator variables and interaction terms were used in models that examined patterns of RNFL thickness across retinal quadrants in MS versus control eyes as well as in MS ON and MS non-ON eyes. A type I error level of $\alpha = 0.05$ was used for statistical significance.

Results

Ninety patients with MS (180 eyes) and 36 disease-free controls (72 eyes) underwent vision testing and OCT imaging. Demographic and clinical characteristics are presented in Table 1. Because patients and disease-free controls in this convenience sample differed with respect to age, statistical models used for analyses included age as a covariate. Multiple sclerosis patients in our cohort were similar to the United States MS population with regard to age, gender, and race (88% Caucasian). Eighty percent of MS patients (72/90) were using standard disease-modifying therapies (median duration of current therapy, 3 years [range, <1–11]). Degree of refractive error (SE), as measured by protocol refractions, did not differ significantly between MS and control group eyes ($P = 0.71$, GEE models accounting for within-patient intereye correlations).

Table 1. Characteristics of Patients with Multiple Sclerosis (MS) and Disease-Free Controls

	MS Patients (n = 90, 180 Eyes)	Disease-Free Controls (n = 36, 72 Eyes)
Age (yrs)* (mean ± standard deviation)	48±8	38±10
Gender [n (% female)]	72 (80)	28 (78)
MS disease duration (yrs) [median (range)]	8 (<1–46)	—
MS disease phenotype† [n (% relapsing remitting)]	76 (84)	—
EDSS score‡ [median (range)]	2 (0–7)	—
MSFC Z score§ [mean ± standard deviation]	−2.49±3.9	—
Refractive error (spherical equivalent, by eyes)‖ [median (range)]	−0.75 (−8.00 to +3.75)	−0.5 (−7.125 to +4.375)
Visual acuity (Snellen equivalent, by eyes) [median (range)]	20/16 (20/12.5–20/200) Mean, 20/20	20/16 (20/12.5–20/20) Mean, 20/15
Average overall retinal nerve fiber layer thickness (μm, by eyes) [median (range)]	93 (36–129)	107 (85–131)

EDSS = Expanded Disability Status Scale; MSFC = MS Functional Composite.
*Age was significantly lower among disease-free controls in this convenience sample ($P<0.0001$, t test); therefore, all statistical models comparing MS and control group eyes accounted simultaneously for participant age.
†Remainder of cohort had secondary progressive MS phenotype.
‡Assigned on an ordinal scale based on the neurological examination, and range in 0.5-increments from 0 (no abnormal findings or disability) to 7.0+ (wheelchair used for mobility).
§The MSFC includes quantitative tests of leg function/ambulation (Timed 25-Foot Walk [T25FW]), arm function (9-Hole Peg Test [9HPT]), and cognition (Paced Auditory Serial Addition Test with a 3-second interstimulus interval [PASAT3]). Z scores represent the number of standard deviations from a disease-free control group mean score, and are calculated as follows: MSFC composite Z score = $(Z_{T25FW} + Z_{9HPT} + Z_{PASAT3})/3.0$.
‖Degree of refractive error, as measured by protocol refractions, did not differ significantly between MS and control group eyes ($P = 0.71$, generalized estimating equation models accounting for within-patient intereye correlations).

Snellen acuity equivalents were 20/20 or better for both MS and disease-free control eyes (Table 1). Although median ETDRS VA scores did not differ from a clinical standpoint (difference of 3 letters, <1 line of acuity), scores for low-contrast letter acuity and contrast sensitivity were significantly worse among eyes of MS patients compared with disease-free controls (Table 2). Scores were lower (worse) for the 1.25% contrast level (lower contrast) compared with 2.5%, with greater differences between patients and controls noted at the 1.25% level. Multiple sclerosis eyes with a history of acute optic neuritis (MS ON eyes) had significantly worse visual function than MS eyes without a history of acute optic neuritis (MS non-ON eyes) for low-contrast letter acuity ($P\leq0.007$) and contrast sensitivity ($P = 0.006$). Eyes of MS patients without a history of acute optic neuritis in either eye (MS non-ON patient eyes) versus fellow eyes of MS patients with a history of acute optic neuritis in one eye (MS ON patient fellow

Table 2. Comparison of Visual Function Test Scores for Eyes of Patients with Multiple Sclerosis (MS), Disease-Free Control Eyes, and MS Eyes with a History of Acute Optic Neuritis (MS ON Eyes)

	All MS Eyes (n = 180, 90 Patients)	Disease-Free Control Eyes (n = 72, 36 Patients)*	MS ON Eyes (n = 63)*	MS Non-ON Eyes (n = 108)
High-contrast VA [ETDRS charts, no. of letters correct, median (range)]†	63 (0–70)	66 (58–70)	62 (0–70)	64 (8–70)
Low-contrast letter acuity [Sloan charts, 1.25% contrast level, no. of letters correct, median (range)]‡	22 (0–41)	32 (15–42)	15 (0–35)	24 (0–41)
Low-contrast letter acuity [Sloan charts, 2.5% contrast level, no. of letters correct, median (range)]‡	36 (0–49)	39 (26–48)	32 (0–44)	37 (0–47)
Contrast sensitivity [Pelli–Robson chart, log contrast, median (range)]§	1.65 (0–1.95)	1.70 (1.45–1.95)	1.65 (0–1.85)	1.65 (1.2–1.95)

ETDRS = Early Treatment Diabetic Retinopathy Study; VA = visual acuity.
*Visual function test scores were significantly lower (worse) among MS eyes than among controls, accounting for age and adjusting for within-patient intereye correlations ($P\leq0.001$ for all comparisons, generalized estimating equation models). Multiple sclerosis ON eyes had significantly worse visual function than MS non-ON eyes for low-contrast letter acuity ($P\leq0.007$) and contrast sensitivity ($P = 0.006$). Eyes of MS patients without a history of acute ON in either eye vs. fellow eyes of MS patients with a history of acute ON in one eye (MS ON patient fellow eyes) did not differ significantly with respect to visual function scores (scores were actually slightly higher, but not significantly so, for MS ON patient fellow eyes; $P\geq0.14$, data not shown). Numbers of MS ON eyes + MS non-ON eyes add to 171 because there were 9 MS eyes for which history of acute ON was not known.
†Charts have 5 letters per line; scores are expressed herein as number of letters identified correctly (range, 0 [0 lines, <20/250 Snellen equivalent]–70 [15 lines, 20/12.5 Snellen equivalent]).
‡Low-contrast charts have a format similar to that of ETDRS VA charts (5 letters per line); scores are expressed herein as number of letters identified correctly (range, 0 [0 lines]–70 [15 lines]). The 2.5% and 1.25% contrast levels were examined in this study.
§Charts, as used in the Optic Neuritis Treatment Trial, consist of 16 groups of 3 large (~20/680 equivalent at 1 m) letters (lines); scores are expressed herein as log contrast (range, 0.00 [1 line/3 letters correct]–2.25 [16 lines/48 letters correct]).

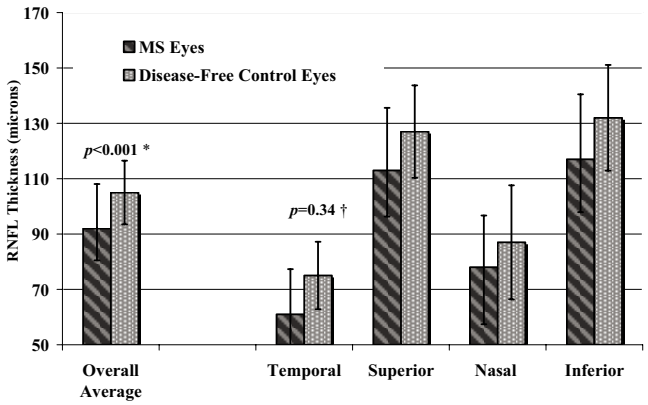

Figure 1. Mean values for overall average retinal nerve fiber layer (RNFL) thickness (360° around the optic disc) and for RNFL thickness in temporal, superior, nasal, and inferior quadrants for patients with multiple sclerosis (MS; n = 90 [180 eyes]) and disease-free controls (n = 36 [72 eyes]). *Average overall RNFL thickness values were significantly lower for MS patients versus controls ($P<0.001$, generalized estimating equation [GEE] models accounting for age and adjusting for within-patient intereye correlations)†Mean RNFL thickness values varied significantly across retinal quadrants ($P<0.0001$), with mean thickness greater in the superior and inferior quadrants. The mean thickness was greater for controls than for MS patients in all quadrants, and the difference between patient groups was of the same magnitude in each quadrant ($P = 0.34$ for interaction terms, GEE models).

eyes) did not differ significantly with respect to visual function scores (scores were actually slightly higher, but not significantly so, for MS ON patient fellow eyes [$P \geq 0.14$, data not shown]).

Average overall RNFL thickness (average thickness for 360° around the optic disc) was significantly reduced in MS eyes (92 ± 16 μm) relative to eyes of disease-free controls (105 ± 12 μm) ($P<0.001$, GEE models accounting for age and adjusting for within-patient intereye correlations) (Fig 1). Although, as expected, MS ON eyes (85 ± 17 μm) had significantly lower RNFL thicknesses than MS non-ON eyes (96 ± 14 μm) ($P<0.001$), values for MS non-ON eyes were also reduced compared with normal controls (105 μm, $P = 0.03$). Using normative data included in the OCT 4.0 processing software for OCT-3, only 40 of 180 eyes of MS patients (22%) had overall average RNFL thickness values that were abnormal in one or both eyes. However, the OCT 4.0 normative database considers the fifth percentile for age to be the cutoff for abnormal values.[49]

Mean RNFL thickness values varied significantly across retinal quadrants ($P<0.0001$), with mean thickness greater in the superior and inferior quadrants. The mean thickness was greater for controls than for MS patients in all quadrants, and the difference between subject groups was of the same magnitude in each quadrant ($P = 0.34$ for interaction terms, GEE models). Within the MS group, comparison of mean RNFL thickness between MS ON eyes and MS non-ON eyes (Fig 2) showed that MS ON eyes had lower mean RNFL thickness ($P<0.001$). The mean thickness was less for MS ON eyes in all quadrants, with a suggestion that the differences between these 2 patient groups were smallest in the nasal quadrant ($P = 0.02$ for interaction terms, GEE models).

To address the question of whether patients with a history of acute optic neuritis that was unilateral may have actually had involvement of the contralateral optic nerve based on reductions in RNFL thickness, additional analyses were performed to compare overall average RNFL thicknesses in eyes of MS patients without a history of acute optic neuritis in either eye (MS non-ON patient eyes) versus fellow eyes of MS patients with a history of acute optic neuritis in one eye (MS ON patient fellow eyes). The overall average RNFL thickness in MS ON patient fellow eyes (99 μm) was similar to that in MS non-ON patient eyes (95 μm) ($P = 0.31$, GEE models accounting for age and adjusting for within-patient intereye correlations). In contrast, eyes with a history of acute optic neuritis (MS ON eyes, Fig 2) had significantly reduced RNFL thickness compared with both groups of non-ON eyes (85 μm, $P<0.001$).

Visual function scores were significant predictors of overall average RNFL thickness among MS eyes ($P<0.001$ for all tests, GEE models accounting for age and adjusting for within-patient intereye correlations). As demonstrated in Table 3, lower visual function scores were associated with reduced average overall RNFL thickness. For every 1-line change in low-contrast letter acuity and in contrast sensitivity scores, RNFL thickness differences of 4 μm on average were noted, accounting for age. Spearman rank correlations between overall average RNFL thickness and visual function scores were highly significant yet modest in magnitude, suggesting that visual dysfunction may occur in some patients in the absence of (or perhaps in advance of) RNFL axonal loss (Spearman r [r_s] = 0.33 and $P<0.0001$ for low-contrast letter acuity, $r_s = 0.31$ and $P<0.0001$ for contrast sensitivity, $r_s = 0.26$ and $P = 0.0005$ for high-contrast VA). Unlike GEE models, however, these simple correlations do not account for factors such as age and disease duration, and do not allow for adjustment for within-patient intereye correlations.

We also examined the relation between RNFL thickness and more global aspects of disease in MS, including duration of disease and scores for overall neurological impairment (EDSS and MSFC [MSFC = T25FW, 9HPT, and PASAT]). Average overall RNFL

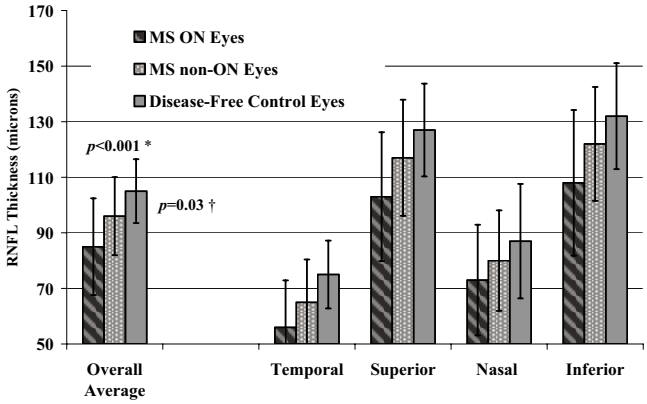

Figure 2. Mean values for overall average retinal nerve fiber layer (RNFL) thickness (360° around the optic disc) and for RNFL thickness in temporal, superior, nasal, and inferior quadrants for multiple sclerosis (MS) eyes with a history of ≥1 episodes of acute optic neuritis (MS ON eyes [n = 63]), MS eyes without an acute ON history (MS non-ON eyes [n = 108]), and disease-free control eyes (n = 72). In a subanalysis comparing eyes of MS patients without a history of acute optic neuritis in either eye (MS non-ON patient eyes) and fellow eyes of MS patients with a history of acute ON in one eye (MS ON patient fellow eyes), overall average RNFL thickness in MS ON patient fellow eyes (99 μm) was similar to that of MS non-ON patient eyes (95 μm) ($P = 0.31$, generalized estimating equation [GEE] models accounting for age and adjusting for within-patient intereye correlations). *Significant differences in average overall RNFL thickness between MS ON eyes and MS non-ON eyes were observed ($P<0.001$, GEE models accounting for age and adjusting for within-patient intereye correlations)†Multiple sclerosis non-ON eyes also had reduced average overall RNFL thickness compared with disease-free control eyes ($P = 0.03$).

Table 3. Association of Worsening in Visual Function Score and Reduction in Retinal Nerve Fiber Layer (RNFL) Thickness (μm), Single Examination

	Decrease in Average Overall RNFL Thickness Associated with 1-Line Decrease in Visual Function Score in MS Eyes (n = 180) (95% CI)*
Low-contrast letter acuity (Sloan charts, 1.25%, 5 letters/line)	3.8 (2.7–4.9)
Low-contrast letter acuity (Sloan charts, 2.5%, 5 letters/line)	3.1 (2.0–4.2)
Contrast sensitivity (Pelli–Robson chart, 3 letters/line)	4.4 (3.5–5.4)
High-contrast VA (ETDRS charts, 5 letters/line)	2.9 (2.1–3.7)

CI = confidence interval; ETDRS = Early Treatment Diabetic Retinopathy Study; MS = multiple sclerosis; VA = visual acuity.
*Visual function scores significantly predicted overall average RNFL thickness, accounting for age and adjusting for within-patient intereye correlations ($P<0.001$ for all tests, generalized estimating equation models). Data are cross-sectional from a single study visit and interpreted as the number of microns reduction in average overall RNFL thickness (360° around the optic disc) associated with a 1-line worsening of visual function test score. For example, a 1-line (5 letters) decrease in low-contrast letter acuity at the 1.25% level was associated with a 3.8-μm reduction in RNFL thickness among all MS eyes in this study.

thickness declined with increasing degrees of overall neurological impairment and disability in our MS cohort and was significantly associated with EDSS score ($P = 0.02$ for linear trend across EDSS tertiles, GEE models) (Fig 3). Multiple Sclerosis Functional Composite scores and RNFL thickness were also significantly related ($P = 0.001$, GEE models), and RNFL thickness declined with increasing disease duration ($P = 0.03$).

Discussion

Results of these investigations demonstrate that low-contrast letter acuity and contrast sensitivity, the two most promising candidate visual outcome measures for MS, correlate well with RNFL thickness. Although eyes with a history of acute optic neuritis (MS ON eyes) demonstrate the greatest reductions in RNFL thickness, MS non-ON eyes are also abnormal (including fellow eyes of MS patients with a history of unilateral optic neuritis), supporting the occurrence of anterior visual pathway axonal loss in MS patients that occurs in the absence of obvious attacks of acute optic neuritis. Retinal nerve fiber layer thickness declines with increasing neurologic impairment and correlates with disease duration. Furthermore, our data strongly support a role for ocular imaging techniques such as OCT in trials that examine neuroprotective and other disease-modifying therapies.

Although MRI is the technique of choice for assessing overall disease burden and atrophy in MS, imaging of RNFL thickness using OCT provides a unique opportunity to measure a central nervous system structure that consists of axons without myelin. Other important characteristics that make RNFL thickness an appealing candidate biomarker include (1) accessibility of the retina for imaging (reliable and feasible in many patients without pupillary dilation),[41] (2) ability to acquire and analyze images quickly and easily (~5 minutes per eye, may be performed by nonphysician personnel), (3) markedly reduced expense compared with MRI techniques that examine optic nerve morphology, and (4) capacity to correlate structure (RNFL thickness) with its corresponding function (vision) directly.

Data on the impact of MS and acute optic neuritis on RNFL thickness are beginning to emerge.[34,35] A small pilot study of patients with MS (n = 14) revealed reductions in overall average RNFL thickness in eyes with a history of acute optic neuritis and in contralateral MS eyes without an acute optic neuritis history.[34] Although average overall RNFL thickness for normal subjects was 111±11 μm, mean values were significantly lower for optic neuritis eyes (60±11 μm, history of acute optic neuritis ≥6 months before study) and for contralateral non–optic neuritis eyes of MS patients (83±10 μm); values for these eyes in our cohort were higher, perhaps due to a larger sample size and differences in selection criteria. In series of patients with a history of acute optic neuritis, decrements in RNFL thickness correlated with high-contrast VA, VF mean deviation, and color vision.[35] Future studies will examine the role of OCT in detecting subtle RNFL edema, establishing rates of decline in RNFL thickness, and detecting corticosteroid treatment response in patients with acute optic neuritis.

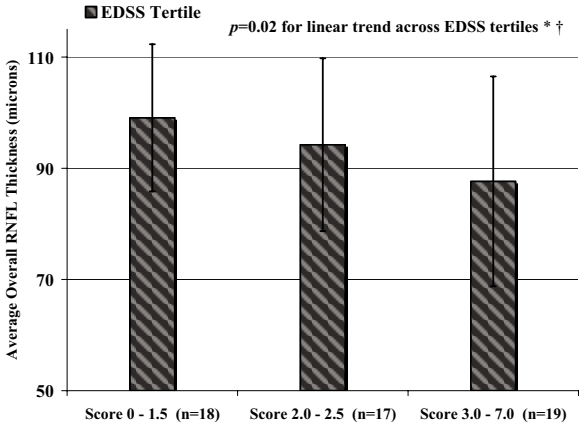

Figure 3. Mean values for average overall retinal nerve fiber layer (RNFL) thickness (360° around the optic disc) across categories (tertiles) for patients with multiple sclerosis who underwent neurologic testing with the Expanded Disability Status Scale (EDSS). Multiple sclerosis patients were divided into 3 approximately equal groups to define EDSS tertiles. *Retinal nerve fiber layer thickness decreased with increasing EDSS scores ($P = 0.02$ for linear trend, accounting for age and adjusting for within-patient intereye correlations), indicating greater degrees of axonal loss in the anterior visual pathways of patients with greater degrees of neurological impairment†Tertile ranges represent (1) minimal abnormalities on neurological examination with no disability (0–1.5), (2) minimal disability in 1 or 2 domains of function (2.0–2.5), and (3) moderate to severe disability (3.0–7.0). Expanded Disability Status Scale scores of 6.0, 6.5, and 7.0 are assigned if a patient requires unilateral assistance (cane), bilateral assistance (walker), or a wheelchair, respectively, for ambulation/mobility.

Analyses in our study demonstrated that fellow eyes of MS patients with a history of unilateral acute optic neuritis were no more likely to have RNFL axonal loss than were eyes of MS patients with no history of acute optic neuritis in either eye. At the same time, compared with disease-free control eyes, RNFL thickness was reduced both in fellow eyes of patients with unilateral optic neuritis and among MS non-ON eyes, supporting the occurrence of axonal loss in MS eyes even in the absence of attacks of acute optic neuritis. Clinical manifestations of MS are caused not only by the effects of acute demyelination on otherwise normal axons, but also by axonal loss (both primary and by Wallerian degeneration), which is now known to occur within the visual pathways and in other areas of the central nervous system.[28] Results of this study support previous observations that many MS patients with no history of acute visual loss (painful or otherwise) complain that vision in one or both eyes is not normal and have evidence of unilateral or bilateral optic nerve dysfunction by clinical or electrophysiologic testing.[5–10,15,16]

Analogous to the RNFL data, scores for visual function tests were reduced most markedly among eyes with a history of acute optic neuritis (Table 2), but also did not differ significantly between fellow eyes of patients with a history of unilateral optic neuritis and eyes of MS patients without a history of optic neuritis in either eye. Patterns of RNFL thickness seen in our investigation are supported by a recently published study of acute optic neuritis and fellow eyes (n = 25 patients).[35] In that investigation, RNFL thickness in fellow eyes was lower than but not significantly different from that in control eyes (94 vs. 103 μm; P = 0.09, 2-sample t test), whereas optic neuritis eyes demonstrated marked reductions in RNFL thickness versus controls (69 μm, $P<0.001$). Multiple sclerosis patients without a history of acute optic neuritis, however, were not included in the study cohort.

In our MS cohort, worse visual function scores were associated with reduced RNFL thickness. A 1-line decline in vision score corresponded to a 4-μm reduction in average overall RNFL thickness (Table 3). Although visual function scores were significant predictors of RNFL thickness, accounting for age, correlations were modest in magnitude. This suggests that clinical tests of low-contrast letter acuity and contrast sensitivity capture visual dysfunction that occurs in the absence of or perhaps in advance of axonal loss. Reduction of visual function test scores without RNFL loss may also reflect MS disease in optic radiations and occipital lobes; lesions in these areas affect function in both eyes and do not produce reductions in RNFL thickness. Low-contrast letter acuity and contrast sensitivity are clinical outcomes that detect visual pathway dysfunction, perhaps in advance of irreversible neuronal/axonal degeneration when the potential for treatment response is greatest.

Retinal nerve fiber layer thickness is considered to be a promising surrogate marker for optic nerve damage in glaucoma, a disorder that is, in part, defined by the presence of axonal loss.[50] However, because axonal degeneration and clinical impairment in MS are not limited to the anterior visual pathways, RNFL thickness has not been proposed as a surrogate marker for disease in MS but represents an attractive biomarker for observing patients with acute and subclinical anterior visual pathway involvement.[51]

Although the relation of age to RNFL thickness remains somewhat controversial, effects of normal aging on overall RNFL thickness as measured by OCT were demonstrated in a recent study.[52] Among 144 normal subjects (144 eyes), ranging in age from 16 to 84 years (mean, 46±18), the following distribution of overall average RNFL thickness was noted: 128±11 μm (age $\leq$ 30 years), 127±11 μm (31–50 years), 120±10 μm (51–70 years), and 114±9 μm (>70 years). These results indicate an estimated decline in RNFL thickness of 0.17% per year, and are consistent with histologic studies demonstrating 0.5% per year declines in human optic nerve fiber counts.[28] Given the potential effects of normal aging on RNFL thickness values, all statistical models in our investigation included participant age as a covariate.

Most patients in therapeutic trials for MS and optic neuritis will be 50 years or younger and, thus, within a range in which the effects of age on RNFL are only slight with regard to absolute differences (see above discussion). Normative reference values based on age have been incorporated into the OCT 4.0 software.[49] This normative database has been approved by the Food and Drug Administration for determining age-based reference values for RNFL thickness, and is represented by green zones on the OCT printout. However, this normative database considers the fifth percentile for age to be the cutoff for abnormal values. In our cohort of MS patients, 40 of 180 eyes (22%) had average overall RNFL thickness values that were lower than the fifth percentile for age. As a result, this investigation and others have included disease-free control subjects to provide additional normative data.[34,41,53]

Although changes in ocular media, such as cataracts or placement of contact lenses (should be removed for OCT imaging), may affect the quality of OCT scans, refractive error itself (SE) did not correlate significantly with RNFL thickness in recent investigations (r = 0.09, P = 0.28).[29,52] Average macular thickness by OCT did not vary with degree of myopia in another recent study,[54] and adding SE as a covariate in our statistical models did not affect the relation of RNFL thickness to visual function.

Among imaging modalities, OCT is comparable to both scanning laser polarimetry (GDx with variable corneal compensation, Carl Zeiss Meditec) and confocal scanning laser ophthalmoscopy (Heidelberg Retina Tomograph II, Heidelberg Engineering GmbH, Heidelberg, Germany) with respect to its capacity to discriminate between healthy eyes and eyes with glaucomatous VF loss.[31] Although comparable for detecting glaucomatous damage, some data suggest that OCT may prove to be the preferred RNFL imaging method in MS. The Heidelberg Retina Tomograph II has a slower acquisition time and provides only an indirect measurement of the RNFL.[29] GDx may be less sensitive for detecting regional RNFL loss in the nasal and temporal quadrants.[55] This differential detection ability may be relevant in MS, particularly if longitudinal studies of acute optic neuritis demonstrate anatomic patterns of RNFL loss. Further studies are underway to examine the role of variable

corneal compensation in GDx techniques for ensuring uniform detection of RNFL losses.

Data from previous cross-sectional and longitudinal studies demonstrate that low-contrast letter acuity (Sloan charts) and contrast sensitivity (Pelli–Robson charts) are vision tests that best distinguish MS patients from disease-free controls and, thus, best capture MS-related visual dysfunction. The potential to demonstrate clinical changes over time was shown for Pelli–Robson charts in the Optic Neuritis Treatment Trial and in ongoing longitudinal analyses of the MS Vision Prospective Cohort Study for Sloan charts (Balcer, unpublished data). Sloan charts have also been incorporated as secondary outcomes in several recent MS clinical trials. Although Sloan chart and Pelli–Robson scores correlate with global measures of brain atrophy, lesion volume, and magnetization transfer ratio (Neurology 64[suppl 1]:A35–6, 2005), the relation shown herein with RNFL thickness is of greater magnitude and is consistent with a major contribution of anterior visual pathway disease to MS-related visual dysfunction. Ongoing longitudinal studies of OCT in MS and optic neuritis cohorts, and incorporation of ocular imaging as secondary outcomes in clinical trials, will further examine patterns of axonal degeneration and visual loss over time and will establish the role for OCT and other ocular imaging modalities as structural biomarkers.

References

1. McDonald WI, Barnes D. The ocular manifestations of multiple sclerosis. 1. Abnormalities of the afferent visual system. J Neurol Neurosurg Psychiatry 1992;55:747–52.
2. Warner J, Lessell S. Neuro-ophthalmology of multiple sclerosis. Clin Neurosci 1994;2:180–8.
3. Leibowitz U, Alter M. Optic nerve involvement and diplopia as initial manifestations of multiple sclerosis. Acta Neurol Scand 1968;44:70–80.
4. Sørensen TL, Frederiksen JL, Brønnum-Hansen H, Petersen HC. Optic neuritis as onset manifestation of multiple sclerosis: a nationwide, long-term survey. Neurology 1999;53:473–8.
5. Regan D, Silver R, Murray TJ. Visual acuity and contrast sensitivity in multiple sclerosis—hidden visual loss: an auxiliary diagnostic test. Brain 1977;100:563–79.
6. Balcer LJ. Multiple sclerosis and related demyelinating diseases. In: Miller NR, Newman NJ, Biousse V, Kerrison JB, eds. Walsh and Hoyt's Clinical Neuro-ophthalmology. Vol. 3. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2004: 3429–525.
7. Cole SR, Beck RW, Moke PS, et al, Optic Neuritis Study Group. The National Eye Institute Visual Function Questionnaire: experience of the ONTT. Invest Ophthalmol Vis Sci 2000;41:1017–21.
8. Kupersmith MJ, Nelson JI, Seiple WH, et al. The 20/20 eye in multiple sclerosis. Neurology 1983;33:1015–20.
9. Balcer LJ, Baier ML, Kunkle AM, et al. Self-reported visual dysfunction in multiple sclerosis: results from the 25-Item National Eye Institute Visual Function Questionnaire (VFQ-25). Mult Scler 2000;6:382–5.
10. Balcer LJ, Baier ML, Pelak VS, et al. New low-contrast vision charts: reliability and test characteristics in patients with multiple sclerosis. Mult Scler 2000;6:163–71.
11. Cutter GR, Baier ML, Rudick RA, et al. Development of a multiple sclerosis functional composite as a clinical trial outcome measure. Brain 1999;122:871–82.
12. Rudick RA, Cutter G, Reingold S. The Multiple Sclerosis Functional Composite: a new clinical outcome measure for multiple sclerosis clinical trials. Mult Scler 2002;8:359–65.
13. Rudick RA, Antel J, Confavreux C, et al. Recommendations from the National Multiple Sclerosis Society Clinical Outcomes Assessment Task Force. Ann Neurol 1997;42:379–82.
14. Cohen J, Cutter G, Lublin F, Schwid S. The MS Co-operative Research (MS-CORE) Group: an alternate approach to fostering multicenter studies [letter]. Mult Scler 2004;10:332–3.
15. Balcer LJ, Baier ML, Cohen JA, et al. Contrast letter acuity as a visual component for the Multiple Sclerosis Functional Composite. Neurology 2003;61:1367–73.
16. Baier ML, Cutter GR, Rudick RA, et al. Low-contrast letter acuity testing captures visual dysfunction in patients with multiple sclerosis. Neurology 2005;64:992–5.
17. Rubin GS. Reliability and sensitivity of clinical contrast sensitivity tests. Clin Vis Sci 1988;2:169–77.
18. Sisto D, Trojano M, Vetrugno M, et al. Subclinical visual involvement in multiple sclerosis: a study by MRI, VEPs, frequency-doubling perimetry, standard perimetry, and contrast sensitivity. Invest Ophthalmol Vis Sci 2005;46: 1264–8.
19. Weinstock-Guttman B, Baier M, Stockton R, et al. Pattern reversal visual evoked potentials as a measure of visual pathway pathology in multiple sclerosis. Mult Scler 2003;9:529–34.
20. Trobe JD, Beck RW, Moke PS, Cleary PA. Contrast sensitivity and other vision tests in the Optic Neuritis Treatment Trial. Am J Ophthalmol 1996;121:547–53.
21. Beck RW, Cleary PA, Anderson MM Jr, et al, Optic Neuritis Study Group. A randomized, controlled trial of corticosteroids in the treatment of acute optic neuritis. N Engl J Med 1992; 326:581–80.
22. Beck RW, Kupersmith MJ, Cleary PA, Katz B. Fellow eye abnormalities in acute unilateral optic neuritis: experience of the Optic Neuritis Treatment Trial. Ophthalmology 1993;100: 691–7, discussion 697–8.
23. Optic Neuritis Study Group. Visual function more than 10 years after optic neuritis: experience of the Optic Neuritis Treatment Trial. Am J Ophthalmol 2004;137:77–83.
24. Optic Neuritis Study Group. Visual function 5 years after optic neuritis: experience of the Optic Neuritis Treatment Trial. Arch Ophthalmol 1997;115:1545–52.
25. Rubin GS, Muñoz B, Bandeen-Roche K, West SK, SEE Project Team. Monocular versus binocular visual acuity as measures of vision impairment and predictors of visual disability. Invest Ophthalmol Vis Sci 2000;41:3327–34.
26. Newman NJ, Wolfe JM, Stewart MI, Lessel S. Binocular visual function in patients with a history of monocular optic neuritis. Clin Vis Sci 1991;6:95–107.
27. Pardhan S. Binocular performance in patients with unilateral cataract using the Regan test: binocular summation and inhibition with low-contrast charts. Eye 1993;7:59–62.
28. Evangelou N, Konz D, Esiri MM, et al. Size-selective neuronal changes in the anterior optic pathways suggest a differential susceptibility to injury in multiple sclerosis. Brain 2001; 124:1813–20.
29. Jaffe GJ, Caprioli J. Optical coherence tomography to detect and manage retinal disease and glaucoma. Am J Ophthalmol 2004;137:156–69.
30. Kanamori A, Nakamura M, Escano MF, et al. Evaluation of the glaucomatous damage on retinal nerve fiber layer thickness measured by optical coherence tomography. Am J Ophthalmol 2003;135:513–20.
31. Medeiros FA, Zangwill LM, Bowd C, Weinreb RN. Comparison of the GDx VCC scanning laser polarimeter, HRT II confocal scanning laser ophthalmoscope, and Stratus OCT

optical coherence tomography for the detection of glaucoma. Arch Ophthalmol 2004;122:827–37.
32. Medeiros FA, Moura FC, Vessani RM, Susanna R Jr. Axonal loss after traumatic optic neuropathy documented by optical coherence tomography. Am J Ophthalmol 2003;135:406–8.
33. Monteiro ML, Leal BC, Rosa AA, Bronstein MD. Optical coherence tomography analysis of axonal loss in band atrophy of the optic nerve. Br J Ophthalmol 2004;88:896–9.
34. Parisi V, Manni G, Spadaro M, et al. Correlation between morphological and functional retinal impairment in multiple sclerosis patients. Invest Ophthalmol Vis Sci 1999;40:2520–7.
35. Trip SA, Schlottmann PG, Jones SJ, et al. Retinal nerve fiber layer axonal loss and visual dysfunction in optic neuritis. Ann Neurol 2005;58:383–91.
36. Bowd C, Zangwill LM, Berry CC, et al. Detecting early glaucoma by assessment of retinal nerve fiber layer thickness and visual function. Invest Ophthalmol Vis Sci 2001;42:1993–2003.
37. Wollstein G, Schuman JS, Price LL, et al. Optical coherence tomography (OCT) macular and peripapillary retinal nerve fiber layer measurements and automated visual fields. Am J Ophthalmol 2004;138:218–25.
38. Schuman JS, Hee MR, Puliafito CA, et al. Quantification of nerve fiber layer thickness in normal and glaucomatous eyes using optical coherence tomography. Arch Ophthalmol 1995;113:568–96.
39. Sanchez-Galeana CA, Bowd C, Zangwill LM, et al. Short-wavelength automated perimetry results are correlated with optic coherence tomography retinal nerve fiber layer thickness measurements in glaucomatous eyes. Ophthalmology 2004;11:1866–72.
40. Pieroth L, Schuman JS, Hertzmark, E, et al. Evaluation of focal defects of the nerve fiber layer using optical coherence tomography. Ophthalmology 1999;106:570–9.
41. Paunescu LA, Schuman JS, Price LL, et al. Reproducibility of nerve fiber thickness, macular thickness, and optic nerve head measurements using StratusOCT. Invest Ophthalmol Vis Sci 2004;45:1716–24.
42. McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the Diagnosis of Multiple Sclerosis. Ann Neurol 2001;50:121–7.
43. Lynch DR, Farmer JM, Rochestie D, Balcer LJ. Contrast letter acuity as a measure of visual dysfunction in patients with Friedreich ataxia. J Neuroophthalmol 2002;22:270–4.
44. Pelli DG, Robson JG, Wilkins AJ. The design of a new letter chart for measuring contrast sensitivity. Clin Vis Sci 1988;2:187–99.
45. Bailey IL, Lovie JE. New design principles for visual acuity letter charts. Am J Optom Physiol Opt 1976;53:740–5.
46. Ferris FL III, Kassoff A, Bresnick GH, Bailey I. New visual acuity charts for clinical research. Am J Ophthalmol 1982;94:91–6.
47. Bodis-Wollner I, Diamond SP. The measurement of spatial contrast sensitivity in cases of blurred vision associated with cerebral lesions. Brain 1976;99:695–710.
48. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983;33:1444–52.
49. Patella VM. *STRATUSOCT*: Establishment of normative reference values for retinal nerve fiber layer thickness measurements. Available at: http://www.meditec.zeiss.com/C125679E00525939/EmbedTitelIntern/Stratus OCT ndb paper/$File/czm ndb paper.pdf. Accessed November 1, 2004.
50. Leung CK, Chan WM, Yung WH, et al. Comparison of macular and peripapillary measurements for the detection of glaucoma. Ophthalmology 2005;112:391–400.
51. Katz R. Biomarkers and surrogate markers: an FDA perspective. NeuroRx 2004;1:189–95.
52. Kanamori A, Escano MF, Eno A, et al. Evaluation of the effect of aging on retinal nerve fiber layer thickness measured by optical coherence tomography. Ophthalmologica 2003;217:273–8.
53. Varma R, Bazzaz S, Lai M. Optical tomography-measured retinal nerve fiber layer thickness in normal Latinos. Invest Ophthalmol Vis Sci 2003;44:3369–73.
54. Lim MC, Hoh ST, Foster PJ, et al. Use of optical coherence tomography to assess variations in macular retinal thickness in myopia. Invest Ophthalmol Vis Sci 2005;46:974–8.
55. Monteiro ML, Medeiros FA, Ostroscki MR. Quantitative analysis of axonal loss in band atrophy of the optic nerve using scanning laser polarimetry. Br J Ophthalmol 2003;87:32–7.