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An investigation of the retinal nerve fibre layer in progressive multiple sclerosis using optical coherence tomography

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Axonal loss is thought to be the predominant cause of disability in progressive multiple sclerosis (MS). The retinal nerve fibre layer (RNFL) is composed largely of unmyelinated axons of retinal ganglion cells, and is accessible to study with optical coherence tomography (OCT), giving a measure of axonal loss. OCT measures of the RNFL thickness (RNFLT) and macular volume were studied in 23 patients with primary progressive multiple sclerosis (primary progressive MS) (13 male; 10 female; mean age 52 years; median EDSS 6.0; mean disease duration 11 years), and 27 patients with secondary progressive multiple sclerosis (secondary progressive MS) (8 male; 19 female; mean age 50 years; median EDSS 6; mean disease duration 22 years). Of the patients with secondary progressive MS, 14 had clinical history of optic neuritis (ON) in a single eye; the remaining patients had not had ON. Twenty healthy controls (11 male; 9 female; mean age 46 years) had RNFLT and macular volume studied. Of the patients' eyes not previously affected by ON, both the mean RNFL thickness and macular volume were reduced when compared with control values. The mean RNFL thickness and macular volume were significantly reduced in secondary progressive MS, but not in primary progressive MS when compared with control RNFL thickness and macular volume. RNFL loss was most evident in the temporal quadrant, where significant reduction was seen in primary progressive MS versus controls and in secondary versus primary progressive MS. There were significant correlations of decreased RNFLT and macular volume with measures of visual acuity, low contrast visual acuity and visual field mean deviation in the MS patients. There are significant global reductions in RNFLT and macular volume in the eyes of secondary progressive MS patients not previously affected by ON, but not in primary progressive MS patients, compared with controls. This may indicate a difference in the extent of the pathological processes that cause axonal loss in the retina, and by inference the optic nerve, in secondary progressive MS and primary progressive MS.

Keywords: retinal nerve fibre layer; optical coherence tomography; multiple sclerosis; axonal degeneration

Abbreviations: MD = mean deviation; MS = multiple sclerosis; OCT = optical coherence tomography; ON = optic neuritis; RNFL = retinal nerve fibre layer; VEP = visual evoked potential.

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Introduction

Axonal loss is considered to be the predominant cause of enduring disability in multiple sclerosis (MS). This is particularly so in the progressive forms of the disease. Pathologically, axonal loss has been demonstrated within both inflammatory lesions (Trapp *et al.*, 1998) and normal appearing white matter in brain and spinal cord (Evangelou *et al.*, 2000; Lovas *et al.*, 2000). Putative magnetic resonance imaging measures of axonal damage or loss such as atrophy (Losseff *et al.*, 1996) and reduced *N*-acetyl aspartate on magnetic resonance spectroscopy (Fu *et al.*, 1998) also indicate the presence of axonal

loss in the spinal cord and brain of patients with progressive MS.

The retinal nerve fibre layer (RNFL) is composed predominantly of unmyelinated axons of retinal ganglion cells. Measurements of the RNFL should, therefore, give relatively direct measures of the number of axons present without the confounding variable of tissue loss due to demyelination. Axonal loss has been recognized in the RNFL in MS by the appearance of abnormalities on fundoscopy (Frisen and Hoyt, 1974), and in a detailed study of the appearance of the RNFL of 20 MS patients using indirect ophthalmoscopy and fundus photography, 73% of all MS eyes examined had visible RNFL defects and 68% of the asymptomatic MS eyes examined had RNFL defects (Elbol and Work, 1990). RNFL loss in MS has also been documented pathologically (Kerrison *et al.*, 1994). However, the abnormalities seen on fundoscopy are not readily quantifiable and the microscopic pathological appearance of the retina cannot be determined in life.

Optical coherence tomography (OCT) (Huang *et al.*, 1991) is a non-invasive technique that allows the quantitative cross-sectional imaging of the RNFL. Its use has been predominantly to investigate retinal axonal loss in glaucoma (Kanamori *et al.*, 2003; Costa *et al.*, 2006) but (Parisi *et al.*, 1999) demonstrated a significant reduction in mean RNFL thickness in the eyes of MS patients that were clinically unaffected by optic neuritis (ON). Trip and colleagues (2005) demonstrated RFNL loss following ON with poor recovery, and Fisher and colleagues (2006) demonstrated a small but significant reduction in RNFL thickness in eyes unaffected by ON in a cohort of patients with MS, the majority of whom had a relapsing remitting course. The present study reports OCT findings from a cohort of patients with progressive forms of MS. It investigates whether axonal loss is present in the RNFL of patients with progressive MS who have not experienced a previous clinical episode of ON. There is evidence from MRI for differences in the extent of brain lesions between primary and secondary progressive MS, with smaller lesion loads and fewer active lesion in the former (Thompson *et al.*, 1990, 1991). Epidemiological studies, however, indicate that once established, the course of progressive MS is similar, regardless of the occurrence of relapses at onset (Confavreux *et al.*, 2000, 2003). The OCT findings in these two subgroups were therefore investigated separately and together.

Methods

Patients

Twenty-three patients with primary progressive multiple sclerosis (primary progressive MS) and 27 patients with secondary progressive multiple sclerosis (secondary progressive MS) were recruited from the MS clinics of the National Hospital for Neurology and Neurosurgery, London, UK. All patients fulfilled the revised McDonald criteria (Polman *et al.*, 2005). Patients were classified into either primary progressive MS or secondary progressive MS according to the Lublin and Reingold criteria (Lublin and Reingold, 1996). None of the subjects were receiving immunomodulatory therapy.

The past history and available medical records were carefully reviewed for evidence of previous ON. In order to remove the known effect of ON upon RNFL thickness, in secondary progressive MS patients, those with a history of episodes of ON that had affected both eyes were excluded from the study, although a single episode or repeated episodes of ON in a single eye were permitted. An episode of ON excluded the classification primary progressive MS. Patients with visual symptoms due to MS—other than those due to ON—were permitted into the study, providing they had had a comprehensive visual assessment to exclude other causes of eye disease. Patients with other eye diseases were excluded.

Ethical approval was obtained from the National Hospital for Neurology and Neurosurgery and Institute of Neurology Joint research ethics committee. Written consent was obtained from all subjects, according to the Declaration of Helsinki.

Control subjects

Twenty control subjects were recruited by advertisement in our institution. The control subjects were known not to have any ophthalmological or neurological diseases.

Clinical assessment

A history was taken from controls and patients to determine the presence of visual symptoms. All subjects were examined for the presence of pupillary and fundoscopic abnormalities. The patients had their level of disability from MS assessed with the Extended Disability Status Score (EDSS) (Kurtzke, 1983). Multiple Sclerosis Severity Scale data was generated according to the method proposed by Roxburgh and colleagues (2005).

Retinal imaging

OCT images were acquired with a Stratus OCT Model 3000 (Carl Zeiss Meditec, Dublin, CA, USA). All OCT imaging was obtained by a single observer (A.H.). Study participants' pupils were dilated if required to obtain good image quality. Images from the OCT device are given a signal strength by the device, with a maximum of 10. Images were rejected if the signal strength obtained by the OCT device was <7, or if the inter-eye signal strength difference was >2. RNFL images were acquired by taking three circular 3.4 mm diameter scans, centred on the optic disc, the mean of which was used to express RNFL thickness (Fast RNFL scanning protocol). The thicknesses of the quadrants of the RNFL were automatically calculated by the OCT device software. Macular thickness maps were acquired by making six radial linear scans, centred on the fovea, and by construction of a map from these scans (Fast macular thickness map scanning protocol). Both OCT protocols were performed twice at all visits in order to obtain data for intra-class correlation coefficients. The two measures were averaged for the purposes of other analyses. Ten patients and 10 controls had repeat measures of RNFLT and macular volume between 1 week and a month after the first visit to obtain measures of reproducibility. In one patient with severe visual loss due to ON, it was not possible to obtain macular volume maps with appropriate foveal centring in the affected eye due to a dense

centrocaecal scotoma, even when fixation with the contralateral eye was attempted. Measures from this eye were excluded from all analyses.

Visual testing

The visual acuity of all primary progressive MS and 21 secondary progressive MS subjects was measured with a retroilluminated ETDRS chart and was recorded as the 4 m logarithm of the minimum angle of resolution (logMAR acuity). Appropriate refraction was used if required. In addition, Sloan 25%, 5% and 1.25% contrast ETDRS charts were used at 4 m to calculate the equivalent low-contrast visual acuity, as low-contrast acuity has been shown to be more sensitive for detection of abnormal visual function in MS (Balcer *et al.*, 2000). If patients were unable to perceive any letters on either the logMAR or Sloan charts at 4 m, the chart was moved to a distance of 1 m and the test repeated, and an adjustment for the change in distance was made according to the method proposed in the paper by Ferris and colleagues (1982). A logMAR acuity of 0.0 is equivalent to a Snellen acuity of 6/6 and a logMAR acuity of 1.0 is equivalent to a Snellen acuity of 6/60. Each 0.1 increase in logMAR acuity represents one line of acuity (comprising five letters) lost. The threshold sensitivity of the central 30 of vision was measured using the full threshold central 30-2 program on a Humphrey visual field analyser (Carl Zeiss Meditec, Dublin, CA, USA). Refractive errors were corrected using wide-angle lenses when needed. The visual field mean deviation (MD), a measure of overall field loss, was calculated by comparison with a reference field provided by the manufacturer. Of the patients with secondary progressive MS, six did not have logMAR or Sloan low-contrast acuity, or Humphrey visual fields measured, but had Snellen acuity and confrontational visual fields. These measures are not included in the analysis as they are not directly comparable to the measures obtained in the other subjects.

Statistical analysis

Eyes not affected by previous ON

For the comparisons of OCT measures between subject groups either a randomly selected eye, or, in patients with a history of acute ON, the fellow eye was selected for analysis. Inter-group comparisons were made by multiple linear regression of the OCT measures on subject group indicators with adjustment for age. In order to reduce the effect of random eye allocation in the comparison between primary progressive and control subjects, the mean of both eyes was compared in a separate analysis.

Eyes affected by ON

Eyes affected by acute optic neuritis were compared with the fellow eye by paired *t*-tests.

Relationships between OCT measures and visual function

The relationships between OCT measures and visual function were investigated with pairwise correlation of the visual function measures of a single ON unaffected eye as described above, and OCT measures.

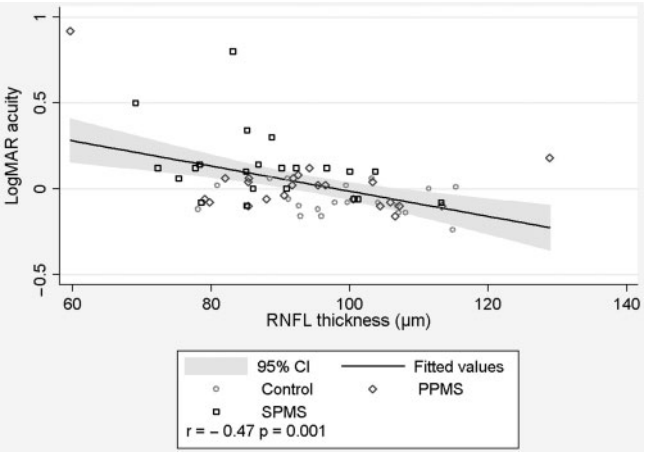

Fig. 1 RNFL thickness plotted against logMAR visual acuity (all subjects: single eye not affected by optic neuritis per subject).

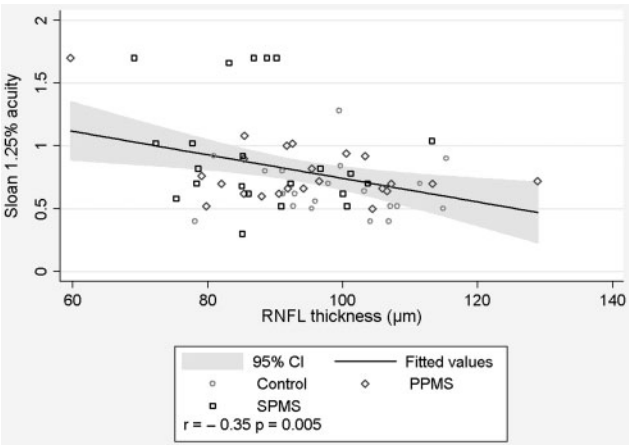

Fig. 2 RNFL thickness plotted against Sloan 1.25% visual acuity (all subjects: single eye not affected by optic neuritis per subject).

Relationships between OCT measures, disease duration and disability

The relationships between OCT measures and disease duration were investigated with multiple linear regression of OCT measures (of a single ON unaffected eye per subject) on the disease duration in years, with adjustment for age. The relationships between OCT measures (of a single ON unaffected eye per subject), and overall disability (as measured by the EDSS) were investigated with Spearman rank correlation. The relationships between mean and quadrant RNFL thickness, macular volume and the MSSS was investigated using linear regression. To ensure that a relationship with disability was not missed by random allocation of one less affected eye, the relationships with EDSS and MSSS were also investigated using the lower value of mean RNFL, quadrant RNFL and macular volume as proposed by Sepulcre and colleagues (2007) (Figures 1–4).

Statistical significance is reported as $P<0.05$. Analyses were conducted in Stata 9.2 (StataCorp, College Station, TX, USA).

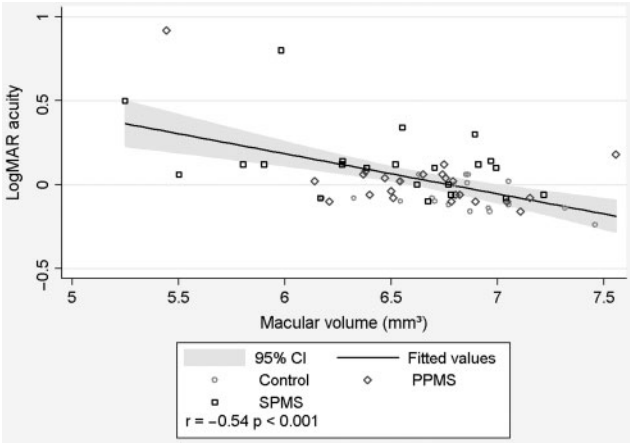

Fig. 3 Macular volume plotted against logMAR visual acuity (all subjects: single eye not affected by optic neuritis per subject).

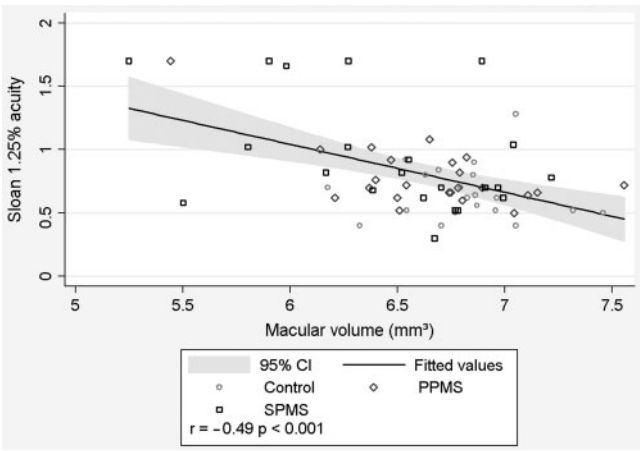

Fig. 4 Macular volume plotted against Sloan 1.25% visual acuity (all subjects: single eye not affected by optic neuritis per subject).

Results

The demographic details and visual assessments of the subjects are described in Table 1.

Clinical visual characteristics

Of the patients with a primary progressive course, three had visual symptoms, predominantly blurred vision, and in two of these three patients glare sensitivity. The visual symptoms developed some time after the initial presentation, which in each case was a progressive spastic paraplegia. All of these patients had undergone a comprehensive visual assessment to exclude other causes of visual dysfunction. One further patient had an episode of difficulty perceiving moving objects that lasted 2 months, where she described moving objects as 'smeared', in the absence of ocular pain, scotoma or change in colour vision. She described her vision at the time of the study as normal. Twelve patients with a primary progressive course were judged on fundoscopy to have bilateral optic disc pallor, and a further four had unilateral disc pallor.

In the 12 patients with secondary progressive course, but no history of acute ON, five were judged to have bilateral optic disc pallor, and a further five patients had unilateral optic disc pallor. One patient had persistent 'bleaching' of vision in bright light. She had noted that a central scotoma would develop if she were in bright light. One further patient had an episode of bilateral Uhthoff's phenomenon without any other symptoms of acute ON.

Of the 15 secondary progressive MS patients with a history of unilateral acute ON, the mean (SD) logMAR acuity of the eye previously affected by optic neuritis was 0.29 (0.52), median 0.1. Three patients had central scotomas detectable on testing, which persisted following ON. One further patient had persistent Uhthoff's phenomenon following ON. Three patients had two episodes of acute ON in the same eye, but only one of these had

Table 1 Demographic details and visual function of subjects

	Control ($n = 20$)	PPMS ($n = 23$)	SPMS ($n = $[a])
Age, years: mean (SD)	46 (14)	52 (12)	50 (9)
Sex (M:F)	11:9	13:10	8:19
Median EDSS	–	6 (range 2–7.5)	6 (range 3–7)
Mean MSSS (SD)	–	7.40 (1.99)	5.78 (1.74)
Disease duration, years mean (SD)	–	11 (6) $p_m < 0.001$	21 (10)
Duration of progressive phase, years: mean (SD)	–	11 (6)	8 (5)
Prior ON (poor recovery)	–	0	15 (3; 1 persistent Uhthoff's)
Time since ON, years Mean (SD)	–	–	19 (11)
Chronic visual symptoms (fellow eye if prior ON)	–	3	1
Mean logMAR acuity (SD)	−0.07 (0.09)	0.03 (0.21)	0.13 (0.21)
Mean Sloan 25% contrast acuity (SD)	0.02 (0.12)	0.12 (0.21)	0.22 (0.24)
Mean Sloan 5% contrast acuity (SD)	0.27 (0.15)	0.41 (0.30)	0.48 (0.33)
Mean Sloan 1.25% contrast acuity (SD)	0.66 (0.22)	0.79 (0.25)	0.96 (0.46)
Mean central 30° visual field mean deviation, dB (SD)	–	−5.87 (2.31)	−6.71 (3.40)

Where P_m = significance in comparison between MS groups.
[a]For SPMS, $n = 21$ for visual function measures, 27 for other measures.

Table 2 Age-adjusted regression coefficients for visual function measures

	Progressive MS – Control (Difference in means)	PPMS – Control (Difference in means)	SPMS – Control (Difference in means)	SPMS – PPMS (Difference in means)
LogMAR acuity (95% CI)	0.13 (0.03, 0.23) $P = 0.014$	0.08 (−0.03, 0.19) $P = 0.143$	0.18 (0.06, 0.29) $P = 0.003$	0.10 (−0.03,0.22) $P = 0.141$
Sloan 25% acuity (95% CI)	0.13 (0.02, 0.24) $P = 0.024$	0.08 (−0.04, 0.21) $P = 0.189$	0.18 (0.05, 0.31) $P = 0.006$	0.10 (−0.04, 0.24) $P = 0.151$
Sloan 5% acuity (95% CI)	0.17 (0.02, 0.32) $P = 0.026$	0.14 (−0.03, 0.31) $P = 0.104$	0.21 (0.03. 0.38) $P = 0.020$	0.07 (−0.12, 0.26) $P = 0.462$
Sloan 1.25% acuity (95% CI)	0.19 (0.01, 0.38) $P = 0.037$	0.12 (−0.08, 0.32) $P = 0.237$	0.28 (0.07, 0.48) $P = 0.010$	0.16 (−0.06, 0.38) $P = 0.158$
Visual field mean deviation, dB (95% CI)	–	–	–	−0.61 (−2.37, 1.13) $P = 0.48$

Table 3 Mean RNFL thickness, quadrant RNFL thickness and macular volume in subject groups: eyes not affected by optic neuritis

	Controls	Progressive MS	PPMS	SPMS
Mean RNFL thickness, μm (SD)	98.8 (10.5)	91.0 (12.6)	93.9 (13.9)	88.4 (10.9)
Macular volume, mm^3 (SD)	6.81 (0.31)	6.54 (0.45)	6.64 (0.42)	6.46 (0.41)
Temporal quadrant RNFL thickness, μm (SD)	76.5 (15.0)	58.2 (14.6)	63.7 (14.6)	53.6 (13.2)
Superior quadrant RNFL thickness, μm (SD)	121.0 (16.3)	112.6 (15.5)	115.0 (14.8)	110.5 (16.1)
Nasal quadrant RNFL thickness, μm (SD)	77.5 (15.6)	77.2 (18.8)	77.7 (23.5)	76.8 (14.0)
Inferior quadrant RNFL thickness, μm (SD)	119.5 (15.2)	116.0 (18.0)	121.2 (21.8)	111.5 (12.7)

persistent visual symptoms (mentioned above). All of the 15 eyes previously affected by ON had disc pallor, and 6 of these 15 patients were judged to have pallor of the contralateral disc. Eight of the patients with a history of ON had a relative afferent papillary defect of the affected eye as evaluated by the swinging flashlight test (Levatin, 1959; Thompson, 1966).

When the analysis was confined to eyes not previously affected by ON, the secondary progressive MS patients had significant reductions in logMAR and low-contrast acuity compared with controls (Table 2). The primary progressive MS patients had slightly worse logMAR and low-contrast acuity than controls but this was not significant. There was no significant difference between secondary progressive MS and primary progressive MS patients in terms of visual function.

Reproducibility of optical coherence tomography measurements

The coefficient of variation for repeat measures of RNFL thickness was 3.66% for controls and 2.56% for MS patients. The coefficient of variation for repeat measures of macular volume was 1.03% for controls and 1.39% for patients. The intra-class correlation coefficient was 0.89 (95% confidence interval 0.75–1.02) for RNFL thickness in controls, and 0.97 (95% confidence interval 0.93–1.00) for RNFL thickness in MS patients. The ICC was 0.96 (95% confidence interval 0.91–1.01) for macular volume in controls and 0.96 (95% confidence interval 0.92–1.00) in MS patients.

OCT measures: comparison of groups

The mean RNFL and macular volume data is summarized in Table 3; age-adjusted coefficients for inter-group comparisons are provided in Table 4.

When both primary and secondary MS patients were analysed as a single group and compared with control values, there were significant reductions in mean RNFL thickness (patient–control difference 7.14 μm, $P = 0.032$, 95% CI: −13.63, −0.65), temporal quadrant RNFL thickness (patient–control difference −17.65 μm, $P < 0.001$, 95% CI: −25.61, −9.69) and macular volume (patient–control difference −0.27 mm^3, $P = 0.019$, 95% CI: −0.50, −0.05). There was no significant difference between the pooled progressive patient group and controls in the superior quadrant RNFL thickness, nasal quadrant RNFL thickness or inferior quadrant RNFL thickness (see Table 3 for raw data and Table 4 for age-adjusted coefficients, *P*-values and 95% confidence intervals).

When secondary progressive MS patients alone were compared to controls, there was a significant reduction in the RNFL thickness (secondary progressive MS–control difference −9.87 μm, $P = 0.007$, 95% CI: −16.97, −2.76) temporal quadrant RNFL thickness (secondary progressive MS–control difference −22.29 μm, $P < 0.001$, 95% CI: −30.79, −13.77), superior quadrant RNFL thickness (secondary progressive MS–control difference −9.61 μm, $P = 0.045$, 95% CI: −19.00, −0.21) and macular volume (secondary progressive MS–control difference −0.36 mm^3, $P = 0.005$, 95% CI: −0.61, −0.11) of the eyes not clinically affected by ON of patients with secondary progressive MS. Primary progressive MS patient eyes had a significant

Table 4 Age-adjusted regression coefficients for retinal measures

	Progressive MS–Control (Difference in means)	PPMS–Control (Difference in means)	SPMS–Control (Difference in means)	SPMS–PPMS (Difference in means)
Mean RNFL thickness, μm (95% CI)	−7.14 (−13.63, −0.65) $P = 0.032$	−3.96 (−11.30, 3.38) $P = 0.286$	−9.87 (−16.97, −2.76) $P = 0.007$	−5.95 (−13.05, 1.15) $P = 0.098$
Macular volume, mm^3 (95% CI)	−0.27 (−0.50, −0.05) $P = 0.019$	−0.17 (−0.43, 0.08) $P = 0.193$	−0.36 (−0.61, −0.11) $P = 0.005$	−0.19 (−0.45, 0.07) $P = 0.145$
Temporal quadrant RNFL thickness, μm (95%CI)	−17.65 (−25.61, −9.69) $P < 0.001$	−12.17 (−21.00, −3.34) $P = 0.008$	−22.29 (−30.79, −13.77) $P < 0.001$	−10.07 (−18.08, 2.07) $P = 0.015$
Superior quadrant RNFL thickness, μm (95%CI)	−7.56 (−16.01, 0.90) $P = 0.079$	−5.13 (−14.87, 4.61) $P = 0.297$	−9.61 (−19.00, −0.21) $P = 0.045$	−4.41 (−13.36, 4.54) $P = 0.327$
Nasal quadrant RNFL thickness, μm (95%CI)	0.04 (−9.67, 9.76) $P = 0.993$	0.51 (−13.75, 11.78) $P = 0.927$	−0.35 (−11.22, 10.51) $P = 0.948$	−0.82 (−11.74, 10.09) $P = 0.880$
Inferior quadrant RNFL thickness, μm (95%CI)	−3.02 (−12.36, 6.31) $P = 0.520$	2.24 (−8.27, 12.74) $P = 0.672$	−7.47 (−17.61, 2.66) $P = 0.146$	−9.65 (−19.72, 0.42) $P = 0.06$

reduction in temporal quadrant RNFL thickness (primary progressive MS–control difference −12.17 μm, $P = 0.008$, 95% CI −21.00, −3.34) but no significant difference in any other individual quadrant of the RNFL. There was a non-significant reduction in both RNFL thickness and macular volume (primary progressive MS–control RNFL thickness difference −3.96 μm, $P = 0.286$, 95% CI: −11.30, 3.39; primary progressive MS–control macular volume difference −0.17 mm^3, $P = 0.193$, 95% CI: −0.43, 0.08) when compared to control eyes. Although there were lower retinal measures in secondary progressive MS compared to primary progressive MS, these differences were only significant when comparing the temporal quadrant of the RNFL (secondary progressive MS–primary progressive MS RNFL thickness difference −10.07 μm, $P = 0.015$, 95% CI −18.08, 2.07). The mean RNFL, individual quadrants other than the temporal quadrant of the RNFL and macular volume were reduced in secondary progressive MS compared with primary progressive MS, but this was not significant.

Given that the eyes of primary progressive MS patients are free of the confounding factor of ON, a comparison was made of the mean of all primary progressive MS eyes, compared with the mean RNFL and MV values for controls to reduce the effect of random differences in eye selection. This comparison approached significance for mean RNFL thickness (primary progressive MS–control RNFL thickness difference −6.81 μm, $P = 0.080$, 95% CI: −14.45, 0.83) and for macular volume (primary progressive MS–control macular volume difference −0.22 mm^3, $P = 0.086$, 95% CI: −0.48, 0.03).

RNFL thickness was significantly reduced in the eyes with a history of ON when compared to the fellow eye (ON affected eye–fellow eye difference −6.64 μm, $P = 0.040$, 95% CI: −12.95, −0.34). Macular volume was also significantly reduced in eyes with a history of prior ON (ON affected eye–fellow eye difference −0.18 mm^3, $P = 0.026$, 95% CI: −0.33, −0.03).

Relationship between retinal measures and visual function

When the relationships between visual function and RNFL thickness were examined with pair wise correlation, these were found to be significant for logMAR acuity ($r = -0.46$ $P = 0.001$), Sloan 25% contrast acuity($r = -0.44$ $P = 0.002$), Sloan 5% contrast acuity ($r = -0.42$ $P = 0.004$), Sloan 1.25% contrast acuity ($r = -0.34$ $P = 0.024$) and visual field MD ($r = 0.31$ $P = 0.038$) in patients (primary progressive MS and secondary progressive MS considered together) but not in controls (Table 5 and figures 1–4). When the relationships between visual function and macular volume were examined with pair wise correlation, these were found to be significant for logMAR acuity ($r = -0.52$ $P < 0.001$), Sloan 25% contrast acuity($r = -0.50$ $P < 0.001$), Sloan 5% contrast acuity ($r = -0.51$ $P < 0.001$), Sloan 1.25% contrast acuity

Table 5 Relationships between retinal measures and visual function in eyes not previously affected by optic neuritis

Visual function measure	Disease status	RNFL thickness		Macular volume	
		r	*P*	*r*	*P*
LogMAR acuity	All subjects	−0.47	<0.001	−0.54	<0.001
	Controls	−0.19	0.414	−0.35	0.125
	PPMS	−0.48	0.021	−0.57	0.004
	SPMS	−0.38	0.084	−0.43	0.043
	All MS patients	−0.46	0.001	−0.52	<0.001
Sloan 25% contrast acuity	All subjects	−0.45	<0.001	−0.50	<0.001
	Control	−0.22	0.347	0.16	0.499
	PPMS	−0.45	0.033	−0.54	0.008
	SPMS	−0.37	0.086	−0.42	0.049
	All MS patients	−0.44	0.002	−0.50	<0.001
Sloan 5% contrast acuity	All subjects	−0.41	<0.001	−0.51	<0.001
	Control	0.01	0.970	−0.08	0.751
	PPMS	0.45	0.033	−0.60	0.003
	SPMS	−0.37	0.086	−0.44	0.039
	All MS patients	−0.42	0.004	−0.51	<0.001
Sloan 1.25% contrast acuity	All subjects	−0.35	0.005	−0.49	<0.001
	Control	−0.07	0.760	−0.04	0.866
	PPMS	−0.46	0.026	−0.63	0.001
	SPMS	−0.22	0.322	−0.44	0.041
	All MS patients	−0.34	0.024	−0.51	<0.001
Visual field mean deviation (dB)	PPMS	0.30	0.159	0.34	0.109
	SPMS	0.32	0.147	0.32	0.142
	All MS patients	0.31	0.038	0.34	0.022

($r = -0.51$ $P < 0.001$) and visual field MD ($r = 0.34$ $P = 0.022$) in patients (primary progressive MS and secondary progressive MS considered together). There were no significant relationships between macular volume and visual measures in the control subjects.

If the primary progressive MS group was considered alone, the relationships remained significant for RNFL thickness and macular volume with all of the measures of visual acuity but not with visual field MD. If the secondary progressive MS group was considered alone, the relationships between macular volume and all acuity measures were significant. There were trends towards significance ($P < 0.1$) for the relationships between RNFL thickness and logMAR acuity, Sloan 25% acuity, Sloan 5% acuity but not for Sloan 1.25% acuity, and the correlation coefficients were similar in value to the values in the whole MS group.

In the eyes not previously affected by ON, no relationship was found between the presence of either optic disc pallor or a relative afferent papillary defect and RNFL thinning or macular volume loss.

Relationship between disability, disease duration and duration of the progressive phase and retinal measures

When a randomly selected eye with no history of ON was used, no significant relationship was found between the duration of disease and either mean RNFL thickness (change in mean RNFL thickness per year of disease −0.12 μm, $P = 0.513$, 95% CI: −0.50, 0.25), or macular volume (change in macular volume per year of disease −0.01 mm^3 $P = 0.125$ 95% CI: −0.02, 0.00). There was a borderline significant relationship between the temporal quadrant of the RNFL and disease duration [change in temporal quadrant RNFL thickness per year of disease (change in temporal quadrant RNFL thickness per year of disease −0.41 μm, $P = 0.056$, 95% CI: −0.84, 0.01)]. There was no significant relationship between any of the other quadrants of the RNFL and duration of disease (change in superior quadrant RNFL thickness per year of disease −0.21 μm, $P = 0.357$, 95% CI: −0.67, 0.25; change in nasal quadrant RNFL thickness per year of disease 0.17 μm, $P = 0.531$, 95% CI: −0.39, 0.74; change in inferior quadrant RNFL thickness per year of disease −0.20 μm, $P = 0.4456$, 95% CI: −0.74, 0.34). No significant relationship was found between duration of the progressive phase and any of the retinal measures (change in mean RNFL thickness per year of progressive disease −0.03 μm, $P = 0.931$, 95% CI: −0.66, 0.61; change in temporal RNFL thickness per year of progressive disease −0.56 μm, $P = 0.258$, 95% CI: −1.56, 0.43; change in superior RNFL thickness per year of progressive disease −0.36 μm, $P = 0.561$, 95% CI: −1.59, 0.88; change in nasal RNFL thickness per year of progressive disease −0.05 μm, $P = 0.920$, 95% CI: −1.14, 1.03; change in inferior RNFL thickness per year of progressive disease −0.36 μm, $P = 0.451$, 95% CI: −1.34, 0.61; change in macular volume per year of progressive disease 0.00 mm^3, $P = 0.784$, 95% CI: −0.03, 0.02).

There was no significant relationship between either measure of disability used in this study (EDSS and MSSS)

Table 6 Relationships between measures of disability and retinal measures

	EDSS	MSSS (Change in retinal measure per unit change in MSSS 95%CI)
Mean RNFL (randomly selected eye)	$\rho = -0.01$ $P = 0.921$	0.18 μm (−1.63 μm, 1.99 μm) $P = 0.846$
Mean RNFL (minimum of either eye)	$\rho = -0.12$ $P = 0.418$	0.33 μm (−1.86 μm, 2.54 μm) $P = 0.759$
Temporal quadrant RNFL (randomly selected eye)	$\rho = -0.19$ $P = 0.175$	0.07 μm (−2.04 μm, 2.18 μm) $P = 0.945$
Temporal quadrant RNFL (minimum of either eye)	$\rho = -0.11$ $P = 0.452$	−0.14 μm (−2.06 μm, 1.78 μm) $P = 0.885$
Superior quadrant RNFL (randomly selected eye)	$\rho = 0.00$ $P = 0.997$	0.60 μm (−1.62 μm, 2.83 μm) $P = 0.589$
Superior quadrant RNFL (minimum of either eye)	$\rho = -0.07$ $P = 0.634$	0.39 μm (−2.18 μm, 2.97 μm) $P = 0.759$
Nasal quadrant RNFL (randomly selected eye)	$\rho = -0.04$ $P = 0.796$	−0.96 μm (−3.66 μm, 1.73 μm) $P = 0.476$
Nasal quadrant RNFL (minimum of either eye)	$\rho = 0.10$ $P = 0.509$	−0.49 μm (−3.14 μm, 2.16 μm) $P = 0.710$
Inferior quadrant RNFL (randomly selected eye)	$\rho = 0.00$ $P = 0.997$	1.07 μm (−1.50 μm, 3.65 μm) $P = 0.405$
Inferior quadrant RNFL (minimum of either eye)	$\rho = -0.07$ $P = 0.651$	1.57 μm (−1.32 μm, 4.47 μm) $P = 0.281$
Macular volume (randomly selected eye)	$\rho = -0.03$ $P = 0.801$	0.01 mm^3 (−0.05 mm^3, 0.08 mm^3) $P = 0.661$
Macular volume (minimum of either eye)	$\rho = -0.03$ $P = 0.801$	0.01 mm^3 (−0.06 mm^3, 0.08 mm^3) $P = 0.760$

and any of the retinal measures. This was true whether a randomly selected eye, or as in the model used by Sepulcre and colleagues (2007), the lower of the two retinal measures was used for comparison. These results are presented in Table 6.

Discussion

The study demonstrates that there is retinal axonal loss in the eyes of progressive MS patients with no history of a previous episode of optic neuritis. The majority of these eyes were without visual symptoms. The mechanism for this axonal loss is not clear, but it may be that clinically silent demyelinating lesions within the optic nerve, associated with axonal damage with retrograde degeneration into the RNFL and macula have occurred. The visual evoked potential (VEP) obtained from the visually asymptomatic eyes of MS patients is frequently abnormal (Halliday, 1993), suggesting that clinically silent demyelinating lesions are common in the optic nerves of patients with MS.

Consistent with previous studies (Trip *et al.*, 2005; Costello *et al.*, 2006; Fisher *et al.*, 2006), both when compared with the fellow eye and healthy controls, there was significant loss of RNFL thickness and macular volume in eyes with a clinical history of ON. The magnitude of the RNFL loss in eyes with a history of ON (mean decrease ~20%) was similar to that seen by Fisher and colleagues (2006) in a study mainly of patients with relapsing remitting MS, and that seen in the unselected cohort studied by Costello and colleagues (2006). In the current study, the RNFL loss seen in eyes with a history of ON was greater than the significant loss of RNFL thickness in the fellow eyes of the secondary progressive MS patients when compared with controls, indicating that a clinically overt episode of ON has adverse consequences for the RNFL over and above that of secondary progressive MS alone. The fact that the severity of RNFL and macular volume loss was independent of the time since the clinical attack of ON suggests that the severity of the initial attack, rather than a subsequent, temporally linked process (e.g. gradual ongoing axonal loss in a persistently demyelinated optic nerve lesion) is more important in determining the extent of axonal loss. There is, however, pathological evidence in other parts of the central nervous system that axonal loss may occur in MS without prior demyelination (Kutzelnigg *et al.*, 2005), and that the degree of axonal loss is independent of the extent of demyelination (DeLuca *et al.*, 2006). The lack of relationship between MS disease duration and RNFL thickness argues against the occurrence of retinal axonal loss which is continuous and progressive from the onset of disease, although there might be different rates of gradual axonal loss between patients that could obscure a relationship with disease duration.

The secondary progressive MS and primary progressive MS groups both had significantly lower temporal RNFL thicknesses than controls, and the secondary progressive MS group had significantly lower temporal quadrant RNFL when compared with the primary progressive MS group. The overall RNFL thickness and macular volume were significantly reduced in the secondary progressive MS group. In the primary progressive group, these measures were reduced, but this was not significantly different from either controls or the secondary progressive MS group. The smaller reductions in the primary progressive MS group than the secondary progressive MS group are in keeping with the clinical profile of primary progressive MS. Visual loss as the presenting symptom in primary progressive MS is uncommon (Riise *et al.*, 1992; Trojano *et al.*, 1995; McDonnell and Hawkins, 1998), whereas in relapsing-remitting MS, and therefore by extension, secondary progressive MS, ON is a common initial event (Confavreux *et al.*, 2003). There is little data that gives any indication of the frequency of optic nerve pathology in primary progressive MS. The studies that established the high frequency of VEP abnormalities in MS

(Halliday *et al.*, 1973) were performed prior to the current division of chronic progressive MS into relapsing onset (i.e. secondary progressive MS) and progressive onset (i.e. primary progressive MS) subtypes. There are published reports of the proportion of VEP abnormalities in patients with a chronic progressive spastic paraparesis, which might be considered a surrogate for what is known now as primary progressive MS, particularly when other diseases were excluded and the cerebrospinal fluid-contained oligoclonal bands. The frequency of VEP abnormalities in such series is higher when there are clinical eye signs or oligoclonal bands or both (Bynke *et al.*, 1977). In a comparative cohort of patients with all forms of MS, Rot and Mesec (2006) found that there was a slightly lower proportion of primary progressive MS patients with delayed VEPs than the secondary progressive MS patients in the same cohort, however the numbers of patients in each group were small (eight secondary progressive MS patients and 14 primary progressive MS patients), impeding reliable conclusions about the relative frequency of VEP abnormalities in these groups. VEPs were not investigated in the present study. In a future study, VEP data would be helpful to complement the present observations, particularly as VEP data in these two groups of patients is scarce.

In addition to any regional differences in pathology between primary progressive MS and secondary progressive MS, the primary progressive MS group in the current study had a significantly shorter duration of disease than the secondary progressive MS group which may have an effect: there is also evidence that silent RNFL thinning occurs in the relapsing phase of the illness, even in the absence of clinically evident ON (Fisher *et al.*, 2006). The degree of RNFL thinning in eyes not affected by ON seen in the secondary progressive MS patients in our study (~10%) is similar to that seen in the group studied by (Fisher *et al.*, 2006). In the current study, no relationship with disease duration was observed. It is possible—and perhaps more plausible—that the mild axonal loss seen in the secondary progressive MS group could be due to clinically silent demyelinating optic nerve lesions that developed in the earlier relapsing phase of the illness, when inflammatory lesions are more likely to occur (although such lesions could also have developed during the secondary progressive MS phase). Serial OCT studies would help determine whether the retinal changes observed in progressive MS are stable or progressing.

Our study was focused on typical cases of primary and secondary progressive MS, and the former tend to have predominant clinical involvement of the spinal cord and smaller brain MRI lesions loads than patients with secondary progressive MS. It would be valuable in a future study to investigate OCT findings in a subgroup of primary progressive patients who are matched to secondary progressive MS patients with regard to clinical and radiological measures of brain involvement such as T2 lesion load, cognitive impairment and ataxia.

As in previously published OCT study in a predominantly relapsing-remitting cohort of patients, (Sepulcre *et al.*, 2007), the temporal quadrant of the RNFL was the most severely affected retinal measure, and was able to distinguish the two different subtypes of progressive MS from controls and each other. In the only pathological study of the retina in MS, Kerrison and colleagues (1994) found that RNFL atrophy was most marked in the 'maculopapillary bundle', which would be included in the temporal quadrant of the RNFL. In their detailed study of the appearance of the retina in MS using red-free photography, Kerrison and colleagues (MacFadyen *et al.*, 1988) found that focal defects of the RNFL were only found in the temporal half of the retina, although diffuse defects were evenly distributed across the whole retina. These findings were supported by the study of Elbol and Work (1990), who found that none of the localized defects they observed were in the nasal half of the retina, and that diffuse defects were seen in all parts of the retina. The current study would support the notion that the temporal nerve fibres of the retina are particularly susceptible to the insults associated with MS.

When considered as a proportion of respective control value, the reductions in macular volume were less than the reductions in RNFL thickness. In the whole patient cohort, mean reduction of the macular volume was 4.0% of the control value and the reduction in RNFL thickness was 7.9% of the control value. The primary insult in progressive MS is likely to be located in the optic nerve, and the RNFL atrophy reflects this. Whereas the ganglion cell bodies that lie within the macula might also be affected by optic nerve insults, the macular volume measurements include the full thickness of the retina, of which only part would be directly affected by an insult to the optic nerve.

The strong correlations observed between RNFL thickness and macular volume with visual function in a predominantly visually asymptomatic group of progressive patients suggests that OCT is capable of detecting functionally important visual impairment that is not clinically overt.

The lack of any such association in the control group suggests that the variation in OCT measures in this group reflects the natural variation in retinal anatomy. The standard deviation of the mean for RNFL in normal eyes is ~11% (Budenz *et al.*, 2007) and in our study the standard deviation of the mean for controls was 10.6% for RNFL thickness. This natural variation reduces the sensitivity of cross-sectional studies such as the current one to group-based change, suggesting that large cohorts will be required in order to detect small but potentially relevant differences. This is particular relevant when considering the OCT findings in primary progressive MS: it is notable that although this group did not have significantly smaller RNFL thickness than controls, there were significant correlations of the retinal and macular measures with visual function in the former but not latter

group, suggesting that subtle but functionally relevant retinal axonal loss occurs in primary progressive MS.

Although there was a strong correlation between visual disability and retinal measures, there was no association between overall disability (as assessed by the EDSS and MSSS) and retinal measures. The relationship between MS functional status and OCT measures has been investigated in other studies. Fisher and colleagues (2006) found an association between RNFL thickness and with EDSS and MSFC in a mainly RRMS cohort, and Sepulcre and colleagues (2007) found an association between EDSS and both overall RNFL and temporal quadrant RNFL also in a mainly RRMS cohort. We note that the current study includes a group of patients with an increased level of disability, and a narrower range of EDSS, which may affect our ability to test such associations. In addition, the higher EDSS associated with progressive MS is also often substantially influenced by spinal cord disease including axonal loss, and this may be relatively dissociated from pathological changes in the optic nerve (and retinal nerve fibre layer) when compared with the earlier relapsing remitting phase of MS where predominantly inflammatory demyelinating lesions determine both OCT abnormality and EDSS.

While acknowledging the limitations in sensitivity of a cross-sectional study, we found that OCT-based measures of RNFL thickness and macular volume were highly reproducible in repeated testing over a short interval; they therefore may be able to detect relatively small longitudinal changes within patients followed over time. OCT may be useful monitoring the efficacy of putative neuroprotective therapies, and observing clinically occult neurodegeneration in MS. Serial studies in MS are required to establish the longitudinal sensitivity and specificity for these measures.

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