ELSEVIER DOYMA

ARCHIVOS DE LA SOCIEDAD ESPAÑOLA DE OFTALMOLOGÍA

www.elsevier.es/oftalmologia

**Original article**

# Effect of treatment in loss of retinal nerve fiber layer in multiple sclerosis patients

*E. García-Martín*[a,*], *V. Pueyo*[a], *J. Fernández*[a], *J. Martín*[b], *J.R. Ara*[b], *C. Almarcegui*[c], *I. Dolz*[c]

[a]*Servicio de Oftalmología, Hospital Universitario Miguel Servet, Zaragoza, Spain*
[b]*Servicio de Neurología, Hospital Universitario Miguel Servet, Zaragoza, Spain*
[c]*Servicio de Neurofisiología, Hospital Universitario Miguel Servet, Zaragoza, Spain*

ARTICLE INFORMATION

*Article history:*
Received on May 30, 2009
Accepted on June 18, 2010

*Keywords:*
Retinal nerve fiber layer
Multiple sclerosis
Interferon
Optical coherence tomography
Scanning laser polarimetry
Visual evoked potentials

A B S T R A C T

*Objective:* To evaluate the effect of pathogenic treatments in the reduction of the retinal nerve fiber layer (RNFL) in patients with Multiple Sclerosis (MS) by means of ocular imaging technologies.

*Material and methods:* A total 155 eyes of 79 patients with MS were enrolled in this study. All patients underwent a complete ophthalmic examination including best corrected visual acuity using Snellen chart, colour vision using Ishihara pseudoisochromatic plates, visual field examination, optical coherence tomography (OCT), scanning laser polarimetry (GDx) and visual evoked potentials. The patients were re-evaluated after a one year period and changes were assessed in order to detect differences between treatments using the Anova statistical test. The patients were divided into four groups: 1) Patients without treatment, 2) Patients treated with interferon beta-1a, 3) Subjects who received interferon beta-1b, 4) Patients treated using glatiramer acetate.

*Results:* There were no statistically significant differences between patients with or without treatment and between the four groups (P>0.05, t test), but functional and structural parameters showed greater loss in RNFL thickness in non-treated patients. Temporal quadrant RNFL thickness measured by OCT was the parameter with the highest variation (reduction of 4.97µm in patients without treatment vs 1.08µm in treated patients).

*Conclusions:* MS pathogenic treatment may be a protective factor in the RNFL loss that is associated to the disease progression. More studies are needed.



*Corresponding author.
*E-mail:* egmvivax@yahoo.com (E. García-Martín).

0365-6691/$ - see front matter 

## Influencia del tratamiento en la pérdida de fibras nerviosas de la retina en pacientes con esclerosis múltiple

*Palabras clave:*
Capa de fibras nerviosas de la retina
Esclerosis múltiple
Interferón
Tomografía de coherencia óptica
Polarimetría láser
Potenciales evocados visuales

R E S U M E N

*Objetivos:* Evaluar el efecto del tratamiento patogénico de la esclerosis múltiple (EM) sobre la pérdida de fibras nerviosas de la retina (CFNR) mediante técnicas de análisis digital de imagen.

*Material y método:* Se incluyeron 155 ojos de 79 pacientes con EM, a los que se exploró la agudeza visual medida con optotipos de Snellen, el defecto de refracción y la visión de colores, y se realizó perimetría automatizada, tomografía de coherencia óptica (OCT), polarimetría láser (GDx) y potenciales evocados visuales (PEV). Este protocolo se repitió al año y los cambios observados fueron comparados según el tratamiento asignado mediante el test de Anova; para lo que se dividió la población en 4 grupos en función del tratamiento recibido: 1) sin tratamiento, 2) interferón beta 1a, 3) interferón beta 1b, 4) acetato de glatirámero.

*Resultados:* No se detectaron diferencias significativas (test t, $p > 0,05$) entre los pacientes con o sin tratamiento ni entre los 4 grupos, pero la mayoría de los parámetros funcionales y estructurales mostraron una tendencia a presentar mayores reducciones de la CFNR en el grupo sin tratamiento. La mayor variación apareció en el cuadrante temporal de la CFNR en la OCT (reducción de 4,97 µm en pacientes sin tratamiento vs 1,08 µm en los tratados).

*Conclusión:* El tratamiento patogénico de la EM puede ser un factor protector para la reducción del espesor de la CFNR que se produce con la progresión de la enfermedad. Se necesitan más estudios.



## Introduction

Multiple sclerosis (MS) can be approached with three different types of treatment. The first is therapy with a pathogenic basis or "fundamental treatment of the disease", which aims at preventing progression and outbreaks. This type of treatment comprises immunosuppressors and immunomodulators such as interferon (IFN), glatiramer acetate, azathioprine, natalizumab and mitoxantrone. The second type of treatment is focused on managing the outbreaks and is based on corticoids, which reduce the intensity and duration of the episodes but do not modify the disability. Finally, the third type of treatment focuses on MS symptoms and endeavors to improve spasticity, fatigue, cognitive deterioration, pain, etc.

To date there is no curative treatment for MS, although pathogenic therapies have demonstrated a reduction in the activity of the disease, diminishing the number of outbreaks and possibly the progression of the disability. All the IFN have exhibited clinical-radiological benefit compared to placebo: IFN beta 1b subcutaneous (Betaferon®),[1-3] IFN beta 1a subcutaneous (Rebif 22® and Rebif 44®)[4-6] e IFN beta 1a intramuscular (Avonex®).[7] IFN beta 1b and 1a are considered safe drugs, well tolerated and efficient for MS RR, both for reducing the number of outbreaks and diminishing their severity.[1,3]

The clinical benefit of glatiramer acetate is similar to the IFN beta[8] with delayed radiological benefits.[9] This has led to ponder a different pathogenic mechanism. It is an agent with immunomodulating activity which induces antigen-specific suppressive cells and interferes in the activation of T-lymphocytes due to competition with the basic myelin protein at the level of the class II major histocompatibility antigen complex. It is administered subcutaneously in a dose of 20 mg per day (Glatiramer®, Copaxone®).

MS is a chronic disease which produces progressive neurological deficit and significant disability. Its etiology is not entirely defined although nowadays most authors consider it is due to inflammation processes, demyelinization and axonal damage. At present there is evidence that the axonal damage is directly related to permanent functional disability[10,11], and some papers go as far as suggesting that said axonal damage can be monitored by measuring the thickness of the retina nervous fiber layer (RNFL) with digital imaging analysis techniques.[12,13] It has also been suggested that said techniques could be useful to monitor the patient response to treatment in clinical trials of this disease.[14-19]

The functional evaluation of the optic nerve is carried out with an exploration of visual acuity and color vision, campimetric techniques and electrophysiological tests, while the structural evaluation utilizes digital imaging analysis techniques such as optic coherence tomography (OCT) or laser polarimetry (GDx).[20-22]

The objective of this study is to determine whether the various types of MS treatments modify the rate of progressive deterioration in the RNFL of MS patients vs. untreated patients.

## Material and methods

### *Patients*

This longitudinal and observational study included 79 patients with MS (155 eyes) that were assessed at baseline and 12 months later.

The inclusion criteria were MS diagnostic confirmed by a neurologist as per Posser's criteria, visual acuity of 0.1 or above in each eye to allow for the correct development of the exploratory protocol and intraocular pressure applanation values under 20mm Hg. The patients who had suffered an optic neuritis outbreak within 6 months before their inclusion in the study or who had an outbreak in the course of their follow-up, as well as those who had a refraction defect over 5 spherical equivalent dioptres or 3 astigmatism dioptres were excluded from the study.

The study was approved by the ethical committee of the hospital and all participants signed an informed consent detailing the objective of the study and the test comprising the exploratory protocol, as well as the possibility of leaving the study at any time. All the subjects had a complete ophthalmological exploration which included visual acuity with Snellen optotype, color test with 20 Ishirara isochromatic sheets, pupil reflexes, ocular motility, refraction defect measurement, anterior pole assessment, applanation tonometry, papillary funduscopic assessment, computerized perimetry made with a Humphrey field analyzer (Carl-Zeiss Meditec, Dublin, Calif. USA) utilizing a 30-2 SITA Standard strategy program; OCT (Stratus OCT 3000. Carl Zeiss Meditec, Dublin) utilizing the *Fast RNFL thickness* protocol (3.4 mm circular scans) and the *Fast macul* protocol, and assessment of the RNFL mean thickness, the thickness of each of the four retinal quadrants and the macular volume; and GDx with variable corneal compensation (GDx VCC, Laser Diagnostic Technologies, San Diego, USA) centering the circular 3.2 mm diameter scans in the papilla in order to measure the RNFL thicknesses (NFI, mean TSNIT, mean superior, mean inferior and TSNIT standard deviation).

The neurological variables about the duration of the disease and the score obtained in the EDSS (*expanded disability status scale*) were also recorded.

The above explorations were made at baseline and one year later to assess changes in the recorded parameters and determine the correlation between said changes and the therapy assigned to each patient.

Twelve months after the first assessment the patients were reassessed applying the same parameters. A comparative statistical analysis was made with the results after dividing the patients in 4 groups on the basis of the treatment they received. Accordingly, group 1 comprised the subjects with MS and without prescribed treatment, group 2 were patients who received INF beta 1a, group 3 included patients with INF beta 1b and group 4 was made up of subjects with MS in treatment with glatiramer acetate.

In said statistical analysis all the aforementioned variables were recorded in a database drawn up with FileMaker Pro 5.0 software. The loss of RNFL thickness and macular volume throughout the follow-up between the 4 groups was compared with the Anova statistical test utilizing the SPSS version 15.0 software (SPSS Inc., Chicago, United States). Prior to the data analysis, the match with normal values was compared with the Kolmogorov-Smirnov test.

**Table 1 – Differences in the mean value of each parameter, with standard deviation in brackets, between the exploration at year 1 minus the baseline assessment, analyzing all the patients in the study and separated in treated and non-treated. Significance of the comparison with the t for Student test between the changes of the treated vs. non-treated groups.**

| | Change at year | Change in non-treated | Change in treated | Patient |
|---|---|---|---|---|
| *VA (Snellen)* | 0.05 (0.15) | 0.07 (0.16) | 0.03 (0.13) | 0.113 |
| *Ishihara Test* | –0.04 (2.90) | –0.06 (2.88) | –0.02 (2.92) | 0.950 |
| *PA: MD (dB)* | 0.31 (3.70) | 0.07 (4.45) | 0.45 (3.17) | 0.537 |
| *EDSS* | 0.13 (0.94) | 0.34 (0.95) | 0.00 (0.91) | **0.029** |
| *VEP Latency (msec)* | –1.49 (7.69) | –2.52 (7.78) | –0.88 (7.64) | 0.328 |
| *OCT* | | | | |
| Medium thickness (µm) | 3.48 (8.65) | 3.42 (10.55) | 3.53 (7.25) | 0.939 |
| Superior thickness (µm) | 2.75 (15.42) | 3.75 (16.28) | 2.12 (14.90) | 0.528 |
| Nasal thickness (µm) | 3.91 (15.59) | 3.42 (15.41) | 4.23 (15.77) | 0.758 |
| Inferior thickness (µm) | 4.70 (14.25) | 3.19 (19.12) | 5.67 (10.02) | 0.297 |
| Temporal thickness l (µm) | 2.59 (13.63) | 4.98 (18.34) | 1.08 (9.32) | 0.085 |
| Macular volume ($mm^3$) | 0.08 (0.22) | 0.06 (0.19) | 0.10 (0.23) | 0.396 |
| *GDX* | | | | |
| NFI | –0.64 (7.50) | –0.75 (6.07) | –0.69 (8.13) | 0.969 |
| Mean TSNIT (µm) | 0.77 (5.03) | –0.42 (5.55) | –0.75 (4.80) | 0.770 |
| Mean superior (µm) | 0.19 (6.47) | 1.40 (5.25) | 0.47 (6.99) | 0.506 |
| Mean inferior (µm) | 1.02 (7.09) | 0.90 (6.82) | –0.15 (7.23) | 0.492 |
| Std. Dev. TSNIT (µm) | –0.64 (3.66) | 1.34 (3.80) | 0.86 (3.61) | 0.542 |

Std. Dev. TSNIT: standard deviation temporal-superior-nasal-inferior-temporal; MD: mean deviation; EDSS: expanded disability status scale; p: significance level; PA: perimetry; msec: milliseconds; µm: microns.

**Table 2 – Differences in the mean value of each parameter, with standard deviation in brackets, between the exploration at year 1 minus the baseline assessment, analyzed on the basis of the group that received treatment.**

| Change at year 1 | No treatment | INF beta 1a | INF beta 1B | Glatiramer acetate |
|---|---|---|---|---|
| *VA (Snellen)* | 0.07 (0.16) | 0.004 (0.11) | 0.09 (0.15) | 0.03 (0.13) |
| *Ishihara Test* | –0.06 (2.88) | 0.18 (3.41) | –0.05 (0.23) | –0.55 (2.79) |
| *PA: MD (dB)* | 0.07 (4.45) | 0.60 (3.02) | 0.48 (2.43) | 0.07 (4.23) |
| *EDSS* | 0.34 (0.95) | 0.16 (0.58) | –0.41 (1.07) | 0.05 (1.24) |
| *VEP Latency (msec)* | –2.52 (7.78) | –0.42 (7.06) | 0.86 (6.25) | –6.44 (10.62) |
| *OCT* | | | | |
| Medium thickness (µm) | 3.42 (10.55) | 3.59 (7.42) | 2.41 (8.44) | 5.32 (6.03) |
| Superior thickness (µm) | 3.75 (16.28) | –0.96 (14.96) | 7.13 (17.07) | 5.40 (12.12) |
| Nasal thickness (µm) | 3.42 (15.41) | 7.19 (15.16) | –0.54 (19.00) | 2.15 (11.67) |
| Inferior thickness (µm) | 3.19 (19.12) | 6.57 (10.91) | 1.27 (8.18) | 9.45 (8.13) |
| Temporal thickness (µm) | 4.98 (18.34) | –0.11 (8.63) | 1.59 (10.97) | 4.25 (8.72) |
| Macular volume ($mm^3$) | 0.06 (0.19) | 0.12 (0.23) | 0.03 (0.27) | 0.12 (0.15) |
| *GDX* | | | | |
| NFI | –0.75 (6.07) | –0.49 (6.53) | 0.43 (9.00) | –3.00 (11.28) |
| Mean TSNIT (µm) | –0.42 (5.55) | –1.16 (3.91) | –1.65 (6.13) | 2.26 (4.81) |
| Mean superior (µm) | 1.40 (5.25) | 0.06 (5.20) | 0.05 (10.90) | 2.24 (5.57) |
| Mean inferior (µm) | 0.90 (6.82) | –1.00 (6.54) | –0.19 (7.99) | 2.73 (8.13) |
| Std. Dev. TSNIT (µm) | 1.34 (3.80) | 0.74 (3.92) | 0.48 (3.64) | 1.07 (2.93) |

Std. Dev. TSNIT, standard deviation temporal-superior-nasal-inferior-temporal; MD, mean deviation; EDSS, Expanded disability status scale; PA, perimetry; msec, milliseconds; µm, microns.

## Results

A total of 79 patients were assessed, 26 male and 53 female (Male/Female proportion of 2:3). Their mean age was 42.32 years (range: 19-66 years) and the mean evolution time of the disease as from diagnostic was of 8.99 years (range: 0.5-26 years). The predominant MS type was relapsing-remitting type with 77 patients (97.46%), while only one patient exhibited primary progressive MS (1.26%) and a further patient had a secondary progressive form (1.26%). The mean score obtained in the EDSS neurological disability scale was of 2.35 (range: 0-8) at the baseline evaluation and of 2.50 (range: 0-8.5) in the one-year assessment of patients.

The results of all the parameters evaluated in this study between the group of subjects with and without treatment, as well as the significance of the comparison of the observed changes are detailed in table 1. As illustrated in said table, no significant differences were identified between the treated and non-treated groups in what concerns functional and structural parameters. However, we observed a tendency in the majority of MS non-treated patients towards a greater loss of RNFL thickness or functionality deterioration.

Table 2 shows the parameters obtained in both assessments for subjects without treatment and the three subgroups of treated patients.

The parameter which exhibited the biggest differences between the treated and non-treated groups was the mean RNFL thickness measured by OCT, with 90.13µm at baseline and 87.86µm in the one-year assessment.

## Discussion

The objective of the study was to quantify the changes observed during one year in the RNFL of MS patients' eyes and identify whether the type of treatment or absence thereof exhibited any correlation with said changes.

The RNFL evaluation in patients with MS was proposed by Frisen and Hoyt in 1974[21] as a useful method to assess the sequels of previous outbreaks of optic neuritis and to detect subclinical episodes of the disease. Typically, ophthalmoscopy and black/white photographs of the retina were utilized because they allowed a qualitative and subjective assessment of the RNFL and optic nerve, but this method only detected defects with losses over 50% of the retina ganglionary cells.[22,23] At present, the application of digital imaging analysis techniques such as OCT and GDx allow a quantitative, objective and reproducible assessment of the RNFL. These techniques, which are non-invasive and not dependent on the analyst, are able to perform highly precise analyses and provide quantitative measurements in a few minutes[24,25] causing a minimum of discomfort to patients.

The reason for not including patients who had suffered optic neuritis within six months prior to the beginning of the study is that this is the period considered as necessary by most authors for the measurements taken with digital imaging analysis techniques such as OCT and GDx to register the retrograde degeneration which occurs after an inflammatory outbreak of the optic nerve, particularly in retrobulbar neuritis cases.[23,26-28]

It must be taken into account that the assessed groups are not homogeneous because, as this is an observational, non-interventionist study, the patients who did not receive treatment and therefore were included in the non-treated group exhibited slighter clinical forms of the disease or longer periods of inactivity and smaller amounts of outbreaks, whereas the patients who were assigned treatment had more acute symptomatic expressions and therefore the aggressiveness of the disease was greater in them than in the control group. This gave rise to a bias which could explain why the differences observed in the RNFL reduction figures did not reach significance between the groups.

In a previous study carried out by our research group on 84 eyes a loss of RNFL in MS patients vs. healthy subjects in the course of one year was observed, even in patients who had not exhibited outbreaks of signs of activity of the disease during the follow-up period. Similar results were observed by Toledo et al.[29] A longitudinal study on 187 eyes of healthy subjects assessed by means of OCT the physiological loss of retinal fibers which takes place with aging and determined that a normal individual has a mean RNFL reduction of 0.16µm per year, this loss being higher over the age of 50.[30] The results of our study demonstrated a loss of 3.48µm per year in patients with MS.

The temporal RNFL thickness diminished a mean of 2.59µm during the follow-up year in patients with MS, of which the subjects who did not receive treatment had a mean reduction of 4.98µm and those who did receive had a reduction of 1.08µm. This leads us to consider the existence of physiopathological mechanisms other than optic nerve inflammation which cause axonal damagel[31-33] and that said damage is smaller in patients who receive pathogenic treatment for MS.

The above results may reinforce the theory of some authors that the evaluation of RNFL by means of digital imaging analysis techniques could be useful for following up the progression of MS and for monitoring the treatment applied in clinical trials.[14-19]

## Conflict of interest

The authors declare they have no conflict of interest.

REFERENCES


1. IFNB Multiple Sclerosis Study Group. Interferon beta-lb is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology. 2001;57:S3-9.
2. The IFNB Multiple Sclerosis Study Group, The University of British Columbia, MS/MRI., Analysis Group. Interferon beta-1b in the treatment of multiple sclerosis: final outcome of the randomized controlled trial. Neurology. 1995;45:1277-85.
3. Paty DW, Li DK, UBC MS/MRI Study Group and the IFNB Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. II. MRI analysis results of a multicenter, randomized, double-blind, placebo-controlled trial. Neurology. 1993;43:662-7.
4. Placebo-controlled multicentre randomised trial of interferon beta-1b in treatment of secondary progressive multiple sclerosis. European Study Group on interferon beta-1b in secondary progressive MS. Lancet. 1998;352: 1491–7.
5. Evidence of interferon beta-1a dose response in relapsing-remitting MS: the OWIMS Study. The Once Weekly Interferon for MS Study Group. Neurology. 1999;53:679–86.
6. Li DK, Paty DW. Magnetic resonance imaging results of the PRISMS trial: a randomized, double-blind, placebo-controlled study of interferon-beta1a in relapsing-remitting multiple sclerosis. Prevention of Relapses and Disability by Interferon-beta1a Subcutaneously in Multiple Sclerosis. Ann Neurol. 1999; 46:197-206.
7. Jacobs LD, Cookfair DL, Rudick RA, Herndon RM, Richert JR, Salazar AM, et al. A phase III trial of intramuscular recombinant interferon beta as treatment for exacerbating-remitting multiple sclerosis: design and conduct of study and baseline characteristics of patients. Multiple Sclerosis Collaborative Research Group (MSCRG). Mult Scler. 1995;1:118-35.
8. Johnson KP, Brooks BR, Ford CC, Goodman A, Guarnaccia J, Lisak RP, et al. Sustained clinical benefits of glatiramer acetate in relapsing multiple sclerosis patients observed for 6 years. Copolymer 1 Multiple Sclerosis Study Group 1. Mult Scler. 2000; 6:255-66.
9. Comi G, Filippi M, Wolinsky JS. European/Canadian multicenter, double-blind, randomized, placebo-controlled study of the effects of glatiramer acetate on magnetic resonance imaging--measured disease activity and burden in patients with relapsing multiple sclerosis. European/Canadian Glatiramer Acetate Study Group 2. Ann Neurol. 2001;49:290-7.
10. Palace J. Inflammation versus neurodegeneration: consequences for treatment. J Neurol Sci. 2007;259:46-9.
11. Hauser SL, Oksenberg JR. The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. Neuron. 2006;52:61-76.
12. Fisher JB, Jacobs DA, Markowitz CE, Galetta SL, Volpe NJ, Nano-Schiavi ML, et al. Relation of visual function to retinal nerve fiber layer thickness in multiple sclerosis. Ophthalmology. 2006;113:324-32.
13. 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;14:609-14.
14. Gordon-Lipkin E, Chodkowski B, Reich DS, Smith SA, Pulicken M, Balcer LJ, et al. Retinal nerve fiber layer is associated with brain atrophy in multiple sclerosis. Neurology. 2007;69:1603-9.
15. Sepulcre J, Murie-Fernandez M, Salinas-Alaman A, Garcia-Layana A, Bejarano B, Villoslada P, et al. Diagnostic accuracy of retinal abnormalities in predicting disease activity in MS. Neurology. 2007;68:1488-94.
16. Sergott RC. Optical coherence tomography: measuring in-vivo axonal survival and neuroprotection in multiple sclerosis and optic neuritis. Curr Opin Ophthalmol. 2005;16:346-50.
17. Zaveri MS, Conger A, Salter A, Frohman TC, Galetta SL, Markowitz CE, et al. Retinal imaging by laser polarimetry and optical coherence tomography evidence of axonal degeneration in multiple sclerosis. Arch Neurol. 2008;65:924-8.
18. Sergott RC, Frohman E, Glanzman R, Al-Sabbagh A. The role of optical coherence tomography in multiple sclerosis: expert panel consensus. J. Neurol. Sci. 2007;263:3-14.
19. Trip SA, Schlottmann PG, Jones SJ, Altmann DR, Garway-Heath DF, Thompson AJ, et al. Retinal nerve fiber layer axonal loss and visual dysfunction in optic neuritis. Ann. Neurol. 2005;58: 383-91.
20. Weinreb RN, Shakiba S, Zangwill L. Scanning laser polarimetry to measure the nerve fiber layer of normal and glaucomatous eyes. Am J Ophthalmol. 1995;119:627-36.

21. Frisen L, Hoyt WF. Insidious atrophy of retinal nerve fibers in multiple sclerosis. Funduscopic identification in patients with and without visual complaints. Arch Ophthalmol. 1974;92:91-7.
22. Honrubia F, Calonge B. Evaluation of the nerve fiber layer and peripapillary atrophy in ocular hypertension. Int Ophthalmol. 1989;13:57-62.
23. Quigley HA, Addicks EM. Quantitative studies of retinal nerve fiber layer defects. Arch Ophthalmol. 1982;100:807-14.
24. Jaffe GJ, Caprioli J. Optical coherence tomography to detect and manage retinal disease and glaucoma. Am J Ophthalmol. 2004;137:156-69.
25. Paunescu LA, Schuman JS, Price LL, Stark PC, Beaton S, Ishikawa H, et al. Reproducibility of nerve fiber thickness, macular thickness, and optic nerve head measurements using Stratus OCT. Invest Ophthalmol Vis Sci. 2004;45:1716-24.
26. Costello F, Coupland S, Hodge W, Lorello GR, Koroluk J, Pan YI, et al. Quantifying axonal loss after optic neuritis with optical coherence tomography. Ann Neurol. 2006;59:963-9.
27. Noval S, Contreras I, Rebolleda G, Muñoz-Negrete FJ. Optical coherence tomography versus automated perimetry for follow-up of optic neuritis. Acta Ophthalmol Scand. 2006;84: 790-4.
28. Kupfer C. Retinal ganglion cell degeneration following chiasmal lesions in man. Arch Ophthalmol. 1963;70:256-60.
29. Toledo J, Sepulcre J, Salinas-Alaman A, Garcia-Layana A, Murie-Fernandez M, Bejarano B, et al. Retinal nerve fiber layer atrophy is associated with physical and cognitive disability in multiple sclerosis. Mult Scler. 2008;14:906-12.
30. Parikh RS, Parikh SR, Sekhar GC, Prabakaran S, Babu JG, Thomas R. Normal age-related decay of retinal nerve fiber layer thickness. Ophthalmology. 2007;114:921-6.
31. Smith KJ, Kapoor R, Hall SM, Davies M. Electrically active axons degenerate when exposed to nitric oxide. Ann Neurol. 2001;49:470-6.
32. Brosnan CF, Battistini L, Raine CS, Dickson DW, Casadevall A, Lee SC, et al. Reactive nitrogen intermediates in human neuropathology: an overview. Dev Neurosci. 1994;16:152-61.
33. Imitola J, Chitnis T, Khoury SJ. Insights into the molecular pathogenesis of progression in multiple sclerosis: potential implications for future therapies. Arch Neurol. 2006;63: 25-33.