NEURO-OPHTHALMOLOGY
2022, VOL. 46, NO. 6, 359–366
https://doi.org/10.1080/01658107.2022.2066699

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Multiple Sclerosis: What Methods are Available for the Assessment of Subclinical Visual System Damage?

Demet Yabanoglu[a], Pinar Topcu-Yilmaz[a], Murat Irkec[a], Belgin Kocer[b], Berna Arli[b], Ceyla Irkec[b], and Sevilay Karahan[c]

[a]Department of Ophthalmology, Hacettepe University Faculty of Medicine, Ankara, Turkey; [b]Department of Neurology, Gazi University Faculty of Medicine, Ankara, Turkey; [c]Department of Biostatistics, Hacettepe University Faculty of Medicine, Ankara, Turkey

ABSTRACT

We aimed to assess the visual fields and optical coherence tomography (OCT) measurements in patients with multiple sclerosis (MS) to detect subclinical visual system disease. The study included 15 MS patients with previous optic neuritis (Group I), 17 MS patients without previous optic neuritis (Group II), and 14 healthy controls (Group III). Each subject underwent standard automated perimetry (SAP), frequency doubling technology perimetry (FDTP), and OCT. The mean deviation of SAP in Group I was lower than those in Groups II ($p = .018$) and III ($p = .001$). The pattern standard deviation of SAP in Group I was higher than those in Group III ($p < .0001$). The mean deviation of FDTP in Groups I and II was lower than those in Group III ($p = .0001$ and $p = .016$, respectively). The temporal quadrant of the retinal nerve fibre layer in Group I was thinner than those in Groups II and III ($p = .005$ and $p = .003$, respectively). The mean macular volume in Group I was thinner than those in Groups II and III ($p = .004$ and $p = .002$, respectively). A single method is inadequate for establishing early and/or mild visual impairment in MS. All conventional and non-conventional techniques are complementary in demonstrating subclinical visual damage in MS.

ARTICLE HISTORY
Received 28 January 2022
Revised 27 March 2022
Accepted 29 March 2022

KEYWORDS
Subclinical multiple sclerosis; optical coherence tomography; optic neuritis; standard automated perimetry; frequency-doubling perimetry; axonal degeneration

Introduction

Visually symptomatic multiple sclerosis (MS) provides only a modest amount of information to assist in our understanding of the disease.[1] When an abnormality of the visual system such as optic neuritis (ON) or cranial nerve palsy occurs in MS, an irreversible neurodegenerative cascade has already begun.[2–4] Recently, researchers have concentrated their efforts on diagnosing MS cases in the subclinical period.[1,5]

Conventional psychophysical techniques are still valid for detecting visual neuronal damage in MS.[3,4,6] However, these approaches frequently fail to uncover clinically meaningful findings until neuronal impairment exceeds a certain threshold. Non-conventional functional approaches and analysis of the retinal nerve fibre layer (RNFL) thickness (RNFLT) are two potential strategies for overcoming this limitation of conventional MS follow-up procedures.[7,8] While frequency doubling technology perimetry (FDTP) has been shown to be effective at detecting early neuronal damage in glaucoma, its usefulness in neurological diseases is debatable.[9] According to a number of researchers, FDTP is as sensitive as standard automated perimetry (SAP) in detecting ON-related nerve damage in MS patients, while others assert that it is insufficient for identifying nerve damage.[5,10] To gain a better understanding of this contentious situation, the present study evaluated conventional and non-conventional perimetry, as well as optical coherence tomography (OCT), for the assessment of subclinical neuronal damage in MS patients.

Materials and methods

This cross-sectional and comparative study was consistent with the Declaration of Helsinki and approved by the Ethics Committee for Clinical Studies of Hacettepe University, Faculty of Medicine (2020/14-36). Informed consent was obtained from the participants.

According to the revised McDonald criteria,[11] MS was diagnosed by the researchers (BK, BA, and CI). The study included patients with MS independent of disease duration, disease-specific therapies, or clinical

CONTACT Demet Yabanoglu demetybngl@gmail.com Department of Ophthalmology Faculty of Medicine, Hacettepe University, Ankara 06230, Turkey

phenotype (relapsing remitting, secondary progressive, and primary progressive). A history of previous ON was determined by the participant and the physician's report. Thirty-two eyes from 32 patients diagnosed with MS were divided into two groups: patients with a previous history of ON (Group I, 15 patients) and patients without a previous history of ON (Group II, 17 patients). There were 42 MS patients at the beginning of this study. However, 10 MS patients (6 without ON and 4 with ON) were excluded since they were unable to complete all of the tests. A control group (Group III) consisted of 14 eyes from 14 healthy subjects. The control group was selected from patients with refractive errors or from healthcare staff. The control group was not recruited from the patients' family members due to the fact that asymptomatic family members of MS patients are also known to have magnetic resonance imaging (MRI) and cerebrospinal fluid abnormalities suggestive of MS at varying rates.[1] Patients with any neurological or inflammatory systemic disease or ocular condition such as amblyopia, any refractive error more than three dioptres, macular degeneration, or glaucoma were excluded from the study due to the possibility of affecting the perimetry and RNFLT test results.

To eliminate bias, the study included one eye from each participant. In Group I, data were collected from the eye with previous ON or from the right eye if the patient had had bilateral ON. Although perimetry and RNFL results of fellow eyes of MS patients with previous ON have been found to be similar to those of MS without ON,[12] our study excluded fellow eyes of Group I from being included in Group II to ensure the study's reliability. Data were obtained from the Group II and III participants' left eyes only.[13] To minimise the potential RNFL oedema associated with acute ON, patients with acute ON episodes that persisted or occurred within 1 month prior to assessment were excluded from the study.

All participants underwent the following consecutive ophthalmological and psychophysical tests: assessment of best corrected Snellen visual acuity (BCVA) and colour vision (CV) using Ishihara colour plates; examination of the anterior segment and measurement of intraocular pressure; perimetry with SAP and FDTP; fundus examination; and RNFL assessment with OCT. All subjects had their pupils dilated with 1% tropicamide prior to fundus examination and OCT.

Perimetry

SAP was performed with a Humphrey Field Analyser II 750 (Carl Zeiss Meditec Inc., Dublin, CA, USA) with a 30–2 Swedish Interactive Thresholding Algorithm (SITA) standard strategy using a white Goldman size III stimulus. FDTP was performed with a Humphrey Matrix Visual Field Instrument (Carl Zeiss Meditec Inc., Dublin, CA, USA) using a 30–2 FDTP threshold strategy. If the SAP or FDTP was not reliable (fixation losses, false-positive or false-negative results more than 33%), the test was repeated. The study included only patients with reliable test results. The mean deviation (MD_{SAP} and MD_{FDTP}) and pattern standard deviation (PSD_{SAP} and PSD_{FDTP}) values were compared between the groups.

Retinal nerve fibre layer analysis

Stratus^{TM} third-generation time-domain (TD) OCT (Carl Zeiss Meditec Inc., Dublin, CA, USA) was used to assess the RNFLT. A certified technician performed all OCT scans in the study. High-quality OCT data were collected by minimising signal strength mistakes and unnecessary/missing scans.[14] Data were obtained using the fast RNFL and fast macular thickness protocols. RNFL images were acquired by the RNFLT 3.4 acquisition protocol and macular thickness maps were acquired by the fast macular thickness map protocol of the OCT. Scans with a signal strength of ≥8 (maximum 10) were included in the analysis. The average (average for 360° around the optic disc) RNFLT ($RNFLT_{avg}$); the thickness of the superior ($RNFLT_S$), inferior ($RNFLT_I$), temporal ($RNFLT_T$), and nasal ($RNFLT_N$) quadrants; foveal thickness; and total macular volume were compared between the groups.

Statistical analysis

The data were analysed using the Statistical Package for the Social Sciences for Windows, version 25 (SPSS, Chicago, IL, USA). The chi-square test was

used to detect any relationship between categorical variables. For independent samples, the Tukey honest significant difference test was used to determine the difference between groups. A two-tailed value of $p < .05$ was considered to be significant.

A post hoc power analysis was conducted using the software package G*Power 3.1.9.7 to assess the difference between three independent group means using a one-way ANOVA test, an effect size of .55, and an alpha of .05. The results indicated that a total of 46 participants, divided into groups of 14, 15, and 17, achieved a power of .88.

Results

The three groups were similar in terms of age ($p = .193$) and gender ($p = .243$) distribution. The patient characteristics and ophthalmological findings are summarised in Tables 1 and 2.

Table 3 presents the perimetry data for SAP and FDTP and the results of comparisons between the groups.

Table 4 summarises the comparisons of RNFL and foveal thickness, and macular volume measurements between the groups.

Table 1. Demographic data for the study groups.

	Group I	Group II	Group III	p value
Gender	13 F, 2 M	11 F, 6 M	12 F, 2 M	0.243
Age in years (range)	37 SD 11.8 (22 to 56)	33 SD 7.8 (24 to 48)	40 SD 12.4 (19 to 59)	0.193

F = female; M = male; SD = standard deviation
Group I: Multiple sclerosis patients with a history of optic neuritis
Group II: Multiple sclerosis patients with no prior history of optic neuritis
Group III: Healthy control subjects

Table 2. Ophthalmological data for the multiple sclerosis patients.

		Group I n (%)	Group II n (%)
Abnormal brain MRI		15 (100%)	17 (100%)
Oligoclonal bands in the CSF		15 (100%)	17 (100%)
BCVA	20/20	11 (73.4%)	17 (100%)
	20/25	2 (13.4%)	-
	20/50	1 (6.6%)	-
	20/200	1 (6.6%)	-
Colour vision	12/12	13 (86.7%)	17 (100%)
	<4/12	2 (13.3%)	-
VEP	Normal	9 (60%)	17 (100%)
	Abnormal	6 (40%)	-
ON episodes/year	< 2	7 (46.7%)	-
	≥ 2	8 (53.3%)	-
OD pallor	Present	6 (40%)	-
	Absent	9 (60%)	-

BCVA = best corrected visual acuity; CSF = cerebrospinal fluid; MRI = magnetic resonance imaging; OD = optic disc; ON = optic neuritis; VEP = visual evoked potentials.
Group I: Multiple sclerosis patients with a history of optic neuritis
Group II: Multiple sclerosis patients with no prior history of optic neuritis.

Discussion

The current study sought to determine which ophthalmological approach was more sensitive in detecting subclinical visual involvement in MS. Based on the results of this study, it seems that FDTP may be more sensitive than SAP in detecting initial visual field (VF) damage in visually asymptomatic MS patients regardless of ON history. There are conflicting results in the literature on the appraisal of neuro-ophthalmological diseases such as MS with FDTP. Sisto et al. claimed that the magnocellular subgroup of retinal ganglion cells was not impaired in MS patients, but even if it was, they provided limited evidence in MS, as FDTP was unable to isolate the function of these cells due to the patients' substantial VF deficits.[5] The magnocellular cell subgroup is known to be present in 3–5% of the retina, and FDTP is a specific test that mainly isolates this group of cells.[15] Corallo et al. highlighted how the fewer fibres in this ganglion cell system

Table 3. The comparison of visual field indices between the groups.

Visual field indices (dB)	Group I (n = 15) Mean SD (range)	Group II (n = 17) Mean SD (range)	Group III (n = 14) Mean SD (range)	p_1	p_2	p_3
MD_{SAP}	−9.69 SD 8.08 (−24.1 to −0.74)	−4.34 SD 4.08 (−14.41 to 0.43)	−1.73 SD 1.02 (−3.29 to 0.01)	**.018**	**.001**	.350
PSD_{SAP}	6.63 SD 3.81 (1.93 to 12.21)	3.71 SD 3.13 (1.22 to 12.23)	1.77 SD 0.32 (1.31 to 2.49)	.159	**< .0001**	.159
MD_{FDTP}	−6.5 SD 5.16 (−14.16 to 0.95)	−4.3 SD 4.5 (−11.05 to 1.66)	0.18 SD 2.5 (−3.41 to 5.69)	.313	**< .0001**	**.016**
PSD_{FDTP}	4.09 SD 1.7 (2.45 to 7.55)	4 SD 1.35 (2.5 to 7.07)	2.98 SD 0.51 (2.19 to 3.82)	> .05	> .05	> .05

FDTP = frequency doubling technology perimetry, MD = mean deviation; PSD = pattern standard deviation; SAP = standard automated perimetry, SD = standard deviation.
Group I: Multiple sclerosis patients with a history of optic neuritis
Group II: Multiple sclerosis patients with no prior history of optic neuritis
Group III: Healthy control subjects
p_1: Comparison of Groups I and II; p_2: comparison of Groups I and III; p_3: Comparisons of Groups II and III.

Table 4. Results of optical coherence tomography and comparisons between the groups.

OCT parameters	Group I (n = 15) Mean SD (range)	Group II (n = 17) Mean SD (range)	Group III (n = 14) Mean SD (range)	p_1	p_2	p_3
$RNFLT_{avg}$ (μm)	96.3 SD 21 (63.72 to 129.72)	107.3 SD 11.7 (83.46 to137.10)	109.5 SD 8.5 (92.7 to 124.68)	.097	.051	.914
$RNFLT_T$ (μm)	56 SD 20.5 (30 to 100)	76 SD 17.4 (39 to 111)	77.8 SD 10.8 (60 to 98)	**.005**	**.003**	.947
$RNFLT_N$ (μm)	77.7 SD 21.5 (46, 121)	83.4 SD 15.7 (55, 115)	89 SD 16.3 (68, 130)	.654	.230	.675
$RNFLT_I$ (μm)	124.3 SD 24.3 (85 to 170)	138.7 SD 21.2 (105 to 201)	136.4 SD 11.8 (120 to 261)	.118	.247	.946
$RNFLT_S$ (μm)	116.7 SD 28.9 (70 to 168)	131.3 SD 15.7 (100 to 157)	134.8 SD 15.6 (108 to 162)	.133	.063	.889
Foveal thickness (μm)	193 SD 17 (159 to 216)	198.5 SD 17.4 (174 to 245)	204.6 SD 23.8 (161 to 239)	.702	.251	.661
Macular volume (mm^3)	6.82 SD 0.36 (6.39 to 7.46)	7.22 SD 0.34 (6.62 to 7.85)	7.27 SD 0.3 (6.85 to 7.81)	**.004**	**.002**	.893

OCT = optical coherence tomography; RNFLT = thickness of retinal nerve fibre layer – avg = average, T = temporal quadrant, N = nasal quadrant, I = inferior quadrant, S = superior quadrant; SD = standard deviation.
Group I: Multiple sclerosis patients with a history of optic neuritis
Group II: Multiple sclerosis patients with no prior history of optic neuritis
Group III: healthy control subjects.
p_1: Comparisons of Groups I and II; p_2: Comparisons of Groups I and III; p_3: Comparisons of Groups II and III.

meant the VF loss manifested earlier.[10] Additionally, they contended that FDTP was more sensitive than SAP in detecting early VF defects. The contribution of the present study to the literature is consistent with this view. Merle et al. also maintained that FDTP is equally sensitive as SAP in MS patients with subclinical optic nerve damage, but not more so.[16] The authors of this study believed that when used in conjunction with other conventional tests, FDTP may be effective in diagnosing subclinical visual involvement in MS patients without a history of ON, but not alone.

Neuro-ophthalmologists generally rate SAP as the gold standard approach for VF evaluation.[17] However, in contrast to FDTP, the functions of the different retinal ganglion cells are not specifically evaluated with SAP.[10] Thus, it is thought that in the presence of mild neuronal damage, VF impairment can be missed, as healthy retinal ganglion cells mask the damaged cells.[10] This hypothesis was supported by the fact that no significant difference was found in patients without an ON episode by SAP in the present study. We suggest that SAP alone might be of little benefit for MS patients with early-stage axonal degeneration and no history of ON.

Psychophysical tests have certain disadvantages. The learning effect, attention factor, and likelihood of patients getting bored have an impact on the rate at which reliable test results are obtained.[18] Sessions with reliability indices above 33% are usually not accepted. In our study, tests were repeated in the event of 10% false-positive or false-negative rates and/or 10% losses of fixation. The strategy selected, the duration of the test, and the current visual field deficiencies of the patients are further factors influencing the test results.[19] FDTP is faster and easier to perform than SAP; thus, the test results are less affected by patient factors such as boredom, learning, and attention. In view of the fact that MS patients are frequently subjected to these tests, regardless of their history of ON, the positive contribution of a test that can be performed more quickly and more easily in the clinic is indisputable. In addition, if consideration is given to the possibility of cognitive functions affected in MS,[20] an easy-to-learn and easy-to-use screening test will facilitate the work of both the clinician and the patient. The present study utilised the Central 30–2 threshold SITA-standard strategy for SAP and the Central 30–2 frequency doubling technology threshold strategy for FDTP. The mean time taken in the study was 8 (standard deviation [SD] 1.9) minutes for SAP and 6.3 (0.4) minutes for FDTP (p = .001). FDTP therefore had a shorter test duration than SAP, which may make it simpler for patients with MS to adjust to psychophysical examinations that require a lot more attention.

The ocular system has long been a focus of research into the neurodegenerative processes associated with MS. Due to the presence of axons in the ganglion cells that comprise the optic nerve and the absence of myelin, the RNFL resembles a component of the central nervous system. In MS, optic nerve demyelination results in retrograde axon degeneration. Due to the fact that these axons originate from retinal nerve fibres, this process results in the thinning of the RNFL. OCT is an objective, quick, non-invasive, office-based imaging technology that enables objective quantification of retinal structures

at high resolution, including estimation of the thickness of the peripapillary RNFL and total macular volume.

OCT technology has made tremendous strides over the last two decades. The technological breakthrough that began with third-generation TD-OCT culminated with the invention of fourth-generation spectral domain (SD) OCT. Numerous investigations have established that SD-OCT provides quicker axial scanning rates, higher axial resolution, and improved repeatability when compared to earlier third-generation TD-OCT technology.[21] However, these promising advances in OCT may create uncertainty in the evaluation of longitudinal data, which is critical in the management of chronic neurodegenerative illnesses like MS.[22] The initial research on RNFL in MS used TD-OCT. Various prior studies have demonstrated a reduction in $RNFLT_{avg}$ in MS patients relative to healthy controls, independent of the ON episode evaluated with TD-OCT.[8,23–25] However, SD-OCT is currently used for a variety of measurements. With this discovery, the debate over the interchangeability of TD-OCT and SD-OCT results has arisen. According to studies, measurements of RNFLT in MS patients demonstrate substantial correlations between the values obtained using the two imaging approaches.[26] Bock et al. evaluated the SD-OCT and TD-OCT imaging techniques in patients with MS and discovered a high association between the two. However, absolute measurements of the RNFL using TD-OCT and SD-OCT equipment were markedly different. SD-OCT measures were "thinner" than TD-OCT at higher RNFL values and "thicker" at lower RNFL values.[27] Several investigations comparing RNFLT values in SD-OCT and TD-OCT have revealed similar results.[28] As a result of these studies, it has been concluded that while TD-OCT is as reliable as SD-OCT in detecting RNFL changes caused by MS, the findings obtained from the two devices should not be used interchangeably due to major discrepancies in the measurements.[21] Because some individuals did not have SD-OCT data, TD-OCT results were used in our study to ensure that all data were comparable.

In contrast to our study, numerous earlier investigations have indicated a decrease in RNFLT in MS patients compared with healthy controls, regardless of ON episode.[29–31] Additionally, the smallest RNFLT values have been reported in eyes previously affected by ON.[12,32] However, even without a history of an acute ON episode, pathological examinations have revealed a reduction in retinal ganglion cell axons in the eyes of patients with MS.[33] Moreover, neuropathologically, this axonal damage and loss may be extensive in chronic MS lesions.[34] Although eyes with a history of acute ON in MS exhibit the highest reduction in RNFLT, certain investigations have shown that OCT can identify anterior visual pathway axonal loss in the absence of these episodes.[25,29] In our study, we observed that the $RNFLT_{avg}$ was lower in MS groups than in healthy controls, but this difference was not statistically significant. We reasoned that such a finding might be due to a variety of factors. Talman et al. reported that a longer follow-up period was associated with a greater degree of RNFL thinning in MS with and without ON and that each 1-year follow-up period resulted in an average 2.0 μm reduction in RNFLT.[31] They contended that increasing RNFL thinning occurs over time in MS.[31] Due to the cross-sectional nature of our study, we would have missed the effect of follow-up time on RNFLT that Talman et al. reported.[31] The disease duration in eyes with a history of ON in MS has been demonstrated to be significant in determining RNFL thinning following an acute ON episode.[31] Again, in the study by Talman et al., the median (minimum – maximum) disease duration of their MS patients was 9 (<1 – 46) years.[31] The mean ± SD (median; minimum – maximum) disease duration in our study was 4.4 ± 4.02 (3; <1 – 12) years for Group I and 4.08 ± 4.9 (1.5; <1 – 12) years for Group II. Due to the short period of disease in our MS patients, the RNFL values may differ from those reported in the literature. The majority of studies included patients at least 3 months after the episode of ON to limit tissue oedema that may persist following an ON episode.[32,35,36] Given that our study included patients at least 1 month after the ON episode, this might account for the absence of a significant reduction in RNFLT in the MS group with ON. Costello et al. also confirmed this hypothesis.[37] They observed that 2 years following an ON episode, $RNFLT_{avg}$ was significantly reduced in MS patients. They discovered a significant drop only in $RNFL_T$ within the first 2 years. As also

demonstrated in our study, $RNFL_T$ reduction in MS has been reported in the literature. While Pro et al. ascribed this observation to interstitial oedema in other sectors (nasal, inferior, and superior),[38] Sergott et al. indicated that the most affected nerve fibres in MS during an ON episode were the papillomacular bundle fibres.[39] Apart from the temporal sector, there are studies demonstrating disparities in other sectors. Parisi et al. reported that the $RNFLT_T$ was retained.[23] Fisher et al., on the other hand, noted that the sectoral difference between groups with and without ON was lowest in the nasal quadrant.[25] Besides this, some researchers believe that there is a possibility that retinal ganglion cell loss occurs in the macula as a result of RNFL axon loss in MS.[24] Macular volume and thickness measurements on OCT can be used to detect this loss. The macular volume measurement involves all neural retinal tissue, including the retinal ganglion cell layer and the RNFL. Studies in which macular volume was also assessed in the literature have demonstrated a decrease in total macular volume in MS patients.[24,31,40] Recent years have seen a greater understanding of the impact of retinal ganglion cell degeneration in MS, owing to thorough segmentation of retinal layers using ultra high-resolution OCT.[41] Saidha et al. revealed that visual impairment in MS was more closely associated to average macular thickness measurements than to RNFLT measurements, implying that the macula may be impacted preferentially in MS.[41] Burkholder et al. also demonstrated an association between decreased macular volume and visual impairment, cortical lesion volume or number, and grey matter atrophy on MRI, as well as increasing levels of disability.[40] In our study, a reduction in total macular volume was observed in MS with ON patients before any change in RNFL thickness. Our MS patients in total had a mean disease duration of 4.27 (4.35) years. Additionally, MS with ON patients were included from the first month of the ON episode. In our study, the macular volume appeared to diminish shortly after an ON episode (1 - 3 months) but before the RNFLT was impaired.

There are some limitations to our study. The first was the small number of participants. A larger number of participants may have changed the statistical results and enabled us to make healthier recommendations for the selection of psychophysical tests. The second was the absence of contrast acuity or sensitivity visual function tests. It has been shown that low contrast letter acuity (Sloan charts) and contrast sensitivity (Pelli-Robson charts) have the greatest capacity to detect visual impairment in MS patients.[25] The third was that high-resolution SD-OCT was not used in RNFL or macular volume assessments. It has been found that SD-OCT provides faster axial scan rates, higher axial resolution, and better repeatability than TD-OCT.[21]

In conclusion, there is no gold standard strategy for diagnosing and monitoring visual involvement in MS from an ophthalmological standpoint. Indeed, a single approach is frequently insufficient to demonstrate mild visual impairment in MS. All approaches, including novel functional techniques and structural analysis, should be used more frequently by neuro-ophthalmologists to aid in unravelling the enigma of axonal degradation in MS. FDTP should be considered in addition to SAP for assessing subclinical visual system involvement.

Acknowledgments

This study was presented in 41th National Congress of Ophthalmology of the Turkish Ophthalmology Society as an oral presentation.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The authors reported that there is no funding associated with the work featured in this article.

References

1. Siva A. Asymptomatic MS. *Clin Neurol Neurosurg*. 2013;115(1):S1–S5. doi:10.1016/j.clineuro.2013.09.012.
2. Costello F. Vision disturbances in multiple sclerosis. *Semin Neurol*. 2016;36(2):185–195. doi:10.1055/s-0036-1579692.
3. Cleary PA, Beck RW, Anderson MM Jr, Kenny DJ, Backlund JY, Gilbert PR. Design, methods, and conduct of the optic neuritis treatment trial. *Control Clin Trials*. 1993;14(2):123–142. doi:10.1016/0197-2456(93)90015-6.

4. Optic Neuritis Study Group. The clinical profile of optic neuritis. Experience of the Optic Neuritis Treatment Trial. *Arch Ophthalmol*. 1991;109(12):1673–1678. doi:10.1001/archopht.1991.01080120057025.
5. Sisto D, Trojano M, Vetrugno M, Trabucco T, Iliceto G, Sborgia C. Subclinical visual involvement in multiple sclerosis: a study by MRI, VEPs, frequency-doubling perimetry, standard perimetry, and contrast sensitivity. *Invest Ophthalmol Vis Sci*. 2005;46(4):1264–1268. doi:10.1167/iovs.03-1213.
6. Ortiz-Perez S, Andorra M, Sanchez-Dalmau B, et al. Visual field impairment captures disease burden in multiple sclerosis. *J Neurol*. 2016;263(4):695–702. doi:10.1007/s00415-016-8034-2.
7. Fong KC, Byles DB, Constable PH. Does frequency doubling technology perimetry reliably detect neurological visual field defects? *Eye (Lond)*. 2003;17 (3):330–333. doi:10.1038/sj.eye.6700376.
8. Britze J, Frederiksen JL. Optical coherence tomography in multiple sclerosis. *Eye (Lond)*. 2018 May;32 (5):884–888. doi:10.1038/s41433-017-0010-2.
9. Alward WL. Frequency doubling technology perimetry for the detection of glaucomatous visual field loss. *Am J Ophthalmol*. 2000;129(3):376–378. doi:10.1016/s0002-9394(00)00352-4.
10. Corallo G, Cicinelli S, Papadia M, Bandini F, Uccelli A, Calabria G. Conventional perimetry, short-wavelength automated perimetry, frequency-doubling technology, and visual evoked potentials in the assessment of patients with multiple sclerosis. *Eur J Ophthalmol*. 2005;15(6):730–738. doi:10.1177/112067210501500612.
11. Polman CH, Reingold SC, Edan G, et al. Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". *Ann Neurol*. 2005;58 (6):840–846. doi:10.1002/ana.20703.
12. Trip SA, Schlottmann PG, Jones SJ, et al. Optic nerve atrophy and retinal nerve fibre layer thinning following optic neuritis: evidence that axonal loss is a substrate of MRI-detected atrophy. *Neuroimage*. 2006;31(1):286–293. doi:10.1016/j.neuroimage.2005.11.051.
13. Karakosta A, Vassilaki M, Plainis S, Elfadl NH, Tsilimbaris M, Moschandreas J. Choice of analytic approach for eye-specific outcomes: One eye or two? *Am J Ophthalmol*. 2012;153(3):571–579.e1. doi:10.1016/j.ajo.2011.08.032.
14. Keltner JL, Cello KE, Balcer LJ, Calabresi PA, Markowitz CE, Werner JS. Stratus OCT quality control in two multi-centre multiple sclerosis clinical trials. *Neuroophthalmology*. 2011;35(2):57–64. doi:10.3109/01658107.2011.557760.
15. Salvetat ML, Zeppieri M, Tosoni C, Parisi L, Brusini P. Non-conventional perimetric methods in the detection of early glaucomatous functional damage. *Eye (Lond)*. 2010;24(5):835–842. doi:10.1038/eye.2009.216.
16. Merle H, Olindo S, Donnio A, Richer R, Smadja D, Cabre P. Anatomic and functional correlation of frequency-doubling technology perimetry (FDTP) in multiple sclerosis. *Int Ophthalmol*. 2011;31 (4):263–270. doi:10.1007/s10792-011-9447-7.
17. Kedar S, Ghate D, Corbett JJ. Visual fields in neuro-ophthalmology. *Indian J Ophthalmol*. 2011;59 (2):103–109. doi:10.4103/0301-4738.77013.
18. Horani A, Frenkel S, Yahalom C, Farber MD, Ticho U, Blumenthal EZ. The learning effect in visual field testing of healthy subjects using frequency doubling technology. *J Glaucoma*. 2002;11(6):511–516. doi:10.1097/00061198-200212000-00011.
19. Geimer SA. Glaucoma diagnostics. *Acta Ophthalmol*. 2013;91:1–32. doi:10.1111/aos.12072. Thesis 1.
20. Dujardin K, Donze AC, Hautecoeur P. Attention impairment in recently diagnosed multiple sclerosis. *Eur J Neurol*. 1998;5(1):61–66. doi:10.1046/j.1468-1331.1998.510061.x.
21. Warner CV, Syc SB, Stankiewicz AM, et al. The impact of utilizing different optical coherence tomography devices for clinical purposes and in multiple sclerosis trials. *PLoS One*. 2011;6(8):e22947. doi:10.1371/journal.pone.0022947.
22. Costello F. Evaluating the use of optical coherence tomography in optic neuritis. *Mult Scler Int*. 2011;2011:148394. doi:10.1155/2011/148394.
23. Parisi V, Manni G, Spadaro M, et al. Correlation between morphological and functional retinal impairment in multiple sclerosis patients. *Invest Ophthalmol Vis Sci*. 1999;40(11):2520–2527.
24. Trip SA, Schlottmann PG, Jones SJ, et al. Retinal nerve fiber layer axonal loss and visual dysfunction in optic neuritis. *Ann Neurol*. 2005;58(3):383–391. doi:10.1002/ana.20575.
25. Fisher JB, Jacobs DA, Markowitz CE, et al. Relation of visual function to retinal nerve fiber layer thickness in multiple sclerosis. *Ophthalmology*. 2006;113(2):324–332. doi:10.1016/j.ophtha.2005.10.040.
26. Vizzeri G, Weinreb RN, Gonzalez-Garcia AO, et al. Agreement between spectral-domain and time-domain OCT for measuring RNFL thickness. *Br J Ophthalmol*. 2009;93(6):775–781. doi:10.1136/bjo.2008.150698.
27. Bock M, Brandt AU, Dörr J, et al. Time domain and spectral domain optical coherence tomography in multiple sclerosis: a comparative cross-sectional study. *Mult Scler*. 2010;16(7):893–896. doi:10.1177/1352458510365156.
28. Klistorner A, Arvind H, Nguyen T, et al. Axonal loss and myelin in early ON loss in postacute optic neuritis. *Ann Neurol*. 2008;64(3):325–331. doi:10.1002/ana.21474.
29. Toledo J, Sepulcre J, Salinas-Alaman A, et al. Retinal nerve fiber layer atrophy is associated with physical and cognitive disability in multiple sclerosis. *Mult Scler*. 2008;14(7):906–912. doi:10.1177/1352458508090221.

30. Zaveri MS, Conger A, Salter A, et al. Retinal imaging by laser polarimetry and optical coherence tomography evidence of axonal degeneration in multiple sclerosis. *Arch Neurol*. 2008;65 (7):924–928. doi:10.1001/archneur.65.7.924.
31. Talman LS, Bisker ER, Sackel DJ, et al. Longitudinal study of vision and retinal nerve fiber layer thickness in multiple sclerosis. *Ann Neurol*. 2010;67(6):749–760. doi:10.1002/ana.22005.
32. Cheng H, Laron M, Schiffman JS, Tang RA, Frishman LJ. The relationship between visual field and retinal nerve fiber layer measurements in patients with multiple sclerosis. *Invest Ophthalmol Vis Sci*. 2007;48 (12):5798–5805. doi:10.1167/iovs.07-0738.
33. Evangelou N, Konz D, Esiri MM, Smith S, Palace J, Matthews PM. Size-selective neuronal changes in the anterior optic pathways suggest a differential susceptibility to injury in multiple sclerosis. *Brain*. 2001;124(Pt 9):1813–1820. doi:10.1093/brain/124.9.1813.
34. Lassmann H, Suchanek G, Ozawa K. Histopathology and the blood-cerebrospinal fluid barrier in multiple sclerosis. *Ann Neurol*. 1994;36(S1):S42–S46. doi:10.1002/ana.410360713.
35. Costello F, Coupland S, Hodge W, et al. Quantifying axonal loss after optic neuritis with optical coherence tomography. *Ann Neurol*. 2006;59(6):963–969. doi:10.1002/ana.20851.
36. Khanifar AA, Parlitsis GJ, Ehrlich JR, et al. Retinal nerve fiber layer evaluation in multiple sclerosis with spectral domain optical coherence tomography. *Clin Ophthalmol*. 2010. 4:1007–1013. Published 2010 Sep 20. doi:10.2147/opth.s13278.
37. Costello F, Hodge W, Pan YI, Metz L, Kardon RH. Retinal nerve fiber layer and future risk of multiple sclerosis. *Can J Neurol Sci*. 2008;35(4):482–487. doi:10.1017/s031716710000915x.
38. Pro MJ, Pons ME, Liebmann JM, et al. Imaging of the optic disc and retinal nerve fiber layer in acute optic neuritis. *J Neurol Sci*. 2006 1;250(1–2):114–119. doi:10.1016/j.jns.2006.08.012.
39. Sergott RC, Frohman E, Glanzman R, Al-Sabbagh A. OCT in MS Expert Panel. The role of optical coherence tomography in multiple sclerosis: expert panel consensus. *J Neurol Sci*. 2007 \;263(1–2):3–14. doi:10.1016/j.jns.2007.05.024.
40. Burkholder BM, Osborne B, Loguidice MJ, et al. Macular volume determined by optical coherence tomography as a measure of neuronal loss in multiple sclerosis. *Arch Neurol*. 2009;66(11):1366–1372. doi:10.1001/archneurol.2009.230.
41. Saidha S, Syc SB, Ibrahim MA, et al. Primary retinal pathology in multiple sclerosis as detected by optical coherence tomography [published correction appears in brain. *Brain*. 2011 Dec;134(Pt 2):518–533. doi:10.1093/brain/awq346.