Microcystic macular oedema, thickness of the inner nuclear layer of the retina, and disease characteristics in multiple sclerosis: a retrospective study

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Summary

Background Microcystic macular oedema (MMO) of the retinal inner nuclear layer (INL) has been identified in patients with multiple sclerosis (MS) by use of optical coherence tomography (OCT). We aimed to determine whether MMO of the INL, and increased thickness of the INL are associated with disease activity or disability progression.

Methods This retrospective study was done at the Johns Hopkins Hospital (Baltimore, MD, USA), between September, 2008, and March, 2012. Patients with MS and healthy controls underwent serial OCT scans and clinical assessments including visual function. OCT scanning, including automated intraretinal layer segmentation, yielded thicknesses of the retinal nerve fibre layer, the ganglion cell layer plus inner plexiform layer, the INL plus outer plexiform layer (the combined thickness of these layers was used as a surrogate measure of INL thickness), and the outer nuclear layer. Patients with MS also underwent annual brain MRI scans. Disability scores were compared with the Wilcoxon rank-sum test. Mixed-effects linear regression was used to compare OCT measures and letter-acyuity scores. Logistic regression was used to examine the relations of baseline OCT thicknesses with clinical and radiological parameters.

Findings 164 patients with MS and 60 healthy controls were assessed. Mean follow-up was 25.8 months (SD 9.1) for patients with MS and 22.4 months (11.4) for healthy controls. Ten (6%) patients with MS had MMO during at least one study visit; MMO was visible at baseline in four of these patients. Healthy controls did not have MMO. Patients with MS and MMO had higher baseline MS severity scores (median 5.93 [range 2.44–8.91]) than those who did not have MMO at any time during the study (151 patients; 3.81 [0.13–9.47]; p=0.032), although expanded disability status scale (EDSS) scores were not significantly different (5.2 [1.0–6.5] for patients with MS and MMO vs 2.5 [0.0–8.0] for those without MMO; p=0.097). The eyes of patients with MS and MMO (12 eyes) versus those without MMO (302 eyes) had lower letter-acyuity scores (100% contrast, p=0.017; 2.5% contrast, p=0.031; 1.25% contrast, p=0.014), and increased INL thicknesses (p=0.003) at baseline. Increased baseline INL thickness in patients with MS was associated with the development of contrast-enhancing lesions (p=0.007), new T2 lesions (p=0.015), EDSS progression (p=0.034), and relapses in patients with relapsing-remitting MS (p=0.008) during the study. MMO was not associated with disease activity during follow-up.

Interpretation Increased INL thickness on OCT is associated with disease activity in MS. If this finding is confirmed, INL thickness could be a useful predictor of disease progression in patients with MS.

Funding National Multiple Sclerosis Society, National Eye Institute, Braxton Debbie Angela Dillon and Skip Donor Advisor Fund.

Introduction Multiple sclerosis (MS) is often regarded as an immune-mediated inflammatory demyelinating disorder of the CNS, in which neurodegeneration is a secondary phenomenon.1 Grey-matter degeneration is, however, common in MS and more closely associated with disability than white-matter degeneration.2,3 Whether grey-matter degeneration in MS occurs only as a result of white-matter injury is unclear.4 Neuronal loss in the retina could occur as a primary process in MS,8 and might be a risk factor for relapses and gadolinium-enhancing lesions.9 Intermediate uveitis, especially pars planitis, also occurs in up to 16% of patients with MS.10 Consistent with clinical observations, post-mortem analyses show retinal inflammation with activated microglia in the eyes of people with MS.11 Collectively, these findings suggest myelin might not be necessary for maintaining or propagating inflammation in MS.

Spectral-domain optical coherence tomography (OCT) renders images with high resolution (<5 μm),12 from which the individual retinal layers can be demarcated, qualitatively assessed, and objectively and precisely
quantified. These layers include the retinal nerve fibre layer (RNFL), ganglion cell layer (GCL), inner nuclear layer (INL), and outer nuclear layer (ONL). The RNFL is the innermost layer of the retina and primarily consists of the axons of ganglion cell neurons, which are located distal to the RNFL. These axons coalesce at the optic disc to form the optic nerve, and exit the eye through the lamina cribrosa, where they acquire myelin.

MS affects the optic nerves both clinically (as optic neuritis) and subclinically, resulting in retrolaminar degeneration of the axons of the optic nerve, culminating in RNFL and GCL atrophy that can be detected and quantified in vivo with OCT. Additionally, deeper retinal layer pathology has been shown to occur in MS, although not in all studies. Consistent with post-mortem demonstration of INL atrophy in 40% of eyes of patients with MS, and the electroretinographic identification of INL and ONL dysfunction, we have previously shown using macular segmentation that INL and ONL thicknesses are decreased in eyes of patients with MS, both with optic neuritis (which we have termed mixed retinal pathology phenotype) and without a history of optic neuritis (which we have termed macular thinning predominant phenotype). These findings could represent primary retinal neuronal mechanisms of pathology, since atrophy of the INL or ONL has not been shown following optic nerve transection in animals. The presence of this deeper retinal neuronal layer pathology in patients with MS was associated with greater disability in a cross-sectional study.

A recent study using OCT identified microcystic macular oedema (MMO) of the INL in about 5% of patients with MS. MMO was thought to represent breakdown of the blood–retina barrier and retinal inflammation, potentially because of subclinical uveitis or retinitis, and was associated with greater disability and visual dysfunction. However, longitudinal data were available for only six patients, and the INL was not quantitatively assessed with OCT segmentation. Therefore, the prevalence of MMO of the INL, and its development over time in patients with MS remains unclear. Since MMO of the INL might be inflammatory, we postulate that some patients with MS could harbour INL inflammation in the absence of visible MMO, resulting in increased thickness of the INL. Indeed, a recent cross-sectional study showed that increased thickness of the INL (measured as the thickness of the combined INL and outer plexiform layer) correlated with higher T2-weighted fluid-attenuated inversion recovery MRI lesion volume in patients with MS, while thicknesses of other retinal layers did not. The clinical relevance of MMO of the INL and increased INL thickness (which could both represent myelin-independent neuronal compartment inflammation) remains to be elucidated.

The primary objectives of our retrospective study were: (I) to confirm the occurrence of MMO in patients with MS, ascertain its prevalence, and assess whether MMO occurs in healthy controls (to determine whether MMO in patients with MS represents a pathological process or a normal phenomenon); (2) to determine whether MMO in patients with MS is associated with disease activity and disability; and (3) to determine whether increased thickness of the INL at baseline, measured as thickness of the combined INL and outer plexiform layer, in patients with MS is associated with disease activity or disability progression.

Methods

Study design and participants

Two cohorts were recruited: an MS cohort (without acute optic neuritis or evidence of optic disc swelling on fundoscopy within 3 months of baseline assessment; about 40% of this cohort have been included in other cross-sectional studies done by our group) and a healthy control cohort. Study participants were recruited between September, 2008, and December, 2010, and studied between September, 2008, and March, 2012.

The MS cohort was recruited by unselected convenience sampling of consecutive eligible patients from one centre (Johns Hopkins MS Center, Baltimore, MD, USA). MS diagnosis was confirmed by the treating neurologist (PAC) on the basis of the 2010 revised McDonald criteria. Participants who had acute optic neuritis during follow-up (which can confound OCT measures owing to oedema), and patients with diabetes, uncontrolled hypertension, glaucoma, refractive errors of more or less than six dioptres, or other ophthalmological or neurological disorders were excluded from the study.

Participants in the control group without known ocular disease, refractive errors of more or less than six dioptres, or neurological disease were recruited from among Johns Hopkins University staff, and invited for annual OCT scans; some of these participants have been included in previous studies done by our group. Johns Hopkins University staff members live in a similar geographic area as our patient population, and were chosen to have a similar range of ages (18–65 years) and sex ratio (roughly 70% female) as in a typical cohort of patients with MS, as well as for their willingness to return for repeat OCT scanning.

The Johns Hopkins University Institutional Review Board approval was acquired and written informed consent was obtained from all participants before study enrolment.

Procedures

Patients with MS underwent clinical assessment and OCT every 6 months and brain MRI annually. Retinal imaging was done with spectral-domain Cirrus HD-OCT (model 4000, software version 5.0; Carl Zeiss Meditec, Dublin, CA, USA), as described in detail elsewhere. Briefly, peripapillary and macular scans were obtained with the Optic Disc Cube 200×200 and Macular Cube 512×128 protocols, respectively. Scans with signal strength less than 7/10 or with artifacts were excluded from analyses.
Macular cube scans were analysed in a masked fashion with segmentation software, as described in detail elsewhere. Briefly, segmentation undertaken in three dimensions yields the thicknesses of the following macular layers: the RNFL, GCL and inner plexiform layer (GCIP), INL including the outer plexiform layer, and ONL including inner and outer photoreceptor segments. The retinal segmentation technique that we used has not yet been extended to separate the INL from the neighbouring outer plexiform layer; therefore, the combined thickness of these two layers is used as a surrogate of INL thickness. This segmentation protocol has been shown to be reproducible in MS and control groups (inter-rater intraclass correlation coefficients: 0.91–0.99 for all segmentation measurements; 0.94 in the MS group and 0.91 in the control group for the INL including the outer plexiform layer).

All macular cube scans were qualitatively assessed for MMO or other retinal abnormalities by two reviewers masked to clinical and radiological status (SS and ESS). MMO was defined as cystic, lacunar areas of hyporeflectivity with clear boundaries, evident on at least two contiguous B scans, or visible in a comparable region on at least two separate acquisitions. Scans designated as fulfilling MMO criteria or showing other retinal abnormalities by either reviewer were reviewed and verified by a retinal specialist, masked to clinical status (QDN).

MS disease subtype was classified as relapsing-remitting (RRMS), secondary progressive (SPMS), or primary progressive MS (PPMS). Disease duration, comorbidities, and history of optic neuritis, including the date and side of occurrence, were recorded. Expanded disability status scale (EDSS) scores were determined by Neurostatus-certified EDSS examiners (SS, JNF, JO, SDN, PAC) at study visits (within 30 days of OCT or MRI examinations). Baseline disease duration and EDSS scores were used to assess participants’ baseline MS severity score (MSSS). Disability progression was defined as a 1 point or more, or a 0.5 point or more increase in EDSS score from baseline to end-of-study EDSS examination, respectively, if the baseline EDSS score was less than six or six or more. Ocular (optic neuritis) and non-ocular relapses during follow-up were verified and recorded.

Standardised 100% high-contrast, 2.5% low-contrast, and 1.25% low-contrast letter-acuity scores were determined at study visits with retroilluminated eye charts, as described in detail elsewhere. High-contrast (100%) and low-contrast (2.5% and 1.25%) visual loss were defined as a decrease of five or more letters or seven or more letters, respectively, during follow-up, in accordance with published data.

Brain MRI was done with a 3 T Philips Intera scanner (Philips Medical System, Best, Netherlands). The following axial, whole-brain sequences were acquired: double-echo fast-spin-echo to obtain T2-weighted scans (acquired resolution 1·1×1·1×2·2 mm; echo time 12 ms and 80 ms; repetition time 4169 ms; sensitivity encoding factor 2; repetitions 1) and a T1-weighted gadolinium-enhanced scan (acquired resolution 0·9×0·9×3·0; echo time 10 ms; repetition time 0·5 s). The same scanner and sequence protocols were used at each study visit. Contrast-enhancing lesions (at baseline and during follow-up) and new T2-hyperintense lesions (defined as the development of one or more T2-hyperintense lesions during follow-up, not evident on baseline imaging) were recorded by a reviewer (SS) masked to both clinical and OCT status.

**Statistical analysis**

Statistical analyses were done with Stata (version 11.0). The Shapiro-Wilk test was used to assess normality of distributions. Comparisons between groups were done with the Wilcoxon rank-sum test (for disease duration, EDSS, and MSSS; these variables were not normally distributed), Student’s t test (for age; normally distributed), and χ² test (for optic neuritis history, sex, immunomodulatory treatment, clinical and radiological disease activity, and proportions of MS subtype). Mixed-effects linear regression, accounting for within-subject inter-eye correlations, was used to compare OCT measures and letter-acuity scores between groups. Logistic regression with robust SE, accounting for
within-subject inter-eye correlations, was used to examine the relations of baseline INL thicknesses with clinical and radiological parameters. Multivariate models were adjusted for age and sex in comparisons of patients with MS with healthy controls, and also disease duration and history of optic neuritis in analyses between MS subgroups. Levene’s variance ratio testing was used to assess differences in INL thickness variance by disease duration. Statistical significance was defined as p<0·05.

Role of the funding source
The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
164 patients with MS and 60 healthy controls participated in the study (figure 1, table 1). Mean follow-up durations were 25·8 (SD 9·1) months for the MS cohort and 22·4 (11·4) months for the control group.

MMO was identified in ten of 164 patients with MS during the study (6%; four of ten had MMO at baseline), and was bilateral in two patients (figure 2). In one of these patients with bilateral MMO, bilateral epiretinal membranes, without associated retinal traction, were also present (epiretinal membranes with retinal traction can appear similar to MMO on OCT images, whereas those without retinal traction do not). Epiretinal membranes, again without associated retinal traction, were identified in an additional four patients with MS (five eyes) without MMO, including one patient with a previous history of uveitis ipsilateral to the epiretinal membranes (no other patients in the study had a history of symptomatic uveitis). Three patients were determined in retrospect to have non-microcystic retinal pathology on OCT and were excluded from subsequent analyses (figure 1). The eyes of healthy controls did not show any qualitative OCT abnormalities, including MMO, during the study.

Only one patient with MMO was exposed to fingolimod (a known cause of macular oedema) during the study, although in this patient (identified in retrospect) bilateral MMO was already evident on OCT, before the exposure (figure 2). Fingolimod treatment was discontinued after 2-5 months because of bilateral visual blurring. Extensive retinal assessment revealed bilateral perivascular sheathing and diffuse bilateral fluorescein leakage on fluorescein angiography. These findings persisted 1 year after visible resolution of the MMO, and despite the short exposure to fingolimod. A complete list of disease-modifying therapies patients were receiving at baseline in the MS cohort (including those with and without MMO) is shown in the appendix. Detailed ophthalmological examination (including fluorescein angiography) was done in two additional patients with MMO and was normal in both cases.

Generally, the distribution of MMO was patchy, and predominantly localised to the INL, although in three eyes microcysts were additionally present in the ONL. MMO was dynamic over time: five eyes had fluctuating MMO (ie, improving and then worsening, or vice versa), four eyes had worsening MMO, and three eyes had improving MMO (figure 2).

Of ten patients with MMO at any time during the study, only four had visible MMO at baseline, and in five of the remaining six patients, the MMO did not become visible during 1 year of follow-up. Despite this finding, all patients who had MMO (regardless of the stage in the study at which the MMO first became evident) had significantly higher baseline (beginning of the study) MSSS compared with patients without MMO at any stage in the study (p=0·032; table 2). Similarly, eyes with MMO at any time during the study (regardless of the stage in the study at which the MMO first became evident) had significantly lower high-contrast and low-contrast letter-acuity scores, decreased GCIP thickness, and increased INL thickness at baseline relative to MS eyes without MMO at any stage in the study (table 3). These findings remained significant for all measures except 2-5% letter acuity when adjusting for age, sex, disease duration, and history of optic neuritis (table 3). Eyes in which MMO

<table>
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<th>Table 1: Demographic, clinical, and radiological characteristics of the study participants</th>
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<td>RRMS</td>
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<tr>
<td><strong>Baseline demographics</strong></td>
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<tr>
<td>Number of participants (number of eyes)</td>
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<tr>
<td>Age, years</td>
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<tr>
<td>Female</td>
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<tr>
<td>Follow-up time in months</td>
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<td><strong>Optic neuritis</strong></td>
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<td>EDSS</td>
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<td>Disease duration in years</td>
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<td>MSSS</td>
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<td><strong>Baseline clinical and radiological characteristics at follow-up</strong></td>
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<td>Non-ocular relapse</td>
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<tr>
<td>EDSS progression†</td>
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<td>New gadolinium-enhancing lesion§</td>
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<td>New T2 lesion§</td>
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<td>Relapse or new gadolinium-enhancing lesion§</td>
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Data are number (%), mean (SD), or median (range). RRMS=relapsing-remitting multiple sclerosis. SPMS=secondary progressive multiple sclerosis. PPMS=primary progressive multiple sclerosis. EDSS=expanded disability status scale. MSSS=multiple sclerosis severity score. †Available for 76 patients with RRMS, 19 with SPMS, and 13 with PPMS. §Available for 120 patients with RRMS, 25 with SPMS, and 15 with PPMS. ¶Available for 121 patients with RRMS, 24 with SPMS, and 14 with PPMS.
developed during follow-up (n=6; ie, was not visible at baseline) had a non-significant trend towards increased INL thicknesses compared with eyes of patients with MS who did not have MMO at baseline (mean difference 2·5 μm; 95% CI 0·0003–4·9000; p=0·050). Figure 2 shows the retinal boundary lines as identified by the automated segmentation technique used in this study in the presence of MMO pathology.

History of optic neuritis was present in a higher proportion in the eyes of patients with MS and MMO at any time in the study (n=6 [50%]) compared with eyes of patients with MS without MMO during the study (n=87 [28%]; p=0·10). The mean time from optic neuritis to identification of MMO was 7·6 years (SD 5·7; range 1·6–17·6). The eyes of patients with MS and a history of optic neuritis had significantly increased INL thicknesses than those without a history of optic neuritis (mean difference 2·1 μm, 95% CI 1·3–2·9; p=0·0001). After excluding eyes that developed MMO, and adjusting for age, sex, and disease duration, this difference remained significant. Only one of ten patients with MMO had steroid therapy in the 30 day period before identification of MMO.

Baseline INL thickness was significantly increased in RRMS, and had a non-significant trend towards being increased across the entire MS cohort, relative to healthy controls (table 3).

Increased baseline INL thickness was associated with a significantly increased risk of developing new gadolinium-enhancing lesions, new T2 lesions, and disability progression during follow-up (table 4). In patients with RRMS (since relapses only occurred in this subtype), increased INL thickness at baseline was additionally associated with a significantly increased risk of developing relapses during follow-up (figure 3, table 4, appendix). Adjusting for characteristics known to be
associated with disease course and activity (including age, sex, disease duration, and history of optic neuritis), baseline INL thickness remained independently predictive of clinical and radiological disease activity and disability progression, both across the cohort and in patients with RRMS (table 4). Other retinal layer thicknesses and development of MMO were not associated with significant inflammatory disease activity (table 5), except GCIP thickness, which was predictive of relapses in patients with RRMS (odds ratio 1·24 per 5 μm, 95% CI 1·04–1·48; p=0·016). Although baseline INL thickness was not predictive of clinically significant visual loss (data not shown), a greater proportion of MMO eyes (n=8) had high-contrast letter-acuity loss during the study compared with non-MMO eyes (n=116; p=0·026).

Scatterplots of baseline INL thicknesses in patients with MS showing disease duration and age were examined to determine whether differences not related to normal ageing existed in INL thickness later versus earlier on in the MS disease course (appendix). A relation between age and INL thickness was not seen, but a trend for greater inter-subject variation of INL thicknesses early in the disease course was visible and Levene’s variance ratio testing showed that INL thicknesses in patients who had MS for less than 20 years versus those who had the disease for 20 years or more had significantly greater variance (p=0·009).

### Discussion

Our study confirms the occurrence of MMO (predominantly of the INL) in patients with MS, shows that MMO is a dynamic process, and highlights MMO as a pathological process that does not seem to occur in healthy controls.19 Although only four of the ten patients with MS and MMO at any stage during the study had visible MMO at baseline, baseline INL thickness was higher in the patients with MS and MMO than in patients with MS who did not have MMO. This finding highlights the potential for earlier and broader identification of this process with OCT segmentation, even when MMO has not become visible. INL thickness in patients with RRMS was also higher than in healthy controls, suggesting...
similar processes occur in the INL in some patients without visible or evident MMO. Furthermore, baseline analyses revealed greater disability (according to MSSS) and visual dysfunction in patients with MS and MMO (regardless of the stage in the study at which the MMO became evident), compared with those who did not have MMO during the study, suggesting a potential phenotype effect. However, disability according to EDSS and visual dysfunction were not worse. A similar phenotype might be present in some patients with MS without MMO, such as those with increased INL thickness. Indeed, analyses using INL thickness to broaden identification of this potential retinal process showed that increased INL thickness at baseline independently predicted the development of relapses, new gadolinium-enhancing lesions, new T2 lesions, and disability progression.

Although the pathobiology underlying MMO or increased INL thickness in patients with MS remains unclear, an inflammatory cause seems plausible; this could be related to subclinical uveitis or retinal periphlebitis, which could be confused with optic neuropathy, especially in the absence of comprehensive ophthalmological assessment. The plexiform layers surrounding the INL contain the primary networks of retinal microglia and act as diffusion barriers, making the INL susceptible to fluid accumulation during inflammation. Consistent with an inflammatory cause, increased INL thickness at baseline in patients with MS predicted inflammatory disease activity. INL swelling was also most striking in patients with RRMS, and earlier in the disease, during which MS tends to be most inflammatory. Conversely, INL neuronal degeneration and loss could predominate later in the disease course, although a prospective longitudinal study is needed to assess whether this is the case.

Another factor that might be relevant to our findings is the presence of anti-KIR4.1 antibodies in about 50% of patients with MS (and no healthy controls) harbouring anti-KIR4.1 antibodies. KIR4.1 is a potassium channel expressed on Müller glia, located in the INL, and is thought to play an important part in regulation of water fluxes in the retina. Additionally, dysregulated KIR4.1-mediated potassium conductance in Müller cells is implicated in impaired water transport by these cells and formation of macular oedema in several

**Table 5:** Comparison of clinical and radiological characteristics between patients with MS and MMO and those without MMO

<table>
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<tr>
<th></th>
<th>Patients with MS and MMO (n=10)</th>
<th>Patients with MS without MMO (n=115)</th>
<th>p value</th>
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<tr>
<td>Baseline gadolinium-enhancing lesion*</td>
<td>2 (25%)</td>
<td>12 (12%)</td>
<td>0.29</td>
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<tr>
<td>EDSS progression†</td>
<td>2 (20%)</td>
<td>41 (28%)</td>
<td>0.59</td>
</tr>
<tr>
<td>New gadolinium-enhancing lesion§</td>
<td>2 (20%)</td>
<td>24 (16%)</td>
<td>0.76</td>
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<tr>
<td>New T2 lesion§</td>
<td>4 (40%)</td>
<td>45 (30%)</td>
<td>0.53</td>
</tr>
<tr>
<td>Non-ocular relapse¶</td>
<td>3 (43%)</td>
<td>32 (28%)</td>
<td>0.41</td>
</tr>
<tr>
<td>Relapse or new gadolinium-enhancing lesion¶</td>
<td>4 (57%)</td>
<td>45 (39%)</td>
<td>0.30</td>
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Data are number (%). MS=multiple sclerosis. MMO=microcystic macular oedema. *Available for eight patients with MS and 100 without MMO. †EDSS progression defined as a ≥1 point increase if EDSS <6.0 and a ≥0.5 point increase if EDSS ≥6.0. ‡Available for ten patients with MMO and 147 without MMO. §Available for ten patients with MMO and 148 without MMO. ¶Only patients with relapsing-remitting multiple sclerosis (seven patients with MMO and 114 without MMO) were included in these analyses.
ocular disorders. Accordingly, a potential relation between MMO and anti-KIR4.1 antibodies in patients with MS warrants further exploration.

About 25% of patients with macular oedema who also show diffuse fluorescein leakage have INL microcysts on OCT, further supporting an inflammatory cause. Indeed, fluorescein angiography revealed bilateral leakage in one of the patients with bilateral MMO in this study, which persisted for more than a year after visible resolution of the MMO, suggesting that MMO might not be visible in the face of ongoing inflammation, and highlighting the potential usefulness of measuring INL thicknesses. Additionally, this patient had bilateral venous sheathing due to retinal periphlebitis. Although this patient was exposed to fingolimod (which can cause macular oedema), this drug was thought to be non-contributory since the exposure was short (2.5 months) and the MMO occurred before exposure.

During acute optic neuritis, the blood–retina barrier is susceptible to breakdown, as shown by fluorescein leakage and features of uveitis in about 25% of patients. This susceptibility could explain the tendency for increased INL thicknesses and MMO to occur in the eyes of patients with a history of optic neuritis. Alternatively, INL aberrations could represent sequelae of retrograde transsynaptic degeneration (instigated by demyelination in the myelinated portions of the optic nerve).

Longitudinal observational studies might be needed to identify such retrograde transsynaptic degeneration. Careful assessment of MMO in other optic neuropathies will be important for the investigation of retrograde transsynaptic processes.

MMO and increased thickness of the INL in patients with MS might not be related to the MS disease process, but rather could be due to concomitant pathology (such as hypertension and diabetes mellitus) or a drug that might be independently associated with increased MS disease activity and the development of macular oedema. This effect seems unlikely, however, since patients with MS and known diabetes or uncontrolled hypertension were excluded, and no such drugs were identified in this study. Alternatively, MMO and increased thickness of the INL could be due to an MS-related autoimmune disorder targeting the retina.

Our study had several limitations. Although detailed ophthalmological assessment, including fluorescein angiography, was done in a subset of patients with MMO, these assessments were not done systematically, as cases were identified retrospectively. Systematic longitudinal ophthalmological assessment is needed in future studies to confirm an inflammatory versus non-inflammatory cause of INL aberrations in patients with MS. Since most patients had RRMS, more accurate characterisation of this potential novel retinal phenotype by MS subtype is warranted, which will require the enrolment of larger numbers of patients with RRMS, SPMS, and PPMS, and also healthy controls. Although the controls in our study had a similar range of ages compared with patients with RRMS, the enrolment of older controls age-matched to patients with SPMS and PPMS, as well as RRMS, is needed in future studies. Since controls underwent OCT annually, and patients with MS underwent OCT every 6 months, less frequent imaging among healthy controls could have impeded detection of MMO in this group. In future studies healthy controls should undergo OCT as often as patients with MS.

Longitudinal studies of acute optic neuritis are warranted to characterise the relation between optic neuritis and MMO and increased INL thickness. Since we did not have a validation cohort, our findings need to be recapitulated in other MS cohorts and with other OCT devices and OCT segmentation techniques. The microcysts recorded in this study were predominantly located in the layer we measured as the INL plus the outer plexiform layer; since INL pathology has been shown to occur in patients with MS whereas outer plexiform layer pathology has not, we strongly suspect that increased

Panel: Research in context

Systematic review

We searched PubMed for articles published in English between June 1, 1960, and June 1, 2012, with the search terms “multiple sclerosis” and “inner nuclear layer” that describe assessment of the inner nuclear layer (INL) in multiple sclerosis (MS). Nine studies were identified, of which one was a large end-of-life ocular pathology study in patients with MS. That study showed qualitative atrophy of the INL in over 40% of the eyes of patients with MS, as well as the presence of activated microglia in the retina of eyes of patients with MS at the end of life. In the remaining eight studies, optical coherence tomography (OCT) was used for the in-vivo assessment of the INL in patients with MS (five of these studies were published by our group). Using segmentation techniques to quantitatively assess the INL, two studies showed decreased thicknesses of the INL in some eyes of patients with MS, and one study showed decreased thickness of the INL across a cohort of patients with primary progressive MS, relative to healthy controls. Two studies reported that aberrations in the thickness of the INL were not the derivative of acute optic neuritis in patients with MS. In two studies, significant differences in INL thickness between patients with MS and healthy controls were not detected, although in one of these studies there was a non-significant suggestion that the thickness of the INL across a large cohort of patients with MS might be higher than in healthy controls. Finally, a recent study identified and described microcystic macular oedema (MMO) of the INL in the eyes of some patients with MS, which was thought to represent breakdown of the blood–retina barrier and retinal inflammation. OCT segmentation techniques were not used in this study to assess the INL quantitatively. The clinical and radiological predictive use of INL thickness in MS has not been previously assessed.

Interpretation

Our findings show a novel and dynamic phenotype of the unmyelinated retina in MS that is characterised by an increase of INL thickness on OCT, with or without accompanying MMO. This phenotype seems to be associated with later inflammatory disease activity and with relapses, new T2 lesions, new gadolinium-enhancing lesions, and disability progression. Our findings raise the possibility that inflammation compartmentalised to the neuronal INL of the retina could be operative in MS, and that myelin might not be necessary for instigating or propagating the MS disease process. These findings have implications for our understanding of the disease process in MS, and for guiding future research endeavours.
thicknesses of this composite measure primarily reflect increased INL thickness. Nonetheless, the possibility of outer plexiform layer pathology cannot be excluded, which if determined to be the case would represent an equally intriguing and novel finding. Future advances in automated OCT segmentation algorithms aiding accurate assessment of the INL alone are needed to confirm our findings. Longitudinal studies are needed to determine whether MMO or increased INL thickness, potentially reflecting INL inflammation, ultimately lead to atrophy of INL or ONL. Finally, some bias in our MS cohort could exist (exemplified by the high rate of disease activity in the patients with RRMS who received treatment) since our centre is a major MS referral centre. This potential source of bias could have implications for the translation of our findings to smaller clinical centres.

In summary, we have identified a novel retinal phenotype in patients with MS characterised by dynamic aberrations of the neuronal INL, in which high-definition OCT shows quantitative swelling of the INL, with or without accompanying microcysts. Our findings raise the possibility that inflammation compartmentalised to the myelin-free neuronal INL of the retina could be operative in MS, suggesting myelin might not be necessary for instigating or propagating inflammation in patients with MS, and that the pathobiology of MS could include neurally targeted inflammation (panel). Moreover, this retinal phenotype seems to be associated with later inflammatory disease activity. In this study, increased INL thickness was associated with the development of relapses, new T2 lesions, new gadolinium-enhancing lesions, and disability progression. The identification of this potentially clinically relevant retinal phenotype in patients with MS could be an important step towards unravelling the elusive pathophysiology of MS, and merits further study.

Contributors
SS, ESS, QDN, and PAC designed the study. SS, ESS, MAS gathered the data. SS, ESS, CMC, QDN, and PAC analysed and interpreted the data. All authors drafted the report, revised subsequent versions, and critically reviewed the report. QDN and PAC were responsible for study supervision.

Conflicts of interest
SS has received consulting fees from MedicalLogics for the development of continuing medical education programmes in neurology, and has received educational grant support from TEVA Neurosciences. CMC has received consulting fees from Merck and On-X. JMG receives grant support from an American Academy of Neurology Clinical Research Training Fellowship, and has received honoraria from the National MS Society for patient education. JNR has consulted for Bristol-Myers Squibb and Diogenix, received a speaking honorarium from Teva, and receives research support from Novartis and Biogen-Idec. JO has received educational grant support from TEVA Neurosciences. SDN is a consultant for Biogen-Idec and Novartis and has received speakers honoraria from Biogen-Idec and Teva Neuroscience. LJB has received consulting, speaking, and travelling honoraria from Biogen-Idec, Bayer, and Novartis. EMF has received speaker honoraria and consulting fees from Biogen Idec, Teva, and Athena. He has also received consulting fees from Abbott Laboratories. AJG has provided consulting services for Prana Pharmaceuticals, Novartis, Biogen, Roche, and Acorda Pharmaceuticals. He has served on an endpoint adjudication committee for a Biogen-sponsored trial and provided expert legal advice for Mylan Pharmaceuticals. QDN serves on the Scientific Advisory Board for Heidelberg Engineering. The Johns Hopkins University has received research support from Heidelberg to conduct studies; however, Heidelberg has had no inputs into the design, conduct, or analyses of the studies. PAC has provided consultation services to Novartis, Teva, Biogen Idec, Vertex, Vaccines, and Genentech, and has received grant support from EMD-Serona, Teva, Biogen Idec, Genentech, Bayer, Abbott, and Vertex. All other authors declare no that they have conflicts of interest.

Acknowledgements
National Multiple Sclerosis Society (TR 3760-A-3 to PAC and RG 4212-A-4 to LJB subcontracted to PAC), National Eye Institute (R01 EY 014993 and R01 EY 019473 to LJB subcontracted to PAC), and Braxton Debbie Angela Dillon and Skip (DADS) Donor Advisor Fund (to PAC, EMF, LJB) funded the study.

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