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Retinal inner nuclear layer volume reflects inflammatory disease activity in multiple sclerosis; a longitudinal OCT study

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Abstract

Background: The association of peripapillary retinal nerve fibre layer (pRNFL) and ganglion cell-inner plexiform layer (GCIPL) thickness with neurodegeneration in multiple sclerosis (MS) is well established. The relationship of the adjoining inner nuclear layer (INL) with inflammatory disease activity is less well understood.

Objective: The objective of this paper is to investigate the relationship of INL volume changes with inflammatory disease activity in MS.

Methods In this longitudinal, multi-centre study, optical coherence tomography (OCT) and clinical data (disability status, relapses and MS optic neuritis (MSON)) were collected in 785 patients with MS (68.3% female) and 92 healthy controls (63.4% female) from 11 MS centres between 2010 and 2017 and pooled retrospectively. Data on pRNFL, GCIPL and INL were obtained at each centre.

Results: There was a significant increase in INL volume in eyes with new MSON during the study ($N = 61/1562$, $\beta = 0.01\,mm^3$, $p < .001$). Clinical relapses (other than MSON) were significantly associated with increased INL volume ($\beta = 0.005$, $p = .025$). INL volume was independent of disease progression ($\beta = 0.002\,mm^3$, $p = .474$).

Conclusion: Our data demonstrate that an increase in INL volume is associated with MSON and the occurrence of clinical relapses. Therefore, INL volume changes may be useful as an outcome marker for inflammatory disease activity in MSON and MS treatment trials.

Keywords: Inflammation, inner nuclear layer, multiple sclerosis, optical coherence tomography

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Introduction

Thinning of the inner retinal layers, as observed with the use of optical coherence tomography (OCT), is a common finding in multiple sclerosis (MS) patients.[1] Retinal OCT has been suggested as a structural imaging biomarker for neuroaxonal degeneration, as reduced thickness of both the peripapillary retinal nerve fibre layer (pRNFL, consisting of axons) and the combined thickness of the ganglion cell layer and inner plexiform layer (GCIPL, consisting of mainly ganglion cells) have shown to be associated with grey and white matter atrophy in patients with MS.[2–6] Although the association of pRNFL and GCIPL thickness with neurodegeneration in MS is well established, a more complex situation is observed for the adjoining inner nuclear layer

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(INL). A histological study demonstrated that the INL, representing a neuronal network of bipolar, amacrine and horizontal cells, shows signs of atrophy but the presence of inflammatory cells was also described.[7] Nevertheless, the INL seems not to be susceptible to retrograde degeneration caused by MS-related optic neuritis (MSON), as it does not show the extensive neuro-axonal injury in eyes with MSON as observed in the pRNFL and GCIPL.[1,8] Rather than reflecting neurodegeneration like the innermost pRNFL and GCIPL, the INL may rather be a biomarker for inflammatory processes. In 2012, Gelfand et al. first described the presence of microcystic macular oedema (MMO) in the INL and the relationship with disability.[9] Furthermore, a retrospective study by Saidha and colleagues reported that increased thickness of the combined INL and outer plexiform layer (OPL) was associated with disease activity in MS.[10] More recently, Knier et al. reported that successful use of disease-modifying treatment (DMT) is associated with sustained reduction of INL volume,[11] suggesting that the INL could serve as a biomarker to monitor central nervous system inflammation. Therefore, the aim of this study was to investigate the relationship of INL volume changes over time with local and global inflammatory disease activity in a large cohort of patients with MS.

Methods

Study design and participants

We used longitudinal data from the International Multiple Sclerosis Visual System Consortium (IMSVISUAL) database (www.imsvisual.org). Patients were recruited from 11 centres in the Netherlands (Amsterdam $N = 165$), Germany (Berlin $N = 81$, Düsseldorf $N = 15$, Munich (Universität München) $N = 11$, Munich (Technische Universität München) $N = 169$), Kuwait (Kuwait-City $N = 98$), Spain (Barcelona CEMCAT $N = 39$, Barcelona IDIBAPS $N = 69$), Italy (Milan $N = 56$), the United States (New York City $N = 10$) and France (Lille $N = 72$). Healthy control individuals (HCs) were recruited from three centres (Amsterdam $N = 41$, Munich $N = 17$ and Berlin $N = 34$). All patients and HCs participated in local observational studies and provided written informed consent for participation in their respective studies. Data were pooled retrospectively. All data from reported and ongoing cohort studies at MS centres were stored in the IMSVISUAL repository. The raw dataset is available from IMSVISUAL on request.

Data were collected between 2010 and 2017. MS patients were included if they were between ages 16 and 80 years, and had a diagnosis of clinically isolated syndrome or MS (including relapsing–remitting (RR), secondary progressive (SP) and primary progressive (PP) subtypes) according to the revised 2010 McDonald Criteria.[12] HCs were included if they were between ages 18 and 80 years and had no history of any neurological disease or ophthalmologic reason for retinal pathology. Regarding the OCT assessments, individuals were included if they had at least two OCT measurements (baseline and at least one follow-up) with INL volume available for at least one eye (minimum follow-up period of six months). Patients were excluded if they had experienced symptomatic MSON within six months preceding the OCT assessment (baseline or follow-up), or if history of MSON was ambiguous or unknown. Inclusion and exclusion of individuals is shown in the flowchart in Figure 1.

OCT

Retinal OCT was performed at each centre by use of spectral-domain OCT with Spectralis (Heidelberg Engineering, Heidelberg, Germany, $N = 10$) or OCT-2000 (Topcon Corp, Itabashi, Japan, $N = 1$). Data on the INL and GCIPL volume (mm^3) in the macular area were acquired using a macular volume scan centred on the fovea, using a 6 mm ring area. Data on global pRNFL thickness (μm) were obtained using a 12-degree ring scan (corresponding to a 3.4 mm diameter) manually placed around the optic disc. At each centre, automated segmentation of OCT scans and quality control (including the assessment whether eyes had signs of MMO) were performed.[13,14] Importantly, the scanning device and protocols were kept identical for all longitudinal measurements within each centre.[15]

Clinical and ophthalmological outcome measures

Demographic data included data on sex, age at baseline and disease duration (from disease onset). Clinical data were collected longitudinally and included MS subtype, occurrence of relapses between visits, Expanded Disability Status Scale (EDSS) score, history of previous MSON and occurrence of new episodes of MSON between visits, presence of MMO and use of DMT. The assessment of history of symptomatic MSON (based on medical history, according to a standard protocol),[16] EDSS score and data on clinical relapses were provided by the individual centres.

Importantly, given the longitudinal design of this study, we made a clear distinction between episodes

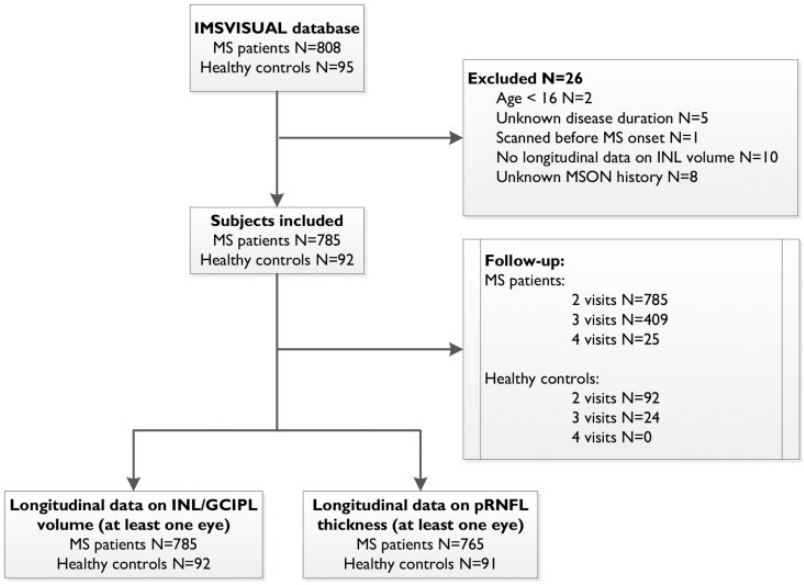

Figure 1. Flowchart of study design. Of the 903 individuals in the initial database, 785 patients and 92 healthy controls were included in this study. All participants had at least two visits, and a subset also had a third or fourth visit. Longitudinal data on inner nuclear layer (INL) and ganglion cell-inner plexiform layer (GCIPL) volume (at least two visits, minimum follow-up >6 months) was available for all included individuals and peripapillary retinal nerve fibre layer (pRNFL) for 765 patients and 91 healthy controls.
IMSVISUAL: International Multiple Sclerosis Visual System Consortium; MS: multiple sclerosis; MSON: multiple sclerosis optic neuritis.

of MSON *before* the study (referred to as 'pre-study MSON') and episodes of MSON *during* the follow-up of the study (referred to as 'MSON during follow-up').

EDSS assessment was performed by a certified examiner and in the absence of acute relapses. Disability progression was defined by an increase in EDSS score of 1.0 point in case EDSS score was less than 5.5 at baseline, or an increase of 0.5 if EDSS score was 5.5 or greater at baseline. This approach is consistent with previous IMSVISUAL collaborative projects.[17]

Statistical analyses

Annualised changes in retinal layer thickness or volume were calculated for every follow-up period. Subsequently, the annualised change scores were averaged over the complete observation period, resulting in one average annualised rate of change for every eye. All analyses were therefore performed on eye level, using generalised estimation equation (GEE) models with a correlation matrix structure that treats the eye measurements as exchangeable to adjust for intra-subject inter-eye dependency.[15] All GEE models were additionally adjusted for relevant confounders (baseline OCT value, pre-study episodes of MSON, disease duration, use of DMT) as indicated. Figures showing longitudinal changes in retinal layer thickness were produced using relative annualised change scores (i.e. baseline was set as 100%).

Regarding the associations between annualised change in retinal thickness and the occurrence of relapses or disease progression, all eyes with a history of MSON were excluded. Short-term effects (clinical event and retinal change assessed within same follow-up period) as well as long-term effects (time-lag analyses, clinical event between baseline and first follow-up visit and change in retinal layer thickness between first and second follow-up visit) were investigated. Consequently, only individuals with at least three visits were included in these analyses. All analyses were adjusted for their respective baseline retinal layer thickness.

Correlations between the different layers were calculated with standardised regression coefficients in GEE models and are therefore also adjusted for inter-eye dependency. Statistical analyses were performed using SPSS V.22.0 (IBM Corp, Armonk, NY, USA) and Stata V.14.1 (StataCorp LP, College Station, TX, USA) with a two-sided statistical significance level of .05.

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Results

Baseline

In total, 1570 eyes from 785 MS patients (68.3% female) and 184 eyes from 92 HCs (63.4% female) were included (Figure 1). MS patients had a median disease duration of 6.4 years (interquartile range (IQR) 1.9–15.0). The majority of patients (80.3%) had an RR disease course. More than half of all patients ($N = 419$, 53.4%) had never experienced a clinically confirmed MSON before baseline. Of all patients with a history of at least one confirmed episode of pre-study MSON ($N = 366$), 281 (77%) patients had unilateral MSON and 85 (23%) a history of MSON in both eyes (not necessarily simultaneously). MMO was present in 2.4% of patients (15/638) and in 1.4% (18/1275) of eyes. An overview of the baseline characteristics is shown in Table 1.

At baseline, MS patients showed significantly higher INL volume compared with HCs (difference of $0.02\,mm^3$, $p = .018$) and lower GCIPL volume and pRNFL thickness (difference of $-0.18\,mm^3$ and –4.4 µm, respectively, $p < .001$ for both comparisons). Eyes with pre-study episodes of MSON showed a higher INL volume compared with eyes without ($0.99 \pm 0.08\,mm^3$ and $0.97 \pm 0.08\,mm^3$, respectively, $p = .001$), whereas GCIPL volume and pRNFL thickness alike were lower in eyes with pre-study MSON compared with eyes without (Table 2).

Change over time in INL, GCIPL and pRNFL thickness and the effect of MSON

The median follow-up duration was 2.1 years (range, 0.5 to 5.2 years) for MS patients and 2.0 years (range, 0.6 to 4.6 years) for HCs. When all eyes of MS patients were analysed together, the INL showed

Table 1. Baseline characteristics.

	All participants $N = 785$	Healthy controls ($N = 92$)
Sex (female, *N*, %)	536 (68.3%)	59 (63.4%)
Age (y)	41.0 (±12.6)	43.4 (±11.5)
Disease duration (y, median (IQR))	6.4 (1.9–15.0)	
EDSS (median (IQR))	2.0 (1.0–3.0)	
Disease type		
CIS	45 (5.7%)	
RRMS	630 (80.3%)	
SPMS	74 (9.4%)	
PPMS	36 (4.6%)	
MSON before baseline, *N* (%)		
No previous MSON	419 (53.4%)	
MSON		
Unilateral MSON	281 (35.8%)	
Bilateral MSON	85 (10.8%)	
MMO before baseline ($N = 638$)		
MMO–	623 (97.6%)	
MMO+	15 (2.4%)	
Disease-modifying treatment at moment of baseline ($N = 743$)		
None	343 (46.2%)	
Interferon beta	172 (23.2%)	
Glatiramer acetate	72 (9.7%)	
Natalizumab	61 (8.3%)	
Fingolimod	53 (7.1%)	
Dimethyl fumarate	20 (2.7%)	
Other[a]	21 (2.8%)	

CIS: clinically isolated syndrome; EDSS: Expanded Disability Status Scale; IQR: interquartile range, reported as 25th and 75th percentile; MMO: microcystic macular oedema; MSON: multiple sclerosis–related optic neuritis; PP: primary progressive; RR: relapsing remitting; SP: secondary progressive.
[a]Rituximab, teriflunomide, azathioprine, mitoxantrone, cyclophosphamide, alemtuzumab and mycophenolate mofetil.

Table 2. Retinal layer thickness at baseline.

	All eyes $N = 1570$	MSON before BL $N = 451$	No MSON before BL $N = 1119$	HCs $N = 184$	p value[a] MSON vs HC	p value[a] No MSON vs HCs	p value[a] No MSON vs MSON
INL (mm^3)	0.98 (0.08)	0.99 (0.08)	0.97 (0.08)	0.96 (0.09)	.001	.066	.001
GCIPL (mm^3)	1.79 (0.26)	1.62 (0.25)	1.86 (0.23)	1.97 (0.19)	<.001	<.001	<.001
pRNFL (μm)	91.4 (15.8)	81.4 (17.5)	95.2 (13.2)	95.8 (9.1)	<.001	.106	<.001

BL: baseline visit; HC: healthy controls; GCIPL: ganglion cell-inner plexiform layer; INL: inner nuclear layer; MSON: multiple sclerosis–related optic neuritis; pRNFL: peripapillary retinal nerve fibre layer.
[a]Generalised estimation equation analyses, unadjusted.

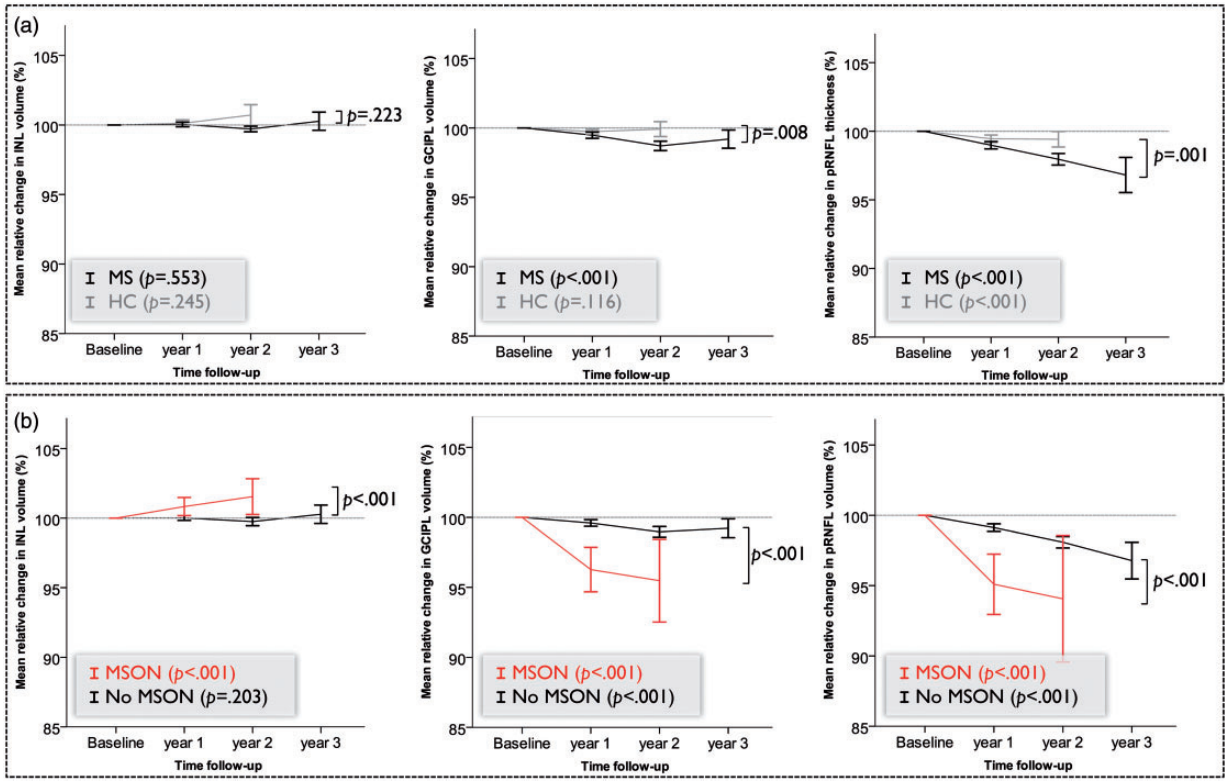

Figure 2. Relative change in retinal layer thickness with 95% confidence interval (based on generalised estimation equation model) for (a) all multiple sclerosis (MS) and healthy control (HC) eyes and (b) stratified by multiple sclerosis optic neuritis (MSON).
GCIPL: ganglion cell-inner plexiform layer; INL: inner nuclear layer; pRNFL: peripapillary retinal nerve fibre layer.

a non-significant average annualised rate of change of $-0.0003\,mm^3$ ($p = .553$, Figure 2(a)). HCs also showed no significant change (annualised rate of change $0.006\,mm^3$, $p = .245$).

Regarding the effect of MSON, there was a clear difference between pre-study MSON (i.e. before the baseline OCT assessment) and MSON occurring during the follow-up period. Pre-study MSON did not affect the rate of change in INL thickness significantly. Eyes with and without pre-study MSON showed similar rates of change of the INL ($\beta = 0.001$, $p = .219$). In contrast, any episode of MSON during the observation period strongly affected INL volume. In eyes with MSON during follow-up ($N = 61/1562$), INL volume showed a significant annualised increase of $0.01\,mm^3$ ($p < .001$). In contrast, in eyes without MSON during the observation period, no significant annualised change in INL was observed ($\beta = -0.001\,mm^3$, $p = .203$, Figure 2(b)). Exclusion of patients with a progressive disease type, or adjustments for use of DMT, disease duration or participating centre, did not change these results (data not shown).

Table 3. (a) Short- and (b) long-term effects of MSON, clinical relapses (other than MSON) and disability progression on annualised change in INL and GCIPL volume and pRNFL thickness.

(a)	β (95% CI) short term	*p* value[a]
	MSON ($N = 26$ eyes) vs no MSON ($N = 1039$ eyes)	
INL	0.01 (0.006 to 0.020)	<.001
GCIPL	–0.13 (–0.18 to –0.08)	<.001
pRNFL	–7.61(–10.8 to –4.3)	<.001
	Relapse ($N = 214$ eyes) vs no relapse ($N = 789$ eyes)	
INL	0.000 (–0.004 to 0.004)	.868
GCIPL	–0.10 (–0.18 to –0.002)	.012
pRNFL	–0.54 (–1.14 to 0.07)	.082
	Progression ($N = 223$ eyes) vs no progression ($N = 673$ eyes)	
INL	0.001 (–0.004 to 0.005)	.774
GCIPL	0.001 (–0.006 to 0.008)	.764
pRNFL	–0.13 (–0.66 to 0.41)	.646
(b)	β (95% CI) long term (time-lag model)	*p* value[a]
	MSON ($N = 11$ eyes) vs no MSON ($N = 581$ eyes)	
INL	–0.006 (–0.026 to 0.013)	.535
GCIPL	0.023 (–0.065 to 0.112)	.604
pRNFL	–1.124 (–3.78 to 1.53)	.406
	Relapse ($N = 148$ eyes) vs no relapse ($N = 440$ eyes)	
INL	0.005 (0.001 to 0.01)	.025
GCIPL	–0.005 (–0.015 to 0.005)	.307
pRNFL	–0.40 (–1.57 to 0.77)	.501
	Progression ($N = 97$ eyes) vs no progression ($N = 409$ eyes)	
INL	0.001 (–0.004 to 0.007)	.609
GCIPL	–0.006 (–0.02 to 0.006)	.329
pRNFL	–0.65 (–0.69 to 1.99)	.342

β = regression coefficient; CI = confidence interval; HC: healthy controls; GCIPL: ganglion cell-inner plexiform layer; INL: inner nuclear layer; MSON: multiple sclerosis–related optic neuritis; pRNFL: peripapillary retinal nerve fibre layer.
[a]Generalised estimation equation model adjusted for inter-eye dependency and baseline retinal thickness.

The annualised rate of change for GCIPL in MS patients was –0.012 mm^3 ($p < .001$), which was significantly more than observed in HCs (–0.004 mm^3, $p = 0.116$, p value for comparison .008). The pRNFL showed significantly more thinning in MS patients (–0.97 µm, $p < .001$) compared with HCs (–0.42 µm, $p < .001$, p value for comparison .001, Figure 2(a)). For both layers, eyes with episodes of MSON during the follow-up period showed significantly more thinning than unaffected eyes (Figure 2(b)).

Short- and long-term effects of clinical disease activity on retinal layer thickness

Table 3 demonstrates the effects of new episodes of MSON during follow-up, other clinical relapses and disease progression on annualised change in INL and GCIPL volume and pRNFL thickness. The short-term (clinical event and retinal change assessed within the same follow-up period, Table 3(a)) as well as the long-term (time-lag analyses, clinical event between t0 and t1 and change in retinal layer thickness between t1 and t2, Table 3(b)) effects are reported. The median duration of t0–t1 was 1.1 year (IQR 1.0–1.9) and for t1–t2 the median duration was 1.0 year (IQR 1.0–1.7).

Clinical episodes of MSON during follow-up demonstrated only a short-term effect on INL (thickening) and GCIPL and pRNFL (thinning). In the time-lag analyses investigating the long-term effects, these effects disappeared. Exclusion of patients with a progressive disease course did not change the statistical findings.

Clinical relapses (other than MSON) during follow-up were present in 24.4% of patients. The occurrence of clinical relapses during the first follow-up was not related to change in INL within the same

period (median 1.1 years from baseline, $\beta = 0.000$, 95% confidence interval (CI) –0.004 to 0.004, $p = .868$) but was significantly associated with an increase in INL volume in the subsequent follow-up (median 2.2 years from baseline, $\beta = 0.005$, 95% CI 0.001 to 0.01, $p = .025$). This effect was similar when only patients with a relapsing disease course were included ($N = 508$ eyes, $\beta = 0.005$ (95% CI 0.00 to 0.01, $p = .049$)). For GCIPL volume and pRNFL thickness, this effect was more pronounced in the short term (i.e. relapse and retinal volume change within the same follow-up period, Table 3(a)).

Disability progression was observed in 17.2% (during the entire follow-up period). Annualised change in INL volume was independent of disability progression both in the short and long term. Likewise, disability progression was not significantly associated with annualised changes in GCIPL or pRNFL (Table 3(a) and 3(b)).

Effect of MMO, disease type and DMT on retinal changes

In the 1.4% of eyes with MMO before or during the study (18/1275 eyes), the INL volume at the last visit was 0.07 mm^3 higher compared with eyes without MMO ($p = .006$, adjusted for new episodes of MSON). Likewise, the average annualised rate of change of INL volume was significantly higher in eyes with MMO compared with eyes without ($\beta = 0.01$, $p = .011$, adjusted for baseline INL and episodes of MSON during follow-up), showing a significant annualised increase over time in MMO eyes (0.01 ± 0.02 mm^3), but no change in eyes without (-0.0002 ± 0.02 mm^3).

Just more than half the patients (53.8%) used DMT during the study. Although the annualised change in INL volume was not influenced by use of DMT, the absolute INL volume was significantly higher in patients using fingolimod compared with RRMS patients who did not use any DMT, independent of history of pre-study MSON and MMO, EDSS at baseline and disease duration (difference 0.03 mm^3, $p = .004$). Other therapies did not show significant differences in INL volume.

Interrelationship between layers

All analyses regarding the interrelationships between the layers demonstrated effect modification by presence of a new episode of MSON during follow-up and are therefore stratified. In eyes with MSON during follow-up, an increase in INL volume was related to a decrease in GCIPL volume (standardised $\beta = -0.42$, $p = .006$, black line in Supplementary Figure 1(a)) and to a lesser extent (although not statistically significant) to a decrease in pRNFL (standardised $\beta = -0.15$, $p = .148$, black line in Supplementary Figure 1(b)). In eyes without new MSON, no significant association with change in INL volume was observed (grey lines in Supplementary Figures 1(a) and (b)). In contrast, GCIPL and pRNFL show positive correlations both in eyes with new MSON (standardised $\beta = 0.41$, $p < .001$) and without MSON (standardised $\beta = 0.26$, $p = .004$, see Supplementary Figure 1(c)).

Discussion

This longitudinal, multi-centre study demonstrates that thickening of the INL as measured with spectral-domain OCT reflects adjacent inflammation of the optic nerve. Besides this association with local inflammation, the INL seems to reflect some degree of global disease activity, as the occurrence of clinical relapses in any functional system was significantly associated with a subsequent increase in INL volume. However, this effect was relatively small (difference of 0.005 mm^3) and should be interpreted with caution, as the sensitivity might be limited.

These findings build on previous findings from other studies, demonstrating the relationship between thickening of the INL and physical disability[9] and disease activity.[10] Saidha et al.[10] reported that INL/OPL thickening at baseline was predictive of clinical relapses, new T2 and contrast-enhancing lesions on magnetic resonance imaging and disability progression during follow-up. In contrast, in the present study we did not observe any predictive value of INL thickening on clinical relapses or disability progression. Our data demonstrated an association between INL volume and clinical relapses only in the time-lag model, in which clinical relapses preceded INL thickening. This would suggest that INL thickening occurred subsequent to inflammatory disease activity. A predictive effect of baseline INL volume on the occurrence of relapses or disability progression, as described by Saidha and colleagues,[10] was not observed in the present study.

When thickening of the INL was first described, it was directly linked to the presence of MMO.[9] Although MMO is present in MS and is related to increased disability,[9,10,18] it is not specific for MS and may vary over time in individual patients.[19,20] Importantly, we have previously demonstrated that MMO was transient in 84% of cases.[19] In the present study, MMO was present in 2.4% of patients,

which is consistent with previous findings.[9–11,21] In the present study we observed increased INL volume in eyes with MMO, which is also consistent with existing literature.[1] Moreover, eyes with MMO also showed a significant increase in INL volume over time. Nevertheless, the findings of the present study did not change when MMO eyes were excluded, suggesting that thickening of the INL can occur in the absence of visually detectable MMO.

The underlying mechanism responsible for thickening of the INL remains unknown. The findings of this study would imply that previously suggested mechanisms such as inflammation-related dynamic fluid shifts and Müller cell dysfunction are more likely than other non-inflammatory mechanisms such as traction and retrograde trans-synaptic degeneration.[21,22] Dynamic retinal layer volume changes can be explained by fluid shifts due to a combination of osmotic and hydrostatic gradients, the retinal glymphatic system.[23–26] The INL is embedded between the superficial vascular plexus and the deep capillary plexus, which can be clearly visualised on OCT angiography.[24] Typically, fluid reaches the retina through the internal limiting membrane and both plexuses, whereas both plexuses and Müller cells can absorb the interstitial fluid. In case of inflammation, diffusion of fluid from the retinal blood vessels increases, leading to an increase in INL volume. Another suggested mechanism is pathology of Müller cells, which would impair the absorption of interstitial fluid, also resulting in increased INL volume. Other suggested non-inflammatory mechanisms such as traction or retrograde trans-synaptic degeneration are also plausible but less well supported by our data, given the clear and direct increase in INL volume following MSON. One approach to further elucidate the underlying mechanism would be the investigation of the retinal vessels using OCT angiography.

Previously, we and others have described the limited susceptibility of the INL to retrograde degeneration caused by MSON.[1] This is in line with our current findings, in which the pRNFL and GCIPL clearly showed significant thinning in eyes with previous episodes of MSON, whereas for the INL no thinning but rather an increase in volume was observed. The opposing effects of local inflammation on the INL on the one hand and pRNFL/GCIPL on the other are clearly demonstrated by the negative correlation between the layers. This further substantiates the potential of pRNFL/GCIPL as a measure for neurodegeneration, whereas INL volume may be a valuable parameter for reflecting inflammatory activity.

A recent study by Knier et al. reported that effective treatment with DMT in patients with MS is associated with a sustained reduction in INL volume in the absence of MSON, and they suggested that INL volume may be a response marker for successful treatment of inflammation.[11] Building on these findings, we investigated the effect of DMT on INL volume changes. Although the absolute INL volume was significantly higher in patients using fingolimod compared with RRMS patients who did not use any DMT, which corroborates previous findings on retinal effects of this drug,[27,28] the annualised change in INL volume was not influenced by use or type of DMT. However, it should be noted that DMT data were available only at baseline for the majority of patients, and that data on the exact duration of treatment or previous DMTs were not available. This lack of detailed information did not permit a thorough investigation of direct effects of DMT or replication of previous results.

Another limitation of the study was that the data did not permit the determination of how acute new episodes of MSON were. A systematic assessment of the early time course of acute MSON would be extremely valuable, and there will need to be a consensus as to what will be defined as onset of an acute episode of MSON. Furthermore, disease activity was recorded only by clinical relapse activity. Data on radiological disease activity (new T2 and/or gadolinium-enhancing lesions during follow-up) were not available. Therefore, no conclusions could be made regarding the relationship of INL volume and radiological disease activity. A common limitation of multi-centre studies is the difference in methodology among the participating centres.[29,30] The OCT device and software were the same for all centres (Spectralis) but one (Topcon). The data on retinal layer thickness of this particular centre were not significantly different from the other centres', and additional adjustment for a potential centre effect did not change any of the results (data not shown).

In summary, our data demonstrate that an increase in INL volume is strongly associated with inflammation of the optic nerve, and to a lesser degree with other clinical relapses. Therefore, INL volume may be a valuable parameter for capturing inflammatory

disease activity and may be considered as an outcome measure for MS and MSON treatment trials.

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Conflict of interests

L.J. Balk has received an institutional research grant from Teva and is a researcher for the OCTIMS study, an observational study for validating OCT as a marker for MS sponsored by Novartis.

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A.U. Brandt is named as inventor on several patent applications describing serum biomarkers in MS, motor function assessments using perceptive visual computing, and methods for analysing retinal images. He is cofounder of shareholder of the companies Motognosis and Nocturne, the latter of which has an interest in using OCT for MS diagnosis.

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Supplemental Material

Supplemental material for this article is available online.

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