eNeurologicalSci 1 (2015) 30–37

Contents lists available at ScienceDirect

eNeurologicalSci

journal homepage: http://ees.elsevier.com/ensci/

Retinal nerve fiber layer sector-specific compromise in relapsing and remitting multiple sclerosis

Anette S. Loughran-Fjeldstad [a,*], Noel G. Carlson [b,c], Cassandra D. Husebye [a], Lawrence J. Cook [d], John W. Rose [a,b]

[a] Department of Neurology, University of Utah, 729 Arapeen Drive, Salt Lake City, UT 84108, USA
[b] Neurovirology Research, VA SLC HCS, GRECC, 500 Foothill Drive, Salt Lake City, UT 84148, USA
[c] Department of Neurobiology & Anatomy, University of Utah, 20 North 1900 East, Salt Lake City, UT 84132 USA
[d] Department of Pediatrics, University of Utah, 295 Chipeta Way, Salt Lake City, UT 84108, USA

ARTICLE INFO

Article history:
Received 11 August 2015
Received in revised form 29 September 2015
Accepted 30 September 2015
Available online 9 October 2015

Keywords:
Axonal loss
Multiple sclerosis
Optic neuritis
Optical coherence tomography
Retinal nerve fiber layer
Total macular volume

ABSTRACT

Objective: To evaluate quadrant and sector retinal nerve fiber layer (RNFL) thickness and total macular volume (TMV) in relapsing–remitting multiple sclerosis (RR-MS) patients.
Methods: Optical coherence tomography measures of RNFL and TMV were studied in 321 eyes without prior optic neuritis (ON) (MS unaffected), 151 eyes with prior ON (MS affected), and 148 healthy control eyes.
Results: Mean RNFL thickness was significantly lower in the MS affected and MS unaffected groups relative to the control group ($p < 0.0001$). RNFL thicknesses in the superior, inferior, and temporal quadrants were significantly reduced in MS unaffected (113 ± 15 μm, 119 ± 17 μm, 63 ± 13 μm) ($p < 0.001$) and MS affected groups (99 ± 19 μm, 103 ± 21 μm, 51 ± 13 μm) ($p < 0.0001$) compared with that in controls (120 ± 14 μm, 128 ± 15 μm, 69 ± 8 μm, respectively). TMV was significantly reduced in both the MS affected and MS unaffected groups compared with that in the controls ($p < 0.0001$).
Conclusion: Quadrant, sector, and PMB RNFL thicknesses are significant individual measures in RR-MS for both affected and unaffected eyes and may prove valuable in future investigations including biomarker and outcomes research.

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory disease that affects the central nervous system (CNS) [1] and is a leading cause of neurological disability affecting young adults [2]. MS leads to progressive axonal loss and degeneration of neurons [3], and often result in permanent disability [4]. Optic neuritis (ON) [5] is the first demyelinating event in approximately 20% of MS patients, and 30–70% of MS patients experience ON during the course of the disease [6]. ON results in thinning of the retinal nerve fiber layer (RNFL) and compromise of the total macular volume (TMV) [7,8]. These detrimental retinal changes result from both axonal thinning with associated loss of neurofilament phosphorylation and subsequent axonal transection with retrograde degeneration [9,10]. Visual problems in MS can result from a range of pathologies, including inflammation, degeneration, demyelination, and axonal loss in the anterior visual pathway (retina, optic nerves, chiasm and tracts) [11]. Alterations in the visual system can be important indicators of CNS neurodegeneration and potential impact of neuroprotective therapy [12]. Optic atrophy and compromise of the RNFL are common findings on ophthalmologic examinations of patients with MS [13], and RNFL thinning in MS patients has been linked to brain atrophy [14] and volumetric changes in the white matter as assessed by magnetic resonance imaging [15]. Other studies found that RNFL and ganglion cell layer (GCL) associated with normalized brain volume, white matter volume and gray matter volume in relapsing and remitting (RR)-MS patients with previous ON [16], whereas Saidha et al. found the strongest relationship between GCL and whole-brain atrophy in progressive MS [17]. RNFL decrease and optic nerve atrophy are more prevalent in those with a prior history of ON [18,19], but can also occur throughout the natural progression of MS in the absence of ON [20].

Optical coherence tomography (OCT) is a non-invasive imaging technique that can quantify RNFL thickness in vivo [20]. Initially, OCT was used to not only examine retinal axonal loss in glaucoma [19,21], but also to demonstrate RNFL loss in the eyes of MS patients with [19,22] and without previous ON [18,19].

Our objective was to use spectral-domain (SD) OCT to examine the RNFL thickness with special attention to analyses of RNFL quadrants and sectors as well as TMV. These measurements were completed in patients diagnosed with RR-MS (with or without a prior history of ON) and compared to results obtained with healthy controls to determine

* Corresponding author at: Department of Neurology, University of Utah, Imaging & Neuroscience Center, 729 Arapeen Drive, Salt Lake City, UT 84108, USA.
E-mail addresses: a.fjeldstad@utah.edu (A.S. Loughran-Fjeldstad), noel.carlson@neuro.utah.edu (N.G. Carlson), dee.husebye@genetics.utah.edu (C.D. Husebye), Larry.cook@hsc.utah.edu (L.J. Cook), jrose@genetics.utah.edu (J.W. Rose).

http://dx.doi.org/10.1016/j.ensci.2015.09.004

the significance of the measurements, their distribution and their suitability as biomarkers for disease state. We hypothesized that when compared to controls, the thickness of the RNFL quadrants and the RNFL sectors may be selectively compromised in MS patients both with and without ON. Here we describe the injury to the RNFL in more detail through quadrant and sector analysis.

2. Materials and methods

2.1. Study design and patient population

A total of 236 patients from the University of Utah with clinically definite RR-MS (ages 49 ± 12 years) according to the revised 2005 McDonald criteria [23] and 74 healthy controls (ages 47 ± 12 years) participated in this cross-sectional study. MS sub-type classification was based on the clinical course and determined by the treating physician (JWR), and the ON attacks were documented in our medical records and determined by the treating physician (JWR). All MS patients underwent a fundoscopic eye exam and a visual acuity test at the time of the clinic visit. Patients with a history of eye disease that may affect OCT measures (e.g. glaucoma, retinal disease, age-related macular degeneration, diabetes, neuromyelitis optica (NMO), Alzheimer's disease or Parkinson's disease) were excluded. Patients had to be free of relapse and ON for at least six months prior to the OCT assessment. None of the patients were taking Gilenya® (fingolimod) at the time of the study.

One goal of this study was also to assess whether there were RNFL differences between the MS patients that had 2 affected eyes compared with 1 affected eye, and similarly, examine potential differences between MS patients with 2 affected eyes compared with 2 unaffected eyes. The rationale behind this partitioned approach is based on evidence from previous studies that suggest a more frequent thinning of the RNFL in eyes with a prior history of ON [18,19], but this can also occur through the natural progression of MS in the absence of ON [20]. Even if the clinical burden of MS is low, a subclinical loss of RNFL in MS patients without prior ON has been suggested [22].

2.2. Ethics

These investigations were approved by the Institutional Review Board of the University of Utah, and all participants provided written informed consent prior to their participation in accordance with the ethical standards of the 1964 Declaration of Helsinki.

2.3. Spectral-domain optical coherence tomography

The subjects in this study underwent SD-OCT examination using the Heidelberg Engineering Spectralis SD-OCT (Heidelberg Engineering, Heidelberg, Germany, Spectralis software version 5.6.3.0, Eye Explorer Software 1.7.1.0). This SD-OCT utilizes a scanning superluminescence diode to emit a scan beam with a wavelength of 870 nm to acquire 40,000 A scans/s with a depth resolution of 7 μm, from which various retinal layers can be identified, and objectively and precisely assessed [24] (Fig. 1). The OCT device also combines OCT and confocal infrared laser ophthalmoscope, which provides a reference infrared fundus image [24]. The combination of eye tracking (TruTrac) and high-speed scanning reduce noise caused by natural eye movement [25]. In addition, TMV scan (30° × 25°, 61 B-scans (121 μm distance between B-scans), ART 9; 768 A-scans each) was centered over the macula after the TruTrac was activated. The following OCT parameters were calculated: global mean RNFL thickness around the optic nerve head from nasal, inferior, temporal, superior to nasal (NITSN) quadrant, and further RNFL sector thicknesses (temporal–inferior; TI, temporal–superior; TS, nasal–inferior; NI, nasal–superior; NS and peripapillary macular bundle (PMB)). The mean retinal thickness was measured in μm per quadrant of the peripapillary area whereas the macular scan provided volume data (mm^3). All scans were performed by an experienced operator (ASL-F). The images were subsequently reviewed for acceptable signal strength (>25), correct placement of the scan ring and appropriate beam placement.

2.4. Expanded disability status scale (EDSS)

The EDSS scale ranges from 0 (no disability) to 10 (death from MS) [26]. EDSS scores for the MS patients participating in the study were determined by the treating neurologist (JWR) and ranged from 0–7 with a mean of 1.7 (Table 2).

2.5. Data analyses

For our studies we have examined the eyes of patients and healthy controls by status as unaffected or affected by ON. Group 1 and group 2 together are labeled the 'MS unaffected group'. Group 3 and group 4 together are labeled the 'MS Affected group'. In more detail: The MS unaffected group consists of patients with 2 eyes not affected by optic neuritis (*number of eyes* = 256) in addition to the patients that have unilateral optic neuritis (*number of eyes* = 65), where the non-optic neuritis eyes are included in the 'MS unaffected group' (*Total number of eyes* = 321) (Table 1). The same grouping procedures were applied when creating the 'MS Affected group' (*Total number of eyes* = 151). Here we included the eyes of patients with bilateral optic neuritis (*number of eyes* = 86) and the eyes that were affected in the unilateral optic neuritis group (*number of eyes* = 65). These five groups of eyes are summarized as;

Here we compare the eyes in the MS unaffected (no prior optic neuritis) and MS affected (prior optic neuritis) groups with the healthy control group. Further, the MS patients that had 2 affected eyes and the MS patients that had 2 unaffected eyes were also compared with the healthy control eyes. Similarly, the MS patients that had 1 affected eye and 1 unaffected eye were compared with the control eyes. In addition, the MS patients that had either 2 affected or 2 unaffected eyes were compared with those MS patients that had only 1 affected or 1 unaffected eye. Only MS patients and healthy controls with complete data for both eyes were included in the analyses.

2.6. Statistical analyses

This was a cross-sectional exploratory study design, where no adjustments for multiple comparisons were made. Kolmogorov and Smirnov test was performed on all dependent variables to test for assumption of normality. Mann–Whitney *U*-tests were performed to examine significant differences in age between the groups. Partial correlation analyses, adjusted for disease duration, were used to examine the relationships between EDSS and OCT parameters in the MS groups. Statistical differences of the various OCT variables between the groups were calculated with a linear model using generalized estimating equations (GEE) with compound symmetry correlation structure to account for gender and within-subject inter-eye differences. Statistical analyses were performed using commercially available software (SPSS 17.0, SPSS Inc., Chicago, IL and Graphpad Software InStat 3.0, La Jolla, CA). All of the data are expressed as mean ± SD. Statistical significance was set as $p \leq 0.05$.

3. Results

3.1. Patient population

We enrolled 310 subjects including 74 healthy controls and 236 patients with relapsing and remitting multiple sclerosis. The RR-MS group included patients without ON, with unilateral ON, and with bilateral ON. The subject demographics are presented in Table 2. There were no significant differences in age between individuals in the MS group as a

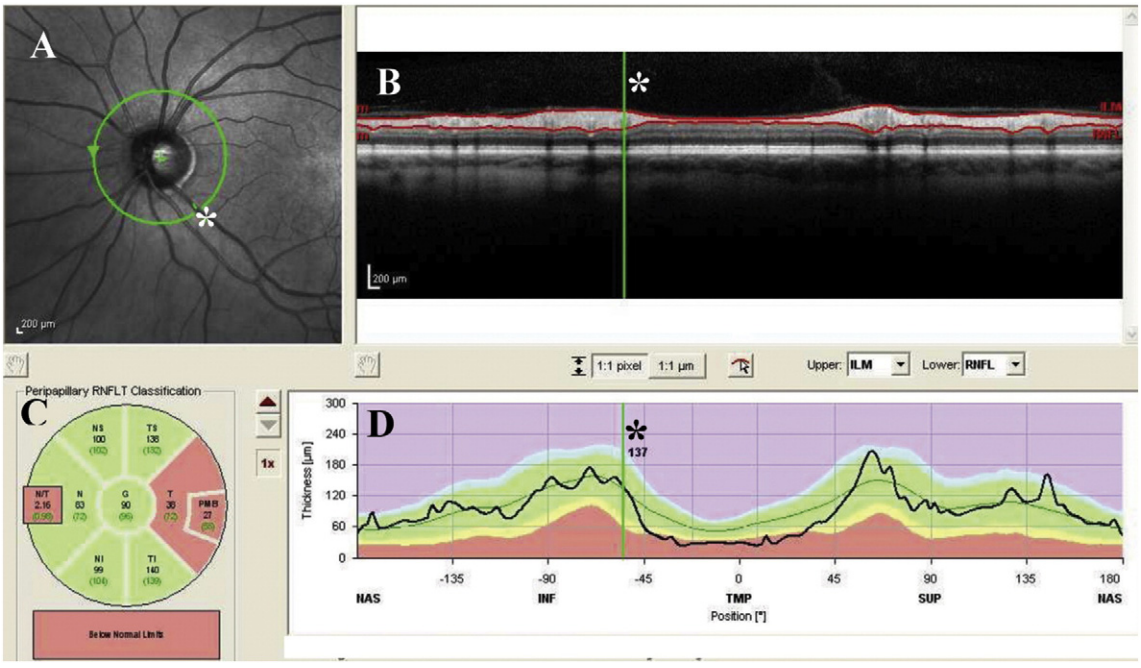

Fig. 1. Example of an OCT scan. Optic nerve head scan. A) A circular scan centered around the optic nerve of a patient diagnosed with RR-MS and with a previous history of ON. The scan starts at the arrow seen to the right of the circle (nasal sector). For orientation, an asterisk is inserted. B) This panel depicts the OCT cross-sectional image of RNFL from the circle scan. The vertical line corresponds to the position of the asterisk shown in image A. C) Data are shown in micrometer of RNFL thickness for all retinal sectors, including the peripapillary macular bundle (PMB). Quantitative data collected by the OCT are overall global (G) (360°) mean RNFL thickness around the optic nerve; quadrant RNFL thickness; temporal (T), superior (S), inferior (I), and nasal (N) (each 90°); sector RNFL thickness; temporal–superior (TS; 45–90°), nasal–superior (NS; 90–135°), nasal–inferior (NI; 225–270°), and temporal–inferior (TI; 270–315°). D) RNFL thickness is measured across 768 locations (A-scans). The black line indicates the patient's score around the optic nerve from nasal, inferior, temporal, superior to nasal (NITSN), while the solid green line is the age-adjusted mean thickness values from the normative database. The green color is within normal limits (values inside 95% normal range), purple is above normal limits (values outside 99% confidence interval (CI)), and blue which is borderline above normal limits (values outside 95%, but within 99% CI of the normal range). Yellow is borderline below (values outside 95%, but within 99% of the CI of the normal distribution) and the red color is below normal limits (values outside the 99% CI of the normal distribution). The green vertical line corresponds to the position of the asterisk in images A, B, and D. Findings of the OCT: the RNFL thickness of this patient is depicted by the black line in image 1D. The thickness chart of this patients shows that the global mean, superior, inferior and nasal quadrants are within normal limits (green color). The temporal quadrant and PMB are below normal limits (red color; values outside the 99% CI of the normal distribution).

whole and the control group, or between the MS patients with optic neuritis and the MS patients without optic neuritis ($p > 0.05$). There was a 3.1:1 ratio of women to men in the MS group and a 1.1:1 ratio of women to men in the control group. Average disease duration was 14 ± 8 years (range 1–45 years). There were no gender differences in RNFL values in the control group or in the MS affected group. However, the males in the MS unaffected group had slightly lower mean RNFL values compared with the females ($p < 0.05$) (data not shown).

3.2. RNFL thickness and TMV in MS affected and MS unaffected groups and controls

Mean RNFL thickness was significantly reduced in the MS affected and the MS unaffected groups relative to the healthy controls ($p < 0.0001$). The MS affected group had a lower RNFL in comparison to the MS unaffected group ($p < 0.0001$). Mean TMV was lower in both MS affected and MS unaffected groups compared to that in the control group ($p < 0.0001$). The MS affected group had a reduced TMV relative to the MS unaffected group ($p < 0.0001$) (Fig. 2A & B).

Table 1
Grouping of eyes for analysis.

1. MS eyes without ON ($n = 256$)	MS unaffected group ($n = 321$)
2. MS eyes unaffected by unilateral ON ($n = 65$)	
3. MS eyes affected by unilateral ON ($n = 65$)	MS affected group ($n = 151$)
4. MS eyes affected by bilateral ON ($n = 86$)	
5. Healthy control eyes ($n = 148$)	

MS; multiple sclerosis, ON; optic neuritis, *n*; number of eyes.

3.3. RNFL quadrants and PMB thicknesses in MS affected and MS unaffected groups and controls

RNFL thickness in the superior (S), inferior (I) and temporal (T) quadrants was reduced in the MS unaffected group compared to that in the control group ($p \leq 0.001$), and different across all four quadrants (S, I, N (nasal), T) relative to the MS affected group ($p < 0.0001$). RNFL thickness for all four quadrants was also significantly lower in the MS affected group compared to that in the MS unaffected group ($p < 0.0001$). The PMB thickness was reduced in the MS affected and

Table 2
Clinical characteristics of study participants.

Characteristics	Controls ($n = 74$)	MS patients ($n = 236$)
Age (yrs)	47 ± 12	49 ± 12
Age range (yrs)	27–73	20–82
Sex		
Male, *n* (%)	34 (46)	54 (23)
Female, *n* (%)	40 (54)	182 (77)
Bilateral unaffected eyes, *n* (%)	148 (100)	256 (54)
Bilateral affected eyes, *n* (%)	n/a	86 (18)
Unilateral unaffected eyes, *n* (%)	n/a	65 (14)
Unilateral affected eyes, *n* (%)	n/a	65 (14)
Disease duration (yrs)	n/a	14 ± 8
DMT, *n* (%)	n/a	214 (90)
EDSS	n/a	1.7 ± 1.7
EDSS range (median)	n/a	0–7 (3)

Mean ± SD, ON; optic neuritis, DMT; disease-modifying therapies, EDSS; expanded disability status scale.

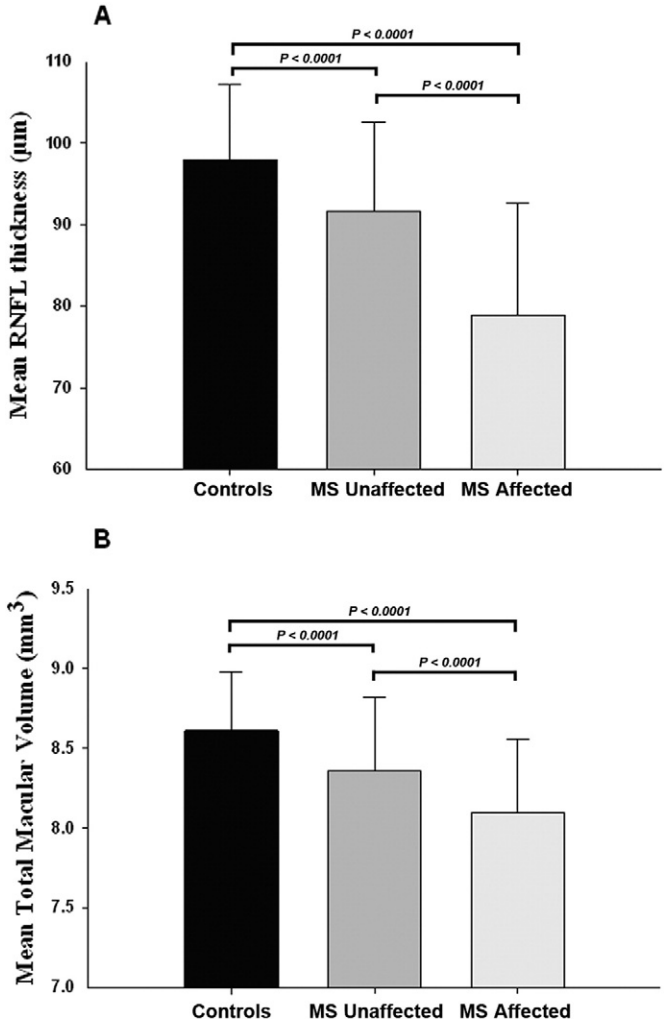

Fig. 2. RNFL and TMV comparisons between controls and MS patients. A) Mean RNFL thicknesses in controlsand MS unaffected and MS affected groups. Significant differences between the groups are indicated with *p*-values. Note that the y-axis starts at 60 µm. B) Mean TMV in controlsand MS unaffected and MS affected groups. Significant differences between the groups are indicated with *p*-values. Note that the y-axis starts at 7 mm^3.

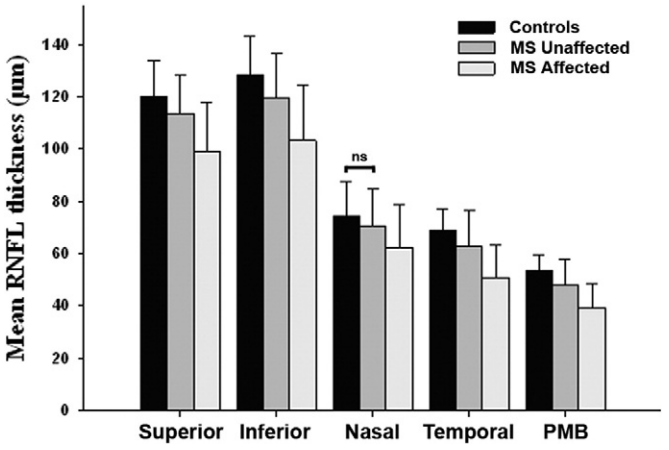

Fig. 3. Comparisons of RNFL quadrants between controls and MS patients. Mean RNFL quadrant thicknesses and PMB sector thickness in controlsand MS unaffected and MS affected groups. All RNFL comparisons were significant between groups at $p < 0.0001$, except for controls compared with the MS unaffected group for the nasal quadrant, as depicted in the graph.

MS unaffected groups relative to the control group ($p < 0.0001$), and lower in the MS affected group compared to that in the MS unaffected group ($p < 0.0001$) (Fig. 3).

3.4. RNFL quadrant and RNFL sector thicknesses in the MS eyes and control eyes

When MS patients' eyes as a pooled cohort were compared to the healthy control eyes, both the RNFL quadrants and the RNFL sectors (TS, NS, NI and TI) were significantly reduced ($p \leq 0.001$). Similar results were observed when comparing MS 2 affected eyes ($p \leq 0.03$) and MS 1 affected eye ($p \leq 0.01$) to healthy controls. The MS 2 unaffected eyes scored significantly lower compared with the control group for mean RNFL, superior, and temporal quadrants ($p \leq 0.02$), in addition to NI, TS, TI and PMB RNFL sectors ($p \leq 0.05$). Further, only the inferior quadrant showed significance when comparing MS patients with 2 affected eyes to MS patients with 1 affected eye ($p = 0.04$). Patients with 2 affected eyes showed significantly lower RNFL quadrant and RNFL sector scores compared with MS 2 unaffected eyes ($p \leq 0.01$), with the exception of the inferior quadrant ($p = 0.2$). The MS patients with 1 affected eye showed significantly lower scores for all RNFL quadrants and RNFL sectors relative to MS patients with 1 unaffected eye ($p \leq 0.003$). Thus, the PMB seems to have a selective vulnerability from a more distinct region of the RNFL (Table 3).

3.5. Expanded disability status scale correlates

There was no difference in EDSS scores between the MS unaffected and the MS affected group ($p > 0.05$). However, when comparing the patients with 1 affected eye (1.9 ± 1.8), EDSS score was significantly lower in the MS patients with 2 affected ON eyes (1.5 ± 1.4) ($p \leq 0.01$), and in patients with 2 unaffected eyes (1.6 ± 1.6) ($p \leq 0.0001$). EDSS correlated with mean TMV ($r = -0.33$, $p = 0.001$) and temporal quadrant ($r = -0.25$, $p = 0.01$) in the MS affected group, while correlations with mean RNFL ($r = -0.16$, $p = 0.03$), superior quadrant ($r = -0.15$, $p = 0.04$), inferior quadrant ($r = -0.15$, $p = 0.04$), and NI RNFL sector ($r = -0.22$, $p = 0.003$) were significant in the MS unaffected group. EDSS correlated with the temporal quadrant ($r = -0.28$, $p = 0.03$) and TMV ($r = -0.26$, $p = 0.05$) in the MS group with 1 unaffected eye and with TMV in the group with 1 affected eye ($r = -0.31$, $p = 0.02$) (data not shown).

4. Discussion

The goal of our study was to examine OCT measurements of the retinal nerve fiber layer in RR-MS patients. The basic investigations of RNFL and TMV in our patient population demonstrated that: 1) there was significant RNFL thinning of 12% in our RR-MS patients regardless of ON status compared to that in healthy controls, 2) there was significant RNFL thinning of about 15% in the MS affected group compared to that in the MS unaffected group, and 3) there was a significant decrease in TMV in the MS patients as a group of about 4% compared to that in healthy controls and a 3% decrease in the MS affected group vs. the MS unaffected group. In our patient population there was significant loss in RNFL thickness and TMV in the eyes with a clinical history of ON compared with that in the unaffected eyes and healthy control eyes which are similar to previous studies [18,27]. These results confirm previous investigations and demonstrate reproducibility of these measurements among comparable patient populations.

4.1. RNFL measures by quadrants and sectors in MS patients and healthy controls

Our study revealed a thinning of the mean RNFL, the RNFL quadrants, PMB and RNFL sectors in MS patients compared to that in the control group. In addition, the MS patients with prior ON showed

Table 3
Controls, MS unaffected and MS affected eyes.

RNFL quadrants & sectors	Control eyes ($n = 148$)	All MS eyes ($n = 472$)	MS 2 unaffected eyes ($n = 256$)	MS 1 unaffected eye ($n = 65$)	MS 2 affected eyes ($n = 86$)	MS 1 affected eye ($n = 65$)	Control vs. all MS *P*-value	Control vs. MS 2 unaffected *P*-value
RNFL (μm)	98 ± 9	87 ± 13	93 ± 10	89 ± 12	77 ± 14	80 ± 13	***0.0001***	***0.004***
Superior (μm)	120 ± 14	108 ± 18	115 ± 14	110 ± 15	97 ± 19	101 ± 19	***0.0001***	***0.02***
Inferior (μm)	128 ± 15	113 ± 20	121 ± 15	116 ± 19	101 ± 20	105 ± 22	***0.0001***	0.3
Nasal (μm)	74 ± 13	68 ± 16	71 ± 13	70 ± 17	61 ± 17	64 ± 16	***0.001***	0.4
Temporal (μm)	69 ± 8	58 ± 14	64 ± 13	60 ± 14	51 ± 12	51 ± 13	***0.0001***	***0.001***
Nasal–superior (μm)	104 ± 17	95 ± 19	101 ± 17	97 ± 17	84 ± 20	89 ± 21	***0.001***	0.3
Nasal–inferior (μm)	112 ± 21	101 ± 22	105 ± 20	106 ± 23	90 ± 23	97 ± 22	***0.0001***	***0.05***
Temporal–superior (μm)	136 ± 14	121 ± 21	128 ± 16	123 ± 18	109 ± 22	113 ± 23	***0.0001***	***0.001***
Temporal–inferior (μm)	145 ± 15	125 ± 25	136 ± 19	125 ± 23	111 ± 23	114 ± 29	***0.0001***	***0.001***
PMB (μm)	53 ± 6	45 ± 11	49 ± 9	45 ± 11	38 ± 9	40 ± 10	***0.0001***	***0.0001***
TMV (mm^3)	8.6 ± 0.5	8.3 ± 0.5	8.4 ± 0.4	8.3 ± 0.5	8.1 ± 0.4	8.1 ± 0.5	***0.0001***	***0.009***

Mean ± SD, MS; multiple sclerosis, RNFL; retinal nerve fiber layer, TMV; total macular volume. (n); number of eyes, significant values are depicted in bold italics. $p \leq 0.05$.

Table 3 (*continued*)

RNFL quadrants & sectors	Control vs. MS 2 affected *P*-value	Control vs. MS 1 unaffected *P*-value	Control vs. MS 1 affected *P*-value	MS 2 affected vs. MS 1 affected *P*-value	MS 2 affected vs. MS 2 unaffected *P*-value	MS 2 affected vs. MS 1 unaffected *P*-value	MS 1 affected vs. MS 1 unaffected *P*-value
RNFL (μm)	***0.0001***	***0.0001***	***0.0001***	0.8	***0.0001***	***0.001***	***0.0001***
Superior (μm)	***0.0001***	***0.0001***	***0.0001***	0.3	***0.0001***	***0.0001***	***0.0001***
Inferior (μm)	***0.03***	***0.04***	***0.01***	***0.04***	0.2	0.3	***0.0001***
Nasal (μm)	***0.004***	0.06	***0.0001***	0.9	***0.005***	0.2	***0.0001***
Temporal (μm)	***0.0001***	***0.0001***	***0.0001***	0.8	***0.0001***	***0.0001***	***0.0001***
Nasal–superior (μm)	***0.0001***	***0.02***	***0.0001***	0.3	***0.0001***	***0.001***	***0.003***
Nasal–inferior (μm)	***0.0001***	0.09	***0.0001***	0.5	***0.01***	***0.02***	***0.0001***
Temporal–superior (μm)	***0.0001***	***0.0001***	***0.0001***	0.5	***0.0001***	***0.001***	***0.0001***
Temporal–inferior (μm)	***0.0001***	***0.0001***	***0.0001***	0.2	***0.0001***	***0.001***	***0.0001***
PMB (μm)	***0.0001***	***0.0001***	***0.0001***	0.2	***0.0001***	***0.0001***	***0.0001***
TMV (mm^3)	***0.0001***	***0.0001***	***0.0001***	1	***0.002***	0.6	0.1

greater thinning than the MS patients with no history of ON. Our results agree with studies by Oberwahrenbrock et al. [18], Bock et al. [19], and Lange et al. [28] who found a pronounced thinning of the RNFL and reduction of TMV [18] in the MS eyes compared to that in the healthy control eyes, and in MS affected eyes compared to that in MS unaffected eyes. However, in contrast to Oberwahrenbrock et al. [18] who only reported the mean RNFL and TMV, our study examined the RNFL quadrants, PMB and sectors of the RNFL in detail. We have identified that the temporal quadrant seems to be a more vulnerable area within the RNFL in MS patients, which is in agreement with the findings of Winges et al. [29]. The temporal quadrant contains fibers of the PMB [30,31] which appeared to be a compromised area with clear distinction between the controls, MS unaffected and MS affected groups.

The temporal quadrant followed by the superior and inferior quadrants in our study demonstrated similar involvement, but in contrast, the nasal quadrant was less impacted. The TS and TI RNFL sectors were the most significant in relationship to controls vs. the MS unaffected group, whereas all quadrants and RNFL sectors were reduced in the MS affected group compared with that in the controls. These results are similar to that of Serbecic et al. [7] who also examined RNFL sector measurements in MS patients with and without previous ON and found a reduced RNFL globally in all MS groups compared to that in controls. In addition, the temporal quadrant, TI and TS RNFL sectors were reduced in the MS group with prior ON, whereas the MS group without previous ON only showed a significant reduction in TS. Garcia-Martin et al. [32] found a decrease in average RNFL, SN (superior–nasal), IN (inferior–nasal), IT (inferior–temporal), ST (superior–temporal) sectors, and temporal quadrant in the MS eyes compared with the healthy control eyes. However, when they compared the MS eyes with ON to the MS eyes without ON, only the temporal quadrant reached statistical significance. The lack of significance in other areas may be a result of the relatively small number of eyes studied. They only studied 75 eyes without prior ON and 25 eyes with previous ON, whereas our study examined 321 unaffected eyes and 151 affected eyes.

Previous studies have shown that RNFL thickness was 46% lower in the MS eyes with prior ON compared to that in 'healthy' control eyes, 26% reduced RNFL in unaffected MS eyes compared with that in control eyes, and 28% lower RNFL in affected eyes compared to that in the unaffected eye of the same patient [6,22]. A retrospective study by Winges et al. found a 34% and 25% reduction in RNFL and TMV, respectively in RR-MS patients that scored less than 5th percentile of normal limits [29]. During the inflammatory process of ON, the optic nerve can swell secondary to inflammation, and in the months following an acute event, the swelling subsides and subsequently the optic nerve decreases in size. It has also been suggested that RNFL thinning becomes more prominent in the 3–6 months following an inflammatory event [33], which is why the patients with ON in the 6 months preceding the measurements were excluded from our study.

4.2. TMV in MS patients and healthy controls

Total macular volume includes all the neuronal layers of the retina [34] and reflects the number of retinal ganglion cell bodies and axons, which have shown to be decreased in the ON eyes [35]. In agreement with other studies [18,36], our study revealed a reduced volume in MS patients compared to that in the control group, and the MS patients with prior ON displayed even lower volumes than the MS patients with no history of ON. In agreement with our results, Henderson et al. showed a lower baseline TMV in their MS patients compared with that in healthy controls. In addition, the same study found no difference in TMV reduction over time between healthy controls and progressive MS patients, though, the small number of subjects in their study and the use of time-domain OCT are limitations. However, these results make us question if TMV changes are more related to normal aging rather than disease changes [36], and if it is appropriate to use TMV as a measurement of neuronal degeneration. It has been suggested that early in the disease course there may be some underlying micro-inflammatory disease activity [37], and the use of disease-modifying therapies [38] may explain why there was no difference in TMV between MS patients with 2 affected eyes and MS patients with 1 affected eye in our study. We will be looking to examine this issue as we divide the MS eyes into 4 groups. This analysis may suggest that the TMV becomes equally compromised both with bilateral and unilateral ON.

4.3. RNFL quadrants and sectors in MS affected and MS unaffected eyes

After separating the MS group according to the number of eyes affected with and without ON, we observed that MS patients with 2 affected eyes had decreased thickness in several RNFL quadrants and in all RNFL sectors compared to MS patients with 2 unaffected eyes. Significant differences in RNFL quadrant and sector values between the patients with 1 affected eye relative to the MS patients with 1 unaffected eye were detected. Although, we observed a trend with lower RNFL scores in the patients with 2 affected eyes compared to the MS patients that had 1 affected eye, only the inferior quadrant reached statistical significance.

4.4. Expanded disability status score correlates

We found significant correlations between EDSS and mean RNFL, inferior quadrant, superior quadrant, and NI RNFL sector in the MS unaffected group. Also, EDSS significantly correlated with the temporal quadrant and TMV in the MS affected group. In addition, the MS group with 1 unaffected eye showed correlations between EDSS and the temporal quadrant and TMV, and the MS group with 1 affected eye revealed a correlation between EDSS and TMV. EDSS scores have previously been shown to be associated with decreased RNFL and TMV in MS patients with and without prior ON [39], which is similar to our findings, while another study reported a correlation between EDSS and RNFL in untreated MS patients [40]. TMV seems to be the best correlate with EDSS in our patient population. Although, several of the comparisons in our study displayed low r values, suggesting a weak association that remained statistically significant after we adjusted for disease duration, was attributed to the large sample size in our study.

4.5. Sex-specific differences in RNFL

There were no significant differences in RNFL between men and women in the control group or in the MS affected group. However, we found that the males had slightly lower RNFL values compared to the females in the MS unaffected group. Costello et al. showed that baseline RNFL values did not differ in the ON eyes between men and women, but found that male MS patients displayed greater RNFL thinning 6 months following ON compared to women, suggesting that there may be a difference in visual recovery following episodes of ON [41]. Men often show worse clinical outcomes and faster disease progression relative to women [42], and this difference may be attributed to sex hormones [43], specifically estrogen [44,45].

5. Limitations

There are some limitations in our study that should be noted. The control group (74 subjects) was relatively small compared to the MS group (236 subjects), and the male proportion was particularly smaller than the women in the MS group. However, this reflects the higher incidence of MS in women, but it is possible that our results were affected, hence we adjusted for gender in the analyses (GEE model). This study was confined to only RR-MS patients, so the results should not be generalized to other MS sub-types. Also, this was a cross-sectional exploratory study design, and cannot infer cause and effect, but this issue will be addressed with a longitudinal study which is currently underway.

6. Conclusions

As OCT begins to emerge as a valuable tool for assessing disease severity, it is important to understand its potential strengths and limitations with respect to sensitivity and fidelity in measuring degeneration in MS patients. This is particularly important for comparing results between different MS centers. Our results demonstrate that OCT measurements can be comparable to those at other institutions. Our study is unique in that we also focused on the various quadrant thicknesses, the PMB and RNFL sector thicknesses, to identify regional differences in contrast to the majority of studies that have primarily focused on mean RNFL thickness. In addition, we validated the results with our patient population compared to other studies and we found similar results for mean RNFL and TMV showing uniformity of this measurement between centers. We also detailed RNFL sector segmentation analysis showing that the nasal quadrant was least affected for significant changes in our MS patients, while other studies have found the nasal quadrant to be more sensitive to change in NMO patients with ON compared with MS ON patients by using the ratio of nasal to temporal RNFL thickness for comparing quadrant-wise thinning [46]. Also, a review study by Bennett et al. showed that NMO patients without a history of ON had normal RNFL thicknesses [47]. Future OCT studies may be important in examining if there are different RNFL injury patterns between NMO and MS patients with ON, and if nasal quadrant injury is more common in NMO and temporal quadrant damage is more prominent in MS. In addition, our study demonstrated that RNFL thickness in the PMB distinguishes MS affected and MS unaffected eyes from controls as well as differentiating MS affected eyes from MS unaffected eyes.

Identifying reproducible and significant measures of neurodegeneration in MS would allow for biomarker development with the potential to assist with diagnosis, ongoing disease course evaluation and pharmacologic disease management [48]. Future studies including large multicenter studies may focus on RNFL changes in quadrants and sectors for investigation of early MS, secondary progressive MS and primary progressive MS, natural history, clinical trials and biomarker correlations.

Funding

VA Merit Review (JWR).

Conflict of interest

The authors have no conflict of interest.

Acknowledgments

The authors thank
Sonja and Kent Davis for their support of the OCT,
Alice and Kevin Steiner for research support, and
James Burns MD, for the review of the manuscript.

References

[1] H. Lassmann, W. Bruck, C.F. Lucchinetti, The immunopathology of multiple sclerosis: an overview, Brain Pathol. 17 (2) (2007) 210–218 (Zurich, Switzerland).
[2] J.H. Noseworthy, C. Lucchinetti, M. Rodriguez, et al., Multiple sclerosis, N. Engl. J. Med. 343 (13) (2000) 938–952.
[3] A. Compston, A. Coles, Multiple sclerosis, Lancet 359 (9313) (2002) 1221–1231.
[4] J. Sepulcre, J.C. Masdeu, J. Sastre-Garriga, et al., Mapping the brain pathways of declarative verbal memory: evidence from white matter lesions in the living human brain, NeuroImage 42 (3) (2008) 1237–1243.
[5] F. Costello, W. Hodge, Y.I. Pan, Exploring the association between retinal nerve fiber layer thickness and initial magnetic resonance imaging findings in patients with acute optic neuritis, Mult. Scler. Int. 2011 (2011), 289785.
[6] E.M. Frohman, J.G. Fujimoto, T.C. Frohman, et al., Optical coherence tomography: a window into the mechanisms of multiple sclerosis, Nat. Clin. Pract. 4 (12) (2008) 664–675.
[7] N. Serbecic, F. Aboul-Enein, S.C. Beutelspacher, et al., Heterogeneous pattern of retinal nerve fiber layer in multiple sclerosis. High resolution optical coherence tomography: potential and limitations, PLoS One 5 (11) (2010), e13877.
[8] B.M. Burkholder, B. Osborne, M.J. Loguidice, et al., Macular volume determined by optical coherence tomography as a measure of neuronal loss in multiple sclerosis, Arch. Neurol. 66 (11) (2009) 1366–1372.
[9] B.D. Trapp, J. Peterson, R.M. Ransohoff, et al., Axonal transection in the lesions of multiple sclerosis, N. Engl. J. Med. 338 (5) (1998) 278–285.
[10] R.T. Naismith, J. Xu, N.T. Tutlam, et al., Radial diffusivity in remote optic neuritis discriminates visual outcomes, Neurology 74 (21) (2010) 1702–1710.
[11] R.E. Sakai, D.J. Feller, K.M. Galetta, et al., Vision in multiple sclerosis: the story, structure–function correlations, and models for neuroprotection, J. Neuroophthalmol. 31 (4) (2011) 362–373.
[12] K. Lidster, D. Baker, Optical coherence tomography detection of neurodegeneration in multiple sclerosis, CNS Neurol. Disord. Drug Targets 11 (5) (2012) 518–527.
[13] C. Fjeldstad, M. Bemben, G. Pardo, Reduced retinal nerve fiber layer and macular thickness in patients with multiple sclerosis with no history of optic neuritis identified by the use of spectral domain high-definition optical coherence tomography, J. Clin. Neurosci. 18 (11) (2011) 1469–1472.
[14] E. Grazioli, R. Zivadinov, B. Weinstock-Guttman, et al., Retinal nerve fiber layer thickness is associated with brain MRI outcomes in multiple sclerosis, J. Neurol. Sci. 268 (1–2) (2008) 12–17.
[15] K.L. Young, A.U. Brandt, A. Petzold, L.Y. Reitz, F. Lintze, F. Paul, et al., Loss of retinal nerve fibre layer axons indicates white but not grey matter damage in early multiple sclerosis, Eur. J. Neurol. 20 (5) (2013) 803–811.
[16] H. Zimmermann, A. Freing, F. Kaufhold, et al., Optic neuritis interferes with optical coherence tomography and magnetic resonance imaging correlations, Mult. Scler. 19 (4) (2013) 443–450.
[17] S. Saidha, O. Al-Louzi, J.N. Ratchford, et al., Optical coherence tomography reflects brain atrophy in MS: a four year study, Ann. Neurol. (2015).
[18] T. Oberwahrenbrock, S. Schippling, M. Ringelstein, F. Kaufhold, H. Zimmermann, N. Keser, et al., Retinal damage in multiple sclerosis disease subtypes measured by high-resolution optical coherence tomography, Mult. Scler. Int. 2012 (2012), 530305.
[19] M. Bock, A.U. Brandt, J. Dorr, et al., Patterns of retinal nerve fiber layer loss in multiple sclerosis patients with or without optic neuritis and glaucoma patients, Clin. Neurol. Neurosurg. 112 (8) (2010) 647–652.
[20] A. Petzold, J.F. de Boer, S. Schippling, P. Vermersch, R. Kardon, A. Green, et al., Optical coherence tomography in multiple sclerosis: a systematic review and meta-analysis, Lancet Neurol 9 (9) (2010) 921–932.
[21] R.A. Costa, M. Skaf, L.A. Melo Jr., et al., Retinal assessment using optical coherence tomography, Prog. Retin. Eye Res. 25 (3) (2006) 325–353.
[22] V. Parisi, G. Manni, M. Spadaro, et al., Correlation between morphological and functional retinal impairment in multiple sclerosis patients, Invest. Ophthalmol. Vis. Sci. 40 (11) (1999) 2520–2527.
[23] C.H. Polman, S.C. Reingold, G. Edan, M. Filippi, H.P. Hartung, L. Kappos, et al., Diagnostic criteria for multiple sclerosis: 2005 revisions to the ""McDonald Criteria"", Ann. Neurol 58 (6) (2005) 840–846.
[24] N. Serbecic, S.C. Beutelspacher, F.C. Aboul-Enein, et al., Reproducibility of high-resolution optical coherence tomography measurements of the nerve fibre layer with the new Heidelberg spectralis optical coherence tomography, Br. J. Ophthalmol. 95 (6) (2011) 804–810.
[25] N. Serbecic, F. Aboul-Enein, S.C. Beutelspacher, et al., High resolution spectral domain optical coherence tomography (SD-OCT) in multiple sclerosis: the first follow up study over two years, PLoS One 6 (5) (2011), e19843.
[26] J.F. Kurtzke, Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS), Neurology 33 (11) (1983) 1444–1452.
[27] M.L. Monteiro, D.B. Fernandes, S.L. Apostolos-Pereira, et al., Quantification of retinal neural loss in patients with neuromyelitis optica and multiple sclerosis with or without optic neuritis using Fourier-domain optical coherence tomography, Invest. Ophthalmol. Vis. Sci. 53 (7) (2012) 3959–3966.
[28] A.P. Lange, F. Zhu, A.L. Sayao, et al., Retinal nerve fiber layer thickness in benign multiple sclerosis, Mult. Scler. 10 (2013) 1275–1281.
[29] K.M. Winges, J.S. Werner, D.J. Harvey, et al., Baseline retinal nerve fiber layer thickness and macular volume quantified by OCT in the north American phase 3 fingolimod trial for relapsing–remitting multiple sclerosis, J. Neuroophthalmol. 33 (4) (2013) 322–329.
[30] A.J. Green, S. McQuaid, S.L. Hauser, et al., Ocular pathology in multiple sclerosis: retinal atrophy and inflammation irrespective of disease duration, Brain 133 (Pt 6) (2010) 1591–1601.
[31] J.M. Gelfand, D.S. Goodin, W.J. Boscardin, et al., Retinal axonal loss begins early in the course of multiple sclerosis and is similar between progressive phenotypes, PLoS One 7 (5) (2012), e36847.
[32] E. Garcia-Martin, V. Pueyo, I. Pinilla, et al., Fourier-domain OCT in multiple sclerosis patients: reproducibility and ability to detect retinal nerve fiber layer atrophy, Invest. Ophthalmol. Vis. Sci. 52 (7) (2011) 4124–4131.
[33] F. Costello, S. Coupland, W. Hodge, G.R. Lorello, J. Koroluk, Y.I. Pan, et al., Quantifying axonal loss after optic neuritis with optical coherence tomography, Ann. Neurol 59 (6) (2006) 963–969.
[34] H. Ishikawa, D.M. Stein, G. Wollstein, et al., Macular segmentation with optical coherence tomography, Invest. Ophthalmol. Vis. Sci. 46 (6) (2005) 2012–2017.
[35] J.N. Ratchford, M.E. Quigg, A. Conger, et al., Optical coherence tomography helps differentiate neuromyelitis optica and MS optic neuropathies, Neurology 73 (4) (2009) 302–308.
[36] A.P. Henderson, S.A. Trip, P.G. Schlottmann, et al., A preliminary longitudinal study of the retinal nerve fiber layer in progressive multiple sclerosis, J. Neurol. 257 (7) (2010) 1083–1091.

[37] J.N. Ratchford, S. Saidha, E.S. Sotirchos, et al., Active MS is associated with accelerated retinal ganglion cell/inner plexiform layer thinning, Neurology 80 (1) (2013) 47–54.
[38] S.B. Syc, S. Saidha, S.D. Newsome, et al., Optical coherence tomography segmentation reveals ganglion cell layer pathology after optic neuritis, Brain 135 (Pt 2) (2012) 521–533.
[39] M. Talebi, M. Nikanfar, R. Sorkhabi, E. Sharifipour, M. Bahrebar, A. Kiavar, Optic coherence tomography findings in relapsing–remitting multiple sclerosis patients of the northwest of Iran, Iran. J. Neurol 12 (3) (2013) 81–86.
[40] R.I. Spain, M. Maltenfort, R.C. Sergott, et al., Thickness of retinal nerve fiber layer correlates with disease duration in parallel with corticospinal tract dysfunction in untreated multiple sclerosis, J. Rehabil. Res. Dev. 46 (5) (2009) 633–642.
[41] F. Costello, W. Hodge, Y.I. Pan, et al., Sex-specific differences in retinal nerve fiber layer thinning after acute optic neuritis, Neurology 79 (18) (2012) 1866–1872.
[42] A. Nicot, Gender and sex hormones in multiple sclerosis pathology and therapy, Front. Biosci 14 (2009) 4477–4515 (Landmark Ed.).
[43] M.J. Eikelenboom, J. Killestein, J.J. Kragt, B.M. Uitdehaag, C.H. Polman, Gender differences in multiple sclerosis: cytokines and vitamin D, J. Neurol. Sci 286 (1–2) (2009) 40–42.
[44] S.M. Gold, R.R. Voskuhl, Estrogen treatment in multiple sclerosis, J. Neurol. Sci. 286 (1–2) (2009) 99–103.
[45] R. Bove, T. Chitnis, The role of gender and sex hormones in determining the onset and outcome of multiple sclerosis, Mult. Scler 20 (5) (2014) 520–526.
[46] E. Schneider, H. Zimmermann, T. Oberwahrenbrock, et al., Optical coherence tomography reveals distinct patterns of retinal damage in neuromyelitis optica and multiple sclerosis, PLoS One 8 (6) (2013), e66151.
[47] J. Bennett, J. de Seze, M. Lana-Peixoto, et al., Neuromyelitis optica and multiple sclerosis: seeing differences through optical coherence tomography, Mult. Scler. (2015).
[48] A.S. Fjeldstad, N.G. Carlson, J.W. Rose, Optical coherence tomography as a biomarker in multiple sclerosis, Expert Opin. Med. Diagn. 6 (6) (2012) 593–604.