REPORT

Retinal inner nuclear layer volume reflects response to immunotherapy in multiple sclerosis

Benjamin Knier,1,2 Paul Schmidt,1,3 Lilian Aly,1,2 Dorothea Buck,1 Achim Berthele,1 Mark Mühlau,1,4 Claus Zimmer,5 Bernhard Hemmer1,4 and Thomas Korn1,2,4

See Petzold (doi:10.1093/aww239) for a scientific commentary on this article.

Assessment of inflammatory disease activity during multiple sclerosis is crucial for selecting appropriate disease-modifying therapies. Previous studies suggested that the retinal inner nuclear layer reflects inflammatory disease severity within the central nervous system. In our study, correlations of longitudinal retinal layer changes as measured by retinal optical coherence tomography with ongoing disease activity were evaluated in 108 multiple sclerosis patients without therapy, on first-line therapy, or on second-line therapy. Healthy subjects served as controls. Inner nuclear layer volume at baseline correlated positively with paraclinical disease activity during the subsequent 12 months. Longitudinal thinning of the inner nuclear layer and thickening of total macular volume were associated with reduced inflammatory disease activity. Reduction in inner nuclear layer volume after 1 year indicated efficient control of inflammatory disease activity including ‘no evidence of disease activity’. In conclusion, the retinal inner nuclear layer could serve as biomarker to monitor sustained control of autoimmune central nervous system inflammation by therapeutic interventions.

1 Department of Neurology, Klinikum rechts der Isar, Technische Universität München, Ismaninger Str. 22, 81675 Munich, Germany
2 Department of Experimental Neuroimmunology, Technische Universität München, Ismaninger Str. 22, 81675 Munich, Germany
3 Department of Statistics, Ludwig-Maximilians-Universität München, Ludwigstr. 33, 80539 Munich, Germany
4 Munich Cluster for Systems Neurology (SyNergy), Feodor-Lynen-Str. 17, 81377 Munich, Germany
5 Department of Neuroradiology, Klinikum rechts der Isar, Technische Universität München, Ismaninger Str. 22, 81675 Munich, Germany

Correspondence to: Thomas Korn
Department of Neurology, Technische Universität München, Ismaninger Str. 22, 81675 Munich, Germany
E-mail: thomas.korn@tum.de

Keywords: neuroophthalmology; neuroimmunology; clinical trials; multiple sclerosis biomarkers; imaging

Abbreviations: DMT = disease-modifying therapy; EDSS = Expanded Disability Status Scale; GCIPL = common layer of ganglion cell layer and inner plexiform layer; Gd+ = gadolinium-enhancing; INL = inner nuclear layer; MMO = microcystic macular oedema; NEDA-3 = no evidence of disease activity; no T2 progress in MRI, no Gd+ lesions, no relapses, no worsening of the EDSS score; OCT = optical coherence tomography; pRNFL = peripapillary retinal nerve fibre layer; RRMS = relapsing remitting multiple sclerosis; TMV = total macular volume
**Introduction**

Multiple sclerosis is an important cause for sustained disability during adulthood, and early and sufficient disease control by appropriate immunotherapy is crucial for long-term outcomes and quality of life. As a consequence, anticipating and monitoring future disease activity is essential for designing appropriate therapeutic strategies.

Optical coherence tomography (OCT) of the retina has been introduced into clinical practice as a non-invasive means to assess retinal changes. OCT enables accurate quantification of the different retinal layer volumes and precise detection of longitudinal changes in layer measures (Leung et al., 2012). OCT has recently been proposed as a method to monitor loss of CNS parenchymal volume as reduced thickness of both peripapillary retinal nerve fibre layer (pRNFL) and common layer of ganglion cell layer and inner plexiform layer (GCIPL) correlated with multiple sclerosis-related brain and spinal cord atrophy (Oh et al., 2015; Saidha et al., 2015). Also, the reduction of GCIPL thickness after optic neuritis has a prognostic value for long-term visual outcome (Gabilondo et al., 2015).

In 2010, the occurrence of inflammatory cells within the inner nuclear layer (INL) of multiple sclerosis patients was described by histological post-mortem retinal analysis (Green et al., 2010) and Gelfand et al. (2012) first described microcystic macular oedema (MMO) in the INL by OCT analysis and found a correlation between microcystic macular oedema and disease severity in multiple sclerosis patients (Gelfand et al., 2012). Further studies confirmed that INL volume correlated with inflammatory disease activity in patients with multiple sclerosis (Saidha et al., 2012) as well as in patients with radiologically isolated syndrome (RIS) and clinically isolated syndrome (CIS) (Knier et al., 2015). Here, higher INL volumes were associated with an increase in T2 and gadolinium-enhancing (Gd + ) lesion load in cerebral MRI, annualized relapse rate (ARR), and progression in Expanded Disability Status Scale (EDSS). In the present study, we characterize longitudinal retinal changes during the course of multiple sclerosis and the impact of immunotherapies on retinal layers. Our data suggest that OCT might serve as a tool for monitoring disease control.

**Materials and methods**

**Study design and participants**

From 2012 to 2015, patients suffering from relapsing remitting multiple sclerosis (RRMS, n = 121) were prospectively recruited at our department. RRMS was defined using 2010 McDonald criteria (Polman et al., 2011). Patients between 18 and 55 years of age were included in the study. The participants were started on first-line disease-modifying therapy (DMT), were recommended to start but declined first-line therapy, or were switched from first- to second-line therapy due to insufficient disease control. The choice of DMT was left to the discretion of the treating physician, who was blinded for the OCT findings. Exclusion criteria were substantial eye disease, a refractive error > 6 dioptre (dpt), insufficient OCT quality, or DMT discontinuation during the study. Eyes with anamnestic optic neuritis before or during the study were excluded from the analysis. Patients with an inter-eye difference in pRNFL of > 20% were assumed to have suffered from subclinical optic neuritis (Petzold et al., 2014) and were also excluded from the analysis.

Forty healthy individuals served as controls. Time of first diagnosis was defined as t₀, starting point of DMT at baseline as t₀, study termination 12 months after t₀ as t₁. At t₀, all patients with multiple sclerosis received cerebral MRI, retinal OCT, and clinical examination. DMT was started within 1 week after the baseline visit. After 12 months (t₁), cerebral MRI, retinal OCT, and clinical examination were repeated. Controls were assessed by OCT only. A subgroup of 20 patients was studied by OCT every 3 months over a period of 18 months. Main outcome parameters were increase in T2 and Gd + lesion load in cerebral MRI, ARR between t₀ and t₁ as well as EDSS change between at t₁ and t₀. Patients presenting with stable T2 and Gd + lesion load at t₁, unchanged or improved EDSS score and absent relapses between t₀ and t₁ were categorized to show NEDA-3 (no evidence of disease activity: no T2 progress in MRI, no Gd + lesions, no relapses, no worsening of the EDSS score). The study was approved by the local ethics committee and conducted following the Declaration of Helsinki.

**MRI**

Brain images were acquired on the same 3 T scanner (Achieva, Philips) at t₀ and t₁ as previously described (Mühlau et al., 2013). Numbers of lesions were counted manually by two independent blinded raters (intraclass correlation 0.986).

**Optical coherence tomography**

OCT examination was performed by a Spectralis SD-OCT (Heidelberg Engineering) on both eyes of each patient as previously described (Knier et al., 2015). Briefly, evaluation of pRNFL was performed by a custom 3.4 mm ring scan centred on the optic nerve head (automatic real time ART 100). The macular area was scanned with 61 vertical B-scans (scanning angle 30° × 25°, 768 A-scans per B-scan) focusing the fovea centralis (ART 13). The automatic rescan mode was used for follow-up OCT scans at t₁. A signal strength > 15 dB was considered as sufficient while the mean signal strength of all ring and volume scans was indeed ≥ 22 dB (ring scan 29.8 ± 0.3 dB, volume scan 28.4 ± 0.2 dB). Every B-scan was segmented automatically into different layers using the company derived software (Eye Explorer version 6.0.9.0). Segmentations were checked manually and corrected if necessary in a blinded manner. Total macular volume (TMV), RNFL, GCIPL, INL, outer plexiform layer, and photoreceptor layer were studied. Layer volumes were calculated by the software’s segmentation algorithm (6 mm diameter circle around the fovea). All examinations were checked for sufficient quality using OSCAR-IB criteria (Tewarie et al., 2012).
Statistics

For statistical analysis R version 3.2.2 (R Core Team 2015) with packages geepack (Højsgaard, 2006), multcomp (Hothorn, 2008) and ICC (Wolak et al., 2012) were used. Demographic differences were analysed using one-way ANOVA and Poisson regression models; multiple comparisons were carried out using the method by Westfall (1997). OCT parameters were compared between treatment groups and time points using generalized estimating equations (GEE). Relative change in OCT layer were calculated as \( \frac{x(t1) - x(t0)}{x(t0)} \). The effect of baseline OCT findings on prospective disease activity was measured by generalized estimating equations with Poisson distributed responses. The correlation of OCT changes with disease progression was analysed using logistic regression models, using mean values of OCT changes. Here, dummy variables were calculated whether progression in T2 lesion load, occurrence of Gd+ lesions or relapses occurred or not. Within all models, we corrected for age, sex right/left eye, disease duration, EDSS, and number of lesions within the optic pathway. Values are given as mean value ± SEM (standard error of the mean) if not stated otherwise. Statistical significance was established at \( P < 0.05 \).

Results

Selection of the study population

Here, we wanted to test whether the assessment of retinal layers by OCT reflected response to treatment in multiple sclerosis patients with inflammatory lesions that were not directly localized in the optic nerve. Twenty-two single eyes and one patient were excluded due to optic neuritis before baseline and one eye due to optic neuritis between \( t_0 \) and \( t_1 \). One female patient suffered from post-traumatic right-sided enucleation. Three patients were excluded due to DMT discontinuation during the study; nine patients and seven single eyes of seven patients were excluded due to poor OCT quality (12/25 eyes: poor signal strength; 11/25 eyes: poor illumination; 2/25 eyes: papilloedema). Of the remaining 108 individuals, 47 RRMS patients started first-line DMT (1st DMT) with interferon beta-1a/1b (22/47), glatiramer acetate (7/47), dimethyl fumarate (14/47), or teriflunomide (4/47) at \( t_0 \). Twenty-five patients started second-line DMT (2nd DMT) such as Natalizumab (8/25), rituximab (3/25), alemtuzumab (3/25), or fingolimod (11/25) at \( t_0 \) due to insufficient disease control under pre-existing first-line DMT. Thirty-six patients denied first-line DMT at \( t_0 \) (no DMT). Taken together 108 patients (1st DMT: 80 eyes; 2nd DMT: 39 eyes; no DMT: 66 eyes) and 40 healthy controls (80 eyes) were enrolled into the analysis.

Baseline cross-sectional optical coherence tomography parameters and disease activity

At baseline (\( t_0 \)), healthy controls and multiple sclerosis patients were of similar age (controls 33.2 ± 1.2; RRMS 36.4 ± 1.3; \( P = 0.19 \)) and showed a comparable sex ratio (female controls 75%; female RRMS 66%). Disease activity patterns and subgroup analyses regarding DMT are shown in Table 1. As expected, multiple sclerosis patients who were switched to second-line DMT had longer disease durations, higher EDSS values, and more new T2 and Gd+ lesions in cerebral MRI scans of the previous year. Multiple sclerosis patients exhibited reduced pRNFL thickness, lower volumes of TMV, RNFL, GCIPL, and higher INL volumes than controls (Table 1). The INL volume correlated positively with outer plexiform layer (\( P < 0.01 \)) and outer nuclear layer (\( P < 0.01 \)), but not with RNFL or GCIPL volumes. We recognized MMO in three eyes of three patients, who suffered from optic neuritis within 6 months prior to baseline examination. As mentioned above, these patients were excluded from the analysis. Taken together, our cohort reproduced known OCT features of multiple sclerosis patients, namely reduced RNFL and increased INL, that had been previously reported (Saidha et al., 2012; Knier et al., 2015).

Next, we evaluated the prognostic value of OCT parameters at \( t_0 \) for disease activity during the subsequent 12 months (\( t_0 \) through \( t_1 \)). Here, the cross-sectional absolute INL volumes at \( t_0 \) correlated positively with an increase in Gd+ T1 lesions (Fig. 1A) and T2 lesions (Fig. 1B) during the subsequent 12 months. pRNFL thickness and GCIPL volume at \( t_0 \) correlated negatively with an increase in T2 lesions (Fig. 1B) during the following year.

Longitudinal changes in optical coherence tomography parameters

To further validate whether OCT parameters reflected disease activity, changes in OCT parameters in the course of various treatment regimens were monitored. At \( t_1 \), multiple sclerosis patients but not controls revealed a decline in INL volumes (controls relative increase 0.001 ± 0.002; RRMS relative reduction −0.008 ± 0.002; \( P = 0.002 \)). No differences were seen in other retinal layers between both groups (data not shown). In the next step, the longitudinal retinal layer changes were correlated with concomitant disease activity estimates. Estimates for regression coefficients of standardized OCT parameters are shown in Fig. 2A; here, changes in INL and TMV were most stringently correlated with disease activity parameters. A reduction of TMV in RRMS patients after 12 months was associated with an increase in T2 lesion load, Gd+ lesions, and relapses. In contrast, a reduction in INL volume was associated with a diminished risk for new T2 lesions and relapses. Moreover, the longitudinal reduction in INL volumes was associated with NEDA-3. In fact, the chance of reaching NEDA-3 was increased by 82% [95% confidence interval (CI) 35–145%] for every per cent point increase in reduction of INL. Even when we excluded all nine patients that suffered from new T2 lesions within the optic tract and radiation between \( t_0 \) and \( t_1 \) (Fig. 2B), the correlation between INL reduction and...
Figure 1 Baseline cross-sectional retinal layer parameters and subsequent disease activity. Correlation of retinal layers as measured by OCT with prospective paraclinical disease activity studied in 108 RRMS patients (185 eyes). (A) Correlation of INL volume at t₀ with prospective annualized increase of Gd⁺ lesions in cerebral MRI between t₀ and t₁. (B) Correlation of INL volume, pRNFL thickness, and GCIPL volume at t₀ with prospective annualized increase of T₂ lesions in cerebral MRI between t₀ and t₁. Generalized estimating equations, Poisson regression.

Table 1 Clinical and OCT characteristics at baseline

<table>
<thead>
<tr>
<th>Baseline characteristics of patient cohort</th>
<th>No DMT</th>
<th>1st DMT</th>
<th>2nd DMT</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38.5 ± 1.7</td>
<td>35.0 ± 1.4</td>
<td>33.5 ± 2.0</td>
<td>0.0693</td>
</tr>
<tr>
<td>Sex (% female)</td>
<td>63.9</td>
<td>66.0</td>
<td>68.0</td>
<td>0.70</td>
</tr>
<tr>
<td>Disease duration (months)</td>
<td>30.4 ± 7</td>
<td>16.5 ± 3.5</td>
<td>42.7 ± 6.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ARR</td>
<td>0.6 ± 0.3</td>
<td>0.3 ± 0.1</td>
<td>1.0 ± 0.2</td>
<td>0.0004</td>
</tr>
<tr>
<td>EDSS</td>
<td>1.0 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.9 ± 0.3</td>
<td>0.0044</td>
</tr>
<tr>
<td>Increase in T₂ lesion load/year (t₀ – t₁)</td>
<td>2.1 ± 0.4</td>
<td>2.7 ± 0.7</td>
<td>5.4 ± 1.2</td>
<td>0.0003</td>
</tr>
<tr>
<td>Increase in Gd⁺ lesion load/year (t₀ – t₁)</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td>1.5 ± 0.5</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Baseline OCT characteristics</th>
<th>HC</th>
<th>RRMS</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pRNFL (μm)</td>
<td>100.2 ± 0.7</td>
<td>97.2 ± 0.9</td>
<td>0.0253</td>
</tr>
<tr>
<td>TMV (mm³)</td>
<td>8.80 ± 0.03</td>
<td>8.64 ± 0.03</td>
<td>0.0033</td>
</tr>
<tr>
<td>RNFL (mm³)</td>
<td>0.91 ± 0.01</td>
<td>0.84 ± 0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>GCIPL (mm³)</td>
<td>2.05 ± 0.02</td>
<td>1.95 ± 0.01</td>
<td>0.0005</td>
</tr>
<tr>
<td>INL (mm³)</td>
<td>0.97 ± 0.01</td>
<td>0.99 ± 0.00</td>
<td>0.0014</td>
</tr>
<tr>
<td>OPL (mm³)</td>
<td>0.83 ± 0.01</td>
<td>0.83 ± 0.01</td>
<td>0.8328</td>
</tr>
<tr>
<td>ONL (mm³)</td>
<td>1.79 ± 0.02</td>
<td>1.79 ± 0.01</td>
<td>0.7111</td>
</tr>
<tr>
<td>PR (mm³)</td>
<td>2.23 ± 0.01</td>
<td>2.24 ± 0.01</td>
<td>0.5027</td>
</tr>
</tbody>
</table>

Baseline characteristics of multiple sclerosis patients without DMT (no DMT, n = 36), first-line DMT (1st DMT, n = 47), and second-line DMT (2nd DMT, n = 25); Findings in retinal OCT at baseline in healthy controls (HC, n = 40, 80 eyes) and patients with multiple sclerosis (RRMS, n = 108, 185 eyes); means ± SEM.

ARR = annualized relapse rate; OPL = outer plexiform layer; ONL = outer nuclear layer; PR = layer of photoreceptors.

One-way ANOVA for log-transformations for disease duration, annualized relapse rate, and EDSS. Poisson models for increase in T₂ and Gd⁺ lesion load.
NEDA-3 status remained robust. Notably, similar results were obtained when including all eyes with former optic neuritis (data not shown), which had been a priori excluded from the analysis according to the inclusion criteria of this study (see ‘Materials and methods’ section).

Influence of disease-modifying therapy on optical coherence tomography parameters and disease activity

Next, we analysed different therapy groups within our patient cohort. At t1, 1st DMT patients had experienced a reduction in their INL while the INL volume remained unchanged in untreated patients and healthy controls. A similar trend was observed in patients with 2nd DMT (Fig. 3A). Notably, 2nd DMT patients exhibited more pronounced pRNFL atrophy than 1st DMT patients (Fig. 3A).

In a second step, patients fulfilling NEDA-3 criteria at t1 were identified within every group. As shown in Fig. 3B, only patients, who were treated with first-line or second-line immunotherapy and who reached NEDA-3 criteria showed a reduction in INL volumes. In contrast, patients without immunotherapy or treated patients with ongoing disease activity were left with constant INL volumes after 12 months (Fig. 3B). Fingolimod was reported to directly

**Figure 2** Correlation of longitudinal retinal layer changes with ongoing disease activity. Standardized effect sizes of retinal layer reductions after 12 months on disease activity as measured by increase in T2 lesions, increase in Gd+ lesions, occurrence of relapses (relapse), and successful achievement of NEDA-3 status. Estimated effects [point estimates and 95% confidence interval (CI)] of standardized retinal layer reduction on disease activity by single logistic regression models as well as odds ratio for a 1% point increase in reduction of TMV or INL volume (non-standardized) on disease activity as measured by the indicated MRI or clinical parameters and respective P-values. (A) All multiple sclerosis patients (n = 108; 185 eyes). (B) Multiple sclerosis patients excluding those patients with new optic tract T2 lesions in MRI between t0 and t1 (n = 99; 171 eyes).
Figure 3  Effect of DMT on disease activity and retinal layer changes. Retinal layer changes under immunotherapy were studied in patients without therapy (no DMT; n = 36; 66 eyes), receiving first-line therapy (1st DMT; n = 47; 80 eyes), and in patients that were switched to second-line therapy (2nd DMT; n = 25; 39 eyes); mean ± 95% CI. (A) Relative changes between t₀ and t₁ are shown for pRNFL, TMV, RNFL, GCIPL, INL, outer plexiform layer (OPL), outer nuclear layer (ONL), and layer of photoreceptors (PR). Generalized estimating equations; **P < 0.01; ***P < 0.001. (B) Retinal changes in patients stratified according to NEDA-3 status. NEDA-3 status was achieved in 14 patients (26 eyes) out of 36 no DMT patients, in 22 patients (37 eyes) out of 47 1st DMT patients, and in 13 patients (20 eyes) out of 25 2nd DMT patients. Generalized estimating equations; *P < 0.05, **P < 0.01; ***P < 0.001 compared to healthy controls (HC); +P < 0.05 compared to no DMT NEDA-3; ++P < 0.01 compared to no DMT NEDA-3. (C) Relative changes in retinal layers as indicated between t₀ and t₁ in patients treated with fingolimod and second-line immunotherapies excluding fingolimod. NEDA-3 was achieved in 4 of 11 fingolimod patients (six eyes) and in 9 of 14 patients (14 eyes) with other second-line DMT. Generalized estimating equations; *P < 0.05. (D) Time course of INL changes in patients with 1st DMT (n = 11; 20 eyes) and 2nd DMT (n = 9; 17 eyes) stratified according to NEDA-3 status.
affect retinal layer volumes (Nolan et al., 2013). However, the fingolimod-treated patients in the 2nd DMT group did not differ from other 2nd DMT patients in their longitudinal retinal layer changes between t₀ and t₁ (Fig. 3C). In fact, INL volume reduction was similar for different types of immunotherapy (P = 0.1334).

The maximum relative INL reduction in treated RRMS patients reaching NEDA-3 was seen after 6 to 9 months of therapy reaching a plateau by 12 months (Fig. 3D). In contrast, patients that failed to reach NEDA-3 under treatment did not experience relevant changes in INL volumes at any follow-up time point. Thus, an efficient treatment response in patients with multiple sclerosis was correlated with a decrease in INL volume after 6 months irrespective of the mode of action of the immunomodulatory agent.

Discussion

In this study we show that longitudinal changes of TMV and INL, as measured by spectral domain OCT, reflect ongoing disease activity in multiple sclerosis. While TMV reduction is associated with increased disease activity, INL reduction correlates with reduced inflammatory disease activity. Notably, DMT affects distinct retinal layers and results in diminished INL volumes within 6 to 9 months of therapy. Thus, changes in INL might indicate response to immunomodulatory treatment regimens.

At baseline, reduced pRNFL thickness, reduced GCIPL volume as well as increased INL volumes correlated with subsequent inflammatory disease activity in multiple sclerosis patients. These data are consistent with findings from other groups (Gelfand et al., 2012; Saidha et al., 2012), who showed a correlation of INL and MRI activity by retrospective analysis. We have previously shown a similar negative correlation of pRNFL thickness and GCIPL volume with disease activity in untreated patients with preclinical and early multiple sclerosis (Knier et al., 2015). A recent multicentre study showed that pRNFL thickness is even prognostic for long term worsening of disability (Martinez-Lapisina et al., 2016). In the present study, reduction of TMV was associated with increased disease activity. TMV loss correlates with disease duration (Oberwahrenbrock et al., 2012) and has also been associated with brain atrophy (Dorr et al., 2011). Sufficient disease control by DMT reduces inflammatory disease activity and slows multiple sclerosis-related brain atrophy (Zivadinov et al., 2008; Frischer et al., 2009). Conversely, it is likely that ongoing inflammatory disease activity results in pronounced brain atrophy causing longitudinal TMV reduction independent of optic neuritis.

INL thickening has been described in the context of MMO. Both INL thickening and occurrence of MMO are associated with disease severity and pronounced disability (Gelfand et al., 2012; Saidha et al., 2012; Kaufhold et al., 2013). In our study, 3/121 (2.5%) patients revealed MMO at baseline, which is consistent with previous reports where MMO was reported in 1% (Balk et al., 2012) to 5% (Gelfand et al., 2012) of all multiple sclerosis patients. It is still unclear whether MMO is a sign of inflammation—similar to T₂ lesions in MRI—or degeneration, similar to ‘black holes’ in MRI T₁ imaging (Petzold, 2012). In our study, patients with MMO were excluded from the analysis as all of them suffered from optic neuritis. Some investigators suggested that MMO and INL thickening occur due to atrophy of inner retinal layers including RNFL or ganglion cell layer. As the vitreous body remains attached to the macula, inner retinal layer atrophy might cause traction on the INL resulting in INL thickening and eventually in formation of MMO (Barboni et al., 2013; Lujan and Horton, 2013; Abegg et al., 2014). Although we cannot entirely refute this notion, we did not find any correlation of INL volume changes with changes of the RNFL or GCIPL, which are mainly affected in the course of retrograde axonal atrophy during multiple sclerosis (Balk et al., 2014, 2015; Saidha et al., 2015).

Another hypothesis suggests that INL thickening might occur as a consequence of retinal periphlebitis. Retinal periphlebitis was first described by Rucker (1944), occurs in ~29% of all multiple sclerosis patients, and is associated with enhanced disease severity (Green et al., 2010; Ortiz-Perez et al., 2013). About 15% of patients reveal infiltrating leucocytes within the INL and opening of tight junctions surrounding retinal vessels has been described (Green et al., 2010). As a consequence, networks of retinal microglia within the inner plexiform layer and outer plexiform layer, which surround the INL, could act as diffusion barriers leading to fluid accumulation and thus swelling within the INL during inflammatory processes (Hume et al., 1983; Saidha et al., 2012). The current study lends further support to the idea of local retinal inflammation in RRMS although we did not formally examine our patients for retinal periphlebitis. Despite similar disease activity at baseline, patients receiving immunomodulatory treatment, but not patients without anti-inflammatory medication, showed a significant relative decline in INL volumes. Among treated RRMS patients, only those individuals with sufficient disease control and absent signs of ongoing disease activity showed a robust INL reduction. In contrast, patients with ongoing inflammation were left with unchanged INL volumes. As we measured similar OCT changes in patients with 1st and 2nd DMT, it is likely that the observed INL declines are due to appropriate control of inflammatory disease activity rather than disease stage-specific neurodegenerative processes in the retina. Our data are consistent with the notion that sufficient immunotherapy might reduce retinal inflammation resulting in declining INL volumes due to reduced INL oedema; this kind of ‘pseudoatrophy’ has also been described in cerebral MRI scans of multiple sclerosis patients after initiation of immunotherapy (Vidal-Jordana et al., 2013, 2015, 2016). True INL atrophy during multiple sclerosis, however, is quite rare (Balk et al., 2014). Although we cannot exclude genuine INL atrophy in our treated multiple sclerosis patients, we did
not observe INL reduction in untreated and still active RRMS patients again supporting the idea that INL changes were associated with changes in inflammatory activity and not with changes in the mass of brain parenchyma. Notably, the observed INL volume changes were very small. However, despite an axial resolution of ~5 μm, the detection limit for retinal layer thickness changes is in the range of ~1 μm (Wolf-Schnurbusch et al., 2009; Oberwahrenbrock et al., 2015). As a consequence, INL volume changes as measured in the present study might reliably be detected in individual patients in standardized longitudinal test protocols using the same OCT device.

In summary, INL as an OCT parameter could be used to confirm the efficacy of anti-inflammatory treatments in multiple sclerosis. Larger studies are needed to confirm and strengthen our findings. If these data are validated, OCT might serve as a cheap and reliable tool for therapy surveillance and might be helpful in designing further therapeutic strategies in patients with multiple sclerosis.

Funding
B.K., M.M., B.H., and T.K. are supported by the Kompetenznetz Multiple Sklerose KKNMS. BK receives intramural funding from the Technische Universität München. B.H. and D.B. receive funding from the Deutsche Forschungsgemeinschaft DFG (TR 128). M.M. is supported by the Gemeinnützige Hertie-Stiftung and the Deutsche Forschungsgemeinschaft DFG. T.K. is supported by the DFG (SFB 1054 and TR 128) and by the European Research Council ERC (CoG 647215 to T.K.).

Acknowledgements
We thank Göran Joost-Zentgraf, Janice Schneider and Kim Obergfell for expert assistance during OCT analysis. We thank Jan Dechent from Heidelberg Engineering for technical support.

References


