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Evaluation of the retinal morphological and functional findings in optic neuritis related to multiple sclerosis

Procena rezultata morfološkog i funkcionalnog ispitivanja mrežnjače kod bolesnika sa optičkim neuritisom u sklopu multiple skleroze

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Abstract

Background/Aim. Unilateral optic neuritis (ON), and its pathological substrate, retrobulbar neuritis (RBN), is a common presentation of multiple sclerosis (MS). The aim of the study was to determine the diagnostic and prognostic value of structural and functional examination using novel 'swept-source' optical coherence tomography (SS-OCT) and OCT angiography (OCTA) techniques in patients with MS who experienced RBN. **Methods.** For examining retinal structural and functional changes in both the affected and nonaffected eye of patients with MS, novel techniques, OCT and OCTA, were used. The obtained results were compared with the results of the same examination on the left and right eye of the healthy controls. **Results.** Using OCT, significant differences in the structural integrity and thickness of retinal layers between the eye in which RBN had been detected and the contralateral, nonaffected eye were found (83.73 ± 18.36 vs. 98.67 ± 11.84; $p = 0.013$). On the other hand, the functional examination of the macular vascular plexus did not show significant differences between the affected and the nonaffected eye in these patients (41.86 ± 1.52 vs. 42.52 ± 1.40; $p = 0.228$). Interestingly, comparing the nonaffected eye of patients with RBN and healthy controls, a significant difference in the thickness of the retinal layers between the contralateral eye of the patient and both healthy eyes of healthy subjects was found. OCT examination showed particularly significant thinning of the macular ganglion cell-inner plexiform layer (mGCIPL) (61.07 ± 5.04 vs. 67.53 ± 4.57; $p < 0.001$). **Conclusion.** Overall, our research showed that OCT and OCTA offer an unprecedented opportunity for a safe, reliable, and repetitive assessment of structural and functional retinal changes as invaluable diagnostic and prognostic tools, paving the way for a better understanding of pathogenic mechanisms underlying inflammatory demyelinating and neurodegenerative diseases. In addition, mGCIPL may be a particularly sensitive and reliable biomarker of pathological changes in MS and perhaps in other neurodegenerative diseases.

Key words:
angiography; diagnosis; multiple sclerosis; optic neuritis; tomography, optical coherence.

Apstrakt

Uvod/Cilj. Jednostrani optički neuritis (ON), kao i njegov patofiziološki supstrat, retrobulbarni neuritis (RBN), česta je manifestacija multiple skleroze (MS). Cilj rada bio je da se utvrdi dijagnostički i prognostički potencijal nove metode funkcijskog i strukturnog ispitivanja oka, tzv. „*swept-source*" optičke koherentne tomografije (SS-OKT) i OKT angiografije (OKTA), kod bolesnika sa prethodnom epizodom RBN u okviru MS. **Metode.** Za ispitivanje strukture i funkcije mrežnjače bolesnika sa MS, kako u oku na kome je dijagnostikovan ON, tako i na kontralateralnom oku, korišćene su nove metode, OKT i OKTA. Dobijeni rezultati upoređivani su sa rezultatima istovetnog ispitivanja na levom i desnom oku zdravih ispitanika. **Rezultati.** Korišćenjem OKT, pokazane su značajne razlike u strukturnom integritetu i debljini pojedinih slojeva mrežnjače u oku bolesnika sa RBN, u poređenju sa kontralateralnim okom (83,73 ± 18,36 vs. 98,67 ± 11,84; $p = 0,013$). Sa druge strane, nisu pokazane značajne razlike u perfuziji horoidnog pleksusa ni u jednom oku tih bolesnika (41,86 ± 1,52 vs. 42,52 ± 1,40; $p = 0,228$). Interesantno, poređenjem oka obolelih koje nije bilo zahvaćeno RBN sa zdravim kotrolama, pronađena je značajna razlika u debljini slojeva mrežnjače između kontralateralnog oka bolesnika i oba zdrava oka zdravih ispitanika. Pregled pomoću OKT pokazao je

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posebno značajno istanjenje unutrašnjeg pleksiformnog makularnog sloja mrežnjače (61,07 ± 5,04 vs. 67,53 ± 4,57; $p < 0,001$). **Zaključak.** Naše istraživanje pokazalo je da OKT i OKTA, kao dragocene metode u dijagnostici i prognozi, pružaju odlične mogućnosti za bezbednu, pouzdanu i ponavljanu procenu strukturnih i funkcijskih promena na mrežnjači, ali i za dalja istraživanja mehanizama koja dovode do takvih promena. Takođe, unutrašnji pleksiformni makularni sloj mrežnjače bi mogao biti naročito osetljiv i vredan biomarker u praćenju patoloških promena kod bolesnika sa MS, a moguće i kod drugih neurodegenerativnih oboljenja.

Ključne reči:
angiografija; dijagnoza; multipla skleroza; tomografija, optička, koherentna; n.opticus, neuritis.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). It is one of the most common neurological diseases of the young population, affecting over two million people worldwide [1, 2]. Optic neuritis (ON) is a presenting manifestation of MS in around 25% of cases and occurs at some point in the disease in over half of the patient population [3, 4].

Optic nerve atrophy and thinning of the retinal nerve fiber layer (RNFL) are typical pathological features in patients with MS. Demyelinating retrobulbar optic neuritis (RBN) refers to inflammation and demyelination affecting the optic nerve. In the absence of pathological features in the ocular fundus, these inflammatory and demyelinating processes in the optic nerve are thought to underlie the production of common symptoms of ON, such as acute unilateral loss of vision, relative afferent pupillary defect, and pain during eye movements [5–8]. Dysfunction and degeneration of the optic nerve axon fibers in the optic nerve are early features of the disease; however, the relationship between inflammation, demyelination, and axon loss is unclear. It has been suggested that axon loss may be a result of the delayed type IV hypersensitivity reaction mediated via cytokines and other inflammatory mediators secreted by infiltrating pathogenic T cells. Namely, during ON, acute inflammation is thought to damage axons within the retrobulbar area of the optic nerve. Degeneration initiated at this area progresses retrogradely (dye back) towards the ganglion cell layer, which manifests as residual paleness of the optic nerve head during routine ophthalmoscopy [9–11].

Indeed, axon loss is considered a major underlying mechanism of disability in MS. However, axon loss is hard to quantify. Modern technologies of optical imaging, such as optical coherence tomography (OCT), offer opportunities for such quantification, at least in the retina and optic nerve [12–15]. Namely, OCT, discovered in 1991 (*in vitro* HUANG, *in vivo* HEE 1995), enables examination of a cross-section of inner ocular structures in high resolution, reaching 1–15 μm. This resolution is higher than that which can be currently achieved using ultrasound, magnetic resonance imaging (MRI), and computed tomography. Furthermore, this technique enables visualization *in situ* and in real-time. Importantly, OCT uses a continual, low-frequency beam (infrared spectrum, 800 nm) delivered at less than 1 mW power, making even repeated exposure safe for both general and patient populations [16, 17].

Thus far, the use of OCT has revealed a decrease in the thickness of peripapillary RNFL (pRNFL) in MS patients who had not suffered from ON compared with controls. This finding is in agreement with a large body of literature describing the association of MS with loss of retinal ganglion cells, reduced thickness of RNFL, loss of metabolic activity, and reduced density of retinal vascular plexus [18, 19]. Furthermore, using an adaptation of OCT, the so-called OCT with angiography (OCTA), a decreased vascular perfusion in the region of the optic nerve head (optic disc) in patients with MS, particularly MS patients with a history of ON, was found [20, 21]. Indeed, combining OCTA with other OCT methods vastly improves the diagnostic accuracy of changes in retinal structures in patients with MS.

In recent years, techniques of digital imaging have been further advanced. The latest improvement in the examination of retinal and choroid structures utilizes the so-called swept-source OCT (SS-OCT) beam technology which uses a longer wavelength (1,050 nm) and minimal reflection of choroid plexus than its predecessor, spectral domain (SD-OCT, 840 nm). Hence, SS-OCT enables visualization of the deepest layers of the eye, even through physical obstacles such as cataract, hemorrhage, etc. In addition, the scan speed of SS-OCT is twice that of SD-OCT devices (100,000 A-scans/s compared with 50,000 A-scans/s), enabling faster acquisition and more accurate 3-D imaging of the retina and choroid [22, 23].

In this study, we measured the thickness of the retina and perfusion of the choroid plexus in patients with MS who experienced a previous episode of ON, in both the affected and nonaffected eye and in healthy controls, using a new, deep-range SS-OCT. Our aim was to establish the diagnostic and prognostic value of structural and functional examination using SS-OCT and OCTA techniques in patients with MS who experienced RBN.

Methods

Study design and study subjects

An observational case study was conducted in patients with relapsing-remitting MS and a history of ON and healthy controls, in the period between 2020 and 2022, at the Department of Functional Ophthalmology of the Eye Clinic at the Military Medical Academy, Belgrade, Serbia. The study protocol was was carried out according to the Declaration of Helsinki. All the participants were informed about the

research protocol before giving their written consent to participate in the study.

We examined 15 patients (4 males and 11 females) with confirmed MS according to the McDonald criteria, who had a history of RBN. The average age of the MS patients was 32 years (range 25–43 years). In the control group, we examined 30 healthy volunteers (7 males and 23 females) chosen by a random sample. The average age in this group was 42.5 years (range 36.5–48 years).

Clinical procedures

All patients were examined using head MRI and visual evoked potentials (VEP) to confirm the diagnosis of MS and ON. Patients with a history of ON were assessed using a standardized ophthalmological examination battery: visual acuity, fundus examination, and visual fields examination.

OCT and OCTA were performed using the Topcon-Triton apparatus (Topcon DRI OCT Triton Swept-source OCT, Topcon, Japan), with a 1,050 nm wavelength beam and a scan speed of 100,000 A-scans/s. We measured pRNFL thickness, macular ganglion cell layer (GCL) thickness, and superficial macular perfusion. The thickness of pRNFL was obtained using circular scanning of the optic disc, whereas the macular ganglion cell-inner plexiform layer (mGCIPL) thickness was obtained by scanning the macular volume in the center of the fovea. Images, maps, and data were displayed directly in Topcon software (Figures 1–3). Numerical data were exported to Microsoft Excel.

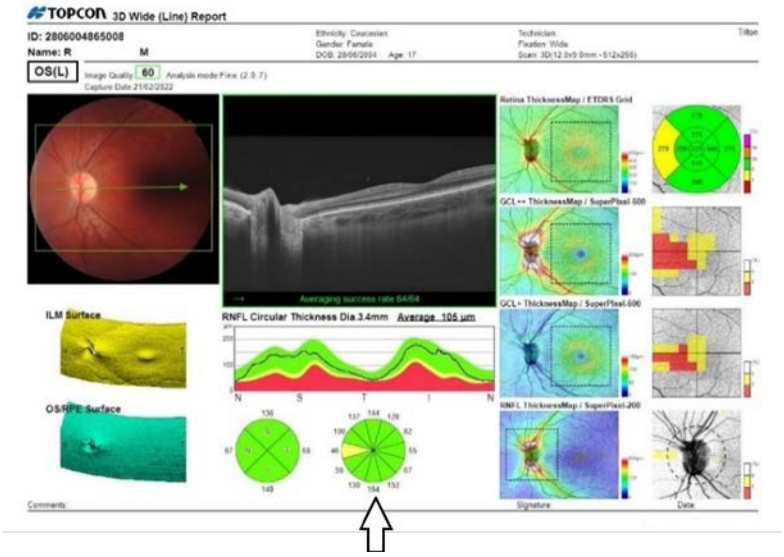

Fig. 1 − Representative images of the retinal nerve fiber layer (RNFL) of the papillary area in the affected eye obtained using Topcon optical coherence tomography (OCT).
Note: The atrophy of papillary retinal nerve fibers in the nasal quadrant.

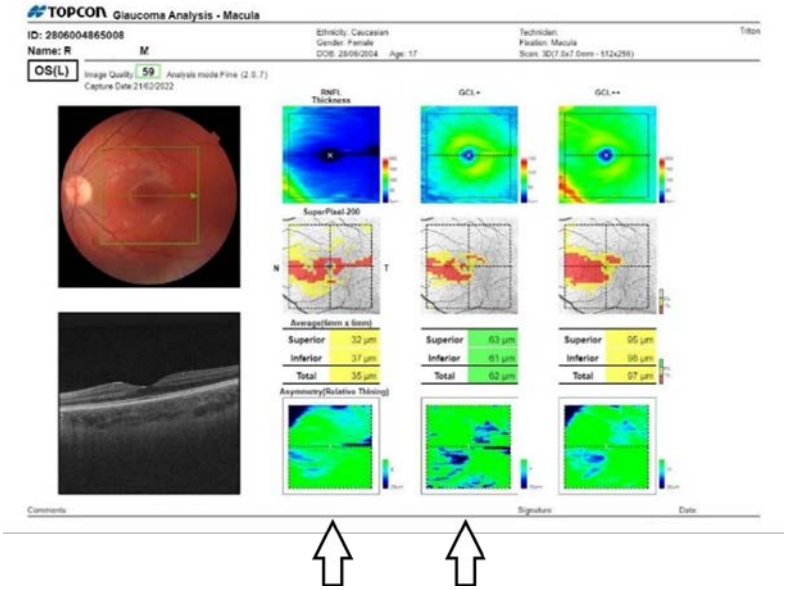

Fig. 2 − Representative images of the ganglion cell layer (GCL) and inner plexiform layer (IPL) in the macula of the affected eye. Bottom left panel shows a cross-section view through the *fovea centralis*, with distinguishable retinal layers.
Note: The GCL and IPL images show a loss of thickness.

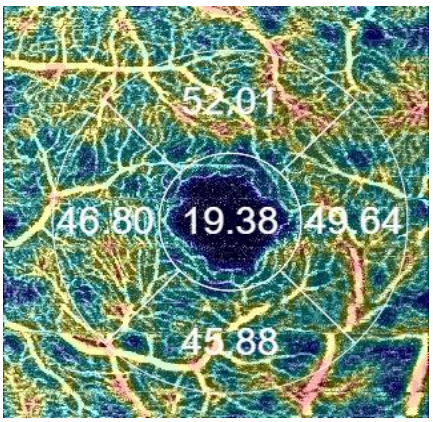

Fig. 3 − Representative image of foveolar and parafoveolar quadrants of the superficial layer of macula examined using optical coherence tomography angiography.

Statistical analysis

The normality of data distribution was tested using the Kolmogorov-Smirnov test. Categorical variables are presented as frequencies and analyzed using the χ^2 test. Continually data are presented as means and standard deviation and analyzed using the matched and nonmatched Student *t*-test. Results are considered statistically significant for a *p*-value lower than 0.05. All analyses were performed using the statistical package IBM SSPS 26.0.

Results

Control group

There were 30 healthy persons in total in the control group (23 females and 7 males), with a mean age of 42.50 years (range 36.50–48.00 years). In order to determine the reference values, we performed OCT and OCTA measurements in healthy controls and compared the values obtained in the left and right eye, as detailed in Table 1. All measurements were within the physiological range. As expected, we found no significant differences between the eyes, apart from a marginal difference in the choroid plexus density in the nasal (N) quadrant (45.97 ± 1.82 vs. 47.922 ± 2.44, right vs. left eye, respectively; $p = 0.028$).

MS patients with RBN

Next, we performed an identical examination in patients with MS, confirmed with the 2017 McDonald criteria, and a loss of vision in one eye, and compared the affected with the nonaffected eye. The average age of these 15 patients (4 males and 11 females) was 32 years. The characteristics of patients with RBN are summarized in Table 2. In

Table 1

Results of OCT and OCTA examination in healthy subjects

Parameter	Right eye	Left eye	*p*-value*
Visual acuity	0.98 ± 0.06	0.99 ± 0.06	0.833
RNFL thickness (μm)	110.27 ± 9.77	110.30 ± 9.61	0.989
superior quadrant	132.73 ± 12.36	134.77 ± 13.82	0.550
inferior quadrant	141.40 ± 16.66	141.70 ± 17.00	0.945
nasal quadrant	85.87 ± 13.68	84.10 ± 13.89	0.622
temporal quadrant	80.33 ± 9.62	78.77 ± 10.04	0.540
mGCIPL (μm)	67.37 ± 4.66	67.70 ± 4.55	0.780
superior	68.10 ± 4.84	68.10 ± 4.78	1.000
inferior	66.63 ± 4.58	67.17 ± 4.42	0.648
Total mRNFL (μm)	41.10 ± 4.54	41.30 ± 5.27	0.875
superior quadrant	39.43 ± 4.69	39.43 ± 5.01	1.000
inferior quadrant	42.70 ± 5.12	43.17 ± 5.74	0.741
OCTA	42.72 ± 1.54	42.87 ± 1.46	0.691
superior quadrant	50.28 ± 2.92	50.42 ± 2.82	0.855
inferior quadrant	49.75 ± 4.40	49.57 ± 3.38	0.859
temporal quadrant	47.65 ± 2.29	46.81 ± 2.42	0.171
nasal quadrant	45.97 ± 1.82	47.22 ± 2.44	0.028
central quadrant	20.05 ± 3.79	20.24 ± 4.17	0.857

OCT – optical coherence tomography; RNFL – retinal nerve fiber layer; mGCIPL − macular ganglion cell-inner plexiform layer; mRNFL – macular RNFL; OCTA – OCT angiography. All values are expressed as mean ± standard deviation.*Independent samples test.

Table 2

General characteristics of patients with RBN

Parameter	Values
Subjects, n	15
Gender, male/female, n (%)	4 (26.7)/11 (73.3)
Age, mean (min-max)	32.00 (25.00–43.00)
Number of patients with loss of VA	15
VA, mean (min-max)	5.00 (1.00–10.00)
Affected eye, left/right, n (%)	10 (66.7)/5 (33.3)

RBN − retrobulbar neuritis; VA – visual acuity; min – minimum; max – maximum; n – number.

these patients, we found that visual acuity was significantly lower before (0.28 ± 0.21) the administration of 1,000 mg of methylprednisolone *iv* as a five-day pulsed treatment than after the treatment was concluded (0.85 ± 0.24).

We found a statistically significant difference in VEP parameters between the affected and nonaffected eye. Namely, the latency was significantly increased in the affected eye (147.20 ± 26.20) compared with the nonaffected eye (119.20 ± 9.61; $p = 0.001$). However, the amplitude of VEP was significantly lower in the affected eye (6.21 ± 3.50) than in the nonaffected eye (9.95 ± 2.78; $p = 0.003$), suggesting neuronal and axonal loss on the affected side. Indeed, further analysis showed a significant loss of RNFL on the affected side compared with the nonaffected side (83.73 ± 18.36 vs. 98.67 ± 11.84; $p = 0.013$). Detailed analysis by quadrants showed that the loss affected the temporal (50.93 ± 13.18 vs. 68.53 ± 14.78; $p = 0.002$), superior (105.13 ± 22.69 vs. 121.87 ± 16.76; $p = 0.029$), and inferior (108.87 ± 27.79 vs. 131.20 ± 19.67; $p = 0.017$) quadrants, but not the nasal quadrant (67.20 ± 14.18 vs. 73.47 ± 14.07; $p = 0.235$).

In addition, we found significant differences in the total thickness of mGCIPL. Namely, total thickness of mGCIPL layers was significantly lower in the affected than the nonaffected eye (54.73 ± 6.06 vs. 61.07 ± 5.04; $p = 0.004$), including that of superior (55.80 ± 6.13 vs. 62.20 ± 5.24; $p = 0.005$) and inferior quadrants (53.47 ± 6.00 vs. 60.20 ± 4.93; $p = 0.002$). Furthermore, the total thickness of macular RNFL was significantly thinner in the affected (31.20 ± 7.64) compared with the nonaffected eye (38.20 ± 5.00; $p = 0.006$). Interestingly, no differences were found in OTCA measurements. Detailed findings in both eyes are presented in Table 3.

Pathological changes within the affected eye in patients with MS compared with the healthy control group

We compared the OCT data (RNFL, mGCIPL, macular RNFL) of the affected eye in MS patients with the healthy controls (both eyes). Indeed, we found that all OCT-derived parameters were significantly lower in the patients than in the healthy controls (Table 4). In addition, the thickness of RNFL and mGCIPL in the nonaffected eye in the patient population was also significantly lower than in healthy controls. Similarly, in the affected eye of patients with RBN, the measurements of the density of choroid plexus obtained using OCTA were significantly lower in the affected eye of the patients compared with the healthy controls (41.86 ± 1.52 vs. 42.80 ± 1.49, respectively; $p = 0.034$), with the single exception for the temporal quadrant.

mGCIPL in the nonaffected eye in patients with RBN compared with the healthy control group

While comparing the nonaffected eye of MS patients with healthy controls, the thickness of RNFL and, in particular, the mGCIPL were significantly reduced (RNFL: 98.67 ± 11.84 for the nonaffected eye in RNB vs. 110.28 ± 9.61 for the healthy controls; $p < 0.001$; mGCIPL 61.07 ± 5.04 for the nonaffected eye vs. 67.53 ± 4.57 for controls; $p < 0.001$). Indeed, the statistically significant reduction between the thickness in all examined quadrants in MS patients vs. controls was replicated (Table 5).

Interestingly, however, the density of the choroid plexus of the nonaffected eye in the patient population was very similar to that of healthy controls (41.86 ± 1.52 vs. 42.52 ±

Table 3

Results of OCT and OCTA examination in MS patients with RBN

Parameter	Affected eye	Nonaffected eye	*p*-value*
VA before methylprednisolone therapy	0.28 ± 0.21	0.99 ± 0.05	< 0.001
VA after methylprednisolone therapy	0.85 ± 0.24	0.99 ± 0.05	0.041
VEP latency	147.20 ± 26.20	119.20 ± 9.61	0.001
VEP amplitude	6.21 ± 3.50	9.95 ± 2.78	0.003
Visual field quadrant degree	3.01 ± 1.08	1.85 ± 1.61	0.028
RNFL (μm)	83.73 ± 18.36	98.67 ± 11.84	0.013
superior quadrant	105.13 ± 22.69	121.87 ± 16.76	0.029
inferior quadrant	108.87 ± 27.79	131.20 ± 19.67	0.017
nasal quadrant	67.20 ± 14.18	73.47 ± 14.07	0.235
temporal quadrant	50.93 ± 13.18	68.53 ± 14.78	0.002
mGCIPL (μm)	54.73 ± 6.06	61.07 ± 5.04	0.004
superior quadrant	55.80 ± 6.13	62.20 ± 5.24	0.005
inferior quadrant	53.47 ± 6.00	60.20 ± 4.93	0.002
Total mRNFL (μm)	31.20 ± 7.64	38.20 ± 5.00	0.006
superior quadrant	29.73 ± 7.55	36.27 ± 4.06	0.006
inferior quadrant	32.53 ± 8.40	39.80 ± 6.55	0.013
OCTA	41.86 ± 1.52	42.52 ± 1.40	0.228
superior	48.43 ± 3.53	49.58 ± 2.33	0.300
inferior	46.84 ± 3.56	48.69 ± 3.14	0.144
temporal	46.77 ± 2.67	46.25 ± 1.98	0.546
nasal	45.18 ± 2.97	46.56 ± 1.68	0.128
central	22.73 ± 4.52	21.64 ± 3.72	0.475

MS – multiple sclerosis; RBN − retrobulbar neuritis; VA – visual acuity; VEP − visual evoked potential. For the abbreviations of other terms see Table 1. All values are expressed as mean ± standard deviation. *Independent samples test.

Table 4

OCT and OCTA findings in the affected eye in patients with RBN and healthy controls (both eyes)

Parameter	Affected eye in RBN	Healthy controls – both eyes	*p*-value*
Visual acuity	0.28 ± 0.21	0.99 ± 0.06	< 0.001
RNFL (μm)	83.73 ± 18.36	110.28 ± 9.61	< 0.001
superior quadrant	105.13 ± 22.69	133.75 ± 13.04	< 0.001
inferior quadrant	108.87 ± 27.79	141.55 ± 16.69	< 0.001
nasal quadrant	67.20 ± 14.18	84.98 ± 13.70	< 0.001
temporal quadrant	50.93 ± 13.18	79.55 ± 9.78	< 0.001
mGCIPL (μm)	54.73 ± 6.06	67.53 ± 4.57	< 0.001
superior quadrant	55.80 ± 6.13	68.10 ± 4.77	< 0.001
inferior quadrant	53.47 ± 6.00	66.90 ± 4.47	< 0.001
Total mRNFL(μm)	31.20 ± 7.64	41.20 ± 4.87	< 0.001
superior quadrant	29.73 ± 7.55	39.43 ± 4.81	< 0.001
inferior quadrant	32.53 ± 8.40	42.93 ± 5.40	< 0.001
OCTA	41.86 ± 1.52	42.80 ± 1.49	0.034
superior quadrant	48.43 ± 3.53	50.35 ± 2.85	0.029
inferior quadrant	46.84 ± 3.56	49.66 ± 3.89	0.013
nasal quadrant	46.77 ± 2.67	47.23 ± 2.38	0.518
temporal quadrant	45.18 ± 2.97	46.59 ± 2.22	0.043
central quadrant	22.73 ± 4.52	20.14 ± 3.95	0.031

RBN − retrobulbar neuritis. For the abbreviations of other terms see Table 1. All values are expressed as mean ± standard deviation. *Independent samples test.

Table 5

OCT and OCTA findings in the nonaffected eye in patients with RBN and healthy controls (both eyes)

Parameter	Nonaffected eye in RNB	Healthy controls – both eyes	*p*-value*
Visual acuity	0.99 ± 0.05	0.99 ± 0.06	0.922
RNFL (μm)	98.67 ± 11.84	110.28 ± 9.61	< 0.001
superior quadrant	121.87 ± 16.76	133.75 ± 13.04	0.004
inferior quadrant	131.20 ± 19.67	141.55 ± 16.69	0.042
nasal quadrant	73.47 ± 14.07	84.98 ± 13.70	0.005
temporal quadrant	68.53 ± 14.78	79.55 ± 9.78	0.001
mGCIPL (μm)	61.07 ± 5.04	67.53 ± 4.57	< 0.001
superior quadrant	62.20 ± 5.24	68.10 ± 4.77	0.001
inferior quadrant	60.20 ± 4.93	66.90 ± 4.47	< 0.001
Total mRNFL (μm)	38.20 ± 5.00	41.20 ± 4.87	0.037
superior quadrant	36.27 ± 4.06	39.43 ± 4.81	0.022
inferior quadrant	39.80 ± 6.55	42.93 ± 5.40	0.058
OCTA	42.52 ± 1.40	42.80 ± 1.49	0.519
superior quadrant	49.58 ± 2.33	50.35 ± 2.85	0.336
inferior quadrant	48.69 ± 3.14	49.66 ± 3.89	0.374
nasal quadrant	46.25 ± 1.98	47.23 ± 2.38	0.144
temporal quadrant	46.56 ± 1.68	46.59 ± 2.22	0.959
central quadrant	21.64 ± 3.72	20.14 ± 3.95	0.189

RBN − retrobulbar neuritis. For the abbreviations of other terms see Table 1. All values are expressed as mean ± standard deviation. *Independent samples test.

1.40, respectively; $p = 0.228$), suggesting that vascular plexus density is a less sensitive parameter than inner ocular layer thickness in the assessment of ocular changes in MS.

Correlation analysis

In order to examine the relationship between the outcome measures obtained using OCT and OCTA, we performed correlation analyses. Indeed, in the affected eye of patients with RBN, we found a highly significant, positive correlation between the RNFL and mGCIPL [Pearson Correlation (r) = 0.833; $p < 0.001$], the tRNFL and macular RNFL ($r = 0.860$; $p < 0.001$), and the mGCIPL and macular RNFL ($r = 0.783$; $p = 0.001$). On the other hand, parameters measured using OCTA did not show a correlation between any variables either in the affected or in the nonaffected eye. In contrast, in healthy controls, a positive correlation between values of OCTA in both the left and right eye was found ($r = 0.491$; $p = 0.006$).

Discussion

Two retinal layers are of considerable importance in MS: the pRNFL, comprised of unmyelinated axons which

form the optic nerve, and the combined mGCIPL which contains the retinal ganglion cells [22, 23]. RNFL is the most proximal part of the afferent optic nerve pathway and a unique pathway in the CNS completely devoid of myelin. Therefore, it is considered one of the most vulnerable regions to pathological and metabolic challenges.

Given that structural changes in these two layers have been associated with visual symptoms and that the retina may be considered an extension of the CNS ("window into the brain"), a high-resolution quantitative assessment of these structures offers an unprecedented opportunity to examine and predict functional optic and wider CNS damage. Indeed, in recent years, the changes affecting the macular inner nuclear layer (INL) have become a major subject of interest as a potential biomarker for CNS inflammation [24–26].

In this study, we examined the thickness of the retina and choroid in both eyes of patients with MS who suffered an episode of ON using a novel SS-deep range imaging OCT technology and compared the results with the healthy control group. Our results show a considerable loss of RNFL in both the affected and nonaffected eye in patients with RBN, compared with a healthy population. Moreover, we show that similar structural changes can be detected in the nonaffected eye of these patients. Although such a finding is not entirely surprising, neurodegeneration is an established and early pathogenic feature of MS. This loss of nerve fibers can be assessed and indirectly quantified by a range of indirect techniques, such as quantification of clinical neurological deficit using the Expanded Disability Status Scale – a gold standard measure of clinical disability in MS patients, MRI, VEP, questionnaires assessing the quality of life, and cognitive ability tests [27–29]. However, precise longitudinal and quantitative measures of axon and neuron loss in MS, with a view to following disease development, progression, and response to therapy and providing prognostic values, is still impractical for most patients and unattainable for the majority of clinics.

Atrophy in pRNFL in MS patients was first described by Parisi et al. [30] in 1999. Since then, several studies have examined the links between the atrophy of inner retinal layers, pRNFL and mGCIPL in particular, and disease characteristics in MS. In patients with ON and confirmed diagnosis of MS, the thickness of pRNFL and mGCIPL was shown to result from retrograde axonal degeneration and retinal ganglion cell loss, which seems to advance up to the INL. On average, the level of atrophy following an episode of ON in MS patients was reported to be 20.1 μm for pRNFL and 16.4 μm for mGCIPL. As the disease progresses, the thinning of these retinal layers increases, and similar degenerative changes have been described in the eye which had not been affected by ON [25, 26].

The ability of OCT to detect structural changes in the retina of patients with MS with a high degree of accuracy and reproducibility propelled this methodology into an irreplaceable tool in differential diagnosis, as well as a reliable high-quality approach to the interpretation of disease progression and therapeutic effects. More specifically, the high precision of OCT in quantifying the loss of thickness of retinal layers within the macula and in peripapillary retinal areas marks a significant advancement in determining the stage of the disease. Moreover, the high safety factor of the technique enabling multiple examinations and longitudinal follow-up has the potential to transform our ability to determine the rate of disease progression in a patient-centered manner. Our results showed that this assessment of the structural integrity of the retina could be refined further using fine subsegmentation of retinal areas, namely, superior, inferior, temporal, and nasal segment assessment. Indeed, our sub-segmental analysis showed that significant loss of retinal thickness occurs in all these segments. Furthermore, we showed that the most severe loss of RNFL thickness affects the temporal segment in both the eye affected by ON in MS patients and the nonaffected eye. This finding is in agreement with results found in other studies [31, 32]. Perhaps surprisingly, the measurements of mGCIPL showed a significant loss of thickness not only in the affected eye of the MS patients but also in the nonaffected eye compared with healthy controls. The latest advancements in the literature confirm our results, suggesting that the thinning in mGCIPL may constitute a particularly sensitive marker of axonal degeneration in MS, indeed more sensitive than the thinning of RNFL [33, 34]. Nonetheless, as the OCT techniques have become more prominent in the clinic, the SS-OCT in particular, further studies are warranted to confirm the exact role of this methodology in the diagnosis and management of neurodegenerative changes [33, 34].

The second part of our study focused on the use of OCTA in the analysis of vascular density within superficial macular layers. Similar to OCT, OCTA is a new, noninvasive method for examination of the vascular perfusion of the macula and optic disc (head). As such, it is of particular importance in the diagnostic assessment of patients with neurodegenerative diseases, particularly of vascular origin, e.g., the small vessel disease of the CNS and its most common consequence, vascular dementia [35]. Indeed, we found a significant reduction in macular perfusion in the eye affected by ON in MS patients, compared with healthy controls. This reduction was particularly pronounced in the temporal segment, which is in agreement with previous studies by Lanzillo et al. [36] and Murphy et al. [37]. However, in contrast with the previous studies, we found no reduction in macular perfusion in the nasal segment of the nonaffected eye in MS patients. This discrepancy is likely to be a result of a relatively small number of participants in our study, a limiting factor in the interpretation of our data. Our correlation analysis showed a strong positive correlation between the analyzed variables, but there was no strong association with OCTA. It can be expected that if we have an increase in one of the indicators, we can expect a jump in other indicators as well because these variables are strongly positively correlated [37, 38].

However, it should be taken into account that the literature describing the use of OCTA in this particular indication is quite scarce. Furthermore, there is a degree of variability in the published literature regarding the dominant location of perfusion deficit in the ON, as well as individual differences in the capillary network, both of which add to the variability encountered in the published work. Nonetheless, the consen-

sus in the literature exists on the fact that macular perfusion deficit, seen in ON and MS, results from pathophysiological characteristics of MS, as opposed to being a consequence of generalized vascular conditions [15, 38]. Of note, however, are epidemiological data suggesting a certain degree of comorbidity between vascular conditions (cardiogenic, cerebrovascular, and peripheral vascular conditions) and MS. Therefore, these systemic conditions may underlie some degree of perfusion changes seen in the macula of MS patients. Further studies to clarify the link between systemic and MS-specific vascular changes are warranted. Importantly, designing such studies should not present a scientific problem in light of the recent advancements in OCT, whereby every patient could be examined for both systemic and macular changes using state-of-the-art techniques. The OCT is perfectly poised in this context, enabling noninvasive, reliable, and reproducible functional measurements of changes in the macular vascular bed. Furthermore, owing to its highly targeted nature of assessment, combined with the ability for frequent reassessment, OCT and OCTA lend themselves to studies where rapid changes in pathogenic and clinical features require techniques with a high degree of sensitivity and specificity in order to provide insight into mechanisms underlying the disease progression.

The limitation of the study was the relatively small number of participants. In addition, the type of study (observational case study) can also be considered a limiting factor.

Conclusion

In this study, we showed that the thinning of the RNFL and GCL layers, as assessed using OCT, represents an accurate and sensitive method for assessing the damage affecting the optic pathway in patients with MS. In addition, we confirmed that, in MS patients, retinal atrophy is present at a certain degree, even in the absence of the previous symptomatic ON. Furthermore, we showed a reduction in vascular plexus density in patients with ON compared with healthy controls. We conclude that OCT represents a novel, noninvasive, reliable, reproducible, and convenient diagnostic tool in general clinical practice, which can be applied to all patients suffering from demyelinating diseases of the CNS. Using this technique, the thickness of retinal layers is quantified in real-time, *in situ*, representing a direct insight into the magnitude of axon and neuron loss in the proximal optic pathway. In addition to the expected thinning of the mGCIPL in the eye affected by ON, we have also detected a thinning in this layer in the nonaffected eye in patients with RBN, compared with healthy controls, which is of great importance. This finding strongly suggests that comparative analysis of RNFL and GCL represents an invaluable biomarker in future research and clinical practice in ON and MS, but also in other vascular and neurodegenerative conditions. Finally, given the well-known neurovascular coupling mechanisms present in the nervous system, the combination of OCT and OCTA promises to lead to a step-change advancement of our understanding and treatment of a wide range of conditions in the eye and CNS as well.

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R E F E R E N C E S

1. *Walton C, King R, Rechtman L, Kaye W, Leray E, Ann Marrie R*, et al. Rising prevalence of multiple sclerosis worldwide: Insights from the Atlas of MS, third edition. Mult Scler 2020; 26(14): 1816–21.
2. *Compston A, Coles A*. Multiple sclerosis. Lancet 2008; 372(9648): 1502–17.
3. *Costello F*. The afferent visual pathway: designing a structural-functional paradigm of multiple sclerosis. ISRN Neurol 2013; 2013: 134858.
4. *Sørensen TL, Frederiksen JL, Brønnum-Hansen H, Petersen HC*. Optic neuritis as onset manifestation of multiple sclerosis: a nationwide, long-term survey. Neurology 1999; 53(3): 473–8.
5. *Shams PN, Plant GT*. Optic neuritis: a review. Int MS J 2009; 16(3): 82–9.
6. *Optic Neuritis Study Group*. The clinical profile of optic neuritis. Experience of the Optic Neuritis Treatment Trial. Arch Ophthalmol. 1991; 109(12): 1673–8.
7. *Dooley MC, Foroozan R*. Optic neuritis. J Ophthalmic Vis Res 2010; 5(3): 182–7.
8. *Costello F*. Inflammatory optic neuropathies. Continuum (Minneap Minn) 2014; 20(4 Neuro-ophthalmology): 816–37.
9. *Plant GT*. Optic neuritis and multiple sclerosis. Curr Opin Neurol 2008; 21(1): 16–21.
10. *Palace J*. Inflammation versus neurodegeneration: consequences for treatment. J Neurol Sci 2007; 259(1–2): 46–9.
11. *Hauser SL, Oksenberg JR*. The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. Neuron 2006; 52(1): 61–76.
12. *Fisher JB, Jacobs DA, Markowitz CE, Galetta SL, Volpe NJ, Nano-Schiavi ML*, et al. Relation of visual function to retinal nerve fiber layer thickness in multiple sclerosis. Ophthalmology 2006; 113(2): 324–32.
13. *Filippi M, Preziosa P, Meani A, Ciccarelli O, Mesaros S, Rovira A*, et al. Prediction of a multiple sclerosis diagnosis in patients with clinically isolated syndrome using the 2016 MAGNIMS and 2010 McDonald criteria: a retrospective study. Lancet Neurol 2018; 17(2): 133–42.
14. *Klistorner A, Arvind H, Nguyen T, Garrick R, Paine M, Graham S*, et al. Multifocal VEP and OCT in optic neuritis: a topographical study of the structure-function relationship. Doc Ophthalmol 2009; 118(2): 129–37.
15. *Garcia-Martin E, Jarauta L, Pablo LE, Bambo MP, Ara JR, Martin J*, et al. Changes in peripapillary choroidal thickness in patients with multiple sclerosis. Acta Ophthalmol 2019; 97(1): e77–e83.
16. *Spaide RF, Fujimoto JG, Waheed NK, Sadda SR, Staurenghi G*. Optical coherence tomography angiography. Prog Retin Eye Res 2018; 64: 1–55.
17. *Zhang Q, Zheng F, Motulsky EH, Gregori G, Chu Z, Chen CL*, et al. A Novel Strategy for Quantifying Choriocapillaris Flow

Voids Using Swept-Source OCT Angiography. Invest Ophthalmol Vis Sci 2018; 59(1): 203–11.
18. *Fjeldstad AS, McDaniel J, Witman MA, Ives SJ, Zhao J, Rose JW*, et al. Vascular function and multiple sclerosis. J Neurol 2011; 258(11): 2036–42.
19. *Feucht N, Maier M, Lepennetier G, Pettenkofer M, Wetzlmair C, Daltrozzo T*, et al. Optical coherence tomography angiography indicates associations of the retinal vascular network and disease activity in multiple sclerosis. Mult Scler 2019; 25(2): 224–34.
20. *Marrie RA, Rudick R, Horwitz R, Cutter G, Tyry T, Campagnolo D*, et al. Vascular comorbidity is associated with more rapid disability progression in multiple sclerosis. Neurology 2010; 74(13): 1041–7.
21. *Keményová P, Siarnik P, Sutovský S, Blaho A, Turcáni P, Kollár B.* Impairment of endothelial function in patients with multiple sclerosis. Neuro Endocrinol Lett 2015; 36(1): 67–71.
22. *Hirata M, Tsujikawa A, Matsumoto A, Hangai M, Ooto S, Yamashiro K,* et al. Macular choroidal thickness and volume in normal subjects measured by swept-source optical coherence tomography. Invest Ophthalmol Vis Sci 2011; 52(8): 4971–8.
23. *Copete S, Flores-Moreno I, Montero JA, Duker JS, Ruiz-Moreno JM.* Direct comparison of spectral-domain and swept-source OCT in the measurement of choroidal thickness in normal eyes. Br J Ophthalmol 2014; 98(3): 334–8.
24. *Sriram P, Wang C, Yiannikas C, Garrick R, Barnett M, Parratt J*, et al. Relationship between optical coherence tomography and electrophysiology of the visual pathway in non-optic neuritis eyes of multiple sclerosis patients. PLoS One 2014; 9(8): e102546.
25. *saidha A, Balcer LJ, Calabresi PA, Costello F, Frohman TC, Frohman EM*, et al. Retinal layer segmentation in multiple sclerosis: a systematic review and meta-analysis. Lancet Neurol 2017; 16(10): 797–812.
26. *Saidha S, Syc SB, Durbin MK, Eckstein C, Oakley JD, Meyer SA*, et al. Visual dysfunction in multiple sclerosis correlates better with optical coherence tomography derived estimates of macular ganglion cell layer thickness than peripapillary retinal nerve fiber layer thickness. Mult Scler 2011; 17(12): 1449–63.
27. *Saidha S, Sotirchos ES, Oh J, Syc SB, Seigo MA, Shiee N*, et al. Relationships between retinal axonal and neuronal measures and global central nervous system pathology in multiple sclerosis. JAMA Neurol 2013; 70(1): 34–43.
28. *Janáky M, Jánossy Á, Horváth G, Benedek G, Braunitzer G.* VEP and PERG in patients with multiple sclerosis, with and without a history of optic neuritis. Doc Ophthalmol 2017; 134(3): 185–93.
29. *Garcia-Martin E, Pueyo V, Pinilla I, Ara JR, Martin J, Fernandez J.* Fourier-domain OCT in multiple sclerosis patients: reproducibility and ability to detect retinal nerve fiber layer atrophy. Invest Ophthalmol Vis Sci 2011; 52(7): 4124–31.
30. *Parisi V, Manni G, Spadaro M, Colacino G, Restuccia R, Marchi S,* et al. Correlation between morphological and functional retinal impairment in multiple sclerosis patients. Invest Ophthalmol Vis Sci 1999; 40(11): 2520–7.
31. *Xu SC, Kardon RH, Leavitt JA, Flanagan EP, Pittock SJ, Chen JJ.* Optical coherence tomography is highly sensitive in detecting prior optic neuritis. Neurology 2019; 92(6): e527–35.
32. *Yau GS, Lee JW, Lau PP, Tam VT, Wong WW, Yuen CY.* Longitudinal Changes in Retinal Nerve Fibre Layer Thickness after an Isolated Unilateral Retrobulbar Optic Neuritis: 1-Year Results. Neuroophthalmology 2015; 39(1): 22–5.
33. *Behbehani R, Al-Moosa A, Sriraman D, Alroughani R.* Ganglion cell analysis in acute optic neuritis. Mult Scler Relat Disord 2016; 5: 66–9.
34. *Syc SB, Saidha S, Newsome SD, Ratchford JN, Levy M, Ford E*, et al. Optical coherence tomography segmentation reveals ganglion cell layer pathology after optic neuritis. Brain 2012; 135(Pt 2): 521–33.
35. *Yoon SP, Grewal DS, Thompson AC, Polascik BW, Dunn C, Burke JR*, et al. Retinal Microvascular and Neurodegenerative Changes in Alzheimer's Disease and Mild Cognitive Impairment Compared with Control Participants. Ophthalmol Retina 2019; 3(6): 489–99.
36. *Lanzillo R, Cennamo G, Criscuolo C, Carotenuto A, Velotti N, Sparnelli F*, et al. Optical coherence tomography angiography retinal vascular network assessment in multiple sclerosis. Mult Scler 2018; 24(13): 1706–14.
37. *Murphy OC, Kwakyi O, Iftikhar M, Zafar S, Lambe J, Pellegrini N*, et al. Alterations in the retinal vasculature occur in multiple sclerosis and exhibit novel correlations with disability and visual function measures. Mult Scler 2020; 26(7): 815–28.
38. *Yilmaz H, Ersoy A, Icel E.* Assessments of vessel density and foveal avascular zone metrics in multiple sclerosis: an optical coherence tomography angiography study. Eye (Lond) 2020; 34(4): 771–8.

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