Dynamics of Retinal Injury after Acute Optic Neuritis

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Objective: We set out to assess the dynamics of retinal injury after acute optic neuritis (ON) and their association with clinical visual outcomes.

Methods: Thirty-one consecutive patients with acute ON were prospectively analyzed over a 6-month follow-up period. Each month, we used optical coherence tomography (OCT) to assess the thickness of peripapillary retinal nerve fiber layer (pRNFL) and segmented macular layers, as well as high-contrast visual acuity, low-contrast visual acuity (LCVA), color visual acuity (CVA), and visual fields (VF).

Results: In this prospective study, we found that 6 months after clinical onset, ON eyes suffered a reduction in pRNFL (-45.3 μm) and macular thickness (-17.3 μm). Macular atrophy was due to the decrease of macular RNFL thickness (-7.8 μm) and that of the ganglion cell layer and inner plexiform layer (GCIP, -11.3 μm), whereas the thickness of the outer retinal layers increased slightly. The macular RNFL and GCIP thickness decreased in parallel, yet it always occurred more rapidly and more severely for the GCIP. The change in the GCIP thickness in the first month predicted the visual impairment by month 6; a decrease $\geq$ of 4.5 μm predicted poor LCVA (sensitivity of 93% and specificity of 88%), and a decrease of ≥ 7 μm predicted poor VF and CVA (sensitivity of 78% and 100% and specificity of 63% and 66%, respectively).

Interpretation: Retinal axonal and neuronal damage develops quickly after ON onset. Assessment of ganglion cell layer thickness by OCT after ON onset can be used as an imaging marker of persistent visual disability.

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Axonal damage is a critical process in several brain diseases and is among the main contributors to permanent neurological disability. As such, understanding the dynamics and processes involved in axonal injury is critical for unraveling the pathogenesis of brain disorders, and also for developing biomarkers and new therapies for neurological conditions. In the case of inflammatory diseases such as multiple sclerosis (MS), axonal damage is also the main cause of persistent disability.[1] In optic neuritis (ON), acute inflammation damages axons at the retrobulbar level, and this injury may extend retrogradely to the body of the retinal ganglion cells (RGC). There is evidence of this retrograde axonal degeneration of RGC in MS patients who suffered ON, such as residual optic nerve head pallor and thinning of the peripapillary retinal nerve fiber layer (pRNFL) in pathological[2] and imaging[3] studies of the retina, and it has also been detected in animal models.[4] Optical coherence tomography (OCT) studies showed that pRNFL atrophy of patients suffering ON developed within 6 months, with an average reduction of 10 to 40 μm, and that this remained stable after 6 months.[5,6] However, the presence of optic disk swelling in the acute phase of ON prevents accurate quantification of pRNFL atrophy. For this reason, quantification of macular RGC layer thickness may be a more specific marker of neuronal damage than pRNFL thickness.

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Studies in animal models of ON[4] and postmortem histological studies of MS patients' eyes[2] offer evidence that RGC loss is a main feature of the irreversible retinal injury associated with ON. Previous studies have observed thinning of the ganglion cell layer and inner plexiform layer complex (GCIP) after ON by OCT,[3] and postmortem MS eyes also reveal significant RGC loss, as well as loss of bipolar neurons.[2] This damage of GCIP has been reported as an early finding after axonal injury in the anterior visual pathway, paralleling RNFL atrophy with swelling of the external retinal layers.[3,7] These observations raise the question of whether damage of the RGC can be transmitted to deeper retinal layers by trans-synaptic degeneration, as observed previously in lesions occurring in the posterior visual pathway.[8]

OCT imaging of the retina provides an important opportunity to study in detail neuroaxonal damage after demyelinating injury. It has the potential to improve our understanding of the dynamics of neuroaxonal injury in MS and offers the opportunity to develop imaging markers that will be of use for clinical decision making in patients after optic neuritis.[9] Thus, the aim of this study was to analyze the dynamics of the injury of retinal layers after axonal damage in the optic nerve triggered by acute ON. This analysis should provide insights about the events that occur after acute axonal damage in inflammatory diseases and other central nervous system (CNS) conditions. Moreover, we also attempted to identify predictors of permanent visual disability based on OCT analysis that could be used in clinical practice and clinical trials.

Subjects and Methods

Study Population

From January 2011 to July 2013, we recruited consecutive patients with acute ON at the Hospital Clinic of Barcelona. ON was defined based on the criteria from the Optic Neuritis Treatment Trial (ONTT).[10] We recruited subjects between the ages of 18 and 55 years with demyelinating ON, including idiopathic ON, clinically isolated syndrome, or previously diagnosed MS,[11] but not neuromyelitis optica (excluded by the detection of anti–aquaporin-4 antibodies). Patients were not included if they had other causes of vision loss not attributable to ON as described before.[8] We excluded all subjects with prior ON in the currently affected eye as reported previously.[12] In the fellow (contralateral) eyes, previous ON was defined as a baseline pRNFL thickness < 78 μm (median of pRNFL thickness at the baseline visit of our MS cohort optic neuritis eyes[8]). Two fellow eyes were classified as eyes with previous ON, 1 based on clinical history and the other on the cutoff value of 78 μm. Patients were recruited by their physician after providing their written informed consent, and the study was approved by the ethics committee of the Hospital Clinic of Barcelona.

Clinical Evaluation

All subjects underwent baseline visual testing and OCT evaluation prior to initiation of steroid treatment. Ophthalmic evaluation was performed every 2 months after the baseline visit up to month 6 (4 assessments in total), measuring the best-corrected high-contrast visual acuity (HCVA) with Early Treatment Diabetic Retinopathy Study (ETDRS) charts, low-contrast visual acuity (LCVA) with Sloan 2.5% and 1.25% charts, and color vision acuity (CVA) using the Hardy–Rand–Rittler (HRR) plates as described elsewhere.[12,13] The scoring of HCVA and LCVA was based on the number of letters correctly identified up to 70, and for CVA, we counted the number of HRR symbols correctly identified up to 36. We classified the vision outcome of ON eyes as good or poor based on the results after 6 months. Recovery was defined as poor as follows: HCVA < 53 of 70 letters (70 letters is 20/20),[14] mean standard deviation (MSD) ≤ -3.00 dB,[14] CVA < 35 symbols,[15] and LCVA < 7 letters.[16]

Visual field (VF) analysis was performed every 2 months from baseline to month 6 (4 assessments in total) using the Humphrey perimetry 30-2 full threshold SITA algorithm (Zeiss Meditech, Dublin, CA), recording the MSD at each visit. We performed brain and orbit magnetic resonance imaging at baseline, and in month 3 and 6, using a 3T scanner (Tim Trio; Siemens Medical Solutions, Erlangen, Germany), including 3-dimensional structural T1-weighted magnetization-prepared rapid gradient echo, fluid-attenuated inversion recovery, and T1 spin echo postgadolinium sequences, as described previously.[8]

Retinal Image Acquisition and Analysis

We obtained spectral-domain OCT (Spectralis; Heidelberg Engineering, Heidelberg, Germany) for each eye every month from baseline visit to month 6 (7 evaluations per eye). All subjects underwent the same acquisition protocol, including a peripapillary scan (3.5mm diameter circle scans with automatic real time [ART] of 90) and a macular raster scan centered on the fovea ($20^\circ \times 20^\circ$ matrix, 25 horizontal of sections with 240 μm of separation and a mean ART of 45), and fulfilled OSCAR-IB criteria.[17] Retinal layer segmentation was performed automatically using the Viewer Module beta v.6.0.0.2. Accordingly, we quantified the thickness of the following layers: (1) pRNFL; (2) macular RNFL (mRNFL); (3) macular GCIP; (4) macular inner nuclear layer (INL); (5) macular outer plexiform layer and outer nuclear layer complex (OPL+ONL); and (6) macular external limiting membrane, inner segments (IS) and outer segments (OS) of photoreceptors, retinal pigment epithelium (RPE), and Bruch membrane (BM) (IS/OS+RPE+BM). We analyzed 8 of the macular ETDRS sectors, excepting the fovea (central 1mm ring), given that this area lacks RGC. All the images from automated segmentation were carefully reviewed by an expert (I.G.) in a blind fashion. Moreover, we performed a separate intersession test–retest variability analysis of OCT segmentation in 14 healthy eyes and in 12 eyes from MS patients.[8] We found good to very good agreement (intraclass correlation coefficient = 0.75–0.9) both in healthy controls

and in MS patients, with GCIP being the layer with the lowest maximum intersession variability (2.65 μm).

To assess the dose–effect relation between optic nerve damage and macular changes, we used the tertiles of pRNFL variation to classify the severity of ON and divided the cohort into 3 groups (mild, moderate, or severe ON). To avoid the effect of pRNFL swelling, we used the tertiles of the difference between pRNFL thickness in the affected eye at month 6 and pRNFL thickness in the contralateral eye at baseline. In the eyes contralateral to the currently affected eye that were classified as having previous ON (n = 2), as well as in cases with bilateral ON (n = 1), the severity of ON was determined using the tertiles of the cohort for the difference between pRNFL thickness in the affected eye at month 6 and pRNFL thickness in the affected eye at baseline.

Statistical Analysis

We used an independent-samples *t* test to compare the means of the OCT measures in eyes with and without ON. We used a logistic regression to test a set of 6 OCT variables (models) obtained at the onset of the study and after 1 month, to predict the following clinical outcomes by month 6: HCVA, LCVA, CVA, and VF. The OCT variables were: (1) pRNFL thickness at baseline in the ON eye; (2) the difference of pRNFL thickness between the ON eye and the fellow eye at baseline; (3) GCIP thickness at baseline in the ON eye; (4) the difference of GCIP thickness between the ON eye and the fellow eye at baseline; (5) the difference in GCIP thickness of the ON eye between baseline and month 1; and (6) GCIP thickness in the ON eye after 1 month. Regression models were adjusted for age, sex, and disease duration, and in models accounting for OCT changes after 1 month, we also adjusted for the use of steroid treatment. We also assessed the effect of the use of disease-modifying therapies (DMT) in this statistical model. We excluded cases with a prior history of ON in the currently affected eye (n = 4) to increase the sensitivity of our analyses. To avoid underestimation of the swelling of retinal layers in the affected eyes, for the intereye asymmetry analyses we also excluded cases with prior history of ON in the contralateral eye (n = 2). All probability values were 2-tailed and they were considered to be significant at $p \leq 0.05$. Statistical analyses were performed using SPSS software v20.0 (IBM, Armonk, NY).

Results

Clinical Characteristics and Overall Retinal Changes of the Cohort

We recruited 31 consecutive patients, and 4 were excluded because of the presence of previous ON in the affected eye, leading to 27 patients for the analysis (Table 1), all except 1 case involving unilateral ON (28 ON eyes). Of 27 cases, 18 (66.7%) had ON in the context of MS, whereas 9 (33.3%) represented idiopathic ON. Seventeen patients were treated with intravenous steroids for 3 days. Four patients were already taking DMT prior to study inclusion (interferon-β); 3 continued with the same treatment during study follow-up, and 1 changed to natalizumab. Seven of the 14 subjects for whom this ON was their first relapse of MS initiated DMT during study follow-up (5 with interferon-β, 1 with glatiramer acetate; 1 participated in a clinical trial with diazoxide [NCT01428726]). All subjects completed every visit of the study except for 3 participants. Lastly, 1 patient with a previous diagnosis of relapsing–remitting MS suffered a relapse of ON in the same eye 4 months after the onset of the current episode. From this subject, we only included the data from the first 3 months of the study in the overall analysis.

TABLE 1. Clinical Characteristics of the Cohort

Characteristic	Value
No.[a]	27
Age, yr, mean ± SD	37 ± 8
Female, No. (%)	22 (81)
ON presentation	26 unilateral; 1 bilateral
Time from clinical onset of ON to baseline visit, days, mean (SD)	
All subjects, n = 27	12.4 (7.8)
Time ≤ 15 days, n = 20	8.7 (4.2)
Time > 15 days, n = 7	22.7 (6.1)
Cause of ON, No. (%)	
Idiopathic ON	9 (33.3)
Previously diagnosed of MS	4 (14.8)
CIS, first relapse of MS	14 (51.8)
Treatment with i.v. steroids, No. (%)	17 (63)
Time from clinical onset to i.v. steroids initiation, days, mean ± SD	12.3 ± 7.8
Patients on DMT during study follow-up, No. (%)	11 (40.7)
Initiated prior to baseline visit	4 (14.8)
Initiated in the course of follow-up	7 (25.9)

[a]31 recruited, 4 excluded because of previous ON.
CIS = clinically isolated syndrome; DMT = disease-modifying therapies; i.v. = intravenous; MS = multiple sclerosis; ON = optic neuritis; SD = standard deviation.

At baseline, the eyes with acute ON displayed significantly higher thickness of pRNFL (+24.4 μm) compared to the fellow eyes, whereas the GCIP thickness was slightly lower (−0.4 μm) and OPL+ONL was slightly

thicker (+0.4 μm) in ON eyes (Table 2, Figs 1 and 2). In contrast to pRNFL, the average macular thickness did not differ significantly between eyes. Regarding visual function, HCVA, LCVA, CVA, and VF were markedly lower in ON eyes than in their fellow eyes (Table 3).

By the end of the follow-up (month 6), the pRNFL thickness in the eyes with acute ON was reduced by 45.3 μm and the macular thickness by 17.3 μm, whereas both measures remained unchanged in the fellow eyes. Reduction of the macular thickness at month 6 was mainly due to the decrease of mRNFL thickness (−7.8 μm, 21.4%) and GCIP thickness (−11.3 μm, 16.2%), whereas the thickness of outer retinal layers (OPL+ONL and IS/OS+RPE+BM) increased slightly (see Table 2).

When considering the clinical outcome of ON eyes at month 6 (see Table 3), HCVA recovered well in 20 of 25 patients (80%). Also, 72% of patients achieved a good recovery of color vision and 64% of patients experienced good VF recovery. However, only 45.5% (10 of 22) of the patients achieved good 2.5% LCVA recovery and only 36.4% of patients achieved good 1.25% LCVA.

Dynamics of Retinal Atrophy in Acute ON

Most of the reduction in pRNFL thickness occurred in the first 2 months, whereas macular thickness decreased in a more progressive manner over the 6-month period (see Table 2, Fig 1). This tendency was also seen when we analyzed the asymmetry between ON eyes and fellow eyes. Macular thickness was slightly higher in the affected eye compared to the fellow eye at baseline, but this difference reverted and became negative after 1 month, decreasing progressively thereafter, with 57.2% of the total reduction of macular thickness happening in the first 2 months and 73.4% in the first 3 months.

To better appreciate the dose–effect relationship between optic nerve damage and macular changes, we analyzed the ON eyes using the tertiles of the difference between pRNFL thickness of the affected eye by month 6 and pRNFL thickness of the fellow eye at baseline visit: mild ON (pRNFL difference ≥ -11 μm, n = 9 eyes), moderate ON (pRNFL difference between −11 and −31 μm, n = 10 eyes), and severe ON (pRNFL difference ≤ -31 μm, n = 9 eyes). In cases with bilateral ON (n = 1) or in cases with fellow eyes with previous ON (n = 2), the severity classification was based on the tertiles of pRNFL change after 6 months (−11 μm and −46 μm). All 3 cases were classified as severe ON (pRNFL change ≥ -46 μm). This analysis revealed that the patterns of pRNFL and macular atrophy in the first 2 months were more evident with increasing severity (see Fig 1).

Regarding the changes in the distinct macular layers, we observed a different response between the inner and outer macular layers that was also clearer with higher severity (see Table 2, Fig 2). Whereas the mRNFL and GCIP thickness decreased each month, which was especially marked in the first 2 months, the thickness of outer layers increased in the first 2 months, and it recovered from month 3 to month 6. Thus, in the first 2 months the severe atrophy of the mRNFL and GCIP was partially compensated by a swelling of the outer retinal layers, explaining why only 57% of the final overall macular thickness reduction happened in this period, although from month 3 to month 6 this compensatory swelling disappeared. We observed a parallel reduction in mRNFL and GCIP thickness, which was always faster and more severe for the GCIP (69% [7.8 μm] of the total average GCIP thinning happened during the first month, compared the 50% [3.9 μm] observed for mRNFL). Regarding the outer layers, the thickness of OPL+ONL and IS/OS+RPE+BM complexes increased until month 2. OPL+ONL increased 3.5 μm (3.8%) in the first month and IS/OS+RPE+BM 2.5 μm (3.2%) in the first 2 months, and by month 6 the thicknesses of both complexes remained slightly above the baseline values. However, the thickness of the INL remained practically unchanged. The swelling of the outer layers was not influenced by the therapeutic use of corticosteroids. As would be appreciated, the thickening of the external retinal layers in the first 2 months was not sufficient to compensate for the GCIP thinning, and for this reason the total macular thickness was reduced (see Figs 1, 2).

To analyze the spatial spread within the retina of the axonal and neuronal damage in ON eyes, we analyzed the thickness of the macular layers for each of the 8 ETDRS retinal sectors (Fig 3). We found that macular atrophy was more severe and homogeneous in the internal sectors, surrounding the fovea, than in the external sectors of the macula. However, in the external sectors, those corresponding to the papillomacular bundle were most strongly affected. The severe thinning of the internal sectors mainly reflected the GCIP thinning, whereas the atrophy of sectors corresponding to the papillomacular bundle was more related to mRNFL thinning. We can observe in Figure 3 that the swelling of the external layers was more diffuse, affecting both the outer and inner sectors, but it tended to affect the sectors covering the papillomacular bundle more intensely. Finally, we did not identify a specific pattern of changes in INL; although it showed a tendency to thicken, the variations detected were minor and without a well-defined spatial distribution. In addition, it should be noted that we did not detect any cases with microcystic macular edema in

TABLE 2. Thickness of Retinal Layers at Baseline and during the 6-Month Follow-up

Measure	pRNFL	Macular Thickness	mRNFL	GCIP	INL	OPL+ONL	IS/OS+ RPE+BM
Average thickness at baseline, μm (SD)							
ON eyes, n = 28	125.9 (40.7)	310.9 (9.2)	36.4 (4.6)	69.7 (5.3)	34.7 (1.8)	93.4 (5.3)	76.7 (2.5)
Intereye asymmetry, n = 24	+24.4 (43.1)	+2.1 (6.7)	+0.7 (3.5)	−0.4 (5.0)	+0.4 (1.1)	+1.4 (2.1)	0.0 (1.8)
Change in average retinal layer thickness from baseline to month 6, μm (SD)							
ON eyes, n = 26	−45.3 (50.9)	−17.3 (12.0)	−7.8 (5.3)	−11.3 (8.5)	+0.4 (1.1)	+0.7 (1.4)	+0.7 (1.8)
Fellow eyes, n = 24	−0.2 (1.8)	−0.7 (2.8)	+0.4 (1.4)	0.0 (0.7)	−0 .4 (0.7)	0.0 (1.1)	−0.7 (1.8)
Monthly change of average retinal layer thickness in ON eyes, μm (SD)							
Baseline to month 1, n = 27	−23.6 (36.8)	−6.7 (6.0)	−3.9 (3.2)	−7.8 (6.0)	+0.4 (0.7)	+3.5 (2.5)	+1.1 (1.8)
Month 1 to 2, n = 27	−14.1 (13.4)	−3.2 (3.5)	−2.8 (2.5)	−1.8 (2.1)	0.0 (0.7)	0.0 (1.4)	+1.4 (1.8)
Month 2 to 3, n = 25	−3.5 (3.7)	−2.8 (2.5)	−0.4 (1.4)	−0.4 (0.7)	0.0 (0.7)	−1.1 (1.4)	−0.7 (1.4)
Month 3 to 4, n = 24	−1.5 (2.2)	−1.4 (2.8)	0.0 (1.4)	0.0 (1.1)	−0.4 (0.7)	−0.7 (1.4)	−0.4 (1.8)
Month 4 to 5, n = 23	−1.1 (2.7)	−1.4 (2.8)	−0.4 (1.1)	−0.4 (1.1)	0.0 (0.7)	−0.4 (1.4)	−0.4 (1.8)
Month 5 to 6, n = 22	−0.6 (2.0)	−0.7 (2.5)	0.0 (1.1)	0.0 (0.7)	0.0 (0.7)	−0.7 (1.1)	−0.4 (1.8)

Intereye asymmetry is the difference in the thickness of each layer in the ON eye with respect to the fellow eye at baseline. This was done considering that the fellow eye does not suffer edema, and for this reason the thickness values are used as a reference. Results are expressed as the mean (SD of the mean) for each measure.
GCIP = ganglion cell layer + inner plexiform layer; INL = macular inner nuclear layer; IS/OS+RPE+BM = inner and outer photoreceptor segments + retinal pigment epithelium + Bruch's membrane; mRNFL = macular retinal nerve fiber layer; ON = optic neuritis; OPL+ONL = outer plexiform layer + outer nuclear layer; pRNFL = peripapillary retinal nerve fiber layer; SD = standard deviation.

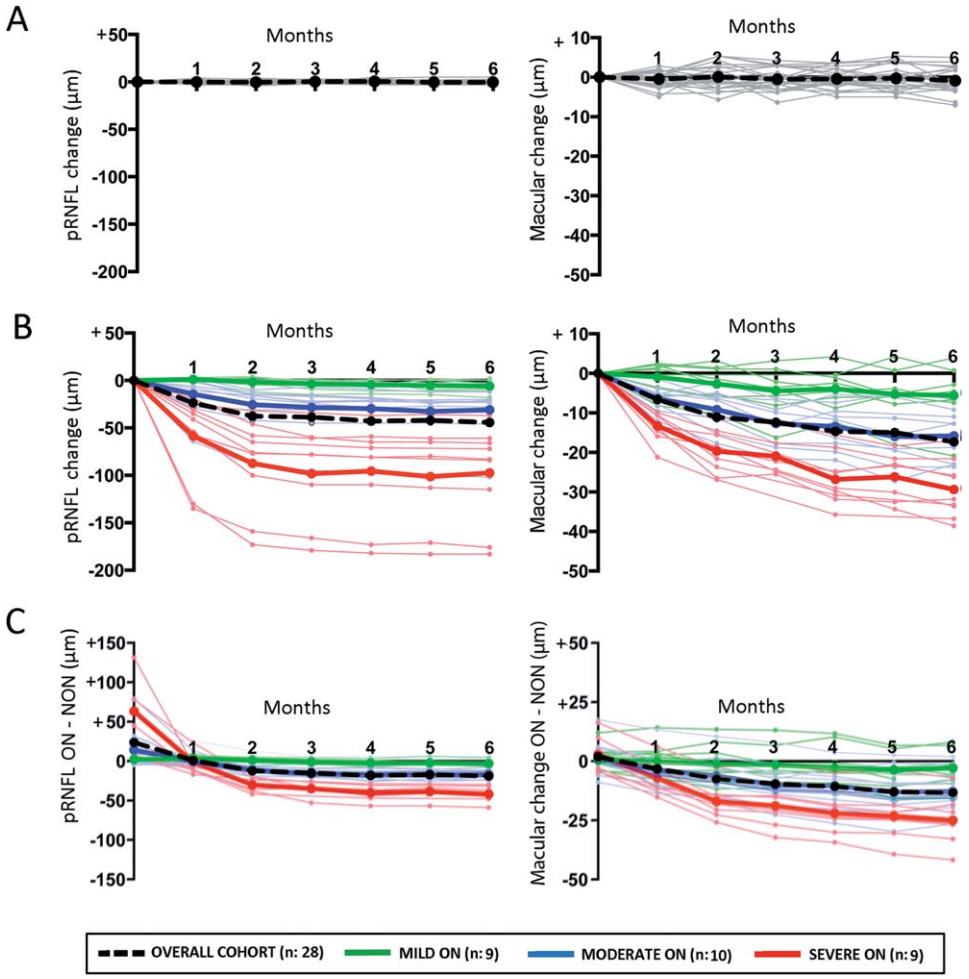

FIGURE 1: Longitudinal changes of the peripapillary retinal nerve fiber layer (pRNFL) and macular thickness after optic neuritis (ON). The results are presented as the monthly change in micrometers with respect to the baseline and for the 3 levels of ON severity: mild (green), moderate (blue), and severe (red) ON (individual cases are shown in thin lines and group means in thick lines). (A) Fellow eye: pRNFL and macular thickness in the non-ON eye (NON); (B) ON eye: pRNFL and macular thickness in the affected eye; (C) asymmetry between ON and NON eyes. The results are presented as the changes instead of the absolute numbers to keep the results in the same scale (for absolute numbers see Table 2). There was a significant decrease in the macular thickness in ON eyes, whereas the pRNFL showed an initial increase due to the edema, with a subsequent decline. Fellow eyes (NON) showed no significant changes during the follow-up. The asymmetry between ON and NON eyes reflects the change from baseline controlling for the effect of the edema (absent in the fellow eye), and for this reason the values in the first month show the presence of edema.

this cohort.[18] However, we cannot exclude the emergence of microcystic edema in later phases.

Predictors of Visual Disability

To identify clinical and imaging markers of permanent visual dysfunction after ON, we analyzed the predictive ability of visual function and OCT measurements at baseline for predicting visual outcome by the end of the study (month 6). First, we evaluated whether each test of visual function (HCVA, 2.5% LCVA, 1.25% LCVA, CVA, and VF) performed at baseline or month 2 predicted poor visual function measured with the same test at month 6. We found that any of these tests performed at baseline visit could not predict visual outcome, and they only started to be associated with poor visual recovery at month 6 when performed later than month 2 (data not shown).

Second, we analyzed the clinical usefulness of measuring the OCT changes at baseline and 1 month after onset to predict poor visual recovery by the end of the study. We evaluated 6 different OCT measures that might predict short-term (6 months) visual outcomes (HCVA, LCVA, CVA, VF) using logistic regression models. We included sex, age, disease duration, and steroid treatment as covariates, although these potential confounders were not statistically significant in any model. We found that the change in the GCIP thickness from baseline to month 1 predicted short-term visual disability

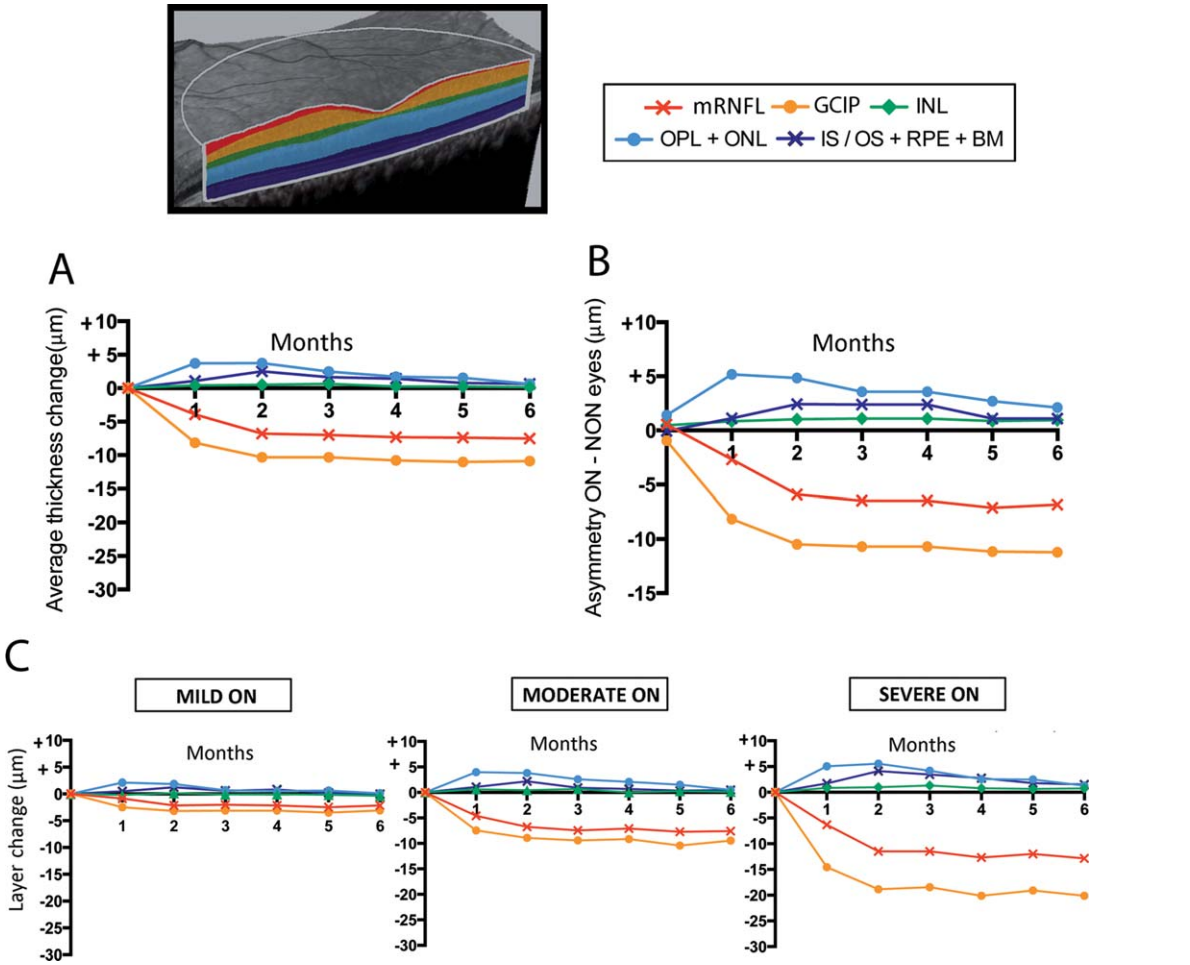

FIGURE 2: Longitudinal changes of macular layers thickness after optic neuritis (ON). The results are presented as the average monthly change (in micrometers) from the baseline of the thicknesses of macular layers in ON eyes (A and C) and as the average of the difference in the thickness of each layer in the ON eyes with respect to the fellow eyes (intereye asymmetry; B). C represents monthly change for the 3 levels of ON severity in ON eyes. GCIP = inner plexiform layer + ganglion cell layer complex; INL = inner nuclear layer; IS/OS+RPE+BM = inner and outer photoreceptor segments + retinal pigment epithelium + Bruch's membrane; mRNFL = macular retinal nerve fiber layer; NON = non-ON; OPL+ONL = outer plexiform layer + outer nuclear layer complex.

as follows: a decrease in GCIP thickness ≥ 4.5 μm predicted poor 2.5% LCVA and 1.25% LCVA recovery with a sensitivity of 92% and 93%, and a specificity of 70% and 88%, respectively. Moreover, a decrease ≥ 7 μm in GCIP thickness predicted poor VF and CVA recovery with a sensitivity of 78% and 100%, respectively, and specificity of 63% and 66%, respectively (Table 4).

We performed sample size calculations to design clinical trials aimed at preventing retina atrophy based on the change of the GCIP thickness from baseline to month 1 in the affected eye, with an alpha error of 0.05 and beta error of 0.20. Because our model indicates that a reduction ≥ 4.5 μm of the GCIP thickness implies a high probability of visual dysfunction (LCVA) at month 6, we defined our endpoint and optimal power as a decrease in the GCIP < 4.5 μm. We found that 25 patients with acute ON per group will have sufficient power to detect differences between the groups using GCIP thickness as the outcome.

Lastly, considering that 11 cases (40.7%) were undergoing DMT for MS during the course of the follow-up (4 started before study inclusion and 7 during follow-up), we evaluated whether the use of DMT influenced the visual prognosis in our statistical model. We found that the use of DMT did not influence the visual prognosis after 6 months.

Discussion

We describe here the dynamics of retinal damage due to ON and we propose the thickness of the ganglion cell layer as an imaging marker to be used as a predictor of short-term visual outcome in clinical settings. Understanding the dynamics of axonal damage after CNS injury is critical to unveil the pathogenesis of axonal

TABLE 3. Visual Function at Baseline and 6 Months after Acute ON

ON Eyes	**Baseline, n = 28**	**Change Baseline to Month 6, n = 26**
HCVA, No. of letters (SD)	34.5 (21.3)	+19.9 (19.9)
2.5% LCVA, No. of letters (SD)	2.3 (7.3)	+19.8 (13.4)
1.25% LCVA, No. of letters (SD)	0.0 (0.0)	+9.5 (8.3)
CVA, No. of figures (SD)	24.1 (9.6)	+8.5 (8.2)
VF, MSD (SD)	−9.0 (11.0)	+6.2 (10.3)
Fellow Eyes	**Baseline, n = 26**	**Change Baseline to Month 6, n = 24**
HCVA, No. of letters (SD)	58.7 (3.0)	+1.2 (2.9)
2.5% LCVA, No. of letters (SD)	31.9 (9.3)	+3.9 (7.2)
1.25% LCVA, No. of letters (SD)	19.1 (8.8)	+4.2 (7.2)
CVA, No. of figures (SD)	35.7 (0.9)	+0.1 (0.4)
VF, MSD (SD)	−1.9 (2.3)	0.1 (2.5)

Results are expressed as the number of letters or figures correctly identified by patients (SD of the mean, SD of the visual fields). CVA = color vision acuity; HCVA = high-contrast visual acuity; LCVA = low-contrast visual acuity; MSD = mean standard deviation; ON = optic neuritis; SD = standard deviation; VF = visual fields.

degeneration in neurological diseases as well as for the development of new therapies targeting this process. In our study, we observed that at the level of the optic nerve head most pRNFL atrophy manifested in the first 2 months, and although edema was present in the optic disk at clinical onset (being almost absent in the macula) it disappeared after month 1. Regarding the changes in the macula and retinal layers, we observed a sustained macular atrophy related to a specific pattern of changes in retinal layers that developed in 2 phases. The first phase occurred in the first 2 months, with atrophy of inner retinal layers (mRNFL and GCIP), accounting for damage of the RGC accompanied by a swelling of the outer layers. In the second phase, from month 3 to month 6, the rate of atrophy in the inner retinal layers was slower and paralleled the disappearance of the swelling of outer retinal layers. At the spatial level (retinal maps), macular atrophy (mainly RGC layer atrophy) was more severe and homogeneous around the fovea (which is the area with the highest RGC density), whereas atrophy of the sectors corresponding to the papillomacular bundle was more closely related to mRNFL thinning. Finally, we show that the atrophy of the GCIP in the first month after onset was a good predictor of short-term visual deficit, with a threshold of a 4.5 μm decrease in the GCIP thickness predicting low-contrast vision recovery and a decrease of 7 μm for VF and color vision.

OCT provides the opportunity to image in vivo retina damage after optic nerve (and brain) insult, and it has been proposed as a candidate biomarker for the prognosis of optic neuropathies and other brain diseases.[9] Previous studies using OCT in ON cases show that pRNFL atrophy develops within the first 3 to 6 months after ON onset and is associated with residual visual acuity deficits.[19,20] However, pRNFL swelling during the first months of ON prevented an accurate measurement of RNFL loss in this period. Studies analyzing macular atrophy in ON show that this damage happens within the first 6 months after clinical onset,[5,7,21] including GCIP atrophy, which occurs in the first 3 months.[7] Our results support the findings in animal models,[22–24] indicating that GCIP damage might occur very rapidly after optic nerve damage, even in a matter of days after the onset of symptoms, as reflected by the asymmetry observed between the affected and contralateral eyes at baseline and the marked atrophy of the GCIP that we have observed 1 month after onset. Moreover, in line with our previous findings[7] and with some studies in experimental autoimmune encephalomyelitis models, we observed that macular RNFL atrophy develops more

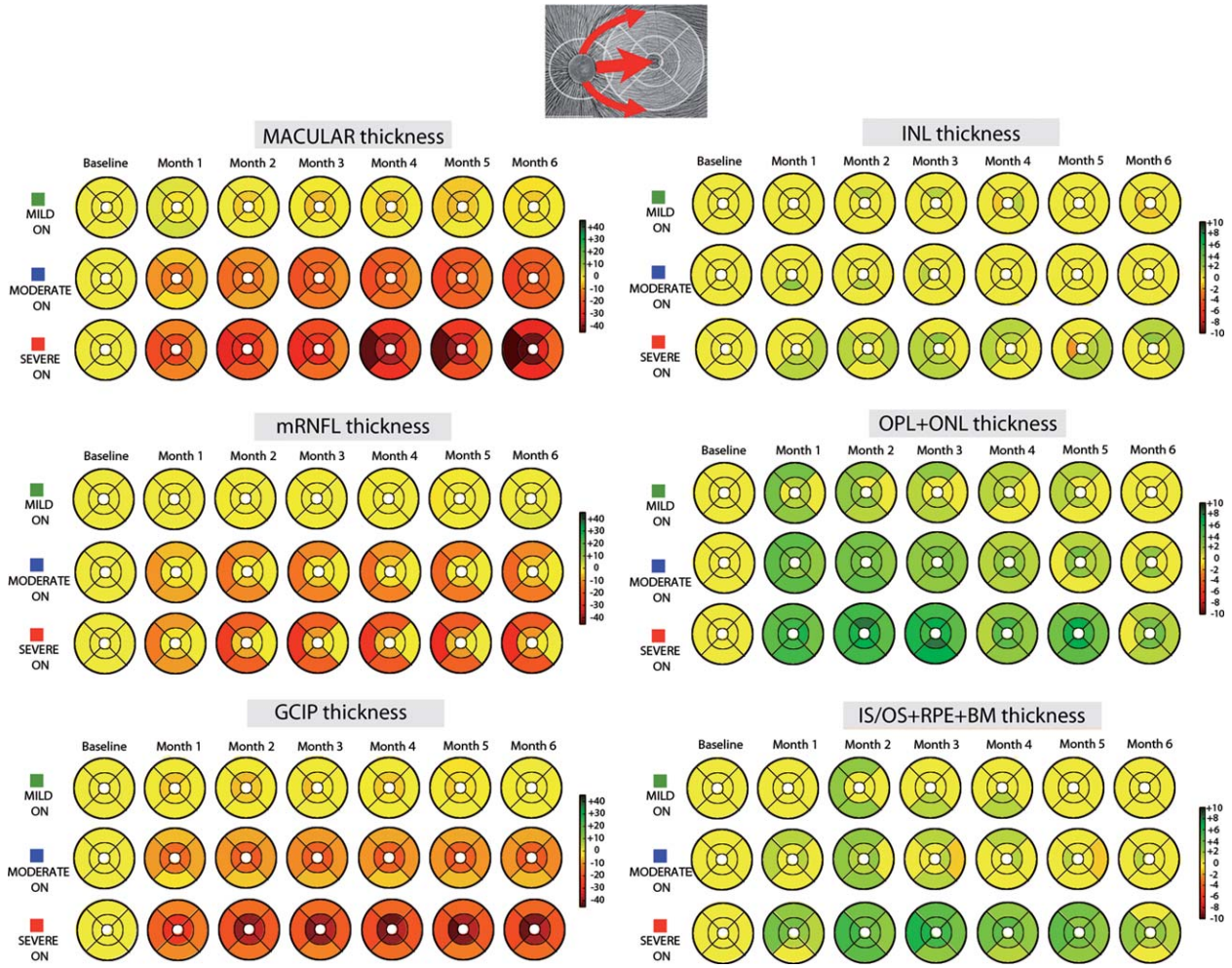

FIGURE 3: Longitudinal changes in macular sectors (spatial dynamics) of retina layer thickness after optic neuritis (ON). The macula was divided into the 8 Early Treatment Diabetic Retinopathy Study (ETDRS) sectors: nasal exterior (Ne), nasal interior, superior exterior (Se), superior interior (Si), temporal exterior, temporal interior, inferior exterior, and inferior interior. The papillomacular bundle is contained in the Ne sector, although it also enters the Se and Si sectors. The thickness change of macular layers is represented as the change in micrometers from baseline visit over time for the 8 ETDRS sectors. Changes for both right and left eyes have been superimposed on a left eye morph. The ETDRS sectors are shown independently for mild, moderate and severe ON. Increased thickness is shown in green, decreased thickness in red, and no change in yellow (see bar on the right for values). GCIP = inner plexiform layer + ganglion cell layer complex; INL = inner nuclear layer; IS/OS+RPE+BM = inner and outer photoreceptor segments + retinal pigment epithelium + Bruch's membrane; mRNFL = macular retinal nerve fiber layer; OPL+ONL = outer plexiform layer + outer nuclear layer complex.

slowly than GCIP atrophy in ON.[22] This finding supports the idea that early functional changes of the axons in the optic nerve may contribute to RGC damage (cell shrinkage or cell loss) even before structural damage of axons occurs. This implies the possible existence of 2 mechanisms of RGC degeneration that seem to be intimately and sequentially related in ON: early signals from the damaged axons initiate RGC damage (first wave of RGC loss) and then further RGC damage occurs by the dying-back process (second wave of RGC loss). Similar dynamic retinal changes were reported in an acute inflammatory lesion of the optic tract[7] and in animal models, showing death of RGC 7 to 60 days after damage to the optic nerve.[23–25] These studies showed that RGC suffer apoptotic-like degeneration within as little as 3 days after optic nerve damage. Pathological study of the retinas of patients with MS also demonstrated that RGC loss is a major feature in MS.[2] We can argue that GCIP atrophy is due to axonal loss and neuronal degeneration, either by dendrite and synapse pruning or by cell body shrinking and apoptosis. Further validation in animal models and in humans is still required to prove these concepts.

The early and transitory swelling of the outer macular layers, followed by a progressive recovery to baseline values without atrophy, suggests that damage to the RGC does not extend to the bipolar cells or photoreceptors, at least in the short term, in agreement with previous observations.[26] The damage to RGC may not extend to

TABLE 4. Diagnostic Accuracy of the Change of GCIP Thickness between Baseline and Month 1 in Predicting Visual Outcome at Month 6

OCT	Visual Outcome	OR (95% CI)	AUC	S, %	SP, %	PPV, %	NPV, %
ΔGCIP ≥ 4.5 μm	2.5% LCVA	1.30 (1.03–1.63)[a]	0.829	92	70	73	86
	1.25% LCVA	1.49 (1.08–2.06)[a]	0.893	93	88	87	86
ΔGCIP ≥ 7 μm	Visual field, MSD	1.19 (1.00–1.42)[a]	0.743	78	63	50	81
	CVA	1.64 (1.10–2.44)[a]	0.940	100	66	50	100

Poor visual outcome was defined as an intereye asymmetry > 7 letters (maximum = 70) for LCVA, MSD ≤ -3.00 dB for visual fields, and Hardy–Rand–Rittler symbols correctly identified < 35 for color vision.
[a] $p < 0.05$.
AUC = area under the curve; CI = confidence interval; CVA = color vision acuity; LCVA = low-contrast visual acuity; MSD = mean standard deviation; NPV = negative predictive value; OCT = optical coherence tomography; OR = odds ratio; PPV = positive predictive value; S = sensitivity; SP = specificity; ΔGCIP = change in the ganglion cell layer + inner plexiform layer complex thickness.

bipolar cells by trans-synaptic degeneration due to the rich connectivity of these cells, with an extensive synaptic tree with amacrine cells and horizontal cells. Outer retinal layers may respond to RGC insult, a response that may be coordinated by microglia or Müller glial cells. Activated microglia accumulate in the retina of animal models with active RGC apoptosis[27] and in the retina of MS patients as shown by pathology.[2] A second explanation may be the presence of a transitory increase in intercellular water (edema) in outer retinal layers. Müller cells, the most abundant glia in the retina, are thought to be in charge of the maintenance of the hydroelectrolytic homeostasis of the retina. The dysfunction of these cells resulting in uncontrolled water clearance has been linked to the presence of retinal edema in pathological conditions.[28] Recent studies have reported swelling of the INL and outer retinal layers in eyes with a history of optic neuropathy.[29] The noncystoid fluid accumulation attributable to Müller cell dysfunction may spread to deeper retinal layers, increasing their thickness. However, it is important to remember that changes in OCT layer thickness do not have pathological specificity (ie, layer thinning equal to cell loss) and that changes may not even occur directly in the layer under study, but could be influenced by changes in adjacent layers. Given that segmentation relies on assessing an idealized version of a biological interface, pathological changes in the retina that influence the appearance of the OCT image and the resulting performance of the segmentation method may impact estimated layer thickness.

Our study has identified an imaging marker based in OCT that will be helpful at the clinical level. Predicting permanent visual dysfunction at the beginning of the disease is important for defining the prognosis and best therapeutic approach. Visual function at presentation or the first months after onset is not a good predictor of permanent visual disability, as revealed by the ONTT as well as by our study. Here we show that by measuring the atrophy of the ganglion cell layer using OCT (a test that takes around 10–15 minutes and is well tolerated), we can predict short-term visual function with high accuracy. Although clinical visual testing may seem to be easier to perform, standardized visual function tests may require 1 hour per patient, and they have to be performed in a room with specific conditions and specialized equipment. Moreover, several studies support the strong association of retinal OCT measures with visual function. It has been demonstrated that pRNFL thickness of 75 μm at month 6 was predictive of poor residual VFs.[5,6] The high- and low-contrast visual acuities at ON onset predict GCIP thickness.[7] This latter finding is supported by the strong correlation between GCIP thickness and visual function[30] and by GCIP atrophy being more severe in MS patients with a more aggressive course.[29] Thus, our findings and previous evidence strongly support the clinical usefulness of measuring GCIP atrophy as an early predictor of permanent visual disability in ON.

Our study may have several limitations. First, we assume that changes in retinal layer thickness are due to axonal loss and neuronal degeneration. Although there is strong support for this assumption from animal studies, new technologies will be required to visualize individual cells to confirm this issue. Similarly, the swelling of the outer retinal layers may be secondary to intercellular edema or a compensatory role of Müller cells, although this has yet to be formally proven. In our experience, the analysis performed with the newest automatic segmentation algorithms is very accurate and consistent, especially for inner retinal layers, although at the price of merging some layers with poorer contrast in the outer retina. In addition, we

performed monthly OCT assessments, and weekly assessments in the first month may provide additional insight into the timing of the early changes in the retina. Although the sample size was limited to 27 cases, the changes were consistent and large enough to be significant and predictive of clinical outcome. However, in clinical settings with less careful inclusion and exclusion criteria, or lower standards for image analysis, the validity of the prediction of visual disability must first be established.

In summary, we provide evidence that after acute axonal damage in the optic nerve, the RGC degenerate rapidly (1–2 months), leading to visual defects. The outer layers of the retina respond transiently to RGC damage by swelling, without being influenced by corticosteroid therapy. Finally, the atrophy of the RGC layer is predictive of vision loss, and the thickness of this layer represents a promising imaging marker to predict permanent clinical disability, possibly serving as a surrogate marker of neuroprotection for clinical trials.

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Authorship

I.G., E.H.M.-L., B.S.-D., and P.V. designed the study. E.F.-P., S.O.-P., R.T.-T., and I.Z. collected the data. A.S., B.S.-D., and P.V. supervised the study. E.H.M.-L. did the statistical analysis. I.G., M.A., and S.L. performed the image analysis. I.G., E.H.M.-L., B.S.-D., and P.V. interpreted the results of the analysis, with subsequent substantial contributions from all the coauthors. I.G. and P.V. drafted the manuscript, to which all the authors contributed revisions. All authors approved the final version.

Potential Conflicts of Interest

S.O.-P.: speaking fees, Novartis. S.L.: grants/grants pending, Red Española de Esclerosis Multiple; speaking fees, Teva, Merck, Biogen. A.S.: consultancy, Bayer Schering, Merck Serono, Biogen Idec, Sanofi Aventis, Teva, Novartis. B.S.-D.: speaking fees, Novartis. P.V.: consultancy, Heidelberg Engineering, Novartis, Roche; patents, trophic factor agonist for the treatment of neurological diseases (WO2012/028959); stock/stock options, Bionure; travel expenses, Novartis.

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