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Longitudinal Study of Vision and Retinal Nerve Fiber Layer Thickness in MS

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Abstract

Objective—Cross-sectional studies of optical coherence tomography (OCT) show that retinal nerve fiber layer (RNFL) thickness is reduced in multiple sclerosis (MS) and correlates with visual function. We determined how longitudinal changes in RNFL thickness relate to visual loss. We also examined patterns of RNFL thinning over time in MS eyes with and without a prior history of acute optic neuritis (ON).

Methods—Patients underwent OCT measurement of RNFL thickness at baseline and at 6-month intervals during a mean follow-up of 18 months at three centers. Low-contrast letter acuity (2.5%, 1.25% contrast) and visual acuity (VA) were assessed.

Results—Among 299 patients (593 eyes) with ≥6 months follow-up, eyes with visual loss showed greater RNFL thinning compared to eyes with stable vision (low-contrast acuity, 2.5%: $p < 0.001$; VA: $p = 0.005$). RNFL thinning increased over time, with average losses of 2.9 μm at 2–3 years and 6.1 μm at 3–4.5 years ($p < 0.001$ vs. 0.5–1-year follow-up interval). These patterns were observed for eyes with or without prior history of ON. Proportions of eyes with RNFL loss greater than test-retest variability ($\geq 6.6 \mu\text{m}$) increased from 11% at 0–1 year to 44% at 3–4.5 years ($p < 0.001$).

Interpretation—Progressive RNFL thinning occurs as a function of time in some patients with MS, even in the absence of ON, and is associated with clinically significant visual loss. These findings are consistent with sub-clinical axonal loss in the anterior visual pathway in MS and support the use of OCT and low-contrast acuity as methods to evaluate the effectiveness of putative neuroprotection protocols.

Visual dysfunction is a common cause of disability and reduced quality of life in multiple sclerosis (MS).¹ The anterior visual pathway is a frequent site for inflammation and demyelination, and axonal degeneration is likely to be a final common pathway to permanent visual loss.²⁻⁵ Recognized by MS experts as a critical dimension for outcomes assessment,⁶ vision has been an important area of investigation. The findings of many studies have supported low-contrast letter acuity as a candidate clinical trial outcome measure. It can capture subtle visual impairment, treatment effects, MRI lesion burden, prolonged visual evoked potential latencies, and quality of life.^{1,7-13} Many ongoing MS trials have incorporated low-contrast acuity as a tertiary outcome.

The emergence of optical coherence tomography (OCT) in MS has brought the anterior visual pathway to the forefront as a model for measuring therapeutic efficacy, particularly for trials involving neuroprotection.¹⁴⁻³² A reliable marker for axonal loss in MS,²⁴ retinal nerve fiber layer (RNFL) thickness by OCT is reduced even in the absence of past episodes of acute optic neuritis (ON).^{15,18-31} While cross-sectional studies have shown strong correlations between RNFL thickness and visual function, disease duration, MS sub-type, and MRI abnormalities, the relation of visual loss to RNFL thinning over time in MS has not been established.¹³⁻³¹

One of the most important characteristics for evaluating outcome measures is the capacity to detect change over time.^{6,32} In this longitudinal study, we determined how change in RNFL thickness relates to visual loss in an MS cohort. We also examined patterns of RNFL thinning that occur over time in MS eyes either with or without prior episodes of ON.

Subjects and Methods

Subjects

Participants were enrolled in an ongoing prospective study of visual outcome measures in MS at the University of Pennsylvania, University of Texas Southwestern Medical Center at Dallas, and Johns Hopkins University. Subjects represented a convenience sample of patients willing to undergo OCT imaging and vision testing for research purposes, and were not selected based on clinical features or extent of symptoms. Following their initial baseline visit, patients were invited to follow-up at six to 12-month intervals. Patients with ≥ 6 months' follow-up were included in these analyses. The present cohort includes $>1,000$ patients with baseline visits, and therefore does not represent substantial overlap with previously published reports of OCT in MS.¹⁸ MS was diagnosed by standard criteria.³³ Patients who had not yet followed-up at the time of these analyses were those who 1) had not been in the study for at least 6 months, 2) were unable to follow-up due to relocation or transportation issues, or 3) chose not to follow-up in the study. Patients with clinically-isolated syndromes were required to fulfill MRI and clinical criteria of dissemination in time and space for diagnosis of MS. Types of disease-modifying therapies were also recorded at study visits.

Patients with comorbid ocular conditions not related to MS were excluded by history, chart review, and examination. A past history (months to years prior to enrollment) of acute optic neuritis (ON) was determined by self- and physician report and confirmed by record review. Eyes with pathologic hyperopia or myopia (spherical correction $\geq +10$ diopters or ≤ -10 diopters) were not included in this study. Patients with an acute attack of ON in either eye that was ongoing or had occurred within three months prior to testing were not included in this study in order to minimize potential optic disc swelling (and RNFL edema) associated with acute ON. Optic disc swelling was not noted among any participants. Eyes that developed acute ON during follow-up or within 3 months before the baseline visit ($n=10$

eyes of 5 patients) were excluded since the primary aim was to examine visual loss and RNFL thinning in MS that occurs outside of the acute phase of ON.

Disease-free control participants were recruited from among staff and family of patients and had no history of ocular or neurologic disease. Control eyes were excluded if best corrected high-contrast visual acuities (Snellen equivalents based on Early Treatment Diabetic Retinopathy [ETDRS] charts) were worse than 20/20 (minimum letter score 70). Institutional Review Board (IRB) approval was obtained for all study protocols, and each subject provided written informed consent. The study was conducted in accordance with HIPAA guidelines.

Optical Coherence Tomography

Optical coherence tomography (OCT) was performed for both eyes using OCT-3, version 4.0 software (Carl Zeiss Meditec, Inc., Dublin, CA). Fast retinal nerve fiber layer (RNFL) thickness protocols were used. OCT was performed by trained technicians following visual function testing. Scans were performed without flash photography to optimize patient comfort. If the pupils were large enough to permit adequate imaging (≥ 5 mm), scanning was completed without the use of mydriatic drops. Dilation has little impact on OCT values and reproducibility, and previous studies of MS patients have been performed without uniform use of mydriatics.^{18,25} Pupils were dilated with 1% tropicamide when necessary. Internal fixation was used, and a patch was placed over the non-tested eye. Good quality scans were defined according to specifications in the OCT-3 User Manual; criteria included signal strength ≥ 7 (maximum 10), centering of the scan, and uniform brightness. This is important since RNFL thickness measurements may vary by signal strength.³⁴ Values for average RNFL thickness (average for 360° around the optic disc) were recorded.

Visual Function Testing

Low-contrast letter acuity testing was performed for each eye using retroilluminated low-contrast Sloan letter charts (2.5% and 1.25% contrast levels at 2 meters, Precision Vision, LaSalle, IL).⁷⁻¹¹ High-contrast visual acuity was assessed using retroilluminated ETDRS charts at 3.2 meters. Low-contrast Sloan letter and ETDRS charts have a similar standardized format with 5 letters per line. Numbers of letters identified (maximum 70/chart) were recorded for each eye. This scoring method provides continuous scale that is equivalent to logMAR (log minimal angle of resolution) yet uses units that are more familiar to neurologists and which have been used in recent MS trials.¹¹ Testing was performed by trained technicians experienced in research examinations. Standardized protocols, including written scripts and instructions, were followed. Snellen acuity equivalents were determined based on scores for ETDRS charts. Participants underwent detailed refractions in order to determine correlations with OCT that reflected best corrected vision.

Visual loss over time was summarized by determining whether changes exceeded the amount that would be expected from repeated testing when there was no real change.³⁵ For high-contrast visual acuity (VA), 2-line, or 10-letter, differences have been used traditionally as criteria for clinically meaningful change, based on studies of test-retest variability.³⁵ However, recent studies have demonstrated that, in patients with relatively good visual acuity, 5-letter or 1-line changes in high-contrast VA are unlikely to be due to testing error.³⁶ Examination of our inter-rater reliability study data has revealed that 5 letters represents two standard deviations of inter-rater difference for VA in patients with MS. Correspondingly, two standard deviations of inter-rater difference for low-contrast acuity were equal to 7 letters.⁷ These values were used as criteria for visual loss in the present study. Since eyes with scores of < 5 letters (for VA) or < 7 letters (for low-contrast acuity) at the baseline visit could not have visual loss during follow-up based on the above definitions,

these eyes were not included in analyses comparing degrees of RNFL thinning or proportions of eyes with visual loss.

Statistical Analysis

Statistical analyses were performed using Stata 10.0 software. Generalized estimating equation (GEE) models, accounting for age and adjusting for within-patient, inter-eye correlations, were used to determine the relation between follow-up period and change in RNFL thickness from baseline. GEE regression models account not only for the effects of age and other covariates on the outcome, but also for within-patient, inter-eye correlation in outcomes. This is necessary since both eyes were included in the analyses for patients in this study. Age was included in the models since this variable may be associated with visual function and RNFL thickness; minimal RNFL thinning (0.16 $\mu\text{m}/\text{year}$) has been reported among normal subjects.^{37,38}

Changes in RNFL thickness from baseline were determined for eyes of patients who had received at least one follow-up visit at the time of analysis. Each one-year follow-up interval was plotted against change in RNFL, with each eye represented only once in the model. For patients with multiple follow-up visits, the change from baseline for the last follow-up visit was used for analyses. Logistic regression models, accounting for age and adjusting for within-patient, inter-eye correlations, were used to assess the association of RNFL thinning with losses of high- and low-contrast acuity (defined by criteria above). Models also examined potential effects of disease duration and disease-modifying therapies on the relation of RNFL thinning to visual loss and length of follow-up. Exploratory analyses examined the relation of treatment type and status to RNFL thinning and visual loss. In terms of univariate analyses, Pearson linear correlation coefficients were calculated. To examine whether eyes with longer follow-up were more likely to have visual or RNFL loss beyond levels expected based on test-retest variability, the chi-square test for trend in binomial proportions was used. For these analyses, eyes were categorized by 1-year follow-up intervals based on last follow-up visit. Type I error for significance was $p < 0.05$.

Results

Characteristics of Baseline and Follow-Up Cohorts

Data for the collaborative study cohort, including 1,005 patients with MS (2,010 eyes) with a baseline or first visit, are shown in Table 1. Patients with at least one follow-up visit and follow-up time of 6 months or greater ($n=593$ eyes of 299 patients) did not differ from the baseline cohort with regard to age, gender mix, or MS sub-type. The follow-up cohort had a slightly greater proportion of eyes with a history of acute optic neuritis (ON) prior to study enrollment. Mean follow-up was 18 months, and ranged from 6 months to 4.5 years for analyses of the follow-up cohort. Eighty-seven percent of patients ($n=518$ eyes) were on disease-modifying therapy at the time of the last follow-up visit. Baseline RNFL thickness and visual function scores were similar between the baseline and follow-up cohorts ($p=0.23$, t-test).

Visual Loss and RNFL Thinning

Based on changes from baseline of ≥ 5 letters for high-contrast VA or ≥ 7 letters for low-contrast acuity, the following percentages of MS eyes had visual loss: high-contrast VA: 12% of eyes (12% of patients); low-contrast acuity, 2.5%: 12% of eyes (13% of patients); low-contrast acuity, 1.25%: 13% of eyes (14% of patients). By our protocol, we excluded patients who had acute ON during the observation period of the study. For eyes with a history of ON prior to study enrollment, percentages of eyes with visual loss were 12% for VA, 12% for low-contrast acuity at 2.5%, and 15% for low-contrast acuity at 1.25%.

MS eyes with visual loss had greater degrees of RNFL thinning during follow-up compared to MS eyes without visual loss (Fig 1). Clinical characteristics were similar between MS eyes with vs. without low-contrast letter acuity loss (2.5% level) during follow-up (Table 2). Mean follow-up and disease duration were greater for eyes with visual loss by low-contrast acuity at 2.5% (Table 2), but accounting for these variables in logistic regression models showed that the association between visual loss and RNFL thinning remained significant ($p=0.004$ accounting for follow-up, $p=0.03$ accounting for disease duration). Importantly, eyes with visual loss during follow-up in the study did not differ with respect to baseline average RNFL thickness from eyes without visual loss (mean 91-92 μm at baseline for VA and both low-contrast levels). Proportions of eyes with visual loss by low- and high-contrast acuity increased with greater length of follow-up; this pattern was most evident for low-contrast letter acuity at the 2.5% level (Table 3). Treatment status (presence of any immunomodulatory therapy) did not predict which eyes developed visual loss ($p=0.26$ -0.86, logistic regression, accounting for age), and did not affect the relation between visual loss and RNFL thinning. Accordingly, exploratory analyses did not suggest associations of specific therapies with different patterns of RNFL thinning in our cohort.

Of interest, longitudinal loss of low-contrast acuity on the 1.25% chart was not associated with greater degrees of RNFL thinning compared to eyes without visual loss at this contrast level (Fig 1C). Percentages of eyes with low or zero scores at baseline were substantially greater for 1.25% low-contrast (23% with baseline scores <7 letters) than for low-contrast acuity at 2.5% (6%) and VA ($<1\%$ with baseline scores <5 letters). These floor effects (low or zero scores at baseline) likely limit the capacity of the 1.25% chart to capture changes in acuity.

Longitudinal Analyses of RNFL Thinning

Among eyes with MS in this longitudinal study, RNFL thinning from baseline increased as a function of time. Graphs in Fig 2 show mean changes from baseline in RNFL thickness by length of follow-up for MS eyes. The triangles represent mean changes in RNFL thickness from baseline for each follow-up interval, and the vertical lines are 95% confidence intervals for the means. Data for all MS eyes in the follow-up cohort are shown in Fig 2A; each eye is represented only once in the graph, with the follow-up interval for that eye determined by the most recent study visit. Accounting for age and adjusting for within-patient, inter-eye correlations, longer length of follow-up was associated with greater degrees of RNFL thinning from baseline ($p<0.001$ for comparisons of ≥ 2 -3-year and ≥ 3 -year follow-up intervals with 0.5-1-year follow-up interval, GEE models, Fig 2A). Eyes with no history of acute ON prior to study enrollment ($n=381$) demonstrated strikingly similar patterns of increasing RNFL thinning over time (Fig 2B). This pattern was also present yet not as well-defined in eyes with a history of ON ($n=208$, Fig 2C), perhaps consistent with the fact that these eyes had already had substantial thinning of the RNFL prior to study enrollment (baseline RNFL thickness $83 \pm 18 \mu\text{m}$ for ON eyes vs. $96 \pm 13 \mu\text{m}$ for MS non-ON eyes). History of ON could not be determined for four eyes in the follow-up cohort. Percent decreases in RNFL thickness compared to baseline in our cohort were 0.4% ($0.40 \mu\text{m}$ [95% CI 1.16, -0.35]) for eyes with 0.5-1-years' follow-up; 1.7% ($1.6 \mu\text{m}$ [95% CI 2.47, 0.70]) for >1 -2 years; 3.2% ($2.9 \mu\text{m}$ [95% CI 4.02, 1.86]) for >2 -3 years; and 6.7% ($6.1 \mu\text{m}$ [95% CI 7.73, 4.41]) for >3 years' follow-up. Average RNFL thinning for disease-free control eyes ($n=119$) was 0.5% ($0.49 \mu\text{m}$ [95% CI 1.36, -0.39]) over 3-year period.

When follow-up time was examined as a continuous variable rather than as discrete time intervals for all MS eyes, longer length of follow-up correlated with greater degrees of RNFL thinning ($p<0.001$ for all MS and MS non-ON eyes, $p=0.005$ for MS ON eyes, GEE models, accounting for age and adjusting for within-patient, inter-eye correlations). The scatter plot and regression line represent group data for degree of RNFL thinning observed

between baseline and the last follow-up visit, and are not meant to define patterns of RNFL thinning over time for individual eyes. Nonetheless, Fig 3 illustrates that there are very few outliers with respect to RNFL thinning in the follow-up cohort for this study.

For the cohort and length of time examined (range 0.5 to 4.5 years), each 1 year of follow-up was associated, on average, with 2.0 μm decreases in RNFL thickness ($p<0.001$). This relation was similar for MS ON eyes (1.4 μm , $p=0.005$) and MS non-ON eyes (2.4 μm , $p<0.001$, GEE models). Thinning was also observed as a function of follow-up time for each of the four retinal quadrants. Percent decreases in RNFL thickness from baseline over the entire follow-up period were 3.1% (95% CI 4.3, 2.0) for the temporal quadrant, 1.9% (95% CI 2.7, 1.0) for the superior quadrant, 2.5% (95% CI 3.8, 1.2) for the nasal quadrant, and 1.0% (95% CI 1.7, 0.2) for the inferior quadrant.

To the extent that disease duration correlates with RNFL thickness and could therefore influence patterns of RNFL thinning over time, we performed additional analyses accounting for this variable. In models accounting for age and disease duration, associations of RNFL thinning with greater length of follow-up remained significant for all MS eyes ($p=0.002$, GEE models with follow-up as a continuous variable). While disease duration did not impact the relation between RNFL thinning and follow-up time among MS non-ON eyes ($p<0.001$, accounting for age and disease duration), adding disease duration to the models for MS ON eyes greatly influenced the association of RNFL thinning with follow-up ($p=0.005$ for models accounting for age; $p=0.45$ for models accounting for age and disease duration), suggesting that perhaps time since the episode of acute ON is important in determining RNFL thinning. In contrast, adding treatment status (presence of any immunomodulatory therapy at last follow-up) or treatment type to the models did not affect the relation between RNFL thinning and length of follow-up ($p<0.001$, accounting for age and treatment). Eyes of patients treated with disease-modifying therapy vs. non-treated eyes also did not differ with respect to RNFL thinning from baseline ($p=0.40$, accounting for age). Non-treated eyes had thinner RNFL at baseline (87 μm vs. 92 μm), but the difference was not significant.

For patients with available scores at baseline and at last follow-up for the EDSS (156 patients) and MS Functional Composite components (MSFC, 83 patients), exploratory analyses were performed to examine the relation between changes in RNFL thickness and changes in EDSS and MSFC. Component scores for the MSFC included the timed 25-foot walk, 9-hole peg test, and Paced Auditory Serial Addition Test with 3-second inter-stimulus interval (PASAT3). Accounting for age, RNFL thinning was associated with changes in the EDSS ($p=0.04$, GEE models) and 9-hole peg test for the non-dominant hand ($p<0.001$).

To provide additional perspective on whether observed changes in RNFL thickness over time are beyond those that would be expected based on values for test-retest variability for the OCT-3 instrument, we calculated proportions of MS eyes in each follow-up interval that had thinning of the RNFL of 6.6 μm or greater. This is an established value for OCT-3 average RNFL thickness³⁹ based on between-session variability in glaucomatous eyes (similar values have not yet been determined for MS eyes). Proportions of eyes with RNFL loss greater than 6.6 μm increased with greater length of follow-up, ranging from 11% for eyes with 0.5-1 years' follow-up to 44% at 3-4.5 years ($p<0.001$, chi-square test for trend in binomial proportions, Table 3). For the follow-up cohort overall ($n=593$ eyes), 18% lost ≥ 6.6 μm . MS ON ($n=208$ eyes) and non-ON eyes ($n=381$) did not differ with regard to these proportions (21% vs. 17%, $p=0.25$, logistic regression models accounting for age and within-patient, inter-eye correlations). Given the potential importance of detecting changes in proportions of eyes with clinically-significant RNFL thinning in future MS clinical trials

that explore this measure, we performed preliminary sample size calculations. These findings are presented as Supplemental Data (available online).

Discussion

Results of this longitudinal study demonstrate that progressive RNFL thinning occurs as a function of time in MS, and is associated with clinically significant visual loss by low-contrast letter acuity and VA. There is a steady decline in RNFL thickness that increases over time in MS, even among eyes without a history of acute ON and even among patients on immunomodulatory therapy. It is noteworthy that more than 4 out of 5 patients were in the relapsing-remitting phase of MS at baseline in this study. These findings suggest that there is sub-clinical axonal loss in the anterior visual pathway in MS, and support OCT and low-contrast acuity as candidate measures for clinical trials, particularly those involving neuroprotection. The degrees to which sub-clinical demyelination and primary degeneration contribute to visual pathway axonal loss over time are uncertain.

Our study is important in demonstrating: 1) significant associations between visual loss and RNFL thinning over time in MS; and 2) progressive declines in RNFL thickness in MS eyes both with and without a prior history of acute ON. These data provide *in vivo* evidence for sub-clinical axonal loss in the anterior visual pathway and add a longitudinal component to established cross-sectional correlations of structure (RNFL thickness by OCT) and function (low- and high-contrast letter acuity).¹⁸ Our results further strengthen the role of the anterior visual pathway as a potential model for assessing treatment efficacy in MS, particularly for neuroprotective agents. While 87% of patients in our study were on disease-modifying treatments, substantial proportions of eyes had sub-clinical RNFL thinning and visual loss, and types of immunomodulatory therapy did not predict outcomes. These findings suggest a significant unmet therapeutic need in MS for treatments that target axonal loss. Numbers of patients not on therapy were relatively small in this study (n=75 eyes, 13%), and our investigation was not powered or designed to determine effects of treatment. While also exploratory, our analyses of RNFL thinning vs. EDSS and MSFC score changes suggest potential associations of RNFL axonal loss with more global measures of impairment. Further analyses of patient subgroups based on EDSS and MSFC will examine visual pathway axonal loss across the spectrum of disease severity. Several ongoing phase 3 MS trials have incorporated low-contrast letter acuity and RNFL thickness by OCT as tertiary outcomes. Data from these trials will provide important information on the performance of OCT measures and high- and low-contrast acuity, complementing the results seen in our study of a heterogeneous cohort.

While the patient mix of our convenience sample cohort was similar to the U.S. MS population with respect to age and gender, the tertiary care setting of our investigation may limit the generalizability of our findings. However, since MS clinical trials are often performed at academic centers, our cohort represents the population of patients who would qualify for enrollment in studies that include visual function tests and OCT as outcomes.

Our findings of progressive RNFL thinning by OCT, strongly suggestive of axonal loss, are analogous to MRI measures of brain atrophy in MS from both a qualitative and quantitative standpoint.^{32,40-45} Change in brain volume on serial MRI scans is recognized as a sensitive candidate measure for neuroprotection trials, and is one of the best-studied methods for quantitating neurodegeneration in MS.³² Brain atrophy occurs even in the absence of clinical manifestations in MS;⁴⁰ similarly, RNFL thinning in our MS cohort was seen even among eyes without a history of acute ON or of progressive visual loss. Brain atrophy occurs at a faster rate in MS than in healthy controls, with decreases in brain volume of 0.5-1.2% per year for patients, and 0.1-0.3% in healthy individuals.^{32,40-42} Average RNFL

thinning for disease-free controls in our study was 0.5% (0.49 μm) over a 3-year period. Patterns of increase in RNFL thinning noted in our investigation are similar to the yearly worsening of whole brain atrophy that has been observed in relapsing-remitting MS.⁴⁰ Average declines in brain parenchymal fraction (BPF) at 2 years in the placebo-controlled trial of interferon beta-1a were similar to our findings for >1-2 years' follow-up (~1-1.5% decreases in BPF). Cross-sectional studies have demonstrated varying degrees of correlation between brain atrophy and reductions in RNFL thickness by OCT.^{23,46} In one study of patients with relapsing-remitting MS, linear correlations between BPF and RNFL thickness were significant ($r = 0.69$, $p=0.001$, accounting for age).²³ RNFL thickness in MS eyes without a history of ON had significant but modest correlations with MRI measures of BPF ($r = 0.40$, $p=0.01$) and gray matter fraction ($r = 0.40$, $p=0.02$).⁴⁶ Ongoing longitudinal studies, including clinical trials, will further examine potential parallels between brain MRI parameters and OCT measures.

While whole brain volume and BPF include both gray and white matter elements, RNFL thickness measures only unmyelinated axons. OCT measures of macular volume and thickness, however, capture neuronal elements including the ganglion cell layer.³⁰ As demonstrated in a recent cross-sectional study, reduced total macular volumes (~34% neuronal cells by average thickness) in MS are associated with peripapillary RNFL thinning, with stronger correlations noted among eyes without a history of acute ON.³⁰ Although the present longitudinal analyses do not include macular volumes (data collection for this measure began much later than for RNFL thickness), continued follow-up of our study cohort and compilation of clinical trial data that include both OCT measures will allow us to determine how neuronal and axonal loss are associated over time in MS.

Brain MRI measures of atrophy demonstrate changes over time that are evident within 1-2 years' follow-up.^{32,40-45} Significant degrees of RNFL thinning were most notable in our study for the >2-3-year and >3-year follow-up intervals. To the extent that our investigation involves a heterogeneous cohort of non-uniformly-treated patients that were not selected for active disease (i.e., generally stable patients), it is likely that clinical trial data for RNFL thickness will demonstrate changes earlier than those observed in our cohort. Although the numbers of eyes decreased with greater length of follow-up, the proportions of eyes with RNFL thinning greater than that expected based on test-retest variability ($\geq 6.6 \mu\text{m}$) increased across follow-up intervals. This finding indicates that the mean changes in RNFL thickness from baseline are not being driven by only a few eyes with extreme RNFL thinning (outliers). The relation of RNFL thinning to follow-up time, demonstrated in Fig 3, also supports these observations. It should be emphasized that the scatter plot and regression line in Fig 3 represent group data for degree of RNFL thinning observed between baseline and the last follow-up visit, and are not meant to define patterns of RNFL thinning over time for individual eyes. Patterns of RNFL thinning in individual eyes may not, in fact, be linear throughout the course of follow-up.

The inter-rater and test-retest reliability of OCT for measuring RNFL thickness and other retinal structures may be enhanced by incorporation of high-resolution spectral domain OCT.^{14,47,48} Recent analyses comparing Cirrus high-resolution OCT with OCT-3 (current technology used in this study) found greater inter-rater reliability for Cirrus RNFL thickness measurements in patients with MS and disease-free controls.^{47,48} While not yet widely available, high-resolution OCT has the advantage of greater reproducibility due to reductions in the operator-dependency of scan placement. Resolution is also 5-7 μm , compared to 8-10 μm for OCT-3. However, unlike the current technology for which a single brand is available (OCT-3 by Zeiss), there are already several manufacturers of high-resolution OCT.¹⁴ Future trials will require continued expertise of established OCT reading

centers and careful consideration of differences between high-resolution and current technologies.

Patterns of RNFL thinning in our longitudinal study, which demonstrated increases in degree of thinning with greater length of follow-up, were strikingly similar between all MS eyes and MS eyes with no history of acute ON. This pattern was also present yet not as well-defined in eyes with a history of ON, perhaps consistent with the fact that these eyes had already had substantial thinning of the RNFL prior to study enrollment (baseline RNFL thickness $83 \pm 18 \mu\text{m}$ for ON eyes vs. $96 \pm 13 \mu\text{m}$ for MS non-ON eyes). The follow-up cohort in our study also had a greater proportion of eyes with a history of ON. Separate analyses of eyes with and without an ON history were performed, however, to help distinguish patterns of RNFL thinning in these groups. While patients were not selected for follow-up based on clinical features, those with visual symptoms may have had a greater tendency to volunteer for follow-up in this study (volunteer bias), potentially overestimating the rate of increase in RNFL thinning over time.

Collectively, these data provide evidence that sub-clinical axonal loss is occurring in the anterior visual pathways in MS even in the absence of acute ON. The relative contributions of sub-clinical demyelination and primary axonal degeneration to this process remain uncertain. These observations are consistent with cross-sectional data that have shown average RNFL thickness values for MS eyes without a history of acute ON to be abnormal (lower than those for disease-free controls, yet greater than those of eyes with a history of acute ON).¹⁴⁻²² In terms of longitudinal studies, our data are unique in showing that progressive RNFL thinning occurs over time in MS non-ON eyes, and also demonstrate that eyes with a distant history of acute ON (prior to study enrollment) have continued RNFL axonal loss. This concept is also supported by our finding that while disease duration did not affect the relation between RNFL thinning and length of follow-up in models examining MS non-ON eyes, accounting for disease duration lessened the strength of association between RNFL thinning and follow-up in MS ON eyes. The amount of time since the episode of acute ON is perhaps important in determining the degree of RNFL thinning observed during any particular follow-up period. While ON remains an attractive model for studying acute effects of neuroprotection, our findings of sub-clinical axonal loss suggest that MS trials could also use the anterior visual pathway as a model.

Our data are also important in demonstrating that clinically significant losses of vision by low-contrast letter acuity and high-contrast VA are associated with RNFL thinning over time. These findings add a longitudinal component to an already strong base of cross-sectional data showing anterior visual pathway structure-function correlations in MS, and support validity for low-contrast letter acuity as a candidate visual outcome measure. In the present study and in phase 3 trials of natalizumab,¹¹ low-contrast letter acuity at the 2.5% level was the most sensitive measure of visual loss. Visual loss by the 2.5% contrast chart in our study was significantly associated with RNFL thinning. Scores from the 1.25% contrast chart, however, correlated less well with RNFL loss. Our results suggest that high-contrast VA, measured with ETDRS charts and using research protocols, may also have a useful role in MS trials, particularly as a correlate of RNFL thinning and as a descriptor of visual function. While floor effects (low or zero scores at baseline---23% of eyes followed up in the present study) may limit the capacity of the 1.25% chart to capture changes in acuity, RNFL thinning in our study did not distinguish eyes with visual loss on the 1.25% chart even after excluding eyes with low or zero baseline scores (<7 letters). It is also possible that low-contrast acuity scores at the 1.25% level reflect disease in the brain and posterior visual pathway to a greater degree than do 2.5% low-contrast acuity or VA. In an investigation of vision and MRI lesion burden in MS, low-contrast acuity scores at 1.25% correlated better with T2 lesion volumes in whole brain than with lesion volumes for Area 17 white matter,

optic radiations, or optic tracts.¹² The opposite pattern was observed for low-contrast acuity at 2.5%, which showed stronger correlations with lesion burden in the visual pathway regions of interest, indicating perhaps greater sensitivity to visual pathway disease. The 2.5% chart is likely to be the most useful in future MS trials; the 1.25% contrast level will yield helpful information in patients who begin the study with non-zero scores. Consistent with data for MS neurologic outcome measures such as the MSFC, our study also showed evidence of greater dysfunction among older patients with longer disease duration.

As a potential new imaging outcome for neuroprotection and other clinical trials in MS, RNFL thickness by OCT fulfills many important criteria, including pathological specificity (measurements are concordant with retinal histology), reproducibility, ease of patient participation, correlation with clinical measures, and sensitivity to changes over time.³² While the final criterion, response to treatment, is fulfilled by low-contrast letter acuity,¹¹ this important characteristic for OCT will be addressed by forthcoming MS clinical trial data. A crucial step forward for the anterior visual pathway as a model for assessing therapies in MS, our longitudinal study demonstrates sensitivity to changes in RNFL thickness and low-contrast letter acuity. As such, it supports these techniques as quantitative of both structure and function of neural tissue, in this case, the retina.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig 1.

Bar graphs showing mean changes from baseline in retinal nerve fiber layer (RNFL) thickness by optical coherence tomography (OCT) for multiple sclerosis (MS) eyes with and without visual loss. The bars represent mean changes in RNFL thickness from baseline and the vertical lines are 95% confidence intervals. Data are presented for MS eyes with no vision loss (left bars), MS eyes with loss of vision (middle bars), and all MS eyes based on (A) high-contrast visual acuity (VA), Early Treatment Diabetic Retinopathy (ETDRS) charts; (B) low-contrast letter acuity, 2.5% contrast level, low-contrast Sloan letter charts; and (C) low-contrast letter acuity, 1.25% contrast level. Definitions of visual loss were based on degrees of change in score that are beyond those expected for test-retest variability (5 letters for visual acuity, 7 letters for low-contrast acuity); these values are based on literature for the two vision testing methods.^{7,35,36} Reductions in average RNFL thickness

were greater for eyes with visual loss for VA and for low-contrast acuity at the 2.5% contrast level.

* Logistic regression models, accounting for age and adjusting for within-patient, inter-eye correlations, were used to determine the association between visual loss and degree of RNFL thinning from baseline.

Fig 2.

Graphs showing mean changes from baseline in retinal nerve fiber layer (RNFL) thickness by length of follow-up for multiple sclerosis (MS) eyes. The triangles represent point estimates for mean changes in RNFL thickness from baseline for each follow-up interval. The vertical lines are 95% confidence intervals. (A) Among all MS eyes in the follow-up cohort, $n=593$, degrees of RNFL thinning increased with increasing follow-up from baseline. Each eye is represented only once in panel (A), with the follow-up interval based on the most recent post-baseline study visit. (B) MS eyes without a past history of acute optic neuritis (ON, $n=381$) showed strikingly similar patterns of RNFL thinning with increasing follow-up from baseline. (C) Eyes with an ON history ($n=208$) were fewer, yet demonstrated a trend toward increasing degrees of RNFL thinning over time; these eyes also had lower values for RNFL at baseline. History of ON could not be determined for 4 eyes in the follow-up cohort; thus $n=589$ for the ON + non-ON eyes in (B) and (C).

* Generalized estimating equation (GEE) models, accounting for age and adjusting for within-patient, inter-eye correlations were used for analyses; p-values are based on

comparisons of RNFL thinning at the >1-year follow-up intervals to 0.5-1-year follow-up interval.

Fig 3.

Scatter plot and fitted linear regression line showing the relation for between RNFL thinning from baseline and follow-up time from baseline to the last visit as a continuous variable in years. The regression line represents fitted values for mean RNFL thinning from baseline for the latest follow-up visit for MS eyes; the gray shaded area shows the 95% confidence intervals on the mean values. The scatter plot and regression line represent group data for degree of RNFL thinning observed between baseline and the last follow-up visit, and are not meant to define patterns of RNFL thinning over time for individual eyes; the pattern of RNFL thinning in individual eyes may not, in fact, be linear throughout the course of follow-up. This graph also illustrates that there are very few outliers with respect to RNFL thinning. Accounting for age and adjusting for within-patient, inter-eye correlations, the relation between RNFL thinning from baseline and follow-up time at the last visit as a continuous variable was significant ($p < 0.001$, GEE models). For the cohort and length of time examined (range 0.5 to 4.5 years), each 1 year of follow-up was associated, on average, with 2.0 μm increases in the amount of RNFL thinning.

Table 1
Clinical Characteristics of Patients with MS in Baseline and Follow-Up Cohorts

	Baseline Cohort n = 1,005 (2,010 eyes)	Follow-Up Cohort: ≥6 months – 4.5 years n = 299 (593 eyes)
Age at baseline, yr, mean ± SD	43 ± 11	43 ± 10
Sex, % female	74%	72%
Race, % Caucasian	90%	92%
MS disease sub-type, % relapsing-remitting	83%	84%
Disease duration, yr, median (range)	9 (<1 – 46)	9 (<1 – 49)
History of ON prior to study, % of eyes ^a	28%	35%
Follow-up from baseline visit, months	—	18 ± 10
Visual acuity at baseline, Snellen equivalent from ETDRS charts, median (range)	20/20 (<20/200 – 20/12.5)	20/20 (<20/200 – 20/12.5)
Low-contrast acuity at baseline, 2.5% contrast, number of letters correct (maximum 70), mean ± SD	28 ± 12	28 ± 13
Low-contrast acuity at baseline, 1.25% contrast, number of letters correct (maximum 70), mean ± SD	15 ± 11	16 ± 12
RNFL thickness at baseline (average for 360° around optic disc), μm, mean ± SD	93 ± 16	91 ± 16

^a ON indicates eyes with a history of acute optic neuritis ≥3 months prior to study enrollment.

MS = multiple sclerosis; ON = optic neuritis; ETDRS = Early Treatment Diabetic Retinopathy Study; RNFL = retinal nerve fiber layer; OCT = optical coherence tomography.

Table 2
Comparison of MS Eyes in Follow-Up Cohort with vs. without Visual Loss by Low-Contrast Letter Acuity (2.5% contrast level)

	Loss of ≥ 7 Letters from Baseline to Last Follow-Up Visit (67 eyes)	No Loss of Vision (493 eyes) ^a
Mean age, yr, mean $\pm$ SD	49 $\pm$ 11	44 $\pm$ 10
MS disease sub-type, % relapsing-remitting	82%	86%
Disease duration, yr, median (range)	13 (3 – 46)	8 (<1 – 49)
Patients on disease-modifying therapy at last visit, %	93%	87%
History of ON prior to study, % of eyes ^b	34%	34%
Follow-up from baseline visit, months, mean $\pm$ SD	26 $\pm$ 13	17 $\pm$ 9
Visual acuity at <i>baseline</i> , Snellen equivalent from ETDRS charts, median (range)	20/20 (20/32 – 20/12.5)	20/20 (<20/200 – 20/12.5)
Low-contrast acuity at <i>baseline</i> , 2.5% contrast, number of letters correct (maximum 70), mean $\pm$ SD	33 $\pm$ 9	31 $\pm$ 9
RNFL thickness at <i>baseline</i> (average for 360° around optic disc), μ m, mean $\pm$ SD	91 $\pm$ 13	92 $\pm$ 17

^a Since eyes with scores of <7 letters at baseline could not have visual loss during follow-up by definition, only eyes with baseline scores ≥ 7 (n = 560 for 2.5% contrast) were included in analyses comparing degrees of RNFL thinning or proportions of eyes with visual loss.

^b ON indicates eyes with a history of acute optic neuritis ≥ 3 months prior to study enrollment.

MS = multiple sclerosis; ON = optic neuritis; ETDRS = Early Treatment Diabetic Retinopathy Study; RNFL = retinal nerve fiber layer.

Table 3

Proportions of MS Eyes with Visual Loss Greater than the Amount Expected Based on Test-Retest Variability and with RNFL Thinning by Follow-Up Time at Most Recent Visit

Type of Measure and Criterion for Loss	Follow-Up Time at Most Recent Visit				<i>p</i>
	All <i>n</i> = 593 % (95% CI)	0.5 – 1 Year <i>n</i> = 201 % (95% CI)	>1 – 2 Years <i>n</i> = 242 % (95% CI)	>2 – 3 Years <i>n</i> = 109 % (95% CI)	
VA on ETDRS charts (≥5 letters) ^a	11.5% (9.0, 14.0)	6.5% (3.1, 10.0)	10.0% (6.2, 13.8)	14.7% (7.9, 21.4)	36.6% (21.2, 52.0) <0.001 <i>b</i>
Low-contrast letter acuity on 2.5% contrast chart (≥7 letters) ^a	12.0% (9.3, 14.7)	6.9% (3.2, 10.5)	7.9% (4.4, 11.5)	17.0% (9.7, 24.2)	47.4% (30.7, 64.0) <0.001 <i>b</i>
Low-contrast letter acuity on 1.25% contrast chart (≥7 letters) ^a	13.3% (10.2, 16.4)	12.8% (7.4, 18.3)	9.6% (5.3, 13.8)	13.5% (6.6, 20.5)	42.3% (22.0, 62.7) 0.01 <i>b</i>
RNFL thickness by OCT (≥6.6 μm)	18.0% (14.9, 21.1)	10.5% (6.2, 14.7)	15.7% (11.1, 20.3)	27.5% (19.0, 36.0)	43.9% (28.0, 59.8) <0.001 <i>b</i>

^aSince eyes with scores of <5 letters (for VA) or <7 letters (for low-contrast acuity) at the baseline visit could not have visual loss during follow-up by definition, only eyes with baseline scores ≥5 letters for VA (*n* = 589) or ≥7 letters for low-contrast acuity (*n* = 560 at 2.5% contrast, *n* = 458 at 1.25% contrast) were included in analyses comparing proportions of eyes with visual loss.

^bProportions of eyes increased with greater length of follow-up, chi-square test for trend in binomial proportions.

MS = multiple sclerosis; CI = confidence interval; ON = optic neuritis; VA = visual acuity; ETDRS = Early Treatment Diabetic Retinopathy Study; RNFL = retinal nerve fiber layer; OCT = optical coherence tomography.