Multiple Sclerosis and Related Disorders 27 (2019) 260–268

Contents lists available at ScienceDirect

Multiple Sclerosis and Related Disorders

journal homepage: www.elsevier.com/locate/msard

Peripapillary retinal nerve fiber layer thickness measured by optical coherence tomography in different clinical subtypes of multiple sclerosis☆

Irmina Jankowska-Lech[a,*], Jaromir Wasyluk[c], Witold Palasik[b], Barbara Terelak-Borys[a], Iwona Grabska-Liberek[a]

[a] Department of Ophthalmology, Medical Centre of Postgraduate Education, Ul. Czerniakowska 231, Warsaw 00-416, Poland
[b] Department of Neurology and Epileptology, Medical Centre of Postgraduate Education, Ul. Czerniakowska 231, Warsaw 00-416, Poland
[c] Department of Ophthalmology, Military Institute of Aviation Medicine, Ul. Krasińskiego 54/56, Warsaw 01-755, Poland

ARTICLE INFO

Keywords:
Multiple sclerosis
Spectral-domain optical coherence tomography
Peripapillary retinal nerve fiber layer

ABSTRACT

Background: Multiple sclerosis (MS) is a chronic inflammatory demyelinating autoimmune disease of the central nervous system (CNS) with axonal degeneration as major determinant of neurological disability. Assessment of unmyelinated retinal nerve fibers using optical coherence tomography (OCT) may be useful for diagnosing the onset and rate of progression of neurodegeneration.

Objective: To assess the incidence and severity of damage of the peripapillary retinal nerve fiber layer (RNFL) in two different MS subtypes: non-progressive [Prog(-)MS] and progressive [Prog(+)MS].

Methods: 48 patients (96 eyes) with MS were included: 13 males, 35 females; aged 22–62 years (mean 38.8; SD ± 10.02) in two subgroups: 26 Prog(-)MS and 22 Prog(+)MS. 3 subtypes of Prog(+)MS were identified by neurologist, according to Lublin criteria: 3 patients had PPMS (14%), 7 had SPMS(32%), 12 had PRMS(54%). RRMS subtype was considered a non-progressive phenotype, designated as Prog(-)MS. All 22 patients with progressive MS phenotypes were included in one group, designated as Prog(+)MS. Progressive disease can be defined over 1 year. The expanded EDSS score was determined by the treating MS specialist and confirmed by the study investigators through the records review. Definition included a 3-strata progression magnitude in the absence of a relapse, confirmed after 3 months within the leading Functional System and required an Expanded Disability Status Scale step ≥ 4 and pyramidal score ≥ 2. 11 Prog(-)MS (16 eyes) and 10 Prog(+)MS (13 eyes) patients had a history of optic neuritis (ON). EDSS score was 1.5–6.5 (mean 3.83 ± 1.62) in the Prog(+)MS group and 1.0–3.5 (mean 1.40 ± 0.57) in the Prog(-)MS. Control group: 31 healthy volunteers (3 males, 28 females), aged 20–62 years (mean 37.4 ± 10.88). Peripapillary RNFL thickness was measured around the optic nerve head (ONH) using spectral-domain OCT (Topcon OCT 1000 MarkII, FastMap v. 3.40, Topcon, Japan). Scans were obtained according to OSCAR-IB consensus criteria.

The generalized estimating equation model (GEE) was used in the statistical analysis to assess differences in RNFL thickness between Prog(-)MS and Prog(+)MS patients, taking into consideration history of ON, EDSS score, immunomodulatory therapy, MS progression, MS duration, age and gender.

The protocol was approved by the Ethical Committee of the Medical Centre of Postgraduate Education, Warsaw, Poland and informed consent was obtained from all subjects.

Results: There was a significant difference between Prog(-)MS and Prog(+)MS groups for mean, nasal and superior quadrant of RNFL thickness. For individuals with a history of ON, significant differences were found between the two MS phenotypes regardless of RNFL thickness measurements.

Conclusions: A significant correlation was established between RNFL thickness and progression of neurodegeneration in MS patients with no regard to history of ON. RNFL thickness may be considered a MS biomarker and potential diagnostic tool for assessment of disease progression.

☆ This clinical trial was sponsored by unrestricted scientific grant from the National Centre for Research and Development (Nr R13 033 03), Warsaw, Poland, www.ncbr.gov.pl.

* Corresponding author.
E-mail addresses: irminajank@onet.eu (I. Jankowska-Lech), witpal3@wp.pl (W. Palasik), b.terelak@message.pl (B. Terelak-Borys).

https://doi.org/10.1016/j.msard.2018.11.003
Received 30 June 2018; Received in revised form 12 October 2018; Accepted 2 November 2018

1. Background

Multiple sclerosis (MS) is an autoimmune disease characterized by inflammatory demyelination, which damages axons and their neurocytes. The disease is usually progressive, leading to irreversible degeneration of the central nervous system (CNS) (Compston and Coles, 2008; Tallantyre et al., 2010).

MS optic neuropathy (MSON) and glaucomatous neuropathy are the two most common types of optic nerve damage. It is estimated that the clinical symptoms of MSON occur in 30–70% of individuals with MS (Balcer, 2006; Frohman et al., 2005) although histological post-mortem examination indicates that demyelination along the optic nerve is seen in as many as 94–99% of MS patients (Ikuta and Zimmerman, 1976). Optic neuritis (ON) is usually the first manifestation and is thought to be caused by inflammation and demyelination in the retrobulbar segment of the optic nerve. The etiology of optic neuropathy is not clear and lesions in demyelinated nerve fibers in the optic nerve head (ONH) and retinal structures (Syc et al., 2012; Walter et al., 2012) invariably are not associated with a history of ON (Fjeldstad et al., 2011; Galetta et al., 2011; Noval et al., 2011; Oberwahrenbrock et al., 2012). This may be explained by observations indicating that two processes occur simultaneously in the CNS: inflammatory demyelination and transsynaptic neurodegeneration (Petzold et al., 2015; Salapa et al., 2017; Syc et al., 2012).

Non-myelinated axons in the anterior visual pathway are easy to examine and therefore measuring the retinal nerve fiber layer (RNFL) to assess the dynamics of CNS degeneration has been proposed (Balk and Petzold, 2014; Petzold et al., 2010). Many published studies confirm associations between RNFL loss and visual acuity (e.g., multifocal visual evoked potentials and low-contrast letter acuity) (Balcer et al., 2015; Balcer and Frohman, 2010; Klistorner et al., 2009; Sriram et al., 2014). Correlation was found between RNFL loss and clinical tests: magnetic resonance imaging [MRI], EDSS score (Dorr et al., 2011; Grazioli et al., 2008; Gordon-Lipkin et al., 2007; Klistorner et al., 2014; Oberwahrenbrock et al., 2012; Sepulcre et al., 2007) and Multiple Sclerosis Functional Composite (Fisher et al., 2006; Oberwahrenbrock et al., 2012; Siepman et al., 2010).

Imaging of early pathological changes involving the RNFL utilizes scanning laser polarimetry (SLP) and optical coherence tomography (OCT) (Huang et al., al.1991; Wojtkowski et al., 2005; Wojtkowski et al., 2004). OCT devices usually have a glaucoma module and can perform measurements of the RNFL and ganglion cell complex – they are used to diagnose and monitor glaucoma progression. Their usefulness for assessing changes in the ONH and retina during the course of MS has not yet been confirmed (Lamirel et al., 2010; Rebolleda et al., 2015).

Spectral-domain OCT (SD-OCT), allows accurate assessment of retinal changes and the following pathologies have been seen using SD-OCT in MS patients: thinning of the peripapillary and macular RNFL (Balk et al., 2014; Gelfand et al., 2012; Seigo et al., 2012; Serbecic et al., 2011; Tatrai et al., 2012; Walter et al., 2012), significant reduction in total macular volume (Oberwahrenbrock et al., 2012; Seidha et al., 2011), reduction in ganglion cell layer (GCL)/inner plexiform layer thickness and thinning of the inner and outer nuclear cell layers, which can be assessed by using the appropriate retinal segmentation algorithm (Alonso et al., 2018; Behbehani et al., 2017; Oberwahrenbrock et al., 2012; Ratchford et al., 2013; Saidha et al., 2011; Syc et al., 2012; Walter et al., 2012). Furthermore, several reports indicate that RNFL loss and GCL thinning in MS patients occur following episodes of acute ON (Chan, 2012; Lamirel et al., 2010; Saidha et al., 2011; Syc et al., 2012). Such association, however, has not been found in studies of the inner and outer nuclear retinal layers at the macula, suggesting that retrograde degeneration may be limited to the inner retinal layers (Syc et al., 2012) and changes observed in the outer retinal layers may represent primary axonal degeneration in the course of MS (Balk et al., 2014; Behbehani et al., 2017).

Numerous clinical studies have attempted to determine the rate of neurodegeneration in MS (Balcer et al., 2015; Henderson et al., 2010; Martinez-Lapiscina et al., 2016; Petzold et al., 2010; Serbecic et al., 2011; Talman et al., 2010). Indeed, use of SD-OCT to monitor transsynaptic retrograde degeneration and to assess the effects of neuroprotective and neurodegenerative MS treatments has been proposed by some authors (Alonso et al., 2018; Balcer et al., 2015; Brakhof et al., 2009; Chan, 2012; Frohman et al., 2008; Lamirel et al., 2010). The search for new diagnostic approaches utilizing OCT has included patients with different MS phenotypes (i.e. progressive relapsing [PRMS], primary progressive [PPMS], secondary progressive [SPMS] and relapsing remitting [RRMS]), as they are heterogeneous and have different pathomechanisms (Behbehani et al., 2017; Costello et al., 2009; Gelfand et al., 2012; Henderson et al., 2008; Oberwahrenbrock et al., 2012; Pulicken et al., 2007; Serbecic et al., 2011; Siepman et al., 2010; Walter et al., 2012). However, one unanswered question is whether examination of retinal structures by high-resolution SD-OCT can be used for their differential diagnosis. The aim of this study was to assess the prevalence, extent and pattern of RFNL loss in patients with either RRMS or progressive MS.

2. Methods

2.1. Patients

Patients undergoing treatment for MS were recruited from the Department of Neurology, Medical Centre of Postgraduate Education, Warsaw, Poland to this observational clinical trial. The study group comprised 48 screened and included patients (96 eyes; 35 females; mean age 38.8 years, participation rate 100%) with MS, the diagnosis of which was consistent with the McDonald criteria (Polman et al., 2011; Polman et al., 2005). Four subtypes of MS were identified by neurologist, according to current diagnostic criteria (Lublin, 2014; Lublin and Reingold, 1996). 3 patients had PPMS, seven had SPMS, 12 had PRMS, and 26 had RRMS. RRMS was considered a non-progressive phenotype, designated Prog(-)MS. All 22 patients with progressive MS phenotypes were included in one group, designated Prog(+)MS, due to the very small sizes of the individual progressive MS subgroups.

Progressive disease can be defined over 1 year. The expanded EDSS score was determined by the treating MS specialist and confirmed by the study investigators through the records review. Definition included a 3-strata progression magnitude in the absence of a relapse, confirmed after 3 months within the leading Functional System and required an Expanded Disability Status Scale step ≥ 4 and pyramidal score ≥ 2. The EDSS score (Kurtzke, 1983) was 1.5–6.5 (mean 3.83 ± 1.62) in the Prog(+)MS group and 1.0–3.5 (mean 1.40 ± .0.57) in the Prog(−)MS group; the mean EDSS score for the entire study group was 2.55 (range 1.0–6.5).

Demographic and clinical particulars for study patients are shown in Table 1. The control group consisted of 90% females vs. 73% in MS group and this difference was accounted in GEE statistical analysis. 21 patients had a history of at least one documented episode of acute ON in one or both eyes (10 patients [13 eyes] in the Prog(+)MS group, 11 patients [16 eyes] in the Prog(-)MS group). All patients were in a clinically inactive stage of the disease: clinical relapse had occurred in the previous 2 years in 12 patients, in the previous 1 year in 9 patients, and in the previous 6 months in 2 patients. None of them had experienced a clinical relapse in the 3 months preceding the study. The mean duration of MS since diagnosis was 4.4 years (range 1–22). Treatment was being received by 45.8% of the study population (16 patients received interferon β- 1b, five received Glatiramer acetate, one received azathioprine).

Inclusion criteria:

- confirmed diagnosis of MS
- signed informed consent for the study.

Table 1
Demographic and clinical data for MS patients (classified by phenotype) and healthy controls.

	Healthy controls	All MS patients	Prog(-) RRMS	Prog(+) SPMS	Prog(+) PRMS	Prog(+) PPMS
Number of patients	31	48	26	7	12	3
Number of eyes (eyes with optic neuritis)	62	96 (29)	52(16)	14(4)	24(9)	6(0)
Number of females	28 (90%)	35 (73%)	19 (73%)	4 (66%)	9 (75%)	2 (66%)
Mean age – years (range)	37.4 (20–62)	38.8 (22–62)	36.5 (22–54)	52.9 (35–62)	41.3 (24–56)	36.8 (24–44)
SD (±)	10.88	10.02	8.35	6.70	10.03	4.85
Mean disease duration in months (range)		52.8 (6–2)	38.8 (6–120)	79.7 (6–264)	45.3 (6–96)	57 (6–180)
Number of patients receiving immunomodulatory therapy		22	16	1	4	1
Mean EDSS score (range)		2.55 (1–6.5)	1.4 (1–3.5)	5.3 (4–6.5)	3.1 (1.5–5.5)	2.5 (1.5–3.5)

MS-multiple sclerosis; PPMS-primary progressive multiple sclerosis; PRMS-progressive relapsing multiple sclerosis; RRMS-relapsing remitting multiple sclerosis; SPMS-secondary progressive multiple sclerosis; Prog(+)-progressive; Prog(-)-non-progressive; ON-optic neuritis; EDSS-Expanded Disability Status Scale; SD-standard deviation.

Exclusion criteria

- any serious eye disorders of the ONH or retina (e.g., glaucoma, age-related macular degeneration, uveitis)
- cataract or corneal disorders causing visual opacities
- abnormalities of the ONH (drusen, congenital anomalies)
- high myopia (spherical equivalent ≥ − 5.0 D) or hyperopia (spherical equivalent ≥ + 2.5 D) or astigmatism (spherical equivalent ≥ ± 2.5 Dcyl)
- history of any intrabulbar surgery intervention during the previous year
- diabetes
- other than MS autoimmune disorders

A control group consisted of 31 healthy volunteers, recruited from hospital personnel (3 males, 28 females), aged 20–62 years (mean 37.4 ± 10.88) (Table 1). BCVA ranged from 0.2 to 1.0 (mean 0.92 ± 0.22) in the RE and from 0.1 to 1.0 (mean 0.91 ± 0.24) in the LE. Intraocular pressure in both eyes ranged between 14–18 mmHg.

2.2. Clinical examinations

Best corrected visual acuity (BCVA) was collected using high contrast test with decimal scale (Snellen) and ranged from 0.5 to 1.0 (mean 0.93 ± 0.13) in the right eye (RE) and from 0.1 to 1.0 (mean 0.83 ± 0.27) in the left eye (LE). Intraocular pressure in both eyes ranged between 9.0 and 21.3 mmHg (mean 15.05).

In all participants the RNFL thickness, the main outcome measure, was obtained after pupil dilation by spectral-domain optical coherence tomography (Topcon OCT 1000 MarkII, FastMap v. 3.40, Topcon, Japan). Measurements were performed in triplicate by two independent investigators and only results meeting the highest quality criteria were included in the subsequent analysis. All OCT scans met OSCAR-IB acquisition criteria (Tewarie et al., 2012). The measurements were performed for mean RNFL thickness in the four peripapillar quadrants.

The study protocol was approved by the Ethical Committee at the Medical Centre of Postgraduate Education, and informed consent was obtained from all participants prior to enrollment.

2.3. Statistical analysis

Calculations were performed using Stata v.14, StataCorp. 2015. *Stata Statistical Software: Release 14*. StataCorp LLC, College Station, Texas, USA.

Generalised estimating equation (GEE) models were used in the statistical analysis to examine associations between the RNFL thickness (mean thickness and thickness of the four retinal quadrants) and MS progression, ON, EDDS score, immunomodulatory therapy, MS duration, age, gender, and refraction. The GEE model takes into account all possible correlations between repeated outcomes for the same patient. The analysis was performed for all MS patients, and separately for MS patients with and without a history of ON. Differences related to age, EDSS, duration, immunomodulatory therapy, gender and refraction between ON and without history of ON MS patients were studied using the Mann-Whitney test and Fisher test. Additionally, comparison of the mean RNFL thickness in MS patients and healthy controls was made using the Kruskal–Wallis test. Statistical significance was assumed at $P = 0.05$.

3. Results

In MS patients (with and without a history of ON) a statistically significant difference between the Prog(-)MS group and the Prog(+)MS group was found in peripapillary RNFL thickness. Differences were found in the mean RNFL thickness and in the RNFL thickness in the nasal and superior quadrants (Tables 2 and3, Fig. 1).

Mean overall RNFL thickness was significantly lower in MS patients compared to healthy controls, in both eyes (Table 2, Fig. 2). Study and control groups were matched by gender, age and refractive error. No losses in observation were seen.

The mean overall RNFL thickness was also significantly lower in MS patients with a history of ON compared with those with no history of ON (Table 2).

Data for Prog(-)MS and Prog(+)MS for groups with/without ON is provided in Table 4 for the following parameters: age, EDSS, duration of MS, immunomodulatory therapy, gender, negative spherical equivalent.

In the subset without a previous episode of ON there was a statistically significant difference in the superior quadrant RNFL thickness between the Prog(-)MS and Prog(+)MS patients (Table 2 and3, Fig. 1).

For individuals with a history of ON, there was a statistically significant difference in mean RNFL thickness between the Prog(-)MS and Prog(+)MS patients (Tables 2 and3, Fig. 1). Statistically significant differences between the Prog(-)MS and Prog(+)MS cohorts were also seen in the superior, inferior, nasal and temporal quadrants (Table 2 and3, Fig. 1).

When EDSS score was taken into account, it was found that for MS patients, with and without a history of ON, there was a statistically significant difference in RNFL thickness in the temporal quadrant between Prog(-)MS and Prog(+)MS patients. Likewise in MS patients without a history of ON, there was a statistically significant difference in RNFL thickness in the temporal quadrant between Prog(-)MS and Prog(+)MS individuals. The temporal RNFL thickness was reduced more in patients with a lower EDSS score when compared with those with a higher EDSS score.

In the MS patients with and without a history of ON, there was no statistically significant difference in mean RNFL thickness between Prog(-)MS and Prog(+)MS patients related to the use of immunomodulatory

Table 2
RNFL thickness in all study groups.

Prog(+) MS **All patients**	Mean RNFL (μm)	Range (μm)	SD (±)	**Prog(-) MS** Mean RNFL (μm)	Range (μm)	SD (±)	***P*-value**
	86.47	69.5–110.75	11.76	98.82	87.00–119.25	12.35	0.025 (a)
ON (+) Prog(+)MS and Prog(-)MS	Mean RNFL (μm)	Range (μm)	SD (±)	**ON (-) Prog(+)MS and Prog(-)MS** Mean RNFL (μm)	Range (μm)	SD (±)	***P*-value**
	92.46	61.88–114.0	14.55	94.97	63.5–115.63	11.38	**0.025** (a)
ON (+)	**Prog(+)MS**			**Prog(-)MS**			
All quadrants	82.88	69.5–98.00	9.6	97.84	89.00–115.5	16.5	**0.001** (a)
Superior	103.30	86–125	11.45	129.31	118–148	9.65	**0.024** (a)
Inferior	101.08	76–128	14.92	128.31	98–151	14.05	**0.0001** (a)
Nasal	70.33	58–85	8.45	83.93	59–100	12.42	**0.022** (a)
Temporal	53.82	45–72	7.81	73.07	53–90	11.03	**0.035** (a)
ON (-)	**Prog(+)MS**			**Prog(-)MS**			
All quadrants	90.05	71.5–110.75	13.95	99.80	87–119.25	8.2	0.138 (a)
Superior	109.77	77–137	15.41	122.69	101–154	12.91	**0.005** (a)
Inferior	111.45	81–135	16.14	123.00	95–153	14.31	0.629 (a)
Nasal	73.11	49–104	12.55	82.57	62–107	12.14	0.166 (a)
Temporal	67.00	52–86	8.25	69.08	49–99	11.33	0.143 (a)
	MS Mean RNFL (μm)	Range (μm)	SD (±)	**Controls** Mean RNFL (μm)	Range (μm)	SD (±)	***P*-value**
RE	95,79	66.50–119.25	12.03	101.56	91.0–119.75	6.40	**0.04** (b)
LE	91.88	53.75–115.0	13.34	99.70	91.0–124.0	6.99	**0.01** (b)

MS-multiple sclerosis; Prog(+)-progressive; Prog(-)-non-progressive; ON-history of optic neuritis; (a) Pearson chi2 test; (b) Kruskal–Wallis test.

Table 3
Difference in the mean and quadrants RNFL thickness between the patients with Prog(+)MS and with Prog(-)MS for groups: all patients, with ON and without ON. The generalized estimating equation (GEE) model was used, taking into consideration progression, duration of MS, history of ON, age, gender, EDSS, immunomodulatory therapy and refraction.

All patients **RNFL**	Model coefficient	Standard error	*P*-value	95% Confidence interval
Mean	−9.377251	4.174773	**0.025**	−17.55966 1.194847
Nasal	−11.8438	5.14028	**0.021**	−21.91856 1.769037
Temporal	1.758288	4.796671	0.714	−7.643014 11.15959
Superior	−19.15584	5.694662	**0.001**	−30.31718 7.994512
Inferior	−10.65656	6.473989	0.100	−23.34535 2.032225
Patients ON (-)				
Mean	−7.051398	4.75238	0.138	−16.36589 2.263095
Nasal	−10.83796	7.818953	0.166	−26.16282 4.486911
Temporal	8.910114	6.080993	0.143	−3.008414 20.82864
Superior	−21.13999	7.583708	**0.005**	−36.00379 6.276197
Inferior	−4.010025	8.305342	0.629	−20.2882 12.26815
Patients ON (+)				
Mean	−19.79276	6.140868	**0.001**	−31.82864 7.756876
Nasal	−14.11805	6.159119	**0.022**	−26.1897 2.046396
Temporal	−17.78644	8.415413	**0.035**	−34.28035 1.292533
Superior	−17.90114	7.944477	**0.024**	−33.47203 2.330255
Inferior	−31.16797	8.882788	**0.0001**	−48.57751 13.75762

MS-multiple sclerosis; Prog(+)MS-progressive; Prog(-)MS-non-progressive; ON-history of optic neuritis; RNFL- retinal nerve fiber layer; Pearson chi2 test.

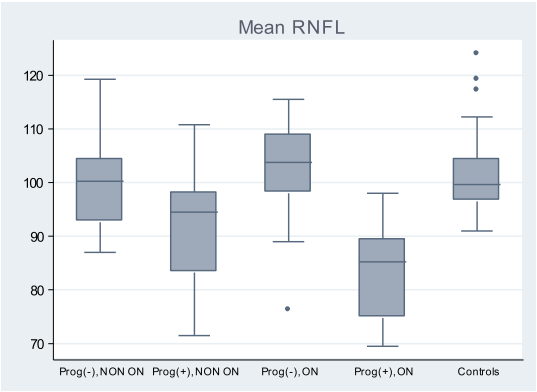
therapy. However, patients who were not receiving immunomodulatory therapy tended to have a lower mean RNFL thickness compared to those receiving immunomodulation.

4. Discussion

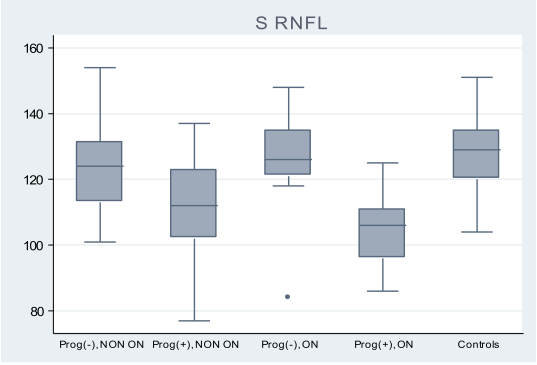
Inflammatory demyelination involving the retrobulbar segment of the optic nerve is considered to be the direct cause of MS ON. In addition to this process, other factors accompanying inflammation, such as vascular dysregulation leading to ischemia, may play a role in the pathogenesis of optic neurodegeneration (Jankowska et al., 2015; Pache et al., 2003). This is supported by pallor of the ONH and an increase in the cup-to-disc ratio (CDR) in MS patients both with and without a history of ON (Rebolleda et al., 2009).

The pathomechanism of axonal loss, observed in MS, has not been fully elucidated, but it is known that ON produces substantial focal

a) Difference in the mean (four quadrants) RNFL thickness between the patients with Prog(+) MS and with Prog(-)MS with a history of optic neuritis (ON) (P=.001).



b) Difference in the superior quadrant RNFL thickness (S RNFL) between the patients with Prog(+) MS and with Prog(-)MS with a history of optic neuritis (ON) (P=.024) and a without a previous episode of ON (P=.005).



c) Difference in the inferior quadrant RNFL thickness (I RNFL) between the patients with Prog(+) MS and with Prog(-)MS with a history of optic neuritis (ON) (P=.0001).

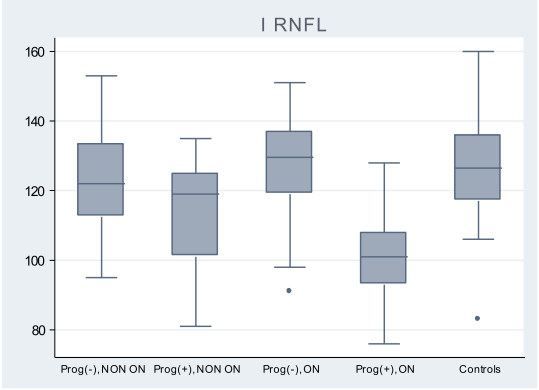
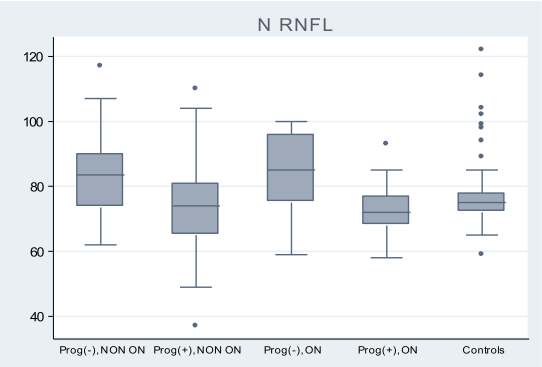


Fig. 1. *x*-axis – Prog(+)MS [progressive multiple sclerosis], Prog(-)MS [non-progressive multiple sclerosis], ON [history of optic neuritis], NON ON [without history of optic neuritis], Control [control group] *y*-axis – RNFL values (μm), **(a)** Difference in the mean (four quadrants) RNFL thickness between the patients with Prog(+) MS and with Prog(-)MS with a history of optic neuritis (ON) ($P = 0.001$). **(b)** Difference in the superior quadrant RNFL thickness (S RNFL) between the patients with Prog(+) MS and with Prog(-)MS with a history of optic neuritis (ON) ($P = 0.024$) and a without a previous episode of ON ($P = 0.005$). **(c)** Difference in the inferior quadrant RNFL thickness (I RNFL) between the patients with Prog(+) MS and with Prog(-)MS with a history of optic neuritis (ON) ($P = 0.0001$)., **(d)** Difference in the nasal quadrant RNFL thickness (N RFLN) between the patients with Prog(+) MS and with Prog(-)MS with a history of optic neuritis (ON) ($P = 0.022$). **(e)** Difference in the temporal quadrant RNFL thickness (T RFLN) between the patients with Prog(+) MS and with Prog(-)MS with a history of optic neuritis (ON) ($P = 0.035$).

d) Difference in the nasal quadrant RNFL thickness (N RFLN) between the patients with Prog(+) MS and with Prog(-)MS with a history of optic neuritis (ON) (P=.022).



e) Difference in the temporal quadrant RNFL thickness (T RFLN) between the patients with Prog(+) MS and with Prog(-)MS with a history of optic neuritis (ON) (P=.035).

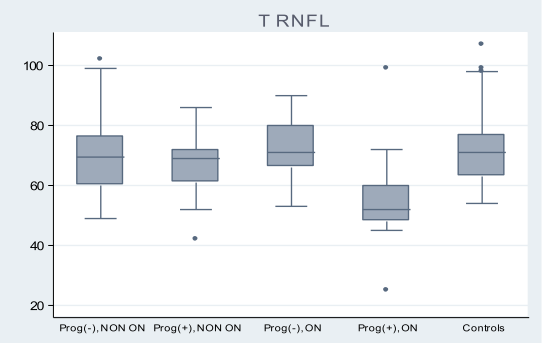


Fig. 1. (*continued*)

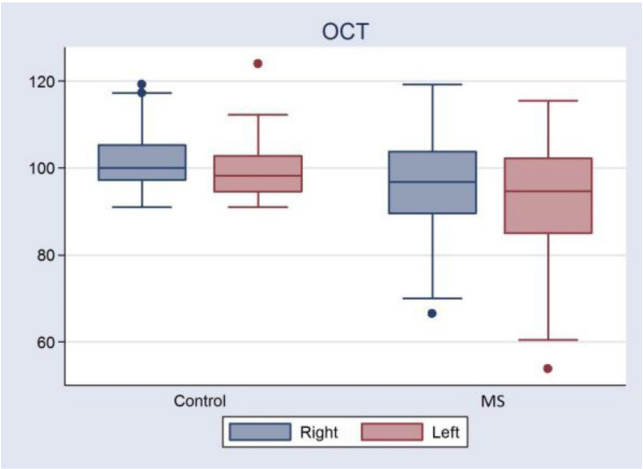


Fig. 2. Mean Peripapillary RNFL Thickness in MS and Healthy Controls. RE: $P = 0.037$ for MS patients vs healthy controls; LE: $P = 0.01$ for MS patients vs healthy controls; Kruskal–Wallis test. (*x*-axis – Control (Control group), MS (Multiple sclerosis); *y*-axis – RNFL values (μm).

damage to the RNFL and GCL (Aktas et al., 2007; Britze et al., 2017; Chitnis et al., 2017; Henderson et al., 2008; Khanifar et al., 2010; Serbecic et al., 2012; Serbecic et al., 2011; Serbecic et al., 2010).

In our study, we assessed peripapillary RNFL thickness in both progressive and non-progressive MS phenotypes. We also compared global RNFL thickness in healthy controls and MS patients, and mean RNFL thickness in MS patients with and without a history of ON. We found a significantly lower RNFL thickness in MS patients compared with healthy controls. This is consistent with data published from. This is consistent with data published from numerous OCT studies (Balk et al., 2014; Cstello et al., 2009; Dorr et al., 2011; Gelfand et al., 2012; Oberwahrenbrock et al., 2012; Pueyo et al., 2010; Retchford et al., 2013; Seidha et al., 2011; Seigo et al., 2012; Serbecic et al., 2011; Suhs et al., 2012; Syc et al., 2012; Tatrai et al., 2012; Walter et al., 2012; Watson et al., 2011). In addition, we found a significantly lower RNFL thickness in MS with a history of ON compared to those without it. Similar findings have also been reported by other authors (Khanifar et al., 2010; Serbecic et al., 2012,).

In the current study, there was a statistically significant difference in superior quadrant RNFL thickness between the Prog(+)MS and Prog(-) MS groups in the subset of MS patients without a history of ON. The differences between Prog(+)MS and Prog(-)MS patients was even more evident in the subset with a history of ON, in which there were statistically significant differences in RNFL thickness in all quadrants.

A significantly lower RNFL thickness has likewise been reported by Oberwahrenbrock in patients with progressive MS compared with individuals with RRMS (Oberwahrenbrock et al., 2012). He used SD-OCT to study a group of 414 MS patients (308 RRMS, 65 SPMS, 41 PPMS), aged 19–59 years with mean disease duration of 9 years and mean EDSS score of 2.5, and he found significantly more pronounced RNFL thinning in 85 eyes of SPMS patients compared with 405 eyes of RRMS patients. There was no statistically significant difference in RNFL thickness between patients with SPMS and those with PPMS.

Gelfand performed SD-OCT on 541 patients with MS (45 CIS, 403

Table 4
Data for Prog(-)MS and Prog(+)MS for groups with/without ON for the following parameters: age, EDSS, duration of MS; refraction (negative spherical equivalent), immunomodulatory therapy, gender.

Prog(+)MS ON(+)	Mean	Range	SD(±)	**ON(-)** Mean	Range	SD(±)	P-value
Age(years)	42	24–58	10.70	46	24–62	11.35	.42 (a)
EDSS	3.17	1.5–6.05	1.64	4.40	2.0–5.5	1.43	0.08 (a)
Duration of MS (years)	6.2	1–22	6.07	3.92	1–15	3.96	.17 (a)
Negative spherical equivalent	1.7	3.0–0.5	0.96	3.1	4.0–1.5	1.13	.11 (a)
RE (right eye) LE (left eye)	1.0	2.75–1.75	1.7	3.1	5.0–0.5	1.9	.14 (a)
Immunomodulatory therapy (%)		30		25		1 (b)	
Gender (females %)		80		67		.65 (b)	

Prog(-)MS ON(+)	Mean	Range	SD(±)	**ON(-)** Mean	Range	SD(±)	P-value
Age (years)	32	22–45	7.46	39	29–54	7.82	.03 (a)
EDSS	1.4	1–3.5	0.77	1.39	1–2.5	0.40	.36 (a)
Duration of MS (years)	3.7	1–10	3.35	2.6	1–10	3.09	.27 (a)
Negative spherical equivalent	1.3	2.5–0.5	0.77	1.0	4.0–0.5	1.22	.09 (a)
RE (right eye) LE (left eye)	1.5	2.5–1.0	0.64	1.1	4.0–0.5	1.29	.05 (a)
Immunomodulatory therapy (%)		81.8		46.6		.11 (b)	
Gender (females %)		82		67		.66 (b)	

MS-multiple sclerosis; ON-history of optic neuritis; Prog(+)MS-progressive; Prog(-)-non-progressive; (a) Mann-Whitney test; (b) Fisher test.

RRMS, 60 SPMS, 33 PPMS) and found that the global and temporal RNFL was thinner in patients with a CIS when compared with controls (Gelfand et al., 2012). RNFL thickness was almost identical between individuals with PPMS and those with SPMS, and there was no difference between the PPMS and RRMS subsets. These observations were even more evident in patients with a history of ON. The authors concluded that retinal axonal loss begins very early in the course of MS, is more prominent in the progressive stages, and does not differ between progressive MS phenotypes.

A review of the literature demonstrates some inconsistencies concerning reported RNFL thickness in different clinical subtypes of MS; two studies using SD-OCT (Oberwahrenbrock and Gelfand) reported different results. We adapted the same definitions used for progressive MS courses as Oberwahrenbrock group, while Galfand didn't specifically mention it in his paper. Our findings are close to those of Oberwahrenbrock et al*;* indeed patient EDSS scores was similar in tese two studies (2.55 *vs* 2.5 respectively) as was patient age range (22–62 years vs 19–59 years, respectively), although the mean duration of disease following diagnosis was shorter in our study (4.4 years vs 9 years, respectively).

A limitation of our study is the small sizes of the study subgroups. To increase the statistical power of the study, we included patients with PPMS or SPMS in the Prog(+) MS group. The PPMS subgroup comprised only 3 patients and could not have affected the final results, particularly given the published reports of no differences in RNFL thickness between patients with PPMS or SPMS (Oberwahrenbrock et al., 2012; Retchford et al., 2013).

We observed a more pronounced difference in RNFL thickness between Prog(-)MS and Prog(+)MS groups in patients with a history of ON, despite possible interference of focal, post-inflammatory RNFL loss, and overall neuronal loss. In the subset without ON, a significant difference between Prog(-)MS and Prog(+)MS patients was found for the superior quadrant. This may have resulted from a combination of compensatory factors, considering that the mean duration of disease in our study was rather short (i.e. half of the duration in the study by Oberwahrenbrock).

We found differences in RNFL thinning in temporal quadrants between the Prog(+)MS and Prog(-)MS that can be explained by number NO incidents, disaese duration and EDSS variations among subjects (Martinez-Lapiscina et al., 2016). The findings we report justify assessment of peripapillary RNFL thickness as another indicator of MS disease progression; however it is uncertain whether it can be considered a marker for MS progression. This uncertainty is due to both the pathomechanisms of ONH degeneration, which have not been fully explained, and the scanning techniques used for RNFL assessment. Results from meta-analyses and long term, observational studies (Henderson et al., 2010; Petzold et al., 2010; Serbecic et al. 2011; Talman et al., 2010) are not consistent. These discrepancies could be explained by the use of different techniques software, degree of pupil dilation, and measurement reproducibility (Bhargava et al., 2015; Costello et al., 2009; Garcia-Martin et al., 2011; Henderson et al., 2008). Indeed a very subtle RNFL loss may be difficult to detect if OCT resolution and signal strength is not sufficient. Discrepancies between results may also be related to the wide variations in RNFL thickness in healthy individuals.

Interpretation of the results may also be influenced by the interference of focal RNFL loss and hypothetical global axonal loss. Assuming global RNFL loss, it is difficult to determine the time of its occurrence. When considering using RNFL lesions as a structural marker for disease activity, one question is whether axons of the optic nerve, which are small in number relative to the billions of axons in the CNS, may be treated as anatomical and functional representatives of CNS axons, however in some studies correlation between ON and brain atrophy has been already found (Saidha et al., 2015). Finally, most patients develop RRMS with clinically stable intervals lasting several months or in some cases decades, but this should not exclude the likelihood of continuous subclinical degeneration.

5. Conclusion

RNFL loss observed in this study in patients with clinically defined subtypes of MS, with and without a history of ON, is consistent with

previous studies. Differences in the prevalence, extent and amount of RNFL thinning, may be related to MS subtype, degree of disability (EDSS), disease duration, time since last relapse, number of relapses, immunomodulatory therapy and other factors. RNFL thickness, with examination of particular retinal segments, may be taken into consideration as a potential biomarker for disease activity, for use in evaluating research results or in assessing disease progression and the effects of neuroprotective or neuroregenerative treatments. In the future, assessment of RNFL thickness may become one of the standard diagnostic tool for estimation of disease prognosis. However, there are numerous limitations of the currently used methods and caution shoul be advised. There have been several attempts already made to unify and standardize both obtaining and reporting of spectral-domain OCT data, as well to keep high quality of clinical trials using this diagnostic tool. To mention the most important of them: OSCAR-IB consensus criteria for retinal OCT quality assessment (Tewarie et al., 2012) and the APOSTEL recommendations for reporting quantitative optical coherence tomography studies (Cruz-Herranz et al., 2016). Long-term studies, further standardisation, and improvement of imaging technique may help to overcome these limitations in future.

Author contributions

All authors meet four authorship ICMJE (International Committee of Medical Journal Editors) criteria.

Jankowska-Lech Irmina and Wasyluk Jaromir had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

All authors declared that there is no conflict of interest.

References

Aktas, O., Ullrich, O., Infante-Duarte, C., et al., 2007. Neuronal damage in brain inflammation. Arch. Neurol. 64 (2), 185–189.

Alonso, R., Gonzalez-Moron, D., Garcea, O., 2018. Optical coherence tomography as a biomarker of degeneration in multiple sclerosis: a review. Mult. Scler. Relat. Disord. 22, 77–82.

Balcer, L.J., 2006. Clinical practice. Optic neuritis. N. Engl. J. Med. 354, 1273–1280.

Balcer, L.J., Frohman, E.M., 2010. Evaluating loss of visual function in multiple sclerosis as measured by low-contrast letter acuity. Neurology 74 (Suppl 3), 16–23.

Balcer, L.J., Miller, D.H., Reingold, S.C., et al., 2015. Vision and vision-related outcome measures in multiple sclerosis. Brain 138, 11–27.

Balk, L.J., Petzold, A., 2014. Current and future potential of retinal optical coherence tomography in multiple sclerosis with and without optic neuritis. Neurodegener. Dis. Manag. 4, 165–176.

Balk, L.J., Tewarie, P., Killestein, J., Polman, C.H., Uitdehaag, B.M.J., Petzold, A., 2014. Disease course heterogeneity and OCT in multiple sclerosis. Mult. Scler. 2014, 1198–1206.

Barkhof, F., Calabresi, P.A., Miller, D.H., Reingold, S.C., 2009. Imaging outcomes for neuroprotection and repair in multiple sclerosis trials. Nat. Rev. Neurol. 5, 256–266.

Behbehani, R., Al-Hassan, A.A., Al-Salahat, A., Sriraman, D., Oakley, J.D., Alroughani, R., 2017. Optical coherence tomography segmentation analysis in relapsing remitting versus progressive multiple sclerosis. PLoS One 12 (2), e0172120.

Bhargava, P., Lang, A., Al-Louzi, O., et al., 2015. Applying an open-source segmentation algorithm to different OCT devices in multiple sclerosis patients and healthy controls: implications for clinical trials. Mult. Scler. Int. 2015, 136295.

Britze, J., Pihl-Jensen, G., Frederiksen, J.L., 2017. Retinal ganglion cell analysis in multiple sclerosis and optic neuritis: a systematic review and meta-analysis. J. Neurol. 264 (9), 1837–1853.

Chan, J.W., 2012. Recent advances in optic neuritis related to multiple sclerosis. Acta Ophthalmol. 90, 203–209.

Chitnis, T, Weiner, HL., 2017. CNS inflammation and neurodegeneration. J. Clin. Invest. 127 (10), 3577–3587.

Compston, A., Coles, A., 2008. Multiple sclerosis. Lancet 372 (9648), 1502–1517.

Costello, F., Hodge, W., Pan, Y.I., Freedman, M., et al., 2009. Differences in retinal nerve fiber layer atrophy between multiple sclerosis subtypes. J. Neurol. Sci. 281, 74–79.

Cruz-Herranz, a., Balk, L.J., Oberwahrenbrock, T., et al., 2016. The APOSTEL recommendations for reporting quantitative optical coherence tomography studies. Neurology 86 (24), 2303–2309.

Dorr, J., Wernecke, K.D., Bock, M., et al., 2011. Association of retinal and macular damage with brain atrophy in multiple sclerosis. PLoS One. 6, e18132.

Fisher, J.B., Markowitz, C.E., Galetta, S.L., et al., 2006. Relation of visual function to retinal nerve fiber layer thickness in multiple sclerosis. Ophthalmology 113, 324–332.

Fjeldstad, C., Bemben, M., Pardo, G., 2011. 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, 1469–1472.

Frohman, E.M., Frohman, T.C., Zee, D.S., McColl, R., Galetta, S., 2005. The neuro-ophthalmology of multiple sclerosis. Lancet Neurol. 4, 111–121.

Frohman, E.M., Fujimoto, J.G, Frohman, T.C., Calabresi, P.A., Cutter, G., Balcer, L.J., 2008. Optical coherent tomography: a window into the mechanisms of multiple sclerosis. Nat. Clin. Pract. Neurol. 4 (12), 664–675.

Galetta, K.M., Calabresi, P.A., Frohman, E.M., Balcer, L.J., 2011. Optical coherence tomography (OCT): imaging the visual pathway as a model for neurodegeneration. Neurotherapeutics 8, 117–132.

Garcia-Martin, E., Pueyo, V., Pinilla, I., Ara, R.A., Martin, J., Fernandez, J., 2011. Fourier-domain OCT in multiple sclerosis patients: reproducibility and ability to detect retinal nerve fiber layer atrophy. Invest. Ophthalmol. Vis. Sci. 52, 4124–4131.

Gelfand, J.M., Goodin, D.S., Boscardin, W.J., Nolan, R., Cuneo, A., Green, A.J., 2012. Retinal axonal loss begins early in the course of multiple sclerosis and is similar between progressive phenotypes. PLoS One 7, e36847.

Gordon-Lipkin, E., Chodkowski, B., Reich, D.S., 2007. Retinal nerve fiber layer is associated with brain atrophy in multiple sclerosis. Neurology 69, 1603–1609.

Grazioli, E., Zivadinow, R., Weinstock-Guttman, B., et al., 2008. Retinal nerve fiber thickness is associated with brain MRI outcomes in multiple sclerosis. J. Neurol. Sci. 268, 12–17.

Henderson, A.P., Trip, S.A., Schlottmann, P.G., et al., 2010. A preliminary longitudinal study of the retinal nerve fiber layer in progressive multiple sclerosis. J. Neurol. 257 (7), 1083–1091.

Henderson, A.P.D., Trip, S.A., Schlottmann, P.G., et al., 2008. An investigation of the retinal nerve fiber layer in progressive multiple sclerosis using optical coherence tomography. Brain 131, 277–287.

Huang, D., Swanson, E.A., Lin, C.P., et al., 1991. Optical coherence tomography. Science 254, 1178–1181.

Ikuta, F., Zimmerman, H.M., 1976. Distribution of plaques in seventy autopsy cases of multiple sclerosis in the United States. Neurology 26, 26–28.

Jankowska-Lech, I., Terelak-Borys, B., Grabska-Liberek, I., Palasik, W., Bik, W., Wolinska-Witort, E., 2015. Decreased endothelin-1 plasma levels in multiple sclerosis patients: a possible factor of vascular dysregulation? Med. Sci. Monit. 21, 1066–1071.

Khanifar, A.A., Parlitsis, G.J., Ehrlich, J.R., et al., 2010. Retinal nerve fiber layer evaluation in multiple sclerosis with spectral domain optical coherence tomography. Clin. Ophthalmol. 4, 1007–1013.

Klistorner, A., Nguyen, T., Garrick, R., et al., 2009. Multifocal VEP and OCT in optic neuritis: a topographical study of the structure- function relationship. Doc. Ophthalmol. 118, 129–137.

Klistorner, A., Sriram, P., Vootakuru, N., et al., 2014. Axonal loss of retinal neurons in multiple sclerosis associated with optic radiation lesions. Neurology 82 (24), 2165–2172.

Kurtzke, J.F., 1983. Rating neurological impairment in multiple sclerosis: an expanded disability scale. Neurology 33, 1444–1452.

Lamirel, C., Newman, N.J., Biousse, V., 2010. Optical coherence tomography (OCT) in optic neuritis in multiple sclerosis. Rev. Neurol. 166, 978–986.

Lublin, F.D., 2014. New multiple sclerosis phenotypic classification. Eur. Neurol. 72 (Suppl 1), 1–5.

Lublin, F.D., Reingold, S.C., 1996. Defining the clinical course of multiple sclerosis: results of an international survey. Neurology 46, 907–911.

Martinez-Lapiscina, E.H., Arnow, S., Wilson, J.A., et al., 2016. Retinal thickness measured with optical coherence tomogtraphy and risk of disability worsening in multiple sclerosis: a cohort study. Lancet Neurol. 15, 574–584.

Noval, S., Contreras, I., Munoz, S., Oreja-Guevara, C., Manzano, B., Rebolleda, G., 2011. Optical coherence tomography in multiple sclerosis and neuromyelitis optica: an update. Mult. Scler. 472790, 1–11.

Oberwahrenbrock, T., Schippling, S., Ringelstein, M., et al., 2012. Retinal damage in multiple sclerosis disease subtypes measured by high -resolution optical coherence tomography. Mult. Scler. Int., 530305.

Pache, M., Kaiser, H., Akhalbedashvili, N., et al., 2003. Extraocular blood flow and endothelin-1 plasma levels in patients with multiple sclerosis. Eur. Neurol. 49, 164–168.

Petzold, A., de Boer, J.F., Schippling, S., 2010. Optical coherence tomography in multiple sclerosis: a systemic review and meta-analysis. Lancet Neurol. 9, 921–932.

Petzold, A., Nijland, P.G., Balk, L.J., et al., 2015. Visual pathway neurodegeneration winged by mitochondrial dysfunction. Ann. Clin. Transl. Neurol. 2 (2), 140–150.

Polman, C.H., Reingold, S.C., Banwell, B., et al., 2011. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann. Neurol. 69, 292–302.

Polman, C.H., Reingold, S.C., Edan, G., et al., 2005. Diagnostic criteria for multiple sclerosis: 2005 revisions to the “McDonald criteria”. Ann. Neurol. 58, 840–846.

Pueyo, V., Ara, J.R., Almarcegui, C., et al., 2010. Sub-clinical atrophy of the retinal nerve fiber layer in multiple sclerosis. Acta Ophthalmol. 88, 748–752.

Pulicken, M., Gordon-Lipkin, E., Balcer, L.J., Frohman, E., Cutter, G., Calabresi, P.A., 2007. Optical coherence tomography and disease subtype in multiple sclerosis. Neurology 69 (22), 2085–2092.

Ratchford, J.N., Saidha, S., Sotirchos, E.S., et al., 2013. Active MS is associated with accelerated retinal ganglion cell / inner plexiform layer. Neurology 80, 47–54.

Rebolleda, G., Diez-Alvarez, L., Casado, A., et al., 2015. OCT: new perspectives in neuro-ophthalmology. Saudi J. Ophthalmol. 29 (1), 9–25.

Rebolleda, G., Noval, S., Contreras, I., Arnalich-Montiel, F., Garcia- Perez, J.L., Munoz-Negrete, F.J., 2009. Optic disc cupping after optic neuritis evaluated with optic coherence tomography. Eye 23, 890–894.

Saidha, S, Al-Louzi, O., Ratchford, J.N., et al., 2015. Optical coherence tomography reflects brain atrophy in multiple sclerosis: a four-year study. Ann. Neurol. 78 (5), 801–813.

Saidha, S., Syc, S.B., Ibrahim, M.A., et al., 2011. Primary retinal pathology in multiple

sclerosis as detected by optical coherence tomography. Brain 134, 518–533.
Salapa, H., Lee, S., Shin, Y., Levin, M., 2017. Contribution of the degeneration of the neuro-axonal unit to the pathogenesis of multiple sclerosis. Brain Sci. 7, 69.
Seigo, M.A., Sotirchos, E.S., Newsome, S., et al., 2012. In vivo assessment of retinal neuronal layers in multiple sclerosis with manual and automated optical coherence tomography segmentation techniques. J. Neurol. 259 (10), 2119–2130.
Sepulcre, J., Murie-Fernandez, M., Salinas-Alaman, A., 2007. Diagnostic accuracy of retinal abnormalities in predicting disease activity in MS. Neurology 68, 1488–1494.
Serbecic, N., Aboul-Enein, F., Beutelspacher, S.C., et al., 2010. Heterogeneous pattern of retinal nerve fiber layer in multiple sclerosis. High resolution optical coherence tomography: potential and limitations. PLoS One 5, e13877.
Serbecic, N., Aboul-Enein, F., Beutelspacher, S.C., et al., 2011. High resolution spectral domain optical coherence tomography (SD-OCT) in multiple sclerosis: the first follow up study over two years. PLoS One. 6, e19843.
Serbecic, N., Beutelspacher, S.C., Kircher, K., Reitner, A., Schmidt-Erfurth, U., 2012. Interpretation of RNFLT values in multiple sclerosis-associated acute optic neuritis using high-resolution SD-OCT device. Acta Ophthalmol. 90, 540–545.
Siepman, T.A.M., Bettink-Remeijer, M.W., Hintzen, R.Q., 2010. Retinal nerve fiber layer thickness in subgroups of multiple sclerosis, measured by optical coherence tomography and scanning laser polarimetry. J. Neurol. 257 (10), 1654–1660.
Sriram, P., Wang, Ch., Yiannikas, C., et al., 2014. Relationship between optical coherence tomography and electrophysiology of the visual pathway in non-optic neuritis eyes of multiple sclerosis patients. PLoS One 9 (8), e102546.
Suhs, K.W., Hein, K., Pehlke, J.R., Kasmann-Kellner, B., Diem, R., 2012. Retinal nerve fiber layer thinning in patients with clinically isolated optic neuritis and early treatment with interferon-beta. PLoS One 7, e51645.
Syc, S.B., Saidha, S., Newsome, S.D., et al., 2012. Optical coherence tomography segmentation reveals ganglion cell layer pathology after optic neuritis. Brain 135, 521–533.
Tallantyre, E.C., Bo, L., Al-Rawashdeh, O., et al., 2010. Clinico-pathological evidence that axonal loss underlies disability in progressive multiple sclerosis. Mult. Scler. 16 (4), 406–411.
Talman, L.S., Bisker, E.R., Sackel, D.J., et al., 2010. Longitudinal study of vision and retinal nerve fiber layer thickness in multiple sclerosis. Ann. Neurol. 67 (6), 749–760.
Tatrai, E., Magdolna, S., Iljicsov, A., Nemeth, J, DeBuc, D.C., Somfai, G.M., 2012. In vivo evaluation of retinal neurodegeneration in patients with multiple sclerosis. PLoS One 7, e30922.
Tewarie, P., Balk, L., Costelo, F., et al., 2012. The OSCAR-IB consensus criteria for retinal OCT quality assessment. PloS One 7 (4), e34823.
Walter, S.D., Ishikawa, H., Galetta, K.M., et al., 2012. Ganglion cell loss in relation to visual disability in multiple sclerosis. Ophthalmology 119, 1250–1257.
Watson, G.M., Keltner, J.L., Chin, E.K., Harvey, D., Nquyen, A., Park, S.S., 2011. Comparison of retinal nerve fiber layer and central macular thickness measurements among five different optical coherence tomography instruments in patients with multiple sclerosis and optic neuritis. J. Neuroophthalmol. 31, 110–116.
Wojtkowski, M., Bajraszewski, T., Gorczyńska, I., et al., 2004. Ophthalmic imaging by spectra opical coherence tomography. Am. J. Ophthalmol. 138, 412–419.
Wojtkowski, M., Srinivasan, V., Fujimoto, J.G., et al., 2005. Three- dimensional retinal imaging with high-speed ultrahigh-resolution optical coherence tomography. Ophthalmology 112, 1734–1746.