Progressive Loss of Corneal and Retinal Nerve Fibers in Patients With Multiple Sclerosis: A 2-Year Follow-up Study

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Introduction

Multiple sclerosis (MS) is a chronic inflammatory neurodegenerative disease characterized by repeated episodes of inflammation with demyelination and axonal degeneration in the central nervous system. There is also evidence of peripheral neuropathy and small fiber damage in patients with MS.¹⁻³ Axonal degeneration is considered to underlie progressive neurologic disability⁴ and can be evaluated by assessing brain atrophy or volume loss on magnetic resonance imaging (MRI) and peripapillary retinal nerve fiber layer (RNFL) thinning measured with optical coherence tomography (OCT).⁵⁻⁸ However, the correlation between MRI measures and clinical disability is poor.⁹,¹⁰ and acute optic neuritis (ON) can increase RNFL thickness.¹¹,¹² Therefore, there is a need for more precise measures of axonal degeneration in MS.

Corneal confocal microscopy (CCM) is a noninvasive imaging technique that allows quantification of the corneal subbasal nerve plexus and dendritic cells (DCs).¹³⁻¹⁵ We and others have previously shown...
that CCM can detect corneal nerve fiber damage in central\textsuperscript{16–20} and peripheral\textsuperscript{21–25} neurodegenerative disorders and increased DCs in diabetic neuropathy, chronic inflammatory demyelinating polyneuropathy, Fabry disease, and Behçet disease.\textsuperscript{14,24–27}

Recently, we and others have shown corneal nerve fiber damage in patients with MS.\textsuperscript{18,19,28} In the current study, we have evaluated longitudinal alterations in corneal nerve fiber morphology, DC density, and peripapillary RNFL thickness in relation to neurologic disability over 2 years in patients with MS.

Methods

Thirty-one patients with relapsing-remitting MS (RRMS) were enrolled in this prospective study at a single tertiary referral university hospital. These patients were part of a larger cohort from a previously published cross-sectional study demonstrating corneal nerve fiber damage and increased DCs in patients with MS.\textsuperscript{28} The patients were diagnosed with RRMS according to the revised McDonald criteria.\textsuperscript{29} A complete neurologic examination was performed for each patient at baseline and after 2 years, and the Kurtzke Expanded Disability Status Scale (EDSS) and the Multiple Sclerosis Severity Score (MSSS) were used to assess disease severity and physical disability. Exclusion criteria were a history of ON within 6 months prior to study entry, previous ocular trauma, surgery, contact lens use, or any other systemic and ocular diseases that might affect corneal nerves, DCs, or RNFL. The study was approved by the Institutional Research Ethics Committee and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants after explanation of the nature and possible consequences of the study.

All patients underwent a complete ophthalmologic examination. The peripapillary RNFL thickness measurements were obtained using a spectral-domain OCT device (Spectralis OCT; Heidelberg Engineering GmbH, Heidelberg, Germany). The OCT scanning circle (3.4 mm in diameter) was manually positioned at the center of the optic disc, and the average peripapillary RNFL thickness was recorded in micrometers. The baseline peripapillary RNFL scans were set as reference for the follow-up measurements.

Laser-scanning CCM (Rostock Cornea Module/Heidelberg Retina Tomograph III; Heidelberg Engineering GmbH) was performed on all patients at baseline and after 2 years of follow-up. The full thickness of the central cornea was scanned using the section mode, and two-dimensional digital images were obtained with a lateral digital resolution of 1 μm/pixel, a depth resolution of 2 μm/pixel, and an image size of 400 × 400 μm. The total duration of examination was approximately 2 minutes per eye. Three to five high-quality subbasal nerve plexus images were selected and analyzed from each patient, and the average of the results was considered. Automated CCMetrics software, version 2.0 (University of Manchester, Manchester, UK), was used to quantify the nerve fibers.\textsuperscript{30} Six corneal nerve measures were quantified: corneal nerve fiber density (CNFD), the total number of major nerves/mm\textsuperscript{2}; nerve branch density (CNBD), the number of branches arising from major nerves/mm\textsuperscript{2}; nerve fiber length (CNFL), the total length of all nerve fibers and branches (mm/mm\textsuperscript{2}); total branch density (CTBD), the total number of branches/mm\textsuperscript{2}; nerve fiber area (CNFA), the total area of nerve fibers (μm\textsuperscript{2}/mm\textsuperscript{2}); and nerve fiber width (CNFW), the average axial diameter of nerve fibers (μm). The number of highly reflective cells with dendriform structures was manually counted in the same images used to quantify the subbasal nerve plexus using the proprietary software within the corneal confocal microscope, and the density was defined as the total number of cells/mm\textsuperscript{2}.

For all patients, data from one eye were used for analyses. Only the right eyes were assessed if the patient had a history of bilateral ON (n = 6) or no ON in either eye (n = 13). The eyes with previous ON were assessed in patients with a history of unilateral ON (n = 12). The same eye was studied for each patient at baseline and follow-up.

Statistical analysis was performed using SPSS version 21.0 (SPSS, Inc., Chicago, IL, USA) software. Basic descriptive statistics were calculated and reported as the mean (SD) or median (interquartile range [IQR]), as appropriate. Normal distribution of continuous variables was confirmed with the Kolmogorov-Smirnov test. Paired samples t-test for normally distributed data and Wilcoxon signed rank test for nonnormally distributed data were used to compare the parameters obtained at baseline and after 2 years of follow-up. Independent samples t-test for parametric data and Mann-Whitney U-test for nonparametric data were used to compare the parameters between different treatment groups. The associations between the change in disease severity scores during follow-up and CCM parameters and RNFL were measured using the Spearman correlation coefficient. For all evaluations, a two-sided P value of less than 0.05 was considered statistically significant.
Table 1. Corneal Confocal Microscopic Parameters, Peripapillary RNFL Thickness, and Disease Severity Scores in Patients With Multiple Sclerosis at Baseline and After 2 Years of Follow-up

| Characteristic | Baseline | Follow-up | P Value |
|----------------|----------|-----------|---------|
| CNFD (No./mm²) | 26.9 ± 7.2 | 25.7 ± 6.6 | 0.38^a |
| CNBD (No./mm²) | 38.1 ± 19.9 | 32.1 ± 14.3 | 0.09^a |
| CNFL (mm/mm²)  | 16.0 ± 3.2  | 15.1 ± 2.8  | 0.05^a  |
| CTBD (No./mm²) | 56.1 ± 25.6 | 48.3 ± 20.0 | 0.08^a  |
| CNFA (μm²/mm²) | 7041.9 ± 1589.9 | 6016.1 ± 1706.8 | 0.003^a |
| CNFW (μm)      | 22.0 ± 1.4  | 21.2 ± 1.0  | 0.005^a |
| DC density (No./mm²) | 26.0 (6.7–37.0) | 21.2 (4.7–75.3) | 0.16^b |
| RNFL thickness (μm) | 84.1 ± 13.8 | 82.8 ± 14.2 | 0.004^a |
| EDSS           | 3.0 (2.0–3.5) | 3.0 (2.5–4.0) | 0.01^b  |
| MSSS           | 4.6 ± 1.8   | 4.3 ± 1.5   | 0.18^a  |

Data are expressed as mean ± SD for CNFD, CNBD, CNFL, CTBD, CNFA, CNFW, RNFL, and MSSS and median (IQR) for DC density and EDSS. The bold P values represent statistically significant differences.

^aPaired samples t-test.
^bWilcoxon signed rank test.

Results

The mean ± SD age of the patients with RRMS at the time of enrollment was 35.0 ± 7.7 years, and 20 (64.5%) of them were female. The mean ± SD duration of MS was 9.5 ± 4.2 years. None of the patients developed confounding ocular disease during the follow-up period. Eighteen patients (58%) had a history of ON, while no patient experienced an ON episode during follow-up. Twenty-eight of the 31 patients (90%) were on teriflunomide, and 3 (10%) were not receiving any disease-modifying therapy. Nine (29%) patients had relapses (8 patients had one, 1 patient had two relapses) during 2 years of follow-up. The median (IQR) value of the EDSS score was 3.0 (2.0–3.5) at baseline and increased significantly to 3.0 (2.5–4.0) after 2 years of follow-up (P = 0.01) (Table 1). During the follow-up period, 15 (48%) patients showed a worsening in EDSS score, 3 (10%) showed an improvement, and 13 (42%) had no change. None of the patients reported a history of trigeminal neuralgia.

There were significant reductions in CNFA (95% confidence interval [CI], −1685.2 to −366.4; P = 0.003), CNFW (95% CI, −1.4 to −0.3; P = 0.005), and RNFL thickness (95% CI, −2.0 to −0.4; P = 0.004) a trend for reduction in CNFL (95% CI, −1.9 to 0.2; P = 0.05) and CTBD (95% CI, −16.5 to 0.9; P = 0.08) and no significant change in CNFD, CNBD, or DC density over 2 years (Table 1, Fig. 1).

The highest percentage of patients with MS showed a decrease in CCM parameters (CNFD [48%], CNBD [65%], CNFL [77%], CTBD [65%], CNFA [74%], and CNFW [71%]), while a proportion showed an increase (CNFD [42%], CNBD [29%], CNFA [26%], and CNFW [29%]) or no change (CNFD [10%], CNBD [6%]). The change in CNFD during follow-up correlated with the change in EDSS (ρ = −0.468; P = 0.008), MSSS (ρ = −0.442; P = 0.01), DC density (ρ = −0.550; P = 0.001), and RNFL thickness (ρ = 0.472; P = 0.007), and the change in CNFL correlated with the change in EDSS (ρ = −0.445; P = 0.01) and MSSS (ρ = −0.490; P = 0.005). The change in DC density correlated with the change in EDSS (ρ = 0.368; P = 0.04) and RNFL (ρ = −0.484; P = 0.006) during the follow-up period (Fig. 2).

There were significant reductions in CNFL (95% CI, −3.1 to −1.1; P < 0.001), CTBD (95% CI, −29.2 to −4.0; P = 0.01), CNFA (95% CI, −2205.1 to −208.2; P = 0.02), CNFW (95% CI, −1.8 to −0.4; P = 0.04), and RNFL thickness (95% CI, −2.8 to −3.0; P = 0.02) and an increase in DC density (median [IQR], 20.3 [6.7–39.3] vs. 52.7 [4.7–111.0]; P = 0.04) in the subset of patients (48%) with a worsening EDSS score over 2 years, with no significant change in any of the study parameters in patients without progression in EDSS (Table 2). Figure 3 illustrates the CCM images of the central corneal subbasal nerve plexus at baseline and after 2 years of follow-up in a patient with RRMS and worsening EDSS score.

The RNFL thickness showed a significant reduction over 2 years in eyes with a history of ON (n = 18; 95% CI, −2.4 to −0.3; P = 0.01) but no change in eyes.
Corneal Nerve Loss in Multiple Sclerosis

**Discussion**

To our knowledge, this is the first study reporting longitudinal changes in corneal nerve morphology in patients with MS. We show a reduction in corneal nerve fiber area, nerve fiber width, and peripapillary RNFL thickness and significant associations between the reduction in CNFD and CNFL and increasing disease severity (EDSS and MSSS) during 2 years of follow-up in patients with RRMS. The increase in DC density was associated with a reduction in CNFD and RNFL and an increase in EDSS. Furthermore, the reduction in CNFD correlated with a reduction in RNFL thickness. In the subset of patients with worsening EDSS, there were significant reductions in CNFL, CTBD, CNFA, CNFW, and RNFL thickness and an increase in DC density during follow-up.

Several studies have demonstrated a reduction in RNFL in early MS and patients without a history of ON.\(^{31-34}\) We have previously reported a lower RNFL thickness in patients with RRMS with and without a history of ON that was related to EDSS and MSSS.\(^{28}\) Furthermore, a progressive thinning of RNFL has been shown in longitudinal studies of patients with
MS, and a recent longitudinal study of 57 patients with MS reported progressive RNFL thinning over 5 years, regardless of a history of ON. Similarly, Graham et al. reported a reduction of the temporal RNFL in 45 patients with RRMS without ON over 3 years. In the current study, we confirm a reduction in RNFL thickness over 2 years only in patients with a history of ON. However, episodes of ON can cause a temporary increase in RNFL thickness, which may confound RNFL measurement in longitudinal studies.

Mikolajczak et al. first reported a significant reduction in corneal nerve density and showed that it was related to the severity of MS but not RNFL thickness. We confirmed and extended this finding by demonstrating significantly lower corneal nerve fiber density, corneal nerve branch density, and length. In this longitudinal study, we now demonstrate the importance of quantifying additional measures of the subbasal nerve plexus and also show that over time, corneal nerve morphology shows dynamic change with concomitant degeneration and regeneration. Although there was no change in the more proximal CNFD, there was a significant reduction in the CNFA and CNFW and a trend for reduction in the more distal CNFL, CNBD, and CTBD. Corneal nerve fiber area is a two-dimensional metric that more fully captures the full spectrum of variation and change in the corneal nerve plexus and may provide a more complete measure of axonal loss and atrophy. Indeed, we have previously shown that CNFA has the greatest utility for assessing longitudinal change, especially in response to therapeutic intervention in patients with diabetic and sarcoid neuropathy. Moreover, the reduction in CNFD and

Figure 2. Scatterplot graphs of significant correlations between corneal confocal microscopic parameters, RNFL thickness, and disease severity scores (EDSS and MSSS) in patients with RRMS.
Table 2. Corneal Confocal Microscopic Parameters, Peripapillary RNFL Thickness, and Disease Severity Scores in Patients With Multiple Sclerosis With and Without a Worsening in EDSS Score Over 2 Years

| Characteristic | Patients with Worsening EDSS (n = 15) | Patients without Worsening EDSS (n = 16) |
|----------------|---------------------------------------|------------------------------------------|
|                | Baseline | Follow-up | P Value | Baseline | Follow-up | P Value |
| CNFD (No./mm²) | 29.6 ± 7.4 | 26.0 ± 7.1 | 0.09 ⁄ | 24.4 ± 6.2 | 25.5 ± 6.2 | 0.43 a |
| CNBD (No./mm²) | 41.1 ± 19.3 | 30.3 ± 14.1 | 0.05 a | 35.3 ± 20.7 | 33.8 ± 14.7 | 0.76 a |
| CNFL (mm/mm²) | 17.1 ± 2.8 | 15.0 ± 2.4 | <0.001 a | 15.1 ± 3.3 | 15.2 ± 3.2 | 0.86 a |
| CTBD (No./mm²) | 61.7 ± 25.4 | 45.1 ± 18.6 | 0.01 a | 50.9 ± 25.6 | 51.4 ± 21.4 | 0.93 a |
| CNFA (μm/mm²) | 7360.0 ± 1141.9 | 6153.3 ± 1860.1 | 0.02 a | 6743.7 ± 1908.6 | 5887.5 ± 1600.4 | 0.08 a |
| CNFW (μm)     | 22.2 ± 1.6 | 21.2 ± 0.9 | 0.04 a | 21.9 ± 1.3 | 21.2 ± 1.7 | 0.07 a |
| DC density (No./mm²) | 20.3 (6.7–39.3) | 52.7 (4.7–111.0) | 0.04 b | 28.4 (4.9–35.5) | 14.5 (4.8–45.5) | 0.68 b |
| RNFL (μm)    | 85.4 ± 12.2 | 83.9 ± 12.4 | 0.02 a | 82.8 ± 15.5 | 81.9 ± 16.0 | 0.09 a |
| EDSS         | 3.0 (2.0–3.5) | 3.5 (2.5–4.0) | <0.001 b | 3.0 (2.0–3.9) | 2.8 (2.0–3.8) | 0.10 b |
| MSSS         | 4.4 ± 1.9 | 4.8 ± 1.4 | 0.08 a | 4.7 ± 1.8 | 3.9 ± 1.6 | <0.001 a |

Data are expressed as mean ± SD for CNFD, CNBD, CNFL, CTBD, CNFA, CNFW, RNFL and MSSS, and median (interquartile range) for DC density and EDSS. The bold P values represent statistically significant differences.

aPaired samples t-test.
bWilcoxon signed rank test.

Figure 3. Representative corneal confocal microscopic images of the subbasal nerve plexus in a patient with multiple sclerosis at baseline (A) and after 2 years (B) showing reduced nerve fibers and increased dendritic cells. This patient had a relapse and an increase of 1.0 point in EDSS score during follow-up.

CNFL, but not RNFL, correlated with worsening neurologic disability in patients with RRMS. This suggests that CCM may have advantage over OCT to assess the impact of axonal degeneration in relation to clinical disability in patients with MS.

In the current study, although we showed no significant change in DC density over time, an increase in DC density was associated with corneal nerve loss (CNFD reduction) and increasing neurologic disability (worsening EDSS). DCs migrate to the central cornea in immune-mediated and inflammatory conditions,14,15,25,26 and we have previously demonstrated an increase in DC density and decrease in the corneal subbasal nerve plexus in patients with MS,28 chronic inflammatory demyelinating polyneuropathy,25 Fabry disease,24 and Behçet disease.27 A study has also reported that DCs may directly mediate corneal nerve fiber damage in a murine model of diabetic neuropathy.39

Different treatments may alter the trajectory of immune-mediated neurodegeneration and disability in MS.40 Although we found no difference in the change in EDSS, MSSS, corneal nerves, and peripapillary RNFL thickness, there was a greater increase in DC
density in patients receiving fingolimod compared with interferon-β.

A limitation of this study is the small sample size and relatively short duration of follow-up. Nevertheless, our study demonstrates a progressive loss of corneal nerves and reduction in peripapillary RNFL thickness over 2 years in patients with RRMS. Furthermore, we show that the reduction in corneal nerves as opposed to RNFL is associated with worsening neurologic disability in RRMS. Further longitudinal studies with a larger sample size and longer follow-up period are required to define the role of CCM as an imaging biomarker of neuronal degeneration and immune activation in patients with MS.

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