Daina Pastare

INVESTIGATION OF AFFERENT VISUAL PATHWAY ANALYZING NEURODEGENERATIVE PROCESS IN MULTIPLE SCLEROSIS PATIENTS

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The Doctoral Thesis was developed in Neurology and Ophthalmology Departments of Pauls Stradins Clinical University Hospital

Scientific supervisors:

Dr. med., Professor **Andrejs Millers**,
Department of Neurology and Neurosurgery
of Rīga Stradiņš University, Latvia

Dr. med., Professor **Guna Laganovska**,
Department of Ophthalmology
of Rīga Stradiņš University, Latvia

Official reviewers:

Dr. med., Professor **Ināra Logina**,
Rīga Stradiņš University, Latvia

Dr.med., Professor **Igors Aksiks**,
University of Latvia

Dr. med., Professor **Katrin Gross-Paju**,
Tallinn University of Technology, Estonia

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The thesis is available in the library of RSU and the RSU website: www.rsu.lv

Secretary of the Promotion Council:

Dr. med., Associate Professor **Angelika Krūmiņa**

TABLE OF CONTENTS

ABBREVIATIONS USED

ANCOVA	–	analysis of covariance
ANOVA	–	analysis of variance
AUC	–	area under the curve
CI	–	confidence interval
CNS	–	central nervous system
EDSS	–	Expanded Disability Status Scale
FLAIR	–	magnetic resonance imaging sequence (*fluid attenuated inversion recovery*)
FS	–	functional system
IQR	–	interquartile range
J	–	Youden index
M	–	arithmetic average
MANOVA	–	multivariate analysis of variance
Me	–	median
mkm	–	micrometers
mkV	–	microvolts
MPR	–	multiplanar reconstruction at MRI investigation
MRI	–	magnetic resonance imaging
ms	–	milliseconds
MS	–	multiple sclerosis
N	–	summary number
NPV	–	negative predictive value
OCT	–	optical coherence tomography
ON	–	optic neuritis
ON (−)	–	eyes without a history of optic neuritis
ON (+)	–	eyes with a history of optic neuritis
p	–	reliability factor
PPV	–	positive predictive value
r	–	Pearson's correlation coefficient
RNFL	–	retinal nerve fiber layer
RNFLN	–	retinal nerve fiber layer in the nasal quadrant
RNFLT	–	retinal nerve fiber layer in the temporal quadrant
ROC curve	–	receiver operating characteristic curve
RRMS	–	relapsing remitting multiple sclerosis

r_s – Spearman's rank correlation coefficient
SD – standard deviation
Se – sensitivity
Sp – specificity
T1 – T1 weighted images at magnetic resonance imaging
T2 – T2 weighted images at magnetic resonance imaging
VEP – visual evoked potentials
φ – dichotomous factor

INTRODUCTION

Topicality of the Problem

Multiple sclerosis (MS) is the most common cause of non-traumatic disability in young people and there are more than 2.3 million MS patients in the world (*Browne et al.*, 2014; *WHO*, 2008).

MS pathogenesis is still not completely clear. For a long time, MS was regarded as a primarily demyelinating central nervous system (CNS) disease; however, recent studies have shown that neurodegenerative processes and axonal damage in MS pathogenesis are more important than demyelination (*Siffrin et al.*, 2010; *Zipp and Aktas*, 2006). It was established that neurodegenerative process in MS patients develops early, even before the development of clinical signs and is a major factor in ensuring the formation of progressive disability and brain atrophy (*Bruck*, 2005; *Fisniku et al.*, 2008).

MS course is highly variable and difficult to predict. Factors that currently affect the pronounced variability of the disease and the transition from relapsing remitting to the treatment resistant and progressive stage are not clear. In addition, MS is not fully curable and the available treatment is primarily based on the reduction of the inflammatory process, but has a little effect on neurodegenerative process (*Fiona Costello*, 2013). There is a growing need for neuroprotective therapy and biological markers, by which to predict and monitor the course of the disease, to classify patients as well as to predict and monitor the effectiveness of treatment (*Fernandez*, 2013).

MS often affects the visual system. For fifteen to twenty percent of patients, visual impairments are the first sign of the clinically defined MS and for almost 70% of MS patients during the disease an acute optic neuritis (ON) develops (*Di Maggio et al.*, 2014). The afferent visual pathway is a suitable clinical model for the research on MS and neuroprotective drugs. Afferent visual

pathway in case of acute ON episode represent an acute focal CNS lesion model, but in case of subclinical, chronic retinopathy and optic neuropathy reflects the diffuse, chronic CNS damage model.

The significant advantage of the afferent visual pathway is that it is available for detailed and direct structural (with magnetic resonance imaging (MRI) and optical coherence tomography (OCT) methods) and functional studies (with visual evoked potentials (VEP) method and determining visual acuity, color vision and visual fields). These methods allow the investigation of different, interrelated processes, such as inflammation, demyelination, axonal damage and neurodegeneration.

In recent years, numerous studies of the changes of the afferent visual pathway in MS patients have been conducted, but the results are very contradictory and difficult to compare. The present research has investigated the possibility of the afferent visual pathway studies for an exploration of neurodegenerative processes in MS patients.

Scientific and Practical Novelty

1. For the first time in Latvia a profound study of the changes of the afferent visual pathway and the possibilities of investigation for multiple sclerosis patients was conducted.
2. For the first time in Latvia precise reference values for the visual evoked potential method were determined.
3. In the study data with the possibilities for the diagnosis of subclinical damage to the afferent visual pathway were obtained and a better method for determination of this damage was suggested.
4. In the study new data with the possibilities to predict structural retinal nerve fiber layer damage were obtained, using functional methods of investigation.

5. The results of the study open the way to a better understanding of structurally-functional changes in the afferent visual pathway and allow to use them as biological markers for neurodegenerative process characterization in patients with multiple sclerosis in the future.

Thesis Structure and the Author's Personal Contribution

The Doctoral Thesis is written in Latvian. The Dissertation has the following structure: introduction, aim of the study, objectives and hypotheses, literature review, materials and methods, results, discussion, conclusions, list of used references. The volume of the Paper is 119 pages including 11 tables, 52 pictures and 5 appendices. List of references includes 121 titles.

Author of the Thesis has carried out neurological and neurophysiological examinations of patients and the control group and has collected, systematized and analyzed the findings of the investigations.

Ethical Aspects

To conduct the study, Rīga Stradiņš University Ethics Committee's permission was received (Appendix 1).

Aim of the Study

The aim was to study the significance of changes in afferent visual pathway regarding the evaluation of neurodegenerative processes caused by multiple sclerosis.

Study Objectives

1. To evaluate the neurological status of multiple sclerosis patients with/without optic neuritis history by using the Expanded Disability Status Scale (EDSS).

2. To analyze ophthalmological condition characteristics of multiple sclerosis patients with/without optic neuritis history, determining visual acuity, *fundus oculi* condition, color vision and visual fields.

3. To determine the reference values of visual evoked potential method and to assess P100 latency and N75/P100 amplitude for multiple sclerosis patients with / without optic neuritis in history.

4. To investigate the retinal nerve fiber layer thickness and localization of the focal defect in multiple sclerosis patients with/without optic neuritis in history by using optical coherence tomography method.

5. To analyze the number, location and activity of demyelinating lesions, as well as brain atrophy using magnetic resonance imaging examination in multiple sclerosis patients with / without optic neuritis in history.

6. To perform statistical analysis of the data collected and to determine the potential correlation of clinical, functional and structural changes, and to analyze the factors influencing the reduced retinal nerve fiber layer.

Hypotheses

1. Analyzing the afferent visual pathway preclinical diagnosis of multiple sclerosis created neurodegenerative process in patients without a history of optic neuritis is possible.

2. Neurodegenerative changes detected in the afferent visual pathway correlate with clinical, radiological and functional parameters.

1. MATERIAL AND METHODS

1.1. Patient Groups and Selection

The cross-sectional study included 76 relapsing-remitting MS patients who were divided into two groups:

1) MS patients with a history of optic neuritis;

2) MS patients without signs of optic neuritis in history.

In the group of MS patients with a history of optic neuritis, ON affected eyes (marked with ON (+)) and the contralateral, ON unaffected eyes (marked with ON (-)) were analyzed separately.

The control group included 28 age-matched and sex-matched healthy spontaneously selected individuals. MS patients were recruited from Pauls Stradins Clinical University Hospital Multiple Sclerosis Center, during the period from October 2011 to April 2014. For all subjects involved in the study evaluation of the clinical neurological and ophalmological condition was performed in the Departments of Neurology and Ophthalmology of Pauls Stradins Clinical University Hospital. Magnetic resonance imaging was performed in the Institute of Diagnostic Radiology of Pauls Stradins Clinical University Hospital, and the examination results were interpreted by a certified radiologist specialized in the diagnosis of MS.

Existence of optic neuritis in history was evaluated on the basis of clinical signs and symptoms of optic neuritis (*The clinical profile of optic neuritis. Experience of the Optic Neuritis Treatment Trial. Optic Neuritis Study Group*, 1991). For a portion, however, not all MS patients, optic neuritis episode was documented. Part of MS patients continued the previously started first-line immunomodulatory therapy (interferon beta-1a, interferon beta-1b or copaxone).

1.1.1. Study Inclusion Criteria

In the study were included patients for whom:

- relapsing-remitting MS diagnosis, based on 2010 McDonald criteria, had been approved;
- ≥ 6 months had passed after a unilateral ON episode to ensure time for remyelination and retrograde degeneration;
- ≥ 30 days after corticosteroid therapy had passed to provide time for the anti-inflammatory activity of the medication.

1.1.2. Study Exclusion Criteria

Patients with:

- acute ON clinical picture;
- diagnoses of clinically isolated syndrome, secondary progressive MS, primary progressive MS, progressive relapsing MS;
- refraction anomalies in excess of ± 6 diopters;
- neurosarcoidosis, lymphoma of the central nervous system, neurosyphilis, diabetes etc. diseases that may affect the afferent visual system;
- ophthalmic diseases – glaucoma, ischemic optic neuropathy, trauma of visual pathway in history, etc;
- inability to participate in tests of visual system and magnetic resonance imaging.

1.2. Research Methods

Examination findings were noted in the study questionnaires (see Appendix 2 “Visual examination questionnaire” and Appendix 3 “Magnetic resonance imaging questionnaire”).

Multiple sclerosis was diagnosed on the basis of 2010 McDonald criteria (*Chris H. Polman et al.,* 2011).

To evaluate MS patients' neurological condition, a unified clinical examination system was used. For standardized, quantitated MS patients' neurological investigation, Kurtzke Functional System (FS) and the Expanded Disability Status Scale (EDSS) scores (See Appendix 4 "Expanded Disability Status Scale" (EDSS) as well as Appendix 5 "EDSS evaluation form") (*Kurtzke*, 1983) have been used. To get the total EDSS score, there were carried out examinations for the vision, brain stem, pyramidal, cerebellar, sensory, bowel and bladder, as well as higher nervous activity functional systems. EDSS scores between 0–3.5 are based on the detailed investigation of neurological FS, but starting from 4 they are based on the ambulation score.

For 50 MS patients, there was performed brain MRI examination with a 1.5 T magnetic field strength MRI apparatus *Siemens Magnetom Avanto 1.5 T*. MRI examinations were performed by sequence FLAIR (*fluid attenuated inversion recovery*) with multiplanar 3D reconstructions (MPR) in axial and/or sagittal, coronal planes, as well as by T1 weighted 3D images (T1 3D IR) with MPR reconstructions in axial and/or sagittal, coronal planes. In MS patients an intravenous contrast substance injection was performed in concentration of 0.5 mmol/ml of the active substance by dose 0.2 ml/kg with waiting the full 10 minutes after contrast substance administration until visualization of active, contrast enhancing lesions. Examinations have been performed for optic nerves in T2 weighted images axially every 4 mm, and in T2 weighted images in FS (fat suppressed) regime coronally every 3 mm, including *chiasma opticum*. In assessing the MRI results, the number of demyelinating lesions, localization, presence of active, contrast enhancing lesions and existence of brain atrophy were analyzed. The results of the study were addressed in the questionnaire (see. Appendix 3 "Magnetic resonance imaging questionnaire").

When ophthalmic clinical evaluation was tested, following visual examinations in all subjects separately for each eye were performed.

1. **Visual acuity determination with visual acuity test characters** using Snellen chart, which is placed 6 meters (20 feet) away from the patient's face. The visual acuity measurement result is expressed as a decimal number by recording the last smallest letter line, in which the patient is able to name at least three letters without mistakes. Visual acuity was tested in each eye separately, the other eye being obscured. Refraction determination by means of corrective lenses was performed. In the calculations made for the purposes of the present study, the corrected visual acuity was used, besides, the visual acuity being ≥ 1, it was considered to be equivalent to the visual acuity of 1.

2. **Intraocular pressure was measured with a non-contact tonometry** using apparatus *Tonoref II Nidex*. 10 to 21 mm Hg was considered to be the normal eye pressure.

3. **The investigations of the anterior part of the eye and anterior part of the vitreous humour** were carried out by using a slit-lamp biomicroscope. *Fundus oculi* examination was performed using a 90 diopter lens. The *Fundus oculi* examination evaluated the optic nerve disc color, borders, the level and diagnosed temporal optic nerve disc decoloration (exists/does not exist).

4. **The computerized visual field perimetry** was performed by means of apparatus *Oculus Centerfield Perimeter* and an appropriate vision correction. Visual fields were investigated by obtaining information on the light points with different intensities distributed in the fields of vision. A straight angle (30 - 2) threshold program was used; and retinal sensitivity was measured at 54 points. The points tested were distributed by 36° from the vertical or horizontal meridian in the central visual field. Different intensities of light stimuli were shown for 200 milliseconds, and the patient, identifying the light stimulus, was asked to press a button. Perimetric threshold, in which a patient identifies the stimulus centrally fixing the view, was analyzed using a decibel (dB) scale. The light

intensity was measured in dB, with a low dB value indicating a larger intensity of the light used and a lower retinal sensitivity. The reference value of this decibel scale was determined by taking into account the maximum stimulus luminance of the perimeter.

5. **For the color vision testing** the Ishihara test was used. The patient, in daylight from a distance of 1 meter for 5 seconds was shown 8 polychromatic tables with hidden numbers and figures. If necessary, a vision correction was performed. The patients who were unable to distinguish the hidden numbers or figures, were diagnosed with disorders in color vision.

6. To all participants of the study **a pattern reversal VEP record** using the hardware *Reti port 21 Roland Consult* was performed. Individuals were located 70 cm away from the screen, fixing the view on the red dot in the center of the screen. Where required, a full refractive correction was made. For acquisition of potential, the vision was repeatedly stimulated in monocular way by the black-white "chess field" video monitor at 1.6 Hz frequency. The record of potentials was made by disc-shaped electrodes placing them on the patient's head according to the International 10–20 system, which is based on the head size measurements (*Odom et al.*, 2010). Oz is an active electrode, which was placed on the midline in the occipital region, 10% from the total distance between the nose and the brow bone connecting place *(nasion)* and the external occipital protrusion *(inion)*. Usually Oz placing distance is 3–4 cm above the external occipital protuberance, which is the closest region to the brain primary visual cortex (17. Brodmann field). Reference disc type electrode was placed in Fz area. Having repeatedly performed 100 stimulations twice for every eye, the average performance potentials were filtered and analyzed. The first negative wave of the action potential was labelled with N75, and the first positive wave of the curve was designated by the P100; the second negative wave was marked with the N135, and these waves were recorded from Oz electrode. So far, the neural structures, which take part in these VEP wave formations are not clearly defined

in literature. It is believed that N75 wave is formed in the primary visual cortex, the P100 wave forms in the dorsal extrastriate occipital cortex but the N135 wave forms at impulse spreading deeper in the brain structures, up to the parietal lobe (*Slotnick, Klein, Carney, Sutter, and Dastmalchi,* 1999). The time period from the stimulus to the beginning of the wave P100 was attributed as P100 latency and measured in milliseconds (ms). N75/P100 amplitude measurements were made in microvolts (mkV) from the N75 maximum negative point to the P100 the maximum positive point.

7. With the **optical coherence tomography (OCT)** method *(Heidelberg Engineering Spectralis)* the retinal nerve fiber layer (RNFL) thickness was measured in the six standard sectors (temporal, temporal upper, temporal lower, nasal, nasal upper and nasal lower), and measurements were expressed in micrometres (mkm). Analyzing the RNFL measurements the temporal upper and nasal upper sector readings were combined and named as the upper quadrant, and the temporal lower and nasal lower sector readings were combined and described as the lower quadrant. For all study participants the *Tru Track* active eye tracking technology was used, which prevents the formation of artefacts due to eye movement. RNFL thickness results were evaluated on the basis of OCT apparatus normative database where green-marked areas were classified as the normal, but the red-marked areas were considered to be abnormally reduced. OCT images of unsatisfactory quality were rejected.

1.3. Statistical Analysis of the Data

Statistical processing of the data was performed with SPSS software *(IBM SPSS Statistics Version 21, SPSS inc., USA)*.

Quantitative variables were described by the arithmetic average and standard deviations (SD). In cases when the distribution radically differed from a normal distribution, the median and the interquartile range distribution were calculated.

Categorical or qualitative variables were described as a number and percentage proportion.

Comparisons of normally distributed quantitative variables were made with t-tests of independent selections between the two groups, or ANOVA *(Analysis of Variance)* method among three or more groups. ANCOVA *(Analysis of Covariance)* was used for multivariate analysis with additional off filtering of parameters. While for analysis of related characteristics, MANOVA *(Multivariative Analysis of Variance)* was used.

In cases when the investigated feature did not meet the normal distribution, an appropriate not parametric test was used (Mann-Whitney test *(Mann-Whitney U test)*).

For the analysis of contiguity of two features, Pearson's correlation analysis (denoted by r) was used. The study adopted the following correlation closeness classification depending on the size of the correlation coefficient r: correlation is weak, if $r \leq 0.30$; correlation is medium if $0.30 < r < 0.70$, but a correlation is strong if $r \geq 0.70$. In cases when the distribution of the two features did not meet the normal distribution, Spearman's correlation factor analysis (denoted by r_s) was used.

Categorical or qualitative variables were compared with the Pearson $\chi 2$ (*Pearson chi square*) test or Fisher exact test according to the conditions of use. Cramer's V or dichotomous factor (denoted by φ) calculated value was used for evaluation of the statistical effect of the analysis of qualitative characteristics. The following categories in the assessment of the statistical effect were used:

- 0.1–0.3 = small;
- 0.3–0.5 = medium;
- > 0.5 = large.

For the assessment of limit values of two comparable groups the ROC *(Receiver Operating Characteristic)* curve analysis was used calculating the sensitivity (Se), specificity (Sp), the maximum Youden index (J), positive and

negative predictive values (PPV and NPV). To assess the size of statistical effect, ROC curves were calculated the area under the curve (AUC) and the following size of effect classification was used:

- 0.90–1 = outstanding;
- 0.80–0.90 = good;
- 0.70–0.80 = medium;
- 0.60–0.70 = weak;
- 0.50–0.60 = no effect.

According to generally accepted principles, p-value < 0.05 will be considered as a statistical significance threshold for bilateral test results. Designation n. s. – *non significant* is used to indicate the size of p-values > 0.05.

To assess the obtained results the limits of 95% confidence interval (CI) for statistical parameters were calculated.

2. RESULTS

2.1. Demographic Characteristics of Study Groups

76 multiple sclerosis patients, whose average age was 38.64 years (SD = 10.60), minimum age 17 years, maximum age 65 years, were included in the study. 28 healthy subjects aged 19 to 65 years were included in the control group and the mean age in this group was 35.78 years (SD = 12.14).

Analyzing the average age of individuals included in the study, on the basis of the t test of independent selections, it was found that the mean age in the control group and in the patients' one differs by 2.58 years, but this difference is not statistically significant (p = 0.1).

Multiple sclerosis patients were divided into two groups: patients with a history of ON and patients without an ON history. MS patients' group with a history of ON included 33 patients. MS patients' group without a history of ON included 43 patients. For each of the study and control subjects all tests were carried out for each eye separately. The number of investigated eyes and percentage distribution for MS patients with / without ON history and control group has been depicted in Figure 2.1.

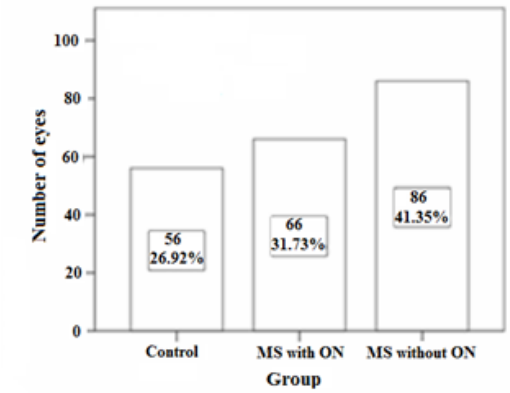

Figure 2.1 **Number of investigated eyes and percentage in study groups**
MS with ON - multiple sclerosis patients with optic neuritis in history
MS without ON - multiple sclerosis patients without optic neuritis in history

Taking into account the impact of every individual's age on the changes in the afferent visual system, it was essential to ascertain the age distribution of the study groups. Basing on the analysis of variance (ANOVA), it was concluded that the three groups analyzed in the study by average age are not statistically significantly different ($p = 0.12$).

In total, the MS patient groups included 45 women and 31 men. In the control group, 19 women and 9 men were included. The summary of gender, age and count of examined eyes in study groups has been represented in Table 2.1.

Table 2.1

Demographic characteristics of study groups

Gender	Group	N, eyes	Mean age, years	SD	Minimum–maximum age, years
Woman	Controls	38	36.68	13.16	19.0–65.0
	MS with ON	38	37.57	12.04	19.0–59.0
	MS without ON	52	40.00	9.81	25.0–55.0
Man	Controls	18	33.88	9.70	25.0–57.0
	MS with ON	28	37.21	7.36	22.0–50.0
	MS without ON	34	38.94	12.31	17.0–65.0

MS with ON - multiple sclerosis patients with optic neuritis in history; MS without ON - multiple sclerosis patients without optic neuritis in history; N - summary number; SD - standard deviation

2.2. Characteristics of Disease Duration and Neurological Status

In the MS patients' group with a history of ON anamnestic mean disease duration was M = 39.56 months (from 6 to 384 months), but in MS patients' group without a history of ON mean disease duration was M = 72.03 months (0 to 400 months). Based on the t-test of independent selections, it was concluded that the average duration of the illness for MS ill patient groups statistically reliably differs ($p = 0.038$).

For MS patients who have had ON, at calculating the period of time after an ON episode, it did not meet the normal distribution; so for characterization of this period the median and interquartile distribution amplitude were used (Me = 12; IQR = 6).

The EDSS score number did not correspond to the normal distribution, and in MS patients' group with a history of ON the EDSS score characterizing indicator Me was 1.50 (IQR = 1.00), but in MS patients without a history of ON in EDSS Me was 1.50 (IQR = 2).

In both patient groups, modal EDSS score number was 1.50 and the maximum total EDSS score was 6. It was concluded that the average EDSS total score ranks for the two patient groups do not differ statistically significantly (Mann-Whitney test, p = 0.40).

For both groups of patients, EDSS score forming functional systems was analyzed separately. The average EDSS score and functional system scores in MS patients groups have been displayed in Table 2.2.

Table 2.2

Average EDSS scores and functional system scores in MS patients groups

Group	Visual FS	Brainstem FS	Pyramidal FS	Cerebellar FS	Sensory FS	Bowel/ bladder FS	Higher nerv. system FS	Ambulation	EDSS
MS with ON	0.79	0.20	1.24	0.53	0.17	0.38	0.45	0.53	1.46
MS without ON	0.44	0.44	1.51	0.52	0.23	0.36	0.45	0.69	1.88
p	< 0.05	< 0.05	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	< 0.05

EDSS - Expanded Disability Status Scale; FS - functional system; MS with ON - multiple sclerosis patients with optic neuritis in history; MS without ON - multiple sclerosis patients without optic neuritis in history; p - reliability factor

Analyzing average scores of the functional systems, it was found that in MS patient groups, they statistically significantly differed in visual, brainstem and pyramidal functional systems ($p < 0.05$).

2.3. Functional Visual System Parameters

2.3.1. Visual Acuity

For MS patients with a history of ON, an average lens-corrected visual acuity was 0.93 (SD = 0.25) and a minimum corrected value of visual acuity was 0.02. In MS patients' group without a history of ON, an average corrected visual acuity was significantly better – 1.02 (SD = 0.21); however, in this group it was possible to correct the visual acuity to 1 only for 30 eyes (35%).

For a relatively small number of individuals from a group of MS patients with a history of ON, for only 7 eyes (10%), the visual acuity adjustment ≥ 1 was possible. In this group also only for 6 contralateral, ON unaffected eyes, complete vision correction was possible.

2.3.2. Visual Evoked Potentials

Following the International Federation of Clinical Neurophysiology recommendations (*Holder, Celesia, Miyake, Tobimatsu, and Weleber, 2010*), in order to avoid inaccurate interpretations of measurements, for VEP apparatus used in the study, it was necessary to determine the precise reference values of N75/P100 amplitude and P100 latency.

Using the ROC curve analysis and determining the precise N75/P100 amplitude threshold for the control group and MS patients, it was concluded that it is 10.52 mkV (AUC = 0.81; 95% CI: 0.76 to 0.86; Se = 0.91; Sp = 0.63, and the maximum Youden index is 0.54). The ROC curve used for N75/P100 amplitude threshold determination has been reflected in Figure 2.2.

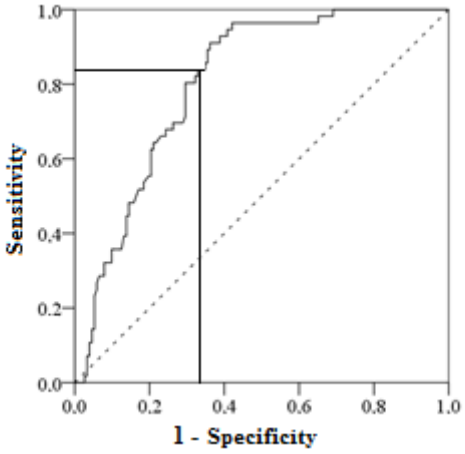

Figure 2.2 **ROC curve for the N75/P100 amplitude reference determination**

Similarly, determining the precise P100 latency threshold for the control group and for MS patients, it was concluded that it was 110.25 ms (AUC = 0.86; 95% CI: 0.80 to 0.91; Se = 0.63; Sp = 0.96, with a maximum Youden index 0.59). This curve has been shown in Figure 2.3.

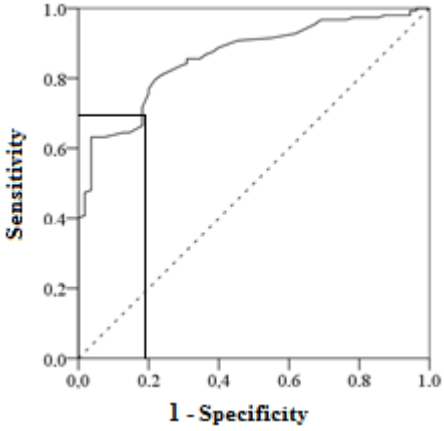

Figure 2.3 **ROC curve for P100 latency threshold determination**

In the VEP examinations carried out, the N75/P100 amplitude, which was lower than 10.52 mkV, was considered to be reduced. P100 latency, which was longer than 110.25 ms, was considered to be prolonged.

Analyzing the average N75/P100 VEP amplitude scores in the study groups, it was found that the lowest amplitude was observed in MS patients with a history of ON, and directly in ON (+) eyes. For these patients' eyes the average N75/P100 amplitude was 8.16 mkV (SD = 4.60). But also in MS patients' group, which had never suffered from the ON clinical episode, a lower average N75/P100 amplitude comparing with the control group was observed. Basing on the t test of independent selections, it was concluded that the average N75/P100 amplitude for the control group (M = 14.51, SD = 3.35) and the group of patients suffering from MS without a history of ON (M = 9.86; SD = 4.63) differs by an average of 4.65 mkV and this difference is statistically significant ($p < 0.001$). According to the ROC curve analysis, assessing the difference between the two groups, it was concluded that it is statistically good (AUC = 0.81; 95% CI: 0.74 to 0.88; $p < 0.001$).

The analysis of both populations suffering from MS showed that for the ON (+) eyes (M = 8.16; SD = 4.60) and the eyes without a history of ON (M = 9.86; SD = 4.63), the average N75/P100 amplitude statistically significantly ($p = 0.03$) differs (by an average of 1.70 mkV). However, having considered the ROC curve analysis and having evaluated the statistical difference between the two groups, it was concluded that it is weak (AUC = 0.65; 95% CI: 0.60 to 0.70; $p < 0.001$).

Analyzing the group of MS patients who have a history of ON, it was concluded that the average N75/P100 amplitude for the ON (+) eyes (M = 8.16; SD = 4.60) and the ON (-) eyes (M = 11.03; SD = 5.40) differs on average by 2.87 mkV, and this difference is statistically significant ($p = 0.01$). Having regard to the ROC curve analysis and the statistical difference between the two groups, it was concluded that it is weak, but statistically significant (AUC = 0.65; 95%

CI: 0.51 to 0.78; $p < 0.001$). Taking into account the established average N75/P100 amplitude threshold – 10.52 mkV, in MS patients' group without a history of ON a reduced amplitude was found in 54 eyes (62.79%). Analyzing the average N75/P100 amplitude for MS patients who have previously suffered from ON, the following results were obtained. For most – 23 ON (+) eyes (69.69% of the ON (+) eyes) a reduced average N75/P100 amplitude was observed, but also in 18 (54.54%) of other – ON directly unaffected eyes, an abnormally reduced average VEP amplitude was observed.

Similarly, analyzing the average VEP P 100 latency, it was found that the most distinct prolongation was observed in MS suffering patients who have experienced ON, for ON (+) eyes – 126.00 ms (SD = 18.26), but also in the group of patients without a history of ON P 100 latency was longer (M = 116.73, SD = 16.00), comparing to the control group (M = 101.81, SD = 5.66).

Comparing the average P100 latency for ON (+) eyes with this indicator in MS patients' group where ON had never been observed, it was found that in the ON affected eyes P100 average latency is for 9.26 ms longer and this difference is statistically significant ($p < 0.01$). When assessing the effect of this difference in both MS patients' groups, it was concluded that it is weak, but statistically significant (AUC = 0.64; 95% CI: 0.55 to 0.72; $p < 0.001$).

The MS patients' group, which previously suffered from ON, being analysed separately, it was concluded that the average P100 latency statistically significantly differs ($p < 0.01$), for an average 11.25 ms for ON (+) eyes (M = 126.00, SD = 18.26) and ON (-) eyes (M = 114.75, SD = 11.94). However, having assessed the statistical effect of this difference, it was concluded that it is weak, but statistically significant (AUC = 0.68; 95% CI: 0.55 to 0.81; $p < 0.001$).

Taking into account the determined P100 latency threshold – 110.25 ms, it was concluded that in MS patients' group without an ON history for 52 eyes (60.47%) this indicator is abnormally prolonged. However, in MS patients' group

who have experienced ON a P100 latency abnormal extension was observed not only in 25 eyes (75.75% of the ON (+) eyes) after ON episodes, but also in 19 eyes (57.57% of the ON (-) eyes), which had not had any ON clinical signs.

The summary of the obtained VEP indicators for the study groups has been reflected in Table 2.3.

Table 2.3

Results of visual evoked potentials

	ON (+), eyes (N = 33)	ON (−), eyes (N = 33)	MS without ON, eyes (N = 86)	Control, eyes (N = 56)
Average N 75/P100 amplitude, mkV, (± SD)	8.16 (4.6)	11.03 (5.4)	9.86 (4.63)	14.51 (3.35)
Average P100 latency, ms, (± SD)	126.0 (18.26)	114.75 (11.94)	116.73 (16.00)	101.81 (5.66)

mkV – microvolts; ms – milliseconds; MS without ON - multiple sclerosis patients without optic neuritis in history; N - summary number; ON (−) - eyes without a history of optic neuritis; ON (+) - eyes with a history of optic neuritis

2.3.3. Changes in Color Vision

While analyzing the changes in color vision, it was established that in MS patients without a history of ON, for 12 eyes (13.95%) an impaired color vision was observed. Slightly more often, in 11 ON (+) eyes (33.33%), color vision was impaired in MS patients with a history of ON. On the basis of Pearson's chi-square statistical analysis, it was concluded that the patient's belonging to a certain group affects their color vision ($p < 0.01$) and the effect size is medium ($\varphi = 0.27$). According to the statistical analysis, it was concluded that the ON presence statistically significantly affects color vision ($p < 0.01$), and the effect size is statistically high ($\varphi = 0.35$). The control group showed no color vision disturbances.

2.3.4. Changes of Visual Fields

When analyzing results of computerized perimetry, it was found that the most frequent type of disturbance of visual fields is paracentral scotomas. Only in one ON affected eye from the MS patients' group with ON history there was observed an arcuate defect of the visual field. In MS patients' group without a history of ON, paracentral scotomas are detected in 55 eyes (63.95%). Such disorders were identified in most of the ON affected eyes (81.81% of the ON (+) eyes) in MS patients with a history of ON. In addition, paracentral scotomas were found also in half of those eyes in which ON episode was not observed (57.57% of the ON (-) eyes). Based on Pearson's chi-square statistical analysis, it was concluded that ON existence does not affect statistically significantly the changes in visual fields ($p = 0.11$), but the statistical effect size is medium ($\varphi = 0.20$). Changes in the visual fields in the control group were not observed.

2.4. Structural Parameters of Visual System

2.4.1. Decoloration of Optic Nerve Disc at the Examination of *Fundus Oculi*

In MS patients' group without a history of ON analyzing changes at *fundus oculi* examination, the decoloration of the optic nerve disc was found in 19 eyes (22.09%). Having analyzed the MS patient population with a history of ON separately, the decoloration of the optic nerve disc was more frequently detected in ON (+) eyes (7 eyes or 24.24%) if compared to the ON (-) eyes (4 eyes or 15.15%). Relying on Pearson's chi-square statistical analysis, it was concluded that the ON existence in a history does not affect the decoloration of the optic nerve disc ($p = 0.80$). Mann-Whitney non-parametric test indicated that the time passed after ON episode has no effect on the appearance of the decoloration of the optic nerve disc ($p = 0.18$).

2.4.2. Retinal Nerve Fiber Layer Measurements by Optical Coherence Tomography

Carrying out investigations with the optical coherence tomography method and basing on covariative multivariate analysis of variance (MANCOVA), it was concluded that simultaneously analyzing the aggregate of the RNFL indicators (RNFL thickness in the temporal, nasal, upper and lower quadrants), it statistically significantly (*Pillai's trace* = 0.26; F (12.340) = 4.41; $p < 0.001$) differs for 2 in the trial included MS patients' groups, besides the age is a statistically significant system influencing factor ($p < 0.01$).

Having analyzed the average RNFL thickness for all research groups, it was found that this layer was the thinnest in the eyes with a history of ON (M = 85.63, SD = 16.51); however, also in MS patients without a previously known ON episode, the average RNFL thickness was thinner (M = 90.16, SD = 11.18) if compared to the control group (M = 98.76; SD = 7.12). Average RNFL thickness readings for study groups have been given in Table 2.4.

Table 2.4

Average RNFL thickness in the study groups

Group	N, eyes	Average RNFL thickness, mkm (± SD)	Minimum, mkm	Maximum, mkm	p value
Controls	56	98.76 (7.12)	88	117	
MS with ON	33	85.63 (16.51)	48	133	< 0.001
MS without ON	86	90.16 (11.18)	66	115	

mkm – micrometers; MS with ON - multiple sclerosis patients with optic neuritis in history; MS without ON - multiple sclerosis patients without optic neuritis in history; N - summary number; p - reliability factor; RNFL - retinal nerve fiber layer; SD - standard deviation

Analyzing the average RNFL thickness values in different quadrants (upper, lower, temporal (RNFLT) and nasal (RNFLN)), the following results were obtained. Having compared the control group and the group of MS patients with a history of ON (only ON (+) eyes), it was concluded that in all quadrants RNFL thickness statistically significantly differs ($p < 0.05$), and on the basis of the ROC curve analysis it was concluded that the biggest RNFL thickness difference was in the temporal quadrant (accordingly AUC = 0.78; 95% CI: 0.67 to 0.89; $p < 0.01$). Average RNFL thickness ROC curves in the temporal, nasal, upper and lower quadrants have been shown in Figure 2.4.

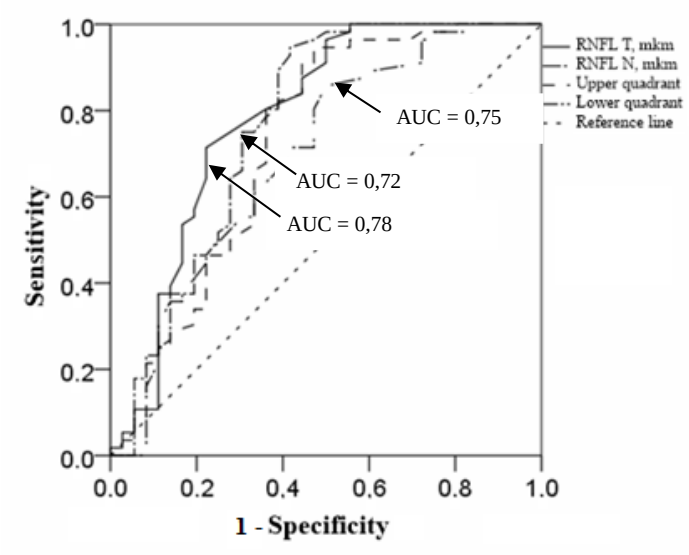

Figure 2.4 **Average RNFL thickness ROC curves in the temporal, nasal, upper and lower quadrants**

AUC - area under the curve; mkm – micrometers; RNFLN - retinal nerve fiber layer in the nasal quadrant; RNFLT - retinal nerve fiber layer in the temporal quadrant

Having compared the control group and the group of MS patients without a history of ON in a similar way, it was concluded that in these groups all RNFL readings differ statistically significantly ($p < 0.05$). In addition, similarly to the above mentioned, for the both groups the greatest RNFL thickness difference was observed in the temporal quadrant (AUC = 0.69; 95% CI: 0.60-0.77; $p < 0.01$).

Average RNFL thickness in mkm in the upper, lower, temporal and nasal quadrants for various study groups has been provided in Table 2.5.

Table 2.5

RNFL average thickness in different quadrants

Group	RNFLT (± SD), mkm	RNFLN (± SD), mkm	Upper quadrant (± SD), mkm	Lower quadrant (± SD), mkm
Control	70.92 (9.49)	75.25 (10.91)	121.01 (12.06)	127.74 (13.66)
MS with ON	56.44 (15.93)	68.30 (20.12)	107.47 (22.63)	110.33 (23.99)
MS without ON	62.12 (13.50)	69.15 (13.05)	111.58 (16.64)	118.70 (18.93)

mkm – micrometers; MS with ON - multiple sclerosis patients with optic neuritis in history; MS without ON - multiple sclerosis patients without optic neuritis in history; RNFLN - retinal nerve fiber layer in the nasal quadrant; RNFLT - retinal nerve fiber layer in the temporal quadrant; SD - standard deviation

In compliance with the normative database included in the OCT apparatus, the number of individuals showing the reduced RNFLT thickness was analyzed. Reduced RNFLT thickness was found in 33% eyes both in the MS patients with a history of ON and MS patients' group without a history of ON.

2.4.3. Demyelination of Optic Nerve and Number of Demyelinating Lesions Found by Magnetic Resonance Imaging

MRI of the brain and spinal cord, and optic nerves was performed in 50 MS patients, so 100 optic nerves were analyzed. MRI was performed for 28 patients with a history of ON and 22 patients without a history of ON. Analyzing the results of MRI optic nerve examinations, it was concluded that in MS patients' group who had experienced ON, in 27 eyes (96.42%) MRI images showed a unilateral optic nerve demyelination. However, also in eyes of 4 MS patients without a history of ON episode (9.09%) were found signs of demyelination at MRI examination. Analyzing the average number of demyelinating lesions in brain and spinal cord, it was found that statistically significantly more lesions in periventricular, juxtacortical, infratentorial parts and spinal cord cervical part were detected in patients with MS without ON history ($p < 0.05$). The average number of demyelinating lesions in various locations for both MS patient groups has been represented in Table 2.6.

Table 2.6

Average number of demyelinating lesions in brain and spinal cord for MS patient groups

Group	Periven-tricular	Juxta-cortical	Infraten-torial	Cervical part	Thoracic part	*Conus medul-laris*	Total number of lesions
MS with ON	5.12	1.67	1.15	1.79	1.15	0.27	11.27
MS without ON	8.37	2.86	2.00	2.67	1.88	0.37	18.33
p	< 0.05	< 0.05	< 0.05	< 0.05	> 0.05	> 0.05	< 0.05

MS with ON - multiple sclerosis patients with optic neuritis in history; MS without ON - multiple sclerosis patients without optic neuritis in history; p - reliability factor

Analyzing demyelinating lesions located in visual pathway separately, it was found that the average number of demyelinating lesions in both MS patient groups did not exhibit any statistically significant difference ($p > 0.05$).

2.5. Interdependence of Afferent Visual Systems and Disease Characterizing Parameters

With a view to explore the impact of disease duration on retinal nerve fiber layer thickness, the Pearson's correlation coefficient analysis was performed. It was concluded that in the group of MS patients with ON history there exists a medium, negative and statistically significant correlation ($r = – 0.38$; $p = 0.02$) between RNFLT thickness and disease duration in months. Similarly, analyzing the MS patient population without a history of ON, it was found that there is a weak, negative but statistically significant correlation ($r = – 0.26$; $p = 0.01$) between RNFL in the temporal quadrant and disease duration in months.

Wishing to investigate whether a patient's neurological status is linked to changes in the retina, Spearman correlation coefficient analysis was used and RNFLT thickness correlation with MS patients' degree of disability was calculated. It was found that in MS patients with a history of ON, there exists a moderate, negative and statistically significant correlation ($r_s = – 0.35$; $p = 0.03$) between RNFLT thickness and degree of disability characterizing EDSS score. A similar mutual relationship ($r_s = – 0.32$; $p < 0.01$) was also observed for other MS patients in the group without signs of ON in history.

When analyzing the correlation of EDSS functional system parameters with RNFLT thickness in both MS patient groups separately, we failed to find a statistically significant correlation ($p > 0.05$).

In order to check the relationship of the retinal nerve fiber layer thickness with the visual acuity function, Pearson's correlation coefficient analysis was performed. It was concluded that in MS patients' group without ON history

between RNFLT thickness and the corrected visual acuity there is a medium, positive and statistically significant correlation ($r = 0.30$; $p < 0.01$). Similarly, it was concluded that in MS patients' group with ON history in the eyes without ON clinical signs among RNFLT thickness and the visual acuity there is a medium, positive and statistically significant correlation ($r = 0.30$; $p < 0.01$). However, in the eyes that had suffered from ON clinical signs a statistically significant correlation between RNFLT thickness and the corrected visual acuity was not demonstrated ($p = 0.57$).

When investigating whether the retinal structure changes affect color vision function, it was found that in MS patients' group between the reduced RNFLT thickness and abnormal color vision there is a statistically significant relationship ($p < 0.05$), but the effect size is small ($\varphi = 0.23$). Overall, in only 16 eyes (10.53%) of all MS patients' eyes the simultaneous RNFLT thickness reduction and color vision disorders were observed.

Analyzing all MS patients, a statistically significant correlation ($p < 0.05$) between the reduced RNFLT thickness and the altered visual fields was observed, but the effect size was small ($\varphi = 0.18$). In total, in 40 (26.32%) MS patients' eyes there was observed both the reduced RNFLT thickness and changes in the computerized visual field perimetry. However, in 61 (40.13%) patients' eyes visual fields were disturbed, but RNFLT thickness was within normal limits.

Analyzing the connection of reduced RNFLT thickness with VEP results in MS patients' group without a history of ON, a decreased N75/P100 amplitude was found in 54 (62.7%) patients' eyes, but decreased RNFLT was observed in only 29 (33.7%) eyes. In this group it turned out that in 29 (33.7%) patients' eyes, which showed a reduced N75/P100 amplitude, RNFLT thickness was within normal limits, but, simultaneosly, the combination of the normal amplitude and the reduced RNFLT thickness was found in only

4 (4.6%) patients' eyes. For 25 (29.07%) eyes in this group of patients both values were abnormally reduced.

Similarly, analyzing the VEP P100 latency in MS patients without a history of ON, it was found that normal RNFLT thickness and prolonged P100 latency was in 27 (31.4%) patients' eyes, but the normal P100 latency and simultaneously reduced RNFLT thickness was found in only 4 (4.6%) patients' eyes.

Analyzing the MS patient population with a history of ON, ON (+) eyes, it was concluded that a normal RNFLT thickness and reduced N75/P100 amplitude was detected in 11 (33.33%) eyes of the patients, but normal VEP amplitude and at the same time reduced RNFLT was found only for 2 (5.56%) patients' eyes. Analyzing P100 latency in ON affected eyes it was concluded that it was prolonged in 12 (36.11%) eyes of those patients who had a normal RNFLT thickness. However, for only two (5.56%) patients with a reduced RNFLT thickness a normal P100 latency was found.

Taking into account Spearman's correlation coefficient analysis, it was concluded that between RNFLT thickness and average N75/P100 amplitude in MS patients' group without ON history, a medium, positive and statistically significant correlation ($r_s = 0.43$; $p < 0.001$) was observed. A similar correlation exists in MS patients who have had ON, in ON (+) eyes ($r_s = 0.45$; $p < 0.001$). In addition, also in the ON (-) eyes between the RNFL thickness and average N75/P100 amplitude, there was found an intermediate, positive and statistically significant correlation ($r_s = 0.35$; $p = 0.04$).

Similarly, analyzing the connection of the average P100 latency with RNFLT thickness, it was found that in both MS patients without a history of ON ($r_s = – 0.40$; $p <0.001$) and in ON (+) eyes, between these indicators there exists a medium, negative and statistically significant correlation ($r_s = – 0.55$; $p < 0.001$). Also in ON (-) eyes a medium, negative and statistically significant correlation ($r_s = – 0.32$; $p = 0.04$) was found.

To elucidate the best method for approving anamnestic ON and using ON clinical signs as the gold standard in the ON diagnosis, the diagnostic utility of VEP and OCT indicators was calculated.

The highest sensitivity (76.19%) for clinical ON validation, as well as a relatively high specificity (95.83%) showed the prolonged P100 latency. Slightly lower was the sensitivity and specificity of the reduced N75/P100 amplitude (72.22% and 91.07%, respectively). Analyzing the reduced RNFLT thickness for the ON clinical validation, its sensitivity was only 44.44%, but this parameter showed a very high specificity – 100%. Reduced RNFLT thickness showed the highest positive expected value for the ON approval, it reached 100% as well. Assessment of the above VEP and OCT indicators has been displayed in Table 2.7.

Table 2.7

Characteristics of sensitivity, specificity and accuracy of the VEP and OCT methods

Indicator	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	Accuracy of diagnostics (%)	Positive predictive value (%) [95% CI]	Negative predictive value (%) [95% CI]
Reduced N75/P100 amplitude	72.22 [54.81–85.78]	91.07 [80.37–97.00]	83.70	83.87 [66.26–94.49]	83.61 [71.91–91.83]
Prolonged P100 latency	76.19 [52.83–91.69]	95.83 [78.81–99.30]	86.00	94.12 [71.24–99.02]	82.14 [63.09–93.87]
Reduced RNFL thickness	44.44 [27.95–61.90]	100 [93.56–100]	78.00	100 [79.24–100]	56 [62.32–83.12]

CI - confidence interval; RNFL - retinal nerve fiber layer

Making assessment of specified VEP and OCT parameters through analysis of ROC curves, it was concluded that the most appropriate diagnostic test for clinical ON approval is the use of average VEP P100 latency (AUC = 0.92; 95% CI: 0.86 to 0.98; $p < 0.001$), followed by the use of average N75/P100

amplitude (AUC = 0.85; 95% CI: 0.76 to 0.94; $p < 0.001$) and the relatively weaker method for the ON approval is the RNFL thickness determination (AUC = 0.78; 95% CI: 0.68–0.90; $p < 0.001$).

To elucidate whether the macroscopic changes in *fundus oculi* are reflected in OCT results, the correlation of the reduced RNFLT thickness with the temporal decoloration of the optic nerve disc was analyzed. Making the above-mentioned calculations, it was concluded that between the two parameters in both groups of MS patients a statistically significant relationship ($p < 0.05$) exists and the effect size is medium ($\varphi = 0.33$). More often simultaneous reduced RNFLT thickness and decoloration of the optic nerve disc were observed in the eyes of patients after the ON episode (in 28 eyes or 87.50% of ON (+) eyes).

Specifying the relationship of the reduced RNFLT thickness to the MRI results and analyzing the data of both groups of MS patients, it was found that only in 15 eyes (15% of the number of MRI investigated eyes), simultaneously with the optic nerve demyelination at MRI investigation, the reduced RNFLT thickness at OCT was also observed.

In addition, in 16 eyes (16% of number of eyes investigated by MRI) in patients with a visually modified optic nerve at MRI, a normal RNFL thickness was found. In its turn, in 24 eyes (24% of the eyes studied by MRI) MRI showed no damage to the optic nerve, but the reduced RNFL thickness was observable.

On the basis of Pearson's chi-square statistical test, it was concluded that between the signs of optic nerve damage in MRI and the reduced RNFLT thickness, there is no statistically significant correlation ($p = 0.24$) and the effect size is small ($\varphi = 0.11$).

Similarly, analyzing the connection of radiological optic nerves modification with the VEP amplitude, it was found that in 17 cases (17% of the number of eyes investigated by MRI) optic nerve damage signs in MRI were found simultaneously with the reduced VEP amplitude, but more often – in 42

cases (42% of the number of eyes investigated by MRI) a reduced amplitude was found in patients with unchanged optic nerves at MRI study.

Similarly to the mentioned above, it was concluded that between the signs of optic nerve damage at MRI and changed VEP amplitude, there is no statistically significant correlation ($p = 0.66$), and the effect size is small ($\varphi = 0.04$).

Taking into account the impact of demyelination on P100 latency in VEP investigation and analyzing the frequency of the prolonged P100 latency and MRI changes in MS patients, it was found that in 21 cases (21% of eyes investigated by MRI) in MS patients with optic nerve damage at MRI, also the P100 latency prolongation was found. In addition, on the basis of Pearson's chi-square statistical test, it was concluded that between the optic nerve demyelination at MRI and P100 latency there is a statistically significant correlation ($p < 0.01$) but the effect size is medium ($\varphi = 0.30$).

Performing the MRI test of the brain and counting demyelinating lesions, it was found that in patients with reduced RNFLT thickness, the number of lesions is statistically significantly higher than that for patients with the normal RNFLT thickness ($p < 0.001$). In addition, the size of this correlation effect as found by ROC curve analysis, is statistically good ($AUC = 0.80$; $p < 0.01$).

In compliance with the Mann-Whitney test and separately analyzing only the number of those demyelinating lesions which affect the visual pathway in the brain (*chiasma opticum*, *tractus opticus* and *radiatio optica* districts), it was found that in patients with reduced RNFLT thickness the number of such lesions is higher than in patients whose RNFLT thickness is normal ($p < 0.001$). However, the effect size is statistically weak ($AUC = 0.67$; $p < 0.01$).

Analyzing brain atrophy at MRI investigation, it was found that in 28 cases (28% of eyes, investigated by MRI) for MS patients with an established atrophy also a reduced RNFLT thickness was detected. In addition, only in 11% of cases with no signs of atrophy in MRI there was observed a reduced RNFLT

thickness. Assessing the interconnection between the cerebral atrophy and the reduced RNFLT, it was found that between these parameters there exists a statistically significant correlation ($p < 0.001$), and the statistical evaluation of the influence is almost large ($\varphi = 0.44$).

Analyzing the existence and number of active, contrast enhancing lesions, it was established that they do not affect the reduced RNFLT thickness ($p = 0.64$).

In total, having assessed all the factors affecting the RNFLT thickness and applying the method of logistic regression analysis, a model was developed enabling the prediction of a reduced RNFLT thickness. Quality factor of the logistic model estimation (*Nagelkerkes R*) is 28%.

Analyzing the chance ratios from the calculated logistic regression model, it can be concluded that for individuals with a reduced N75/P100 amplitude, chance to have a reduced RNFLT thickness is 4.25 fold larger than for patients with normal VEP amplitude. Conversely, for individuals who have a prolonged P100 latency, the probability to have a reduced RNFLT thickness is 6.81 times higher than that for the patients whose VEP amplitude is within normal limits.

In the model developed, there were included only mutually independent, and statistically significant RNFLT thickness influencing factors. The resulting equation is as follows:

$$\text{Logit (to reduced RNFLT thickness)} = -3.09 + 1.44 \times \text{N75/P100 amplitude} + 1.92 \times \text{P100 latency}$$

By means of performing mathematical transformations, in each particular case it is possible to calculate the exact probability of the reduced RNFLT thickness.

Into the logistic regression model, there were inserted also other features that could affect the reduced RNFLT thickness, such as age, EDSS, visual acuity, color vision, visual field changes as well as ON episode in history, but it failed

to gain a statistically significant prediction model of the reduced RNFLT thickness from these signs.

3. DISCUSSION

3.1. Functional Changes in Afferent Visual Pathway

In the conducted study analyzing the visual acuity as a simple diagnostic functional parameter of the afferent visual pathway, we found that a large proportion of MS patients (72%) had significant visual acuity disorders. For MS patients with ON episode in history, the average corrected visual acuity was relatively good – 0.93, but the complete vision correction (≥ 1.0) was possible only for a minority of patients (10%). In this MS patients' group only patients with unilateral ON were included, but also for the ON directly unaffected fellow eyes only in 6 cases (18% of the ON (-) eyes) a total visual acuity correction was possible, indicating that there exists a visual dysfunction in both eyes.

Analyzing the MS patients' group without a history of ON, we found that, despite the absence of clinical ON, only in 35% of the cases in this group the visual acuity ≥ 1 was possible. For most of patients of this group, visual acuity was not fully adjustable, although ON clinical signs had never been observed. These results point to a subclinically proceeding visual dysfunction in MS patients.

It is emphasized in the sources of literature that in many MS patients a significant visual impairment is observed, even while maintaining visual acuity 1.0 (*Fiona Costello,* 2013; *Fisher et al.,* 2006; *Sakai et al.,* 2011). Several studies showed that in MS patients compared with healthy subjects, a reduced image contrast makes a significant impact on the visual function and in patients' investigation a low contrast letter acuity testing is preferable; however, it is not included in the EDSS total score and in the daily practice (*Balcer and Frohman,* 2010; *Bermel and Balcer,* 2013). This test identifies the difficulties to perceive reduced contrast images also in patients without previous ON episode and indicates impaired quality of life and difficulties in everyday activities, such as

reading, recognizing of faces and car driving (*Fiona Costello,* 2013; *Sakai et al.,* 2011). In addition, literature points out that despite the acuity of 1.0, in the eyes, which had ON episode, the low contrast vision was worse than in the eyes, which did not suffer from ON (*Frohman, Frohman, Zee, McColl, and Galetta,* 2005). Although currently it is believed that visual acuity is a relatively negligible informative indicator for the evaluation of the function of the afferent visual system, in addition, making a low contrast letter test several cases of visual dysfunction still would be diagnosed.

For the functional diagnostics study of the axonal demyelinating process of visual pathway average latency P100 and N75/P100 amplitude measurements by VEP method were used. We found that a significantly lower average N75/P100 amplitude was observed in MS patients with a history of ON, and directly in ON affected eyes the average amplitude was for 6.35 mkV lower compared with the control group ($p < 0.001$), indicating to axonal integrity impairment after ON episode, presumably because of prior demyelination. However, in this group in 51% of cases were found abnormally reduced average N75/P100 amplitude also in ON directly unaffected fellow eyes. In this case we need to think about other functional axonal tissue damage, unrelated to acute episode of demyelination.

Similar changes of axonal tissue dysfunction were observed also in the group of MS patients without a history of ON episode. In this group, the average N75/P100 amplitude was statistically significantly for 4.65 mkV lower than in control subjects. Moreover, determining the exact N75/P100 amplitude reference value in this MS patient population, a reduced amplitude was observed in most patients – 63%.

Analyzing the the average P100 latency separately, similar results were obtained. The most distinct average P100 latency prolongation was observed in MS patients who had had ON, in ON affected eyes it was 126.0 ms, which is on average 24.19 ms longer than for the control group. But also in patients without

a history of ON, the average P100 latency was for 14.92 ms longer than that found in the control group. In addition, similarly to the case of a subclinical axonal damage, 60% patients without clinical signs of ON in history, were found a subclinical P100 latency prolongation.

Although the examination of visual evoked potentials was initially mentioned as an additional criterion for diagnosis of primary progressive MS (*McDonald et al.,* 2001; *Polman et al.,* 2005), in 2010 it was not repeatedly included in the revised McDonald's criteria (*Polman et al.,* 2011) and the VEP role in the diagnosis of MS has decreased. Relatively limited number of studies about the role of VEP in subclinical optic nerve damage diagnostics have been performed in MS patients. From experimental autoimmune ON model it is known that the VEP amplitude reduction indicates an axonal tissue damage, but the latency prolongation is an early sign of demyelination (*You et al.,* 2011). From this point of view, of particular relevance could be the studies of VEP amplitude carried out in patients for which axonal damage could not occur secondary to demyelination of the optic nerve, so in patients without a clinical ON history.

Our results demonstrate that the VEP examination can provide additional information on the integrity of visual pathway, especially in cases where there are no definite clinical signs of ON. In addition, it can confirm an involvement of visual system in clinically uncertain situations and in cases when the patient is not able to define his history clearly due to cognitive impairments. Abnormal VEP findings in the clinically unaffected eyes provide information about subclinical lesions in visual pathway which can help to identify the dissemination of lesions in the space, providing additional criteria for the diagnosis of MS (*Sakai et al.,* 2011); however, it is unclear whether these lesions are not consequences of an alternative, with inflammatory activity unbound process.

In recent years, in the literature the multifocal VEP method is observed that allows the simultaneous stimulation of multiple fields of visual field parts and study individual small axonal groups by topographical analysis (*Klistorner et al.,* 2009). Recently, a study has been published in which, the hypothesis on retrogenicular demyelination in ON unaffected eyes is proved using multifocal VEP analysis (*Alshowaeir et al.,* 2014). Currently multifocal VEP technique is not available in Latvia.

To evaluate the integrity of functional vision pathway we used investigation of the color vision. Our results showed that the color vision affects ON existence in the history, the impairment develops in 19% cases after ON episode. However, in 14% of patients without a prior history of ON, color vision was affected as well. This indicates that color vision disturbances may occur without acute optic neuritis in history. Similar results were published in a recent study, where it was found that color vision was altered in 19% of eyes without prior ON, in addition, these patients, conducting a longitudinal observation for a year, developed serious disorders of other functions and disability progressed faster compared to patients which color vision was unhindered (*Martinez-Lapiscina et al.,* 2014). So, probably, changes in color vision are of prognostic significance.

We observed a convincing functional visual defect at the examination of visual fields in patients without an ON in history. In our study paracentral scotomas were evolved even in 64% of this group of patients. However, relatively more often (in 85% cases), a defect of visual fields developed in the patients with a history of ON in those eyes, which had suffered from ON. Although restrictions of the visual fields are one of the most characteristic ON clinical signs and in onset of the disease they develop almost in all patients (*Balcer,* 2006; *Fiona Costello,* 2013), our results allow to suggest that the defect of the visual fields arises regardless of the ON clinical signs. A similar trend was also observed in a longitudinal study, which analyzed ON affected eyes 15 years

after the ON episode and the visual fields defect was retained in 39.5% cases, but in ON unaffected fellow eyes this defect developed in 26.3% cases as well (*Keltner et al.,* 2010). In this study, similarly to our observations, it was found that the most frequent type of the visual fields defect are partial disorders, including paracentral scotomas. Although the visual field defects convincingly point to a damage of functional integrity of the afferent visual pathways, which, in addition, using automated perimetry, is easy to be diagnosed and interpreted, the subjective factor during the exam should be taken into account. Characteristic fatigue and attention disorders of MS patients can lead to the false positive distortions of vision fields.

3.2. Structural Changes of Afferent Visual Pathway

Since the introduction of an ophthalmoscope, the structure and damage of the optic nerve most often are analyzed directly, at the examination of *fundus oculi*. During the acute phase of optic neuritis one third of patients experience edema of the optic nerve, i.e. papillitis followed by bleaching of the optic nerve disc. Two thirds of patients do not show the acute changes in *fundus oculi* examination because the damage is evolved in the retrobulbar part of the optic nerve (*Balcer,* 2006; *Voss et al.,* 2011). In our study, analyzing the structural macroscopic changes in the *fundus oculi* examination we found that more frequently optic nerve disc bleaching in the temporal part was found in the MS patients with the history of ON; however, it was observed only in 24% of the ON affected eyes. Likewise, in 15% of patients without a history of ON such changes were observed. Taking into account the obtained results, it can be concluded that the ON existence in the history substantially does not affect the optic nerve disc temporal decoloration and is probably associated with neurodegenerative processes. This possibility is also confirmed by our findings

that in both patient groups reduced RNFLT is associated with temporal optic nerve disc decoloration.

Analyzing the literature, we have come to conclusion that the etiology of temporal decoloration of optic nerve disc is not fully clear. It is believed that this is due to axonal degeneration and damage of the nerve fiber layer thickness and the architecture (*Neuro-Ophthalmology Review Manual* (*7th Edition*), 2012). Localization of the temporal optic nerve disc bleaching implicates to the most expressed changes in the temporal region, i.e. in the area where nerve fibers move towards macula, and form papillomacular bundle. It is likely that such a defect is developing also in patients without an acute inflammatory episode; however, the *fundus oculi* inspection results should be analyzed prudently, because even though tests for all patients in our study were made by one ophthalmologist, optic nerve disc decoloration is a subjectively interpretable sign.

More expressed involvement of the temporal area in our study was confirmed by microscopic RNFL measurements conducted by optical coherence tomography examination. Basing on our results, RNFL thickness directly in temporal segment (RNFLT) showed the greatest differences between the study groups and similar results had been obtained also in other studies (*Fjeldstad, Bemben, and Pardo,* 2011; *Gelfand et al.,* 2012; *Klistorner et al.,* 2013; *Tatrai et al.,* 2012; *Wang et al.,* 2010). However, in some studies in MS patients' eyes a diffuse RNFL reduction in all quadrants was observed (*Serbecic et al.,* 2010; *Urano et al.,* 2011). In addition, longitudinal studies were carried out in which this reduction was found to be progressing and more pronounced in the upper quadrant (Herrero et al., 2012). Certain authors put forward the assumption that the temporal RNFL reduction is observable in patients after the ON episode; however, if ON had not been RNFL diminishes diffusively (*Bock et al.,* 2010), but in our study this hypothesis was not confirmed.

In our study the thinnest RNFLT layer was in the ON affected eyes (average of 56.44 microns), but also in the eyes without ON in history, it was statistically considerably thinner (average of 62.12 microns) than in the control group (average of 70.92 microns). Such subclinical RNFLT damage has been observed by several research groups (*Fjeldstad et al.,* 2011; *Garcia-Martin et al.,* 2010; *Parisi et al.,* 1999; *Pueyo et al.,* 2008; *Quelly, Cheng, Laron, Schiffman, and Tang,* 2010; *Zaveri et al.,* 2008), and the hypothesis is stated that a reduction of RNFLT in ON affected eyes is caused by a retrograde axonal degeneration as a consequence of retrobulbar optic nerve demyelination. Similar results have been published in a recent meta analysis summarizing the data from 14 studies, and it was calculated that, comparing ON affected eyes with the control subjects, the mean RNFL decrease is 20 microns, but in ON unaffected eyes RNFL is on average about 7 microns thinner compared with the control group (*Petzold et al.,* 2010).

In order to examine the changes in the afferent visual system as MS characterizing parameter, in our study it was essential to clarify reciprocal links both of these changes and other disease characterizing indicators. This point is clinically relevant and literature data on it is very different.

Analyzing the impact of disease duration on the retinal nerve fiber layer thickness, we concluded that in the MS patient population with ON history between RNFLT and disease duration in months there is a statistically significant correlation, so RNFLT after the acute ON episode continues to diminish. A study is performed in which influence of the duration of the disease on RNFL is confirmed and estimated that it continues to become thinner by about 3.7 microns/year after the ON episode (*F. Costello et al.,* 2006).

However, there are also studies claiming that the RNFL thickness is not affected by the duration of the disease (*Serbecic et al.,* 2010). Experimental models demonstrate that after a total optic nerve transection, RNFL reduction develops within two months (*Fiona Costello,* 2013). While longitudinal studies

showed that RNFL continues to decrease within 6 months after onset of symptoms and stabilizes within 7–12 months (*F. Costello et al.,* 2008). In our study also in MS patients' group without a history of ON there is a correlation between RNFLT and disease duration observed. The results published in the literature indicate that for MS patients without an acute ON episode RNFL reduction takes place more rapidly than it is in the normal aging process and RNFL decreases on average by 2 microns/year (*Talman et al.,* 2010).

In addition, the fact that in published studies a variety of OCT techniques (*Heidelberg Spectralis* and *Cirrus HD-OCT*) were used, causes ambiguities, so these results must be the compared with caution. In any case, in the eyes without an acute ON episode in history RNFL damage could not cause an acute demyelination, and it should be considered as a primary event that arose as a result of other mechanisms. However, such explanation of the results is ambiguous, as also a subclinically ongoing demyelination process is possible, not noticed by the patient. Currently it is unclear whether really the clinically defined optic neuritis causes RNFL reduction, because it is described that even more pronounced reduction in RNFL is observed in the primary progressive MS patients without any ON episodes than that for relapsing-remitting MS patients after the repeating ON episodes (*Oberwahrenbrock et al.,* 2012).

In light of these considerations, we cannot say for sure whether retinal damage is of a primary character, or it forms secondary to demyelination. In this respect, there are many uncertainties, but the results of examinations performed allow to cast doubt on the theory raised in the past that MS is a primarily demyelinating pathology, and only secondary to demyelination an axonal tissue damage arises.

Important information about the clinical significance of the pathology of afferent visual pathways provides interconnection between the RNFLT changes and the patient's overall neurological status and disability. In our study RNFLT

thickness for both MS patient groups statistically significantly correlates with EDSS scores, hence decreasing RNFLT increases the level of disability and neurological disorders in the functional systems. However, it should be taken into account that in the EDSS total score calculations the visual system is included as well. For a patient with severe visual impairments EDSS score will be higher, but if there are no significant changes in other systems, EDSS overall assessment cannot exceed 4.

RNFL contains only unmyelinated axons and it is known that directly axonal pathology is related to the severity of the disability and irreversible progression in *post mortem* studies (*Trapp et al.,* 1998), which points to the need for a closer analysis of this layer tissue and the factors affecting it. Taking into account the correlation obtained, the hypothesis can be put forward that changes in retinal axonal layer represent the same MS related damage manifestations observable diffuse in CNS elsewhere. However, analyzing the literature, we found that data are not unambiguous. Some authors, similar to our results, describe negative RNFLT and EDSS correlation (*Grazioli et al.,* 2008; *Tatrai et al.,* 2012; *Toledo et al.,* 2008); however, other studies do not confirm this relationship (*Moghaddasi et al.,* 2011; *Pueyo et al.,* 2008).

In addition, in some studies there is a hypothesis proposed that EDSS correlates with RNFLT only in patients without a history of ON (*Albrecht, Frohlich, Hartung, Kieseier, and Methner,* 2007). Also RNFL relationship with EDSS progression was studied and had shown that in patients without prior ON, RNFL atrophy correlates with the more rapid progression of the disease (*Sepulcre et al.,* 2007). For further exploration of this issue the target population could be MS patients without a history of ON; however, it should be taken into account that the overall assessment of the EDSS is little informative at the beginning of the disease and for patients of the clinically isolated syndrome.

Although EDSS include measurements of the visual acuity, in our study we were additionally seeking the RNFLT relationship with visual acuity

function, and it was concluded that in the eyes of MS patients, which have not had an episode of ON, these factors are mutually statistically significantly correlated. However, on the basis of our results, a statistically significant correlation between RNFLT and corrected visual acuity in ON affected eyes was not established. Possibly, this result occurred because the visual acuity was measured by Snellen table, which is used to determine the EDSS total score. It is described in the literature that RNFL decrease by 1 micron provides the decrease of the visual acuity by 0.01 log MAR using the ETDRS table (*Trip et al.,* 2005). In most published studies, RNFL thickness correlates with the visual acuity, as determined by the ETDRS tables (*Wang et al.,* 2010), which are recommended as the "gold standard" for MS clinical trials (*Fiona Costello,* 2013). In addition, it is described in literature that the retinal nerve fiber layer changes in the eyes following the ON, significantly affect the results of the low-contrast letter acuity test (*Fisher et al.,* 2006; *Merle et al.,* 2010; *Talman et al.,* 2010).

Analyzing the changes in color vision, it was found that in all MS patients there is a statistically significant relationship between the reduced RNFLT and altered color vision. A study is carried out, which describes a better correlation of abnormal color vision with reduced RNFL compared with that of visual acuity (*Villoslada et al.,* 2012). In our study, in only 10% of all MS patients simultaneous RNFLT reduction and color vision disorders were observed. It is mentioned in literature that probably in patients with persistent color vision impairment MS will progress more rapidly if compared to the patients whose color vision is unhindered (*Martinez-Lapiscina et al.,* 2014).

In our study also changes in visual field perimetry are statistically significantly associated with the reduced RNFLT, although in 40% of patients the visual field defect and unchanged RNFLT were observed. Literature data often deal with an impact of the reduced RNFL on the irreversible visual field defect prognosis; for the majority of patients the visual field defect developed

within 3–6 months after the ON episode and in cases when the RNFL was reduced below 75 microns, visual field defect was irreversible (*F. Costello et al.*, 2008).

Similarly to previous studies (*Fisher et al.*, 2006; *Klistorner et al.*, 2008; *Naismith et al.*, 2009; *Talman et al.*, 2010; *Trip et al.*, 2005), our results confirm that both VEP and OCT measurements can be used to determine a subclinical damage in ON unaffected eyes. Taking into account the obtained results, we may state that there is a moderate correlation between RNFLT and average N75/P100 amplitude, as well as the average P100 latency both in the eyes suffered from ON, and in ON unaffected eyes. Literature data are similar (*Di Maggio et al.*, 2014; *Naismith et al.*, 2009), although some authors approve isolated connection of RNFLT with the P100 latency (*Fatehi et al.*, 2012), while others with VEP amplitude (*Trip et al.*, 2005).

Taking into account various aspects of damage (structural and functional), we may recommend to take both of these tests, even in patients without complaints of visual impairments, especially in clinically ambiguous situations where the ON symptoms are weak, or the patient is not able to define them adequately.

In our study we found that the OCT method diagnoses almost twofold less optic nerve damage cases, compared to the VEP and only in 4% of the cases normal VEP results and reduced RNFLT were observed simultaneously. Analyzing the ROC curve parameters, the most appropriate diagnostic test for the clinical ON approval is P100 latency, followed by N75/P100 amplitude, while RNFLT determination is a relatively weaker method. RNFLT is a parameter with a relatively lower sensitivity but very high specificity and positive predictive value which is explained by the well created normative database. One of the possible explanations for the low RNFLT sensitivity is the fact that this test characterizes only the initial part of the afferent visual pathways, but the VEP indicates the whole integrity from the retina to the visual

cortex. Besides, it should be taken into consideration that in the situations after the ON episode, RNFL reduction is formed secondary to demyelination of the optic nerve; it proceeds retrograde, and this damage may be incomplete. In addition, the erroneously thicker RNFL could cause replacement of axonal tissue by glial tissue. To improve the sensitivity of OCT, recently some authors (*Davies et al.,* 2011; *Seigo et al.,* 2012; *Walter et al.,* 2012) have proposed to undertake separate ganglion cell layer and inner reticular layer measurements that could even more accurately indicate to the axonal damage, because these structures include no additional glial tissue and blood vessels (*Hood, Salant, Arthur, Ritch, and Liebmann,* 2010). However, it should be borne in mind that the ganglion cell layer and inner reticular layer measurements are currently possible only manually, which is a time consuming and not applicable in the clinical practice process. In the sources of literature there are no data with precise VEP and OCT sensitivity measurements; moreover, in publications with the number of cases detected, VEP method revealed pathology more frequently compared with OCT (*Di Maggio et al.,* 2014; *Naismith et al.,* 2009). So, if due to circumstances only one method is applicable, the visual evoked potentials examination is preferable; however, the combination of both methods increases the chances to diagnose the optic nerve damage. Furthermore, basing on the model developed in our study, it is possible to predict the reduced RNFL using VEP parameters.

Analyzing other methods available for the investigation of the structure of optic nerve, we found that the decoloration of optic nerve in *fundus oculi* examination correlates with the reduced RNFLT, especially in cases after ON clinical episode.

Although the magnetic resonance imaging examination was not carried out for all MS patients, virtually for all patients (97% of the ON (+) eyes) who had experienced a clinical episode of ON, optic nerve demyelination at MRI was observed. However, only in 15% cases, simultaneously with demyelinating changes of optic nerve, MRI examination observed also a reduced RNFLT, but

in 24% cases MRI showed no damage of the optic nerve, and RNFLT was reduced. The results of our study confirm that a statistically significant correlation between the optic nerve demyelination in MRI and reduced RNFLT does not exist. Similarly, such a correlation could not be proved between the optic nerve changes in MRI and a reduced VEP amplitude. However, a statistically significant correlation was obtained between the optic nerve demyelination in MRI and P100 latency. Presumably, this relationship confirms the fact that the P100 latency changes are primarily related to the myelin sheath damage in the optic nerve.

Significant results were obtained by counting demyelinating lesions at MRI examination of the brain, and finding that in patients with a reduced RNFLT, the number of lesions is statistically significantly higher than in patients with normal RNFLT thickness. Moreover, in patients with the reduced RNFLT, the number of lesions affecting the visual pathways in the brain is greater than in patients whose RNFLT is normal. By contrast, the number of active, contrast enhancing lesions does not influence the RNFLT.

Important conclusions can be drawn after the results on brain atrophy in MRI examination. Although precise measurements of brain atrophy in Latvia currently are not performed, signs of atrophy observed in the brain by a radiologist, statistically significantly correlate with the reduced RNFLT. Making accurate measurements of brain atrophy, in Gordon-Lipkin etc. authors' work (*Gordon-Lipkin et al.,* 2007) a reduced brain parenchymal fraction correlated with the reduced RNFL putting forward the hypothesis that the atrophic changes in the RNFL reflect atrophic changes in the brain. Recently some data have been published on the relation of the reduced RNFL to the atrophy of the *thalamus* (*Zivadinov et al.,* 2014) and demonstrated that the brain atrophy simultaneously with the RNFLT correlates to permanent disability (*Abalo-Lojo et al*., 2014) and fatigue as well (*Cruz Gomez, Ventura Campos, Belenguer, Avila, and Forn,* 2013). It is known that not only atrophic changes, but also the number of lesions

in the brain correlate with the level of disability (*Popescu et al.*, 2013), and these changes may be used in order to predict the disease course. These observations are of great importance. The assumption made underpins the belief of RNFL atrophy being the sign of the central nervous system atrophy, and using non-invasive and rapid eye examinations is possible to follow the course of the disease and to changes in different situations, for example, to evaluate the therapeutic efficacy of neuroprotective drugs. Some authors also have expressed the assumption that early RNFL reduction may foresee a more rapid development of brain atrophy (*Chan*, 2012), but it has not been approved by longitudinal studies yet. Furthermore, investigating the patients with clinically isolated syndrome, the results of RNFL thickness measurements did not exert impact on the risk of MS development in the future (*Outteryck et al.*, 2009).

3.3. Directions for Future Studies

The afferent vision pathway opens the opportunity to investigate closer disease mechanisms that cause neurological disorders and disability in MS patients. Monitoring the acute and chronic consequences of the clinical ON episode, it is possible to obtain information about the factors that affect the relapse process, as well as to model the functional-structural recovery after relapse, which probably has taken place also in other parts of CNS. Conducting longitudinal observations and choosing patients without ON episode in history as the study objects, it would be possible to obtain an important information about whether and how axonal and neuronal damage takes place, regardless of the inflammatory process in the afferent vision pathway, consequently also in CNS. The trials of neuroprotective medication, observing dynamic neuroprotective, neuroregenerative and remyelination changes in the afferent vision pathway could be particularly targeted and important in this patient population.

5. CONCLUSIONS

1. For multiple sclerosis patients, in the afferent visual system there are observed functional (visual acuity, amplitude and latency of the visual evoked potentials, color vision, visual fields) changes and structural changes (temporal decoloration of the optic nerve disc, retinal nerve fiber layer reduction), which are not associated with the prior clinical episode of optic nerve inflamation.
2. In multiple sclerosis patients, regardless of the clinical optic nerve inflammation episode in history, a mutual correlation of the structural and functional changes in the afferent visual pathway is observable.
3. Neurodegenerative changes in the retina correlate with the disease duration, degree of disability, visual acuity (in patients without optic neuritis in history), colour vision, visual field defects, temporal optic nerve disc decoloration, N75 /P100 amplitude, P100 latency as well as the number and localization of the demyelinating lesions in the brain and brain atrophy.
4. Prolonged P100 latency of the visual evoked potentials, better than the reduced N75/P100 amplitude and with the optical coherence tomography diagnosed reduced retinal nerve fiber layer, confirm a clinical episode of optic neuritis.
5. Structural damage of the retinal nerve fiber layer could be prognosed using functional investigation methods – N75/P100 amplitude and P100 latency measurements at the visual evoked potentials.

6. PRACTICAL RECOMMENDATIONS

Considering subclinical changes in the afferent visual pathway, all multiple sclerosis patients are recommended to perform detailed functional and structural investigations of visual system. If there are observed any deviations, it is recommended to monitor these changes in dynamics, obtaining information about futher development of neurodegenerative processes.

In future the usage of afferent visual system findings as biological markers of neurodegenerative processes is possible, that may be included as secondary outcome measurements in clinical trials of neuroprotective and immunomodulating treatment.

PUBLICATIONS AND REPORTS ON THE STUDY THEME

Publications on the study theme:

Pastare, D., Kire, I., Laganovska, G., Millers, A. *Use of optical coherence tomography to monitor multiple sclerosis. A review.* Neurologijos Seminarai, 2012. 16 (54): p. 301–310.

Pastare, D., Kire, I., Erts R., Laganovska, G., Millers, A. *Evaluation of axonal optic nerve damage using visual evoked potentials and optical coherence tomography in patients with multiple sclerosis.* Medicina (Kaunas), 2013. 49 (11): p. 474–478.

Pastare, D., Kire, I., Laganovska, G., Millers, A. *Diagnostics of subclinical optic nerve damage by optical coherence tomography in multiple sclerosis patients.* Rīga Stradiņš University, Collection of Scientific Papers, 2014. p. 29–33.

Pastare, D., Kire, I., Laganovska, G., Millers, A. *Functional and structural evaluation of afferent visual system in multiple sclerosis patients.* Proceedings of the Latvian Academy of Sciences. Section B, Vol. 69 (2015), No. 5 (698), p. 20–30.

Pastare, D., Kire, I., Laganovska, G., Millers, A. *Reduced retinal nerve fiber layer prediction for multiple sclerosis patients.* Rīga Stradiņš University, Collection of Scientific Papers, 2015. p. 60–66.

Reports in international conferences and congresses:

Kire, I., Laganovska, G., Pastare, D., Millers, A. *Assesment of optic nerve axonal pathology in multiple sclerosis patients using optical coherence tomography.* European Society of Ophthalmology, Copenhagen, Denmark, June 8–11, 2013 (poster).

Pastare, D. *Optical coherence tomography in multiple sclerosis*. The 7th Baltic Congress of Neurology, Tartu, Estonia, May 9–12, 2012 (oral report).

Pastare, D., Kire, I., Laganovska, G., Millers, A. *Assesment of optic nerve axonal pathology in multiple sclerosis patients using optical coherence tomography*. European Federation of Neurological Societies, Stocholm, Sweden, September 8–11, 2012 (poster).

Pastare, D. *Optic nerve as multiple sclerosis model*. II Baltic Conference on Multiple Sclerosis and Autoimmune disorders, September 24, 2015 (oral report).

Reports in Latvian conferences and congresses:

Pastare, D., Ķire, I. Laganovska, G., Šepetiene, S., Millers, A. *Redzes izsaukto potenciālu parametru izmaiņas pacientiem ar multiplo sklerozi.* 2012. gada RSU Zinātniskā konference (poster).

Pastare, D., Ķire, I., Laganovska, G., Millers, A. *Redzes nerva aksonāla bojājuma izvērtēšana, izmantojot optisko koherences tomogrāfiju pacientiem ar multiplo sklerozi*. 2012. gada RSU Zinātniskā konference (poster).

Pastare, D., Ķire, I., Laganovska, G., Millers, A. *Funkcionālu un strukturālu redzes nervu izmeklējumu korelācija multiplās sklerozes pacientiem.* 2013. gada RSU Zinātniskā konference (poster).

Pastare, D., Ķire, I., Erts, R., Laganovska, G., Millers, A. *Redzes nerva aksonāla bojājuma diagnostika, izmantojot VEP un OCT metodes multiplās sklerozes pacientiem.* 2014. gada RSU Zinātniskā konference (poster).

REFERENCES

1. Abalo-Lojo, J. M., Limeres, C. C., Gomez, M. A., Baleato-Gonzalez, S., Cadarso-Suarez, C., Capeans-Tome, C. and Gonzalez, F. Retinal nerve fiber layer thickness, brain atrophy, and disability in multiple sclerosis patients. *J Neuroophthalmol.* 2014, 34 (1), 23–28. doi: 10.1097/wno.0000000000000057
2. Albrecht, P., Frohlich, R., Hartung, H. P., Kieseier, B. C., and Methner, A. Optical coherence tomography measures axonal loss in multiple sclerosis independently of optic neuritis. *J Neurol.* 2007, 254 (11), 1595–1596. doi: 10.1007/s00415-007-0538-3
3. Alshowaeir, D., Yiannikas, C., Garrick, R., Parratt, J., Barnett, M. H., Graham, S. L., and Klistorner, A. Latency of multifocal visual evoked potentials in nonoptic neuritis eyes of multiple sclerosis patients associated with optic radiation lesions. *Invest Ophthalmol Vis Sci.* 2014, 55 (6), 3758–3764. doi: 10.1167/iovs.14-14571
4. Balcer, L. J. Clinical practice. Optic neuritis. *N Engl J Med.* 2006, 354 (12), 1273–1280. doi: 10.1056/NEJMcp053247
5. Balcer, L. J. and Frohman, E. M. Evaluating loss of visual function in multiple sclerosis as measured by low-contrast letter acuity. *Neurology.* 2010, 74 Suppl 3, S 16–23. doi: 10.1212/WNL.0b013e3181dbb664
6. Bermel, R. A. and Balcer, L. J. Optic neuritis and the evaluation of visual impairment in multiple sclerosis. *Continuum* (*Minneap Minn*). 2013, 19(4 Multiple Sclerosis), 1074–1086. doi: 10.1212/01.CON.0000433282.00221.7e
7. Bock, M., Brandt, A. U., Dorr, J., Kraft, H., Weinges-Evers, N., Gaede, G. et al. Patterns of retinal nerve fiber layer loss in multiple sclerosis patients with or without optic neuritis and glaucoma patients. *Clin Neurol Neurosurg.* 2010, 112 (8), 647–652. doi: 10.1016/j.clineuro.2010.04.014
8. Browne, P., Chandraratna, D., Angood, C., Tremlett, H., Baker, C., Taylor, B. V., and Thompson, A. J. Atlas of Multiple Sclerosis 2013: A growing global problem with widespread inequity. *Neurology.* 2014, 83 (11), 1022–1024. doi: 10.1212/WNL.0000000000000768
9. Bruck, W. The pathology of multiple sclerosis is the result of focal inflammatory demyelination with axonal damage. *J Neurol.* 2005, 252, Suppl 5, v3–9. doi: 10.1007/s00415-005-5002-7
10. Chan, J. W. Recent advances in optic neuritis related to multiple sclerosis. *Acta Ophthalmol.* 2012, 90 (3), 203–209. doi: 10.1111/j.1755-3768.2011.02145.x
11. Costello, F. The Afferent Visual Pathway: Designing a Structural-Functional Paradigm of Multiple Sclerosis. *ISRN Neurology.* 2013, 17. doi: 10.1155/2013/134858
12. Costello, F., Coupland, S., Hodge, W., Lorello, G. R., Koroluk, J., Pan, Y. I. et al. Quantifying axonal loss after optic neuritis with optical coherence tomography. *Ann Neurol.* 2006, 59 (6), 963–969. doi: 10.1002/ana.20851

13. Costello, F., Hodge, W., Pan, Y. I., Eggenberger, E., Coupland, S., and Kardon, R. H. Tracking retinal nerve fiber layer loss after optic neuritis: a prospective study using optical coherence tomography. *Mult Scler.* 2008, 14 (7), 893–905. doi: 10.1177/1352458508091367
14. Cruz Gomez, A. J., Ventura Campos, N., Belenguer, A., Avila, C., and Forn, C. Regional brain atrophy and functional connectivity changes related to fatigue in multiple sclerosis. *PLoS One.* 2013, 8 (10), e77914. doi: 10.1371/journal.pone.0077914
15. Davies, E. C., Galetta, K. M., Sackel, D. J., Talman, L. S., Frohman, E. M., Calabresi, P. A. et al. Retinal ganglion cell layer volumetric assessment by spectral-domain optical coherence tomography in multiple sclerosis: application of a high-precision manual estimation technique. *J Neuroophthalmol.* 2011, 31 (3), 260–264. doi: 10.1097/WNO.0b013e318221b434
16. Di Maggio, G., Santangelo, R., Guerrieri, S., Bianco, M., Ferrari, L., Medaglini, S., et al. Optical coherence tomography and visual evoked potentials: which is more sensitive in multiple sclerosis? *Mult Scler*. 2014. doi: 10.1177/1352458514524293
17. Fatehi, F., Shaygannejad, V., Mehr, L. K., and Dehghani, A. Optical coherence tomography versus visual evoked potential in multiple sclerosis patients. *Iran J. Neurol.* 2012, 11 (1), 12–15.
18. Fernandez, O. Integrating the tools for an individualized prognosis in multiple sclerosis. *J Neurol Sci.* 2013, 331 (1–2), 10–13. doi: 10.1016/j.jns.2013.04.021
19. Fisher, J. B., Jacobs, D. A., Markowitz, C. E., Galetta, S. L., Volpe, N. J., Nano-Schiavi, M. L.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
20. Fisniku, L. K., Chard, D. T., Jackson, J. S., Anderson, V. M., Altmann, D. R., Miszkiel, K. A. et al. Gray matter atrophy is related to long-term disability in multiple sclerosis. *Ann Neurol.* 2008, 64 (3), 247–254. doi: 10.1002/ana.21423
21. Fjeldstad, C., Bemben, M., and Pardo, G. 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.* 2011, 18 (11), 1469–1472. doi: 10.1016/j.jocn.2011.04.008
22. Frohman, E. M., Frohman, T. C., Zee, D. S., McColl, R. and Galetta, S. The neuro-ophthalmology of multiple sclerosis. *Lancet Neurol.* 2005, 4 (2), 111–121. doi: 10.1016/s1474-4422(05)00992-0
23. Garcia-Martin, E., Pueyo, V., Martin, J., Almarcegui, C., Ara, J. R., Dolz, I. et al. Progressive changes in the retinal nerve fiber layer in patients with multiple sclerosis. *Eur J Ophthalmol.* 2010, 20 (1), 167–173.
24. Gelfand, J. M., Goodin, D. S., Boscardin, W. J., Nolan, R., Cuneo, A. and Green, A. J. Retinal axonal loss begins early in the course of multiple sclerosis and is similar between progressive phenotypes. *PLoS One.* 2012, 7 (5), e36847. doi: 10.1371/journal.pone.0036847
25. Gordon-Lipkin, E., Chodkowski, B., Reich, D. S., Smith, S. A., Pulicken, M., Balcer, L. J. et al. Retinal nerve fiber layer is associated with brain atrophy in

multiple sclerosis. *Neurology.* 2007, 69 (16), 1603-1609. doi: 10.1212/01.wnl.0000295995.46586.ae

26. Grazioli, E., Zivadinov, R., Weinstock-Guttman, B., Lincoff, N., Baier, M., Wong, J. R. et al. Retinal nerve fiber layer thickness is associated with brain MRI outcomes in multiple sclerosis. *J Neurol Sci.* 2008, 268(1–2), 12–17. doi: 10.1016/j.jns.2007.10.020
27. Herrero, R., Garcia-Martin, E., Almarcegui, C., Ara, J. R., Rodriguez-Mena, D., Martin, J. et al. Progressive degeneration of the retinal nerve fiber layer in patients with multiple sclerosis. *Invest Ophthalmol Vis Sci.* 2012, 53 (13), 8344–8349. doi: 10.1167/iovs.12-10362
28. Holder, G. E., Celesia, G. G., Miyake, Y., Tobimatsu, S., and Weleber, R. G. International Federation of Clinical Neurophysiology: recommendations for visual system testing. *Clin Neurophysiol.* 2010, 121 (9), 1393–1409. doi: 10.1016/j.clinph.2010.04.010
29. Hood, D. C., Salant, J. A., Arthur, S. N., Ritch, R. and Liebmann, J. M. The location of the inferior and superior temporal blood vessels and interindividual variability of the retinal nerve fiber layer thickness. *J Glaucoma. 2*010, 19 (3), 158–166. doi: 10.1097/IJG.0b013e3181af31ec
30. Keltner, J. L., Johnson, C. A., Cello, K. E., Dontchev, M., Gal, R. L., and Beck, R. W. Visual field profile of optic neuritis: a final follow-up report from the optic neuritis treatment trial from baseline through 15 years. *Arch Ophthalmol.* 2010, 128 (3), 330–337. doi: 10.1001/archophthalmol.2010.16
31. Klistorner, A., Arvind, H., Nguyen, T., Garrick, R., Paine, M., Graham, S. 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
32. 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), 129–137. doi: 10.1007/s10633-008-9147-4
33. Klistorner, A., Garrick, R., Barnett, M. H., Graham, S. L., Arvind, H., Sriram, P., and Yiannikas, C. Axonal loss in non-optic neuritis eyes of patients with multiple sclerosis linked to delayed visual evoked potential. *Neurology.* 2013, 80 (3), 242–245. doi: 10.1212/WNL.0b013e31827deb39
34. Kurtzke, J. F. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology.* 1983, 33 (11), 1444–1452.
35. Martinez-Lapiscina, E. H., Ortiz-Perez, S., Fraga-Pumar, E., Martinez-Heras, E., Gabilondo, I., Llufriu, S. et al. Colour vision impairment is associated with disease severity in multiple sclerosis. *Mult Scler.* 2014, 20 (9), 1207–1216. doi: 10.1177/1352458513517591
36. McDonald, W., Compston, A., Edan, G., Goodkin, D., Hartung, H., Lublin, F. et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the Diagnosis of Multiple Sclerosis. *Ann Neurol.* 2001, 50, 121–127.

37. Merle, H., Olindo, S., Donnio, A., Beral, L., Richer, R., Smadja, D. and Cabre, P. Retinal nerve fiber layer thickness and spatial and temporal contrast sensitivity in multiple sclerosis. *Eur J Ophthalmol.* 2010, 20 (1), 158–166.

38. Moghaddasi, M., Hosseini Nasab, A., Shirali, A., and Soltan Sanjari, M. Evaluation of relation between the retinal nerve fiber layer and Expanded Disability Status Scale (EDSS) in patients with relapsing-remitting form of multiple sclerosis referring to Rasool Akram Hospital. [Research]. *Razi Journal of Medical Sciences.* 2010, 18 (87), 36–43.

39. Naismith, R. T., Tutlam, N. T., Xu, J., Shepherd, J. B., Klawiter, E. C., Song, S. K., and Cross, A. H. Optical coherence tomography is less sensitive than visual evoked potentials in optic neuritis. *Neurology.* 2009, 73 (1), 46–52. doi: 10.1212/WNL.0b013e3181aaea32

40. *Neuro-Ophthalmology Review Manual.* 7th ed. Thorofare, NJ, USA: SLACK Incorporated, 2012.

41. Oberwahrenbrock, T., Schippling, S., Ringelstein, M., Kaufhold, F., Zimmermann, H., Keser, N. et al. Retinal damage in multiple sclerosis disease subtypes measured by high-resolution optical coherence tomography. *Mult Scler Int.* 2012, 530305. doi: 10.1155/2012/530305

42. Odom, J. V., Bach, M., Brigell, M., Holder, G. E., McCulloch, D. L., Tormene, A. P., and Vaegan. ISCEV standard for clinical visual evoked potentials (2009 update). *Doc Ophthalmol.* 2010, 120 (1), 111–119. doi: 10.1007/s10633-009-9195-4

43. Outteryck, O., Zephir, H., Defoort, S., Bouyon, M., Debruyne, P., Bouacha, I. et al. Optical coherence tomography in clinically isolated syndrome: no evidence of subclinical retinal axonal loss. *Arch Neurol.* 2009, 66 (11), 1373–1377. doi: 10.1001/archneurol.2009.265

44. 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), 2520–2527.

45. Petzold, A., de Boer, J. F., Schippling, S., Vermersch, P., Kardon, R., Green, A. et al. Optical coherence tomography in multiple sclerosis: a systematic review and meta-analysis. *Lancet Neurol.* 2010, 9 (9), 921–932. doi: 10.1016/s1474-4422(10)70168-x

46. Polman, C. H., Reingold, S. C., Banwell, B., Clanet, M., Cohen, J. A., Filippi, M. et al. Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria. *Annals of Neurology.* 2011, 69 (2), 292–302. doi: 10.1002/ana.22366

47. Polman, C. H., Reingold, S. C., Edan, G., Filippi, M., Hartung, H. P., Kappos, L. 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

48. Popescu, V., Agosta, F., Hulst, H. E., Sluimer, I. C., Knol, D. L., Sormani, M. P. et al. Brain atrophy and lesion load predict long term disability in multiple sclerosis. *J Neurol Neurosurg Psychiatry.* 2013, 84 (10), 1082–1091. doi: 10.1136/jnnp-2012-304094

49. Pueyo, V., Martin, J., Fernandez, J., Almarcegui, C., Ara, J., Egea, C. et al. Axonal loss in the retinal nerve fiber layer in patients with multiple sclerosis. *Mult Scler*. 2008, 14 (5), 609–614. doi: 10.1177/1352458507087326
50. Quelly, A., Cheng, H., Laron, M., Schiffman, J. S. and Tang, R. A. Comparison of optical coherence tomography and scanning laser polarimetry measurements in patients with multiple sclerosis. *Optom Vis Sci.* 2010, 87 (8), 576–584. doi: 10.1097/OPX.0b013e3181e3dcb3
51. Sakai, R. E., Feller, D. J., Galetta, K. M., Galetta, S. L., and Balcer, L. J. Vision in multiple sclerosis: the story, structure-function correlations, and models for neuroprotection. *J Neuroophthalmol.* 2011, 31 (4), 362–373. doi: 10.1097/WNO.0b013e318238937f
52. Seigo, M. A., Sotirchos, E. S., Newsome, S., Babiarz, A., Eckstein, C., Ford, E. et al. In vivo assessment of retinal neuronal layers in multiple sclerosis with manual and automated optical coherence tomography segmentation techniques. *J Neurol.* 2012, 259 (10), 2119–2130. doi: 10.1007/s00415-012-6466-x
53. Sepulcre, J., Murie-Fernandez, M., Salinas-Alaman, A., Garcia-Layana, A., Bejarano, B., and Villoslada, P. Diagnostic accuracy of retinal abnormalities in predicting disease activity in MS. *Neurology.* 2007, 68 (18), 1488–1494. doi: 10.1212/01.wnl.0000260612.51849.ed
54. Serbecic, N., Aboul-Enein, F., Beutelspacher, S. C., Graf, M., Kircher, K., Geitzenauer, W. et al. Heterogeneous pattern of retinal nerve fiber layer in multiple sclerosis. High resolution optical coherence tomography: potential and limitations. *PLoS One.* 2010, 5 (11), e13877. doi: 10.1371/journal.pone.0013877
55. Siffrin, V., Vogt, J., Radbruch, H., Nitsch, R. and Zipp, F. Multiple sclerosis – candidate mechanisms underlying CNS atrophy. *Trends Neurosci.* 2010, 33 (4), 202–210. doi: 10.1016/j.tins.2010.01.002
56. Slotnick, S. D., Klein, S. A., Carney, T., Sutter, E., and Dastmalchi, S. Using multi-stimulus VEP source localization to obtain a retinotopic map of human primary visual cortex. *Clin Neurophysiol.* 1999, 110 (10), 1793–1800.
57. Talman, L. S., Bisker, E. R., Sackel, D. J., Long, D. A., Jr., Galetta, K. M., Ratchford, J. N. 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
58. Tatrai, E., Simo, M., Iljicsov, A., Nemeth, J., Debuc, D. C., and Somfai, G. M. In vivo evaluation of retinal neurodegeneration in patients with multiple sclerosis. *PLoS One.* 2012, 7(1), e30922. doi: 10.1371/journal.pone.0030922
59. The clinical profile of optic neuritis. Experience of the Optic Neuritis Treatment Trial. Optic Neuritis Study Group. *Arch Ophthalmol.* 1991, 109 (12), 1673–1678.
60. Toledo, J., Sepulcre, J., Salinas-Alaman, A., Garcia-Layana, A., Murie-Fernandez, M., Bejarano, B., and Villoslada, P. 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
61. Trapp, B. D., Peterson, J., Ransohoff, R. M., Rudick, R., Mork, S., and Bo, L. (1998). Axonal transection in the lesions of multiple sclerosis. *N Engl J Med.* 338 (5), 278–285. doi: 10.1056/NEJM199801293380502

62. Trip, S. A., Schlottmann, P. G., Jones, S. J., Altmann, D. R., Garway-Heath, D. F., Thompson, A. J., Miller, D. H. (2005). Retinal nerve fiber layer axonal loss and visual dysfunction in optic neuritis. *Ann Neurol.* 58 (3), 383–391. doi: 10.1002/ana.20575

63. Urano, T., Matsuura, T., Yukawa, E., Arai, M., Hara, Y., and Yamakawa, R. Retinal nerve fiber layer thickness changes following optic neuritis caused by multiple sclerosis. *Jpn J Ophthalmol.* 2011, 55 (1), 45–48. doi: 10.1007/s10384-010-0902-1

64. Villoslada, P., Cuneo, A., Gelfand, J., Hauser, S. L., and Green, A. Color vision is strongly associated with retinal thinning in multiple sclerosis. *Mult Scler.* 2012, 18 (7), 991–999. doi: 10.1177/1352458511431972

65. Voss, E., Raab, P., Trebst, C., and Stangel, M. Clinical approach to optic neuritis: pitfalls, red flags and differential diagnosis. *Ther Adv Neurol Disord.* 2011, 4 (2), 123–134. doi: 10.1177/1756285611398702

66. Walter, S. D., Ishikawa, H., Galetta, K. M., Sakai, R. E., Feller, D. J., Henderson, S. B. et al. Ganglion cell loss in relation to visual disability in multiple sclerosis. *Ophthalmology.* 2012, 119 (6), 1250–1257. doi: 10.1016/j.ophtha.2011.11.032

67. Wang, X. L., Yu, T., Xia, D. Z., Zhang, J. S., Yan, Q. C., and Luo, Y. H. Measurement of retinal nerve fiber layer thickness in optic atrophy eyes of patients with optic neuritis using optical coherence tomography. *Graefes Arch Clin Exp Ophthalmol.* 2010, 248 (7), 1013–1018. doi: 10.1007/s00417-010-1326-9

68. WHO. *Atlas Multiple Sclerosis Resources in the World 2008*. Albany, NY, USA: WHO, 2008.

69. You, Y., Klistorner, A., Thie, J., and Graham, S. L. Latency delay of visual evoked potential is a real measurement of demyelination in a rat model of optic neuritis. *Invest Ophthalmol Vis Sci.* 2011, 52 (9), 6911–6918. doi: 10.1167/iovs.11-7434

70. Zaveri, M. S., Conger, A., Salter, A., Frohman, T. C., Galetta, S. L., Markowitz, C. E. 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

71. Zipp, F. and Aktas, O. The brain as a target of inflammation: common pathways link inflammatory and neurodegenerative diseases. *Trends Neurosci.* 2006, 29 (9), 518–527. doi: 10.1016/j.tins.2006.07.006

72. Zivadinov, R., Bergsland, N., Cappellani, R., Hagemeier, J., Melia, R., Carl, E. et al. Retinal nerve fiber layer thickness and thalamus pathology in multiple sclerosis patients. *Eur J Neurol.* 2014, 21 (8), 1137–e1161. doi: 10.1111/ene.12449

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