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ORIGINAL COMMUNICATION

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Optical coherence tomography in multiple sclerosis

Thickness of the retinal nerve fiber layer as a potential measure of axonal loss and brain atrophy

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Abstract *Background* Axonal distribution within the retinal nerve fiber layer (RNFL) measured by optical coherence tomography (OCT) correlates with axonal viability and integrity. *Objective* To investigate correlations between RNFL and MRI measures of axonal loss in MS patients. *Methods* Fifty one remitting-relapsing MS patients, 20 with a history of optic neuritis (MS-ON), 31 without optic neuritis (MS N-ON), and 12 healthy control subjects (HC) were included in the study. RNFL was measured by OCT and brain atrophy was assessed by MRI. *Results* The average RNFL in the affected eye (AE) in the MS-ON group was significantly lower than the RNFL in the MS N-ON ($p = 0.01$) and in HC ($p = 0.01$). The average RNFL in the unaffected eye (UE) and RNFL in MS N-ON were also lower than HC, but this value did not achieve significance. In MS N-ON a lower average RNFL was associated with an increased T1 lesion volume ($p = 0.03$) and T2-lesion volume ($p = 0.001$). The RNFL in MS N-ON was also associated with a reduction of BPF and %gm fraction ($p = 0.01$, $p = 0.02$ respectively). In MS-ON there was a much weaker, non-significant correlation between RNFL thickness and T1, T2 volume, BPF, %gm and %wm fractions that might have resulted from a pronounced post-inflammatory local optic nerve atrophy in AE. *Conclusion* The RNFL measured by OCT may be useful as a surrogate marker for assessment of brain atrophy in MS

Key words multiple sclerosis · optical coherence tomography

Introduction

Multiple sclerosis (MS) is a primary demyelinating disease of the central nervous system with a variable spectrum of clinical and pathological presentation. Although pathologic damage in MS involves the myelin sheath, recent data showed that axonal degeneration might be equally important and occur during early phases of the disease [2]. For assessment of axonal loss *in vivo* in MS patients several MRI techniques, such as T1 lesion load [21], atrophy measures, and brain parenchymal fraction (BPF) [12], have been applied. All these techniques require advanced technology and extensive examination times.

The retina represents the most proximal part of optic nerve, with a unique structure composed of unmyelinated axons and with some contribution from glial cells [8, 13]. This axonal distribution creates a retinal nerve fiber layer (RNFL) and its thickness correlates with changes in axonal function [4]. A reduction in the RNFL has been observed in patients with several optic neuropathies [1]. Qualitative changes in RNFL in MS patients was first reported by Frisen and Hoyt [7]. Quantitative changes in RNFL in optic neuritis were first described by Parisi et al. [14], Trip et al . [19] and Fisher

et al [6]. Recently published data have also shown a reduction of RNFL in patients with optic neuritis, one of the primary clinical presentations of MS in MS patients without a history of optic neuritis [17]. Although there are a few methods for retinal imaging, including Scanning Laser Polarimetry (GDx with variable corneal compensation) and Confocal Scanning Laser Ophthalmoscopy (Heidelberg retinal tomography), results from several studies have suggested that OCT is the best method for assessment RNFL in MS [8]. This is a non-invasive diagnostic technique, based on echo time delay of back-scattered infrared light, that is used in ophthalmology to investigate the retina and optic nerve [3]. Thus, the application of OCT for the assessment of MS patients is a reliable method that can be used for the direct quantification of axonal loss in the optic nerve.

The goal of this study was to estimate whether RNFL as measured by OCT may represent a new structural parameter in the evaluation of global axonal loss in MS patients. We hypothesized that axonal loss in RNFL would correlate with brain axonal damage and that RNFL would correlate with neurological impairment and disease duration in MS. In essence we searched for a correlation between OCT measurements of RNFL and brain tissue damage as assessed by MRI.

Methods

Subjects

We included 51 patients with a diagnosis of relapsing-remitting MS (RR-MS) based on McDonald criteria [15] in the study. None of these patients had been treated with immunomodulatory or immunosuppresive drugs. We divided our patient population into two subgroups: MS with optic neuritis (MS ON) in the past and without optic neuritis (MS N-ON). Most of our MS-ON patients had a single attack of unilateral ON and four have subsequent optic neuritis in both eyes. In the MS ON group we compared OCT measures between the affected eye (AE) and the unaffected eye (UA). Age- and sex-matched healthy control subjects (HC), with no history of neurological and ocular disease and unrelated to the MS participants, were included in the study as a control group. We excluded from analysis subjects with high myopia. The neurological status of all MS patients was determined and included the Expanded Disability Status Scale (EDSS) [10]. The study was approved by the local ethics committee.

Magnetic resonance imaging

MRI scans were obtained on a 1.5 Tesla scanner (Vision Plus, Siemens, Erlangen, Germany). The MRI protocol included dual-echo (TR = 5000 ms, TE = 20/80 ms; 50 slices, thickness = 3 mm gap = 0.0 mm, matrix 154 × 256, and FOV = 250 × 250 mm), T1-weighted Magnetization Prepared Rapid Gradient Echo (MPRAGE – TR = 9.7 ms, TE = 4 ms; eff thick 1.5 mm, no partitions 164, matrix 192 × 256).

MRI data analysis included assessment of total lesion volume on T2- and T1-weighted images using semi-automated techniques (Java Image, Xinapse, UK). Brain atrophy was measured using T1-3D MPRAGE and SMP 99 software (Wellcome Department of Cognitive Neurology, University College of London, UK). White matter (%wm), gray matter (%gm) fraction and brain parenchyma fraction (BPF) were calculated according to an accepted formula [5].

Optical coherence tomography

After mydriasis of both eyes with 1 % tropicamide, the fast RNFL protocol included three 3.4 mm diameter scans, with samples of 256 points. The OCT 4.0 software (Stratus version 3, Carl Zeiss Meditec Inc, Dublin, CA) was used. For analysis only scans with a signal strength ≥7 and uniform brightness across the scan circumference were used. The average and quadrants (temporal, superior, nasal and inferior) RNFL were measured.

Statistical analyses

Statistical analyses were performed with SPSS (version 14.0) package. The normal distribution of all variables was tested using the Kolmogorov-Smirnov test. Using the t-test we compared EDSS and disease duration between two group of MS patients. Spearman's rank test was used to assess correlation between EDSS and RNFL and Pearson's correlation was applied to examine correlations between RNFL and MRI data. We used mean value of RNFL of both control eyes to analyze the data. All statistical tests were regarded as significant at $p < 0.05$.

Results

The clinical characteristics of the MS patients and control groups are shown in Table 1 and confirm appropriate matching in relation to age, EDSS and disease duration ($p > 0.05$).

RFNL in patients with optic neuritis

The average RNFL in the AE was lower compared with the UE (83.92 ± 17.63 versus 91.08 ± 19.3). Accordingly, RNFL in the AE of MS ON patients was significantly lower compared to the average RNFL in MS N-ON patients (83.92 ± 17.63 versus 94.38 ± 15.0, $p = 0.01$) and in HC (83.92 ± 17.63 versus 100.3 ± 12.1, $p = 0.01$) (Table 2). In addition, RNFL of the UE in MS ON patients was also reduced, albeit not significantly, compared with average RNFL in MS N-ON patients (91.08 ± 19.3 versus 94.38 ± 15.0) and with HC (91.08 ± 19.3 versus 100.3 ± 12.1). Sectoral analysis showed that, compared with MS N-ON, RNFL thickness in the AE was significantly lower in the

Table 1 Clinical characteristic of MS patients and HC

	MS ON (n = 20)	MS N-ON (n = 31)	HC (n = 12)
Age, mean (SD), y	33 (6.6)	34 (4.6)	31 (3.5)
EDSS, median (SD)	**2.49 (1.03)**	**2.40 (1.1)**	
Disease duration, mean (SD), y	7.1 (2.2)	6.9 (3.2)	

SD standard deviation; *y* year

temporal quadrant (51.4 ± 14.3 versus 63.2 ± 13.5 p = 0.002) (Table 2). Compared with HC, RNFL in the AE was significantly lower in the temporal (51.4 ± 14.3 versus 74.0 ± 10.0 p = 0.0001), nasal (70.1 ± 18.9 versus 81.2 ± 14.2 p = 0.05) and inferior sector (120.5 ± 24.5 versus 142.0 ± 14.8 p = 0.002) (Table 2). In the UE, a significant reduction of RNFL compared with HC was observed in the temporal (58.6 ± 20.7 versus 74.0 ± 10.0, p = 0.02) and inferior (126.4 ± 22.1 versus 142.0 ± 14.8, p = 0.03) sector.

RNFL in MS patients without a history of optic neuritis

The average RNFL in MS N-ON was also lower when compared with RNFL in HC (94.38 ± 15.0 versus 100.3 ± 12.1) (Table 2). There was no difference in RNFL thickness between the right and left eye in MS N-ON patients. Sectoral analysis detected significantly lower RNFL in MS N-ON compared to HC in temporal (63.2 ± 13.5 p = 0.01) and inferior quadrant (125.4 ± 19.9 versus 142.0 ± 14.8 p = 0.01) (Table 2).

RNFL and disease duration and disability

In MS ON patients, reduction of the RNFL in the UE correlated with disease duration (p = 0.02; r = –0.6) (Fig. 1 a). Similarly, in MS N-ON there was a correlation between the average RNFL and disease duration (p = 0.04; r = –0.4) (Fig. 1 b). In MS ON, a lower RNFL in the UE correlated with more advanced neurological disability as assessed by EDSS (p = 0.003; r = –0.7) (Fig. 2 a). Interestingly, there was no correlation between RNFL in the AE of MS

Table 2 Sectoral and average RNFL in AE, UE, MS N-ON and HC

	AE	UE	MS N-ON	HC
superior, mean (SD), µm	115.8 (24.1)	123.4 (21.6)	118.9 (18.1)	125.5 (24.5)
inferior, mean (SD), µm	120.5 (24.5)	126.4 (22.1)	125.4 (19.9)	142.0 (14.8)
nasal, mean (SD), µm	70.1 (18.9)	76.1 (11.5)	74.3 (15.8)	81.2 (14.2)
temporal, mean (SD), µm	51.4 (14.3)	58.6 (20.7)	63.2 (13.5	74.0 (10.0)
average RNFL (SD), µm	83.92 (17.63)	91.08 (19.3)	94.38 (15.0)	100.3 (12.1)

SD standard deviation; *µm* micrometer

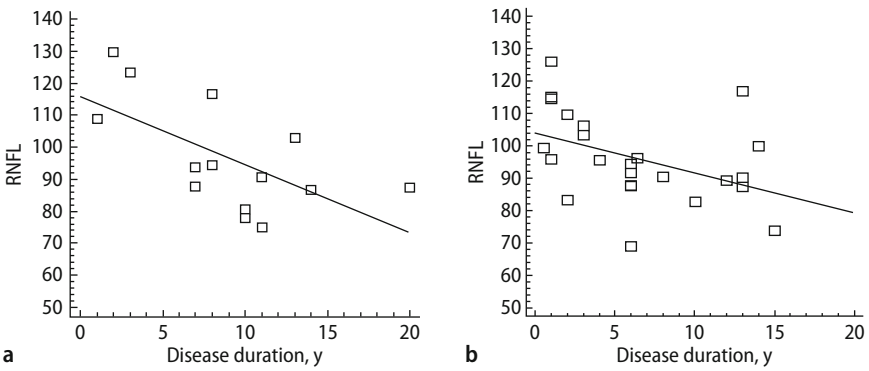

Fig. 1 Correlation between RNFL thickness and disease duration as shown by regression model: **A** in UE of MS ON (p = 0.02; r = –0.6); **B** in MS-NON (p = 0.04; r = –0.4)

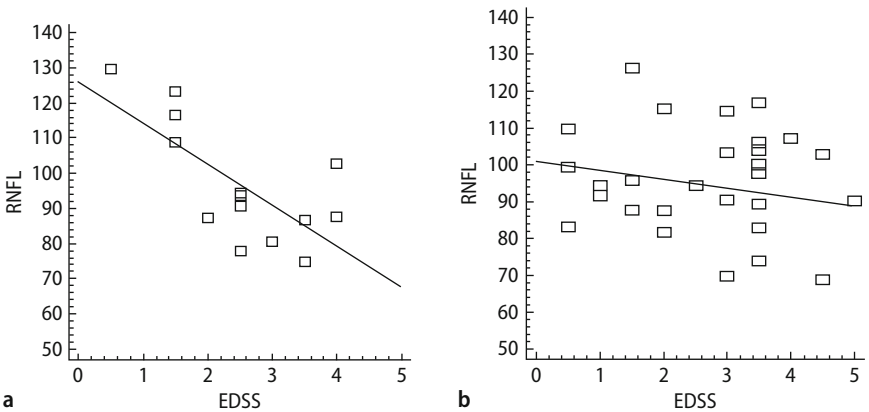

Fig. 2 Correlation between RNFL thickness and EDSS as shown by regression model: **A** in UE of MS ON (p = 0.003; r = –0.7); **B** in MS N-ON (p = 0.2; r = –0.2)

ON patients and disease duration (p = 0.09; r = –0.3) and neurological disability (p = 0.3; r = –0.2.). Similarly, there was no correlation between the average RNFL in MS N-ON and disability (p = 0.2; r = –0.2) (Fig. 2 b).

Correlations between RNFL and MRI measures

MRI characteristics of the MS groups are shown in Table 3. T1-, T2-lesion volume and segmentation measures were comparable in MS ON and MS N-ON patients (p > 0.05). In MS N-ON a lower average RNFL was associated with a higher T1 lesion volume (p = 0.004; r = –0.4) (Fig. 3 a). A reduction of RNFL also correlated with the T2-lesion volume in MS N-ON (p = 0.03; r = –0.3) (Fig. 3 b). In contrast, in MS ON there was no correlation between the average RNFL and T1 or T2 lesion volume for both AE and UA.

Assessment of brain atrophy parameters and correlation with RNFL thickness showed that the average RNFL in MS N-ON was associated with a reduction of the BPF and a reduction of the %gm fraction (p = 0.01; r = 0.4; p = 0.02; r = 0.4, respectively) (Fig. 4), but not with the %wm fraction (p = 0.3). Interestingly, in MS ON patients there was no correlation between the average RNFL and BPF (p = 0.1; r = 0.3) and the %gm fraction (p = 0.2; r = 0.2) and the %wm fraction (p = 0.1; r = 0.3), for both the AE and UE.

Table 3 MRI characteristic of MS group. Mean value and standard deviation (SD)

	MS ON	MS N-ON
T1 V mm^3	4564.06 (6692.7)	4797.68 (5514.8)
T2 V mm^3	8855.64 (7257.3)	9458.8 (9904.56)
BPF	0.81 (0.04)	0.80 (0.03)
% wm	0.27 (0.01)	0.26 (0.01)
% gm	0.53 (0.03)	0.53 (0.02)

T1 V lesion volume on T1-weighted images; *T2 V* lesion volume on T2-weighetd images; *BPF* brain parenchyma fraction; *% wm* fraction of white matter; *% gm* fraction of gray matter

Discussion

In this study we present an analysis of the thickness of RNFL in RR MS patients in relation to clinical and MRI parameters. A reduction of RNFL in patients with optic

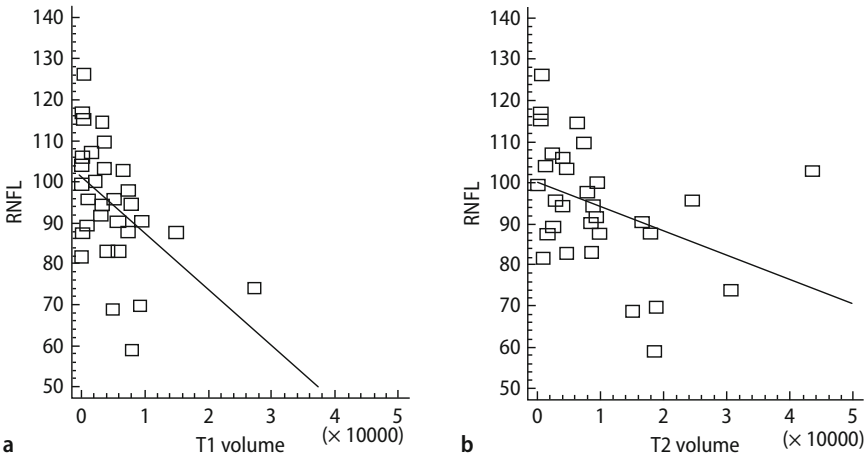

Fig. 3 Correlation between RNFL thinning and MRI measures as shown by regression model in MS N-ON: **A** with T1 lesion volume (p = 0.004; r = –0.4); **B** with T2 lesion volume (p = 0.03; r = –0.3)

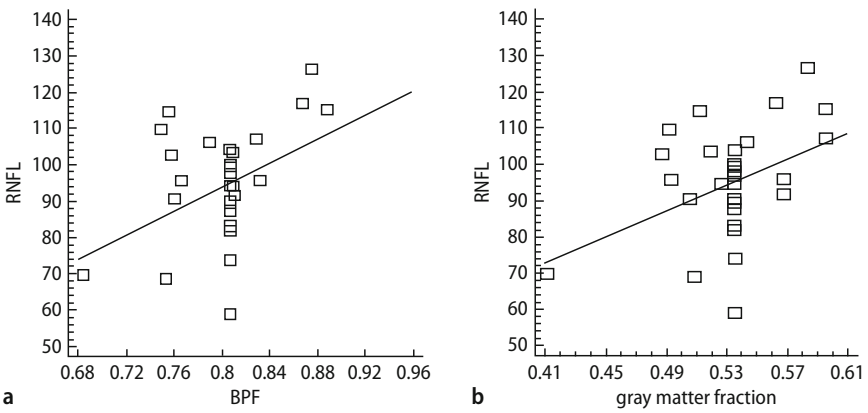

Fig. 4 Correlation between RNFL thickness and MRI brain atrophy measures as shown by regression model in MS N-ON patients: **A** with BPF (p = 0.01; r = 0.4); **B** with gray matter fraction (p = 0.02; r = 0.4)

neuritis correlated with disease duration, neurological disability, and in MS N-ON patients with MRI brain atrophy measures.

All of the previously published studies showed a reduction of RNFL in MS as a result of optic neuritis [4, 14, 19]. Some publication have also detected a reduction of RNFL in the non-affected MS eye, which was interpreted as evidence of subclinical damage in the optic pathway [4]. Only very recently has it been shown that MS patients without optic neuritis also have a reduced RNFL compared to healthy controls and that a reduction of RNFL was a predictor of disease activity and progression [17]. A reduction of RNFL was detected in the AE compared with the UA, as well as with MS N-ON over HC [6]. Our results of RNFL reduction in MS ON patients corroborates the findings of previously published data, which showed RNFL thinning in this population compared with the control group [18].

The main goal of our study was to search for correlations between RNFL reduction and brain atrophy as assessed by MRI. In a previously published report, RNFL thickening was correlated with lesions visible in the optic nerve on MRI, during acute optic neuritis but no correlation was found with lesion load and location [16]. In another recent cross-sectional study, the optic nerve atrophy measured by MRI correlated with RNFL thinning [18]. In our study, in MS N-ON thinning of RNFL correlated with more extensive brain damage, as measured by T2 and T1- lesion volume. T2-lesions are pathologically heterogeneous involving axonal damage, demyelination and gliosis. However, a high correlation between RNFL thinning and T1-lesion volume, an established MRI marker of brain axonal damage, points to RNFL as a predictive factor for assessment of brain atrophy. This conclusion is strongly supported by the demonstration of correlations between RNFL reduction in MS N-ON with the loss of BPF. We have also applied a segmentation method to search for a correlation between RNFL and brain atrophy. Interestingly, only the gray matter fraction showed a correlation with a reduction in RNFL in MS N-ON patients. Gray matter pathology in MS is directly related to axonal damage, whereas white matter pathology is more heterogeneous and depends more on tissue inflammation [11]. Thus, our results suggest a correlation between a reduction of RNFL and brain axonal loss.

RNFL also showed a correlation with disease duration and neurological disability, two clinical parameters consistently linked with progressive axonal loss [20]. The only paper published so far of the correlation between OCT and T1- and T2-lesion volume showed no correlation [17]. Most of the patients included in that study, in contrast to ours, were treated with immunomodulatory drugs. Therefore, our results might provide more accurate data of the natural history of RNFL in MS. Moreover, our cohort of MS patients represents a very homogeneous relapsing-remitting subpopulation, whereas Sepulcre et al [17] included a very heterogeneous group of patients with CIS, SP MS, and PP MS. Both papers, by Sepulcre [17] and ours, showed however good correlation between brain atrophy measure and RNFL thinning. Another very recent paper [9] also confirmed a correlation between BPF and RNFL.

Surprisingly, we found only weak correlations between RNFL and T1, T2-lesion volume, BPF, and grey and white matter fractions in MS ON patients. On the whole, MS ON patients had a significantly higher reduction of RFNL than MS N-ON patients. Lack of correlations between RNFL and T1, T2 lesion volume and segmentation measures in MS ON patients indicate that axonal loss in the retina resulting from optic neuritis occurred independently from distant brain MRI lesions and atrophy. In contrast, in MS N-ON patients there should be no lesions in the optic nerve and therefore brain axonal loss may perhaps better correlated with RNFL.

In summary, the results of this study indicate that measurement of RNFL by OCT in MS N-ON patients might be used as a structural surrogate marker of axonal destruction in brain tissue injury. This technically easy-to-perform examination can be included in the standard evaluation of MS patients to assess axonal loss. However, further studies should be performed to replicate and extend our findings

Conflict of interest The authors declare no conflict of interest.

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