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The Inner Nuclear Layer in Pediatric Multiple Sclerosis

Hannah Hummel-Abmeier,[1,2] Sabine Naxer,[3] Ella Maria Kadas,[4,5] Hanna Zimmermann,[4,5] Bianca Knaack,[4] Peter Huppke,[1,6] Antonia Kowallick,[3] Kolja Meier,[1] Alexander Ulrich Brandt,[4,7] Friedemann Paul,[4,8] Michael Schittkowski,[3] Frederike Cosima Oertel,[4,8] and Jutta Gärtner[1]

Correspondence
Dr. Hummel-Abmeier
hannahmaria.hummel@googlemail.com

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Abstract

Background and Objectives

Pediatric onset multiple sclerosis (POMS) leads to optic nerve and retinal damage from optic neuritis (ON) and potential subclinical disease activity. Neuroaxonal retinal damage manifests in peripapillary retinal nerve fiber layer (pRNFL) and macular ganglion cell and inner plexiform layer (GCIP) thinning. Inner nuclear layer (INL) thickness has been suggested to increase with inflammatory activity or after acute ON, and decrease from chronic neurodegeneration. Macular microcysts in the INL have been described in patients with adult MS. The objective of this study was to investigate the INL in a large cohort of POMS as a potential biomarker for evaluation of disease course and therapeutic success.

Methods

For this cross-sectional case-control study, we prospectively recruited 153 patients with POMS and 92 controls, including asymptomatic healthy volunteers and children admitted to the hospital with nonretinal disorders. Optical coherence tomography was performed including intraretinal segmentation. Visual function was determined as best corrected visual acuity (BCVA).

Results

Eyes of children with POMS with prior ON had increased INL thickness (44.31 μm) compared with control eyes (42.96 μm, $p = 0.014$), whereas pRNFL (83 μm, $p < 0.001$) and GCIP thickness (68.42 μm, $p < 0.001$) were reduced compared with control eyes (pRNFL 97 μm, GCIP 78.53 μm). In eyes without history of ON, INL and other layer thicknesses were not different from controls. pRNFL (B = −2, $p < 0.001$) and GCIP loss (B = −1.6, $p < 0.001$), but not INL, were associated with worse BCVA. We found macular microcysts in 1 eye of 1 patient with a history of severe ON (0.3%). INL thickness was not associated with age, sex, disease duration, immunotherapy, disability or the MRI parameters T2 lesion count, T2 lesion volume, contrast-enhancing lesions, or contrast-enhancing lesion volume.

Discussion

The INL in POMS shows changes similar to what has been reported in adults, with macular microcysts being much rarer. A lack of cross-sectional association between INL thickness and disease severity may represent the early disease stage with neuroinflammation instead of neurodegeneration being in focus.

[1]Division of Pediatric Neurology, Department of Pediatrics and Adolescent Medicine, University Medical Center Göttingen, Georg-August-Universität Göttingen, Germany; [2]Department of Pediatric Neurology, Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Germany; [3]Department of Ophthalmology, Section for Strabismus, Neuroophthalmology and Oculoplastics, University Medical Center Göttingen, Georg-August-Universität Göttingen, Germany; [4]Experimental and Clinical Research Center, Charité–Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, and Max Delbrück Center for Molecular Medicine, Germany; [5]Nocturne GmbH, Berlin, Germany; [6]Division of Pediatric Neurology, Department of Pediatrics, University of Jena, Germany; [7]Department of Neurology, University of California, Irvine, CA; and [8]Department of Neurology, Charité—Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Germany.

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Glossary

BCVA = best corrected visual acuity; **CELC** = contrast-enhancing lesions; **CELV** = contrast-enhancing lesion volume; **DFG** = deutsche forschungsgemeinschaft; **EDSS** = Expanded Disability Status Scale; **GCIP** = ganglion cell and inner plexiform layer; **INL** = inner nuclear layer; **IQR** = interquartile range; **MS** = multiple sclerosis; **MSSS** = MS Severity Scale; **NON** = eyes without ON; **OCT** = optical coherence tomography; **ON** = optic neuritis; **POMS** = pediatric onset multiple sclerosis; **pRNFL** = peripapillary RNFL; **RNFL** = retinal nerve fiber layer; **SE** = standard error; **T2LC** = T2 lesion count; **T2LV** = T2 lesion volume; **VEP** = visual evoked potential.

Introduction

Pediatric onset multiple sclerosis (POMS) is typically defined as MS with an onset before an age of 16 years and affects 3%–10% of all patients with MS.[1-4] Patients with POMS usually start with a relapsing-remitting course, take longer to reach a progressive disease course than patients with adult MS onset, but do so at a younger age.[5] Nearly a quarter of patients with POMS present with optic neuritis (ON) as initial symptom,[6,7] and ON is a common relapse symptom during the further disease course.[3] Optical coherence tomography (OCT) can be used to monitor retinal and optic nerve damage, and OCT measurements can be used to quantify acute multiple sclerosis optic neuritis (MS-ON) damage and to predict visual outcomes.[8]

The retinal nerve fiber layer (RNFL) contains unmyelinated axons before they exit the eye through the optic nerve head, and its thickness is typically measured in a peripapillary ring scan (pRNFL). The ganglion cell and inner plexiform layer (GCIP) contains corresponding ganglion cells and dendrites, and is typically measured in the macula.[9] A few studies have investigated the retina using OCT in POMS: like in adult onset MS, the pRNFL thickness is reduced as a consequence of axonal loss from ON.[10-15] Neurodegeneration also presents as GCIP thickness reduction, and here, non–ON-related damage was also reported.[12,13] It is important that vision impairment in POMS is associated with pRNFL and GCIP, suggesting OCT as a relevant structural correlate of visual dysfunction.[12,16]

The inner nuclear layer (INL) contains retinal interneurons (i.e., bipolar cells, horizontal cells, and amacrine cells) and cell bodies of Mueller cells. An early histopathologic study has shown ubiquitous neuronal loss in the INL in MS.[17] In adult MS, the INL reacts to neuroaxonal damage from acute ON by swelling.[18] In later disease stages, degeneration and thinning of the INL have been reported.[19] Macular microcysts may form in the INL, possibly as an extreme form of inflammatory processes.[20,21] Although macular microcysts are not specific to MS,[22] their occurrence and INL thickening are associated with a worse disease course in adults with MS.[19] As such, the INL may be an interesting biomarker for tracking disease course or therapeutic success.[23,24] Against this background, we aimed to investigate the INL in a large cohort of POMS in comparison with controls.

Methods

Patients and Controls

Patients and controls for this cross-sectional study were prospectively recruited from the German Center for Multiple Sclerosis in Childhood and Adolescence in Göttingen, Germany. Data were collected from March 29th, 2011, to January 1st, 2019. We screened 177 patients for inclusion in the study. An inclusion criterion was a confirmed diagnosis of relapsing-remitting MS as defined by the McDonald criteria 2010 and the criteria of the International Pediatric MS Study Group.[25,26] Exclusion criteria for this study were age older than 18, incomplete clinical data, acute ON with clinical onset within 6 months of OCT examination, refractive error > −5 dpt, comorbid eye disorders, or insufficient OCT image quality as defined below. In suspected cases, we tested for Myelin-Oligodendrozyten-Glykoprotein-antibodies (MOG antibodies) and Aquaporin4 antibodies (17/153 and 67/153).

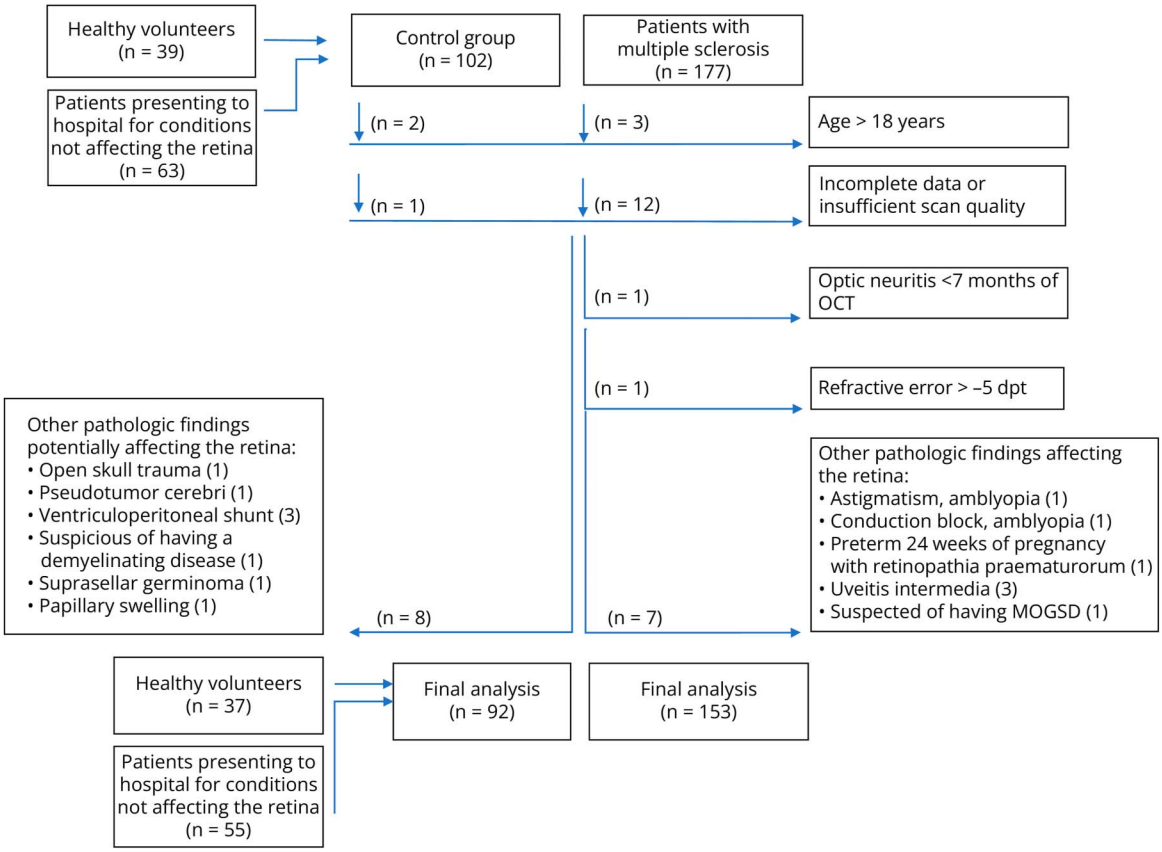
We screened 102 children as a control group, consisting of healthy volunteers and patients who presented to the hospital with disorders that did not affect the retina and were not suspected of having a demyelinating disorder. Thirty-nine children of the control group were healthy volunteers. Nineteen children presented with cephalgia and were seen by a child neurologist. They received an EEG and a neurologic examination with no pathologic findings. Four patients had epilepsy. Five patients had rheumatologic disorders, 12 had psychological disorders, 4 had oncologic disorders, 4 had unrelated neurologic symptoms, 1 had cardiologic symptoms, 2 had type I diabetes, 3 had gastrointestinal symptoms, and 1 had a traumatic brain injury.

Twenty-four patients and 10 controls were excluded (Figure 1).

Standard Protocol Approvals, Registrations, and Patient Consents

Institutional review board approval was obtained from the ethics committee of the University Medicine Göttingen, and the study was conducted in accordance with the Declaration of Helsinki in its currently applicable form and applicable German laws. All participants gave written informed consent. For underage participants, parents or legal guardians gave additional consent.

Figure 1 Study Flowchart Inclusion and Exclusion of Data From the Study



From the initially 177 patients and 102 controls, data of 153 patients and 92 controls were included in the final analysis.

Visual Acuity and Clinical Disability

For each patient, disease duration, Expanded Disability Status Scale (EDSS) score, MS Severity Scale (MSSS), and disease-modifying therapy were recorded. The EDSS score was taken from patient's case files on the same study visit as the OCT was performed, and MSSS was calculated using EDSS and disease duration.

Concurrently to the OCT, all children underwent a detailed eye examination. Visual assessment included refraction, best corrected visual acuity (BCVA) measured in decimal units using Snellen charts, visual field, relative afferent pupillary defect, color vision, slitlamp examination and inspection of the optic disk by fundoscopy, and visual evoked potentials (VEP). In 7 patients and 4 healthy controls, we only report vision at near because the vision at distance could not be evaluated because of a lack of compliance.

OCT

All OCT scans were performed with dilated pupils by an experienced and certified ophthalmic photographer. Spectral domain OCT was performed using the Cirrus HD-OCT 4000 (Carl Zeiss Meditec, Dublin; instrument software version 6.5.0.772). Using the optic disk cube setting (6 × 6 × 2 mm, 200 B-Scans with 200 A-Scans per B-Scan), we determined the thickness of the peripapillary RNFL (pRNFL) with device's own segmentation. Macular scan parameters were determined using a macular cube measuring 6 × 6 × 2 mm centered on the fovea (128 B-scans and 512 A-scans per B-scan over 1,024 samplings). Intraretinal segmentation of macular scans was performed using the SAMIRIX pipeline as described previously,[27] and corrected by an experienced grader, if necessary. We determined the thickness of GCIP and INL in a 5 mm diameter anulus around the fovea, excluding the 1 mm diameter circle around the foveal center. All scans were quality controlled according to the OSCAR-IB criteria by an experienced grader (H.Z.)[28] and reported in conformity with APOSTEL recommendations.[29] Occurrence of macular microcysts was defined as clearly delineated cystoid structures in the INL, using shadowing artifacts and half-moon shaped regional presentation on scanning laser ophthalmoscopy as supporting evidence. An experienced grader (H.Z.), blinded to diagnosis and group, performed the assessment.[18]

MRI

Clinical MRI was available from 154 patients (99.3%). From 2 patients, T2-weighted sequences were not useable because of motion artifacts; for 2 patients, no postcontrast T1-weighted sequences were available. All scans were quality controlled,

and T2-weighted and contrast-enhancing lesions were counted (T2 lesion count [T2LC] and contrast-enhancing lesions [CELC]) and segmented to derive volume in ml (T2 lesion volume [T2LV] and contrast-enhancing lesion volume [CELV]) by 2 trained experienced graders using routinely established manual segmentation pipelines with ITK-SNAP.

Statistical Analysis

Group differences in age and sex were tested using the Welch *t* test and $\chi 2$ test, respectively. We compared OCT and visual field parameters between patients with POMS and controls as well as eyes without ON [NON] and ON eyes using linear mixed models as implemented in the *R* package *lme4*, accounting for within-participant correlations by including participant ID as random effect with intercept. Model significance and *p* values were estimated using *t* tests of fixed terms with Satterthwaite approximation for degrees of freedom. To account for minor age and sex differences, we corrected all models with sex and age as fixed interaction and main effects. Pairwise comparisons of 3-group contrasts were corrected through Tukey. For best corrected visual acuity (BCVA), the statistical analyses were performed using the logMAR [-lg(BCVA]) in linear mixed models. We investigated correlations between OCT parameters and logMar using the marginal R^2 and conditional R^2 to asses for both fixed and random factors. Analyses investigating possible associations with INL, as well as sensitivity analyses, were performed in a similar fashion. Correlations between OCT and MRI lesion count and volume were performed using nonparametric testing, because of lesion counts' and volumes' not normal distributions. All statistical analyses were performed with *R* Project version 4.2.2 and RStudio 2022.12.0 + 353 (RStudio, PBC, Boston, MA, US). *p* values below 0.05 were considered significant. INL was primary outcome, and we applied no correction for multiple testing outside post hoc pairwise analyses in secondary analyses.

Data Availability

Data used for this research study are available from the corresponding author on reasonable request.

We used the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) cross-sectional checklist when writing our report.

Results

INL Is Thicker in Eyes With a History of ON

After excluding patients and controls with relevant exclusion criteria, we analyzed a final cohort of 153 patients (101 female/52 male, age 14.69 ± 2 years) with POMS and 92 controls (55 female/37 male, age 13.64 ± 2.7 years, Figure 1 and Table 1). Patients were significantly older than controls ($t = -3.1$, $p = 0.002$), but well-matched regarding sex ($X^2 = 0.7$, $p = 0.398$).

There were 44 children with ON (28.8% of children with POMS): 27 with unilateral ON (27 eyes, 17.6% of all patients with POMS, 61.4% of children with ON) and 17 with bilateral ON (34 eyes, 11.1% of all patients with POMS, 38.6% of children with ON), of whom 13 had bilateral involvement within 30 days (26 eyes, 8.5% of all patients with POMS, 29.5% of children with ON).

POMS eyes with a history of ON had a significantly increased INL, but reduced pRNFL and GCIP thickness as well as a decreased BCVA compared with control eyes (Table 2, Figure 2). This was confirmed in a sensitivity analysis for GCIP using only the asymptomatic controls (ON: B = 11.01, standard error [SE] = 1.42, $p < 0.001$; NON: B = 1.65, SE = 1.22, $p = 0.371$). By contrast, in NON eyes, layer thicknesses, including INL, were not significantly different from controls (Table 2, Figure 2). Fingolimod treatment is known to lead to increased INL thickness in adult MS.[30] To exclude the possibility that eyes from patients under fingolimod therapy influenced these results, we repeated the analysis excluding these patients (n = 3), which confirmed a significant thickening in ON eyes vs healthy controls (B = 1.08, SE = 0.39, $p = 0.018$) but not NON eyes vs controls (B = 0.22, SE = 0.34, $p = 0.798$). In a sensitivity analysis, removing the outlier with very high INL measurement in the ON group (N = 1; Figure 2), the INL result was confirmed for both ON eyes vs controls ($p = 0.012$) and NON eyes vs controls ($p = 0.655$). Despite increased INL thickness in ON eyes, the INL was not associated with the thickness of pRNFL (B = −0.01, SE = 0.01, $p = 0.132$) and GCIP (B = −0.01, SE = 0.01, $p = 0.261$) as markers for neuroaxonal damage.

INL Thickening Is Not Associated With Visual Function

pRNFL ($B = -3*10^{-3}$, SE < 0.001, $p < 0.001$, $R^2_{marginal} = 0.164$, $R^2_{conditional} = 0.459$) and GCIP loss (B = −0.01, SE < 0.001, $p < 0.001$, $R^2_{marginal} = 0.236$, $R^2_{conditional} = 0.566$) were associated with worse BCVA, but not INL ($B = 1*10^{-3}$, SE < 0.001, $p = 0.675$, $R^2_{marginal} = 0.013$, $R^2_{conditional} = 0.243$). To make sure that INL may not contribute to visual dysfunction as a consequence of ON, we investigated ON and NON eyes separately, which confirmed no association between BCVA in ON ($B = -3*10^{-3}$, SE < 0.001, $p = 0.536$, $R^2_{marginal} = 0.091$, $R^2_{conditional} = 0.993$) and NON eyes ($B = 6*10^{-4}$, SE = 0.002 $p = 0.770$, $R^2_{marginal} = 0.014$, $R^2_{conditional} = 0.760$). Similarly, pRNFL (B = 9, SE = 2, $p < 0.001$) and GCIP thinning (B = 6.61, SE = 1.07, $p < 0.001$) were associated with prolonged p100 latency in VEP, but not INL thickness (B = 0.47, SE = 0.26, $p = 0.070$).

INL Thickening Is Not Associated With Disability or With MRI Lesion Load

Furthermore, INL thickness was not associated with age (B = 0.01, SE = 0.07, $p = 0.856$), sex (B = 0.03, SE = 0.34, $p = 0.922$), time since onset (B = 0.02, SE = 0.01, $p = 0.166$), and MSSS (B = 0.14, SE = 0.13, $p = 0.293$).

To investigate whether INL thickness is associated with lesion load, we analyzed T2-weighted lesion load and contrast-

Table 1 Cohort Overview

	Patients with POMS	Controls	Statistic	*p* Value
N	153	92		
Age, y (mean [SD])	14.69 (2.28)	13.64 (2.70)	$t = -3.1$	0.002
Sex (n [%])	F 101 (66)	F 55 (60)	$X^2 = 0.7$	0.398
	M 52 (34)	M 37 (40)		
Time since diagnosis, mo (mean [SD])	17 (19)			
EDSS (median [min-max])	0.0 (0.0–4.0)			
MSSS (mean [SD])	2.3 (1.9)			
Therapy (%)				
Dimethyl fumarate	2 (1.3)			
Fingolimod	3 (2.0)			
Glatiramer acetate	11 (7.2)			
β-interferons	103 (67.3)			
Natalizumab	12 (7.8)			
Treatment naive	22 (14.4)			

Abbreviations: EDSS = Expanded Disability Status Scale; F = female; M = male; MSSS = MS Severity Scale; POMS = pediatric onset multiple sclerosis.

enhancing lesion load using clinical MRI. Median T2LC was 16 (interquartile range ([IQR] 7–35), T2LV 3.04 mL (IQR 0.99–6.79 mL), CELC 0 (IQR 0–1), and CELV 0.00 mL (IQR 0.00–0.05 mL). There was no correlation between INL thickness and T2LC (B = $-2*10^{-3}$ SE = 0.01, $p = 0.864$), T2LV (B = 0.06, SE = 0.05, $p = 0.235$), CELC (B = $-2*10^{-3}$, SE = 0.09, $p = 0.985$), and CELV (B = 0.67, SE = 0.74, $p = 0.368$).

Macular Microcysts

Only 1 of 290 eyes (0.3%) from patients with POMS showed signs of macular microcysts (Figure 3). This eye was from a 15-year-old girl with a history of ON under glatiramer acetate treatment. Disease duration was 36 months, and this eye had ON at disease onset. The eye showed profound neuroaxonal damage with pRNFL = 67 μm, GCIP = 57.5 μm, and INL = 51.46 μm. VEP p100 latency was prolonged, and BCVA was 0.9.

Discussion

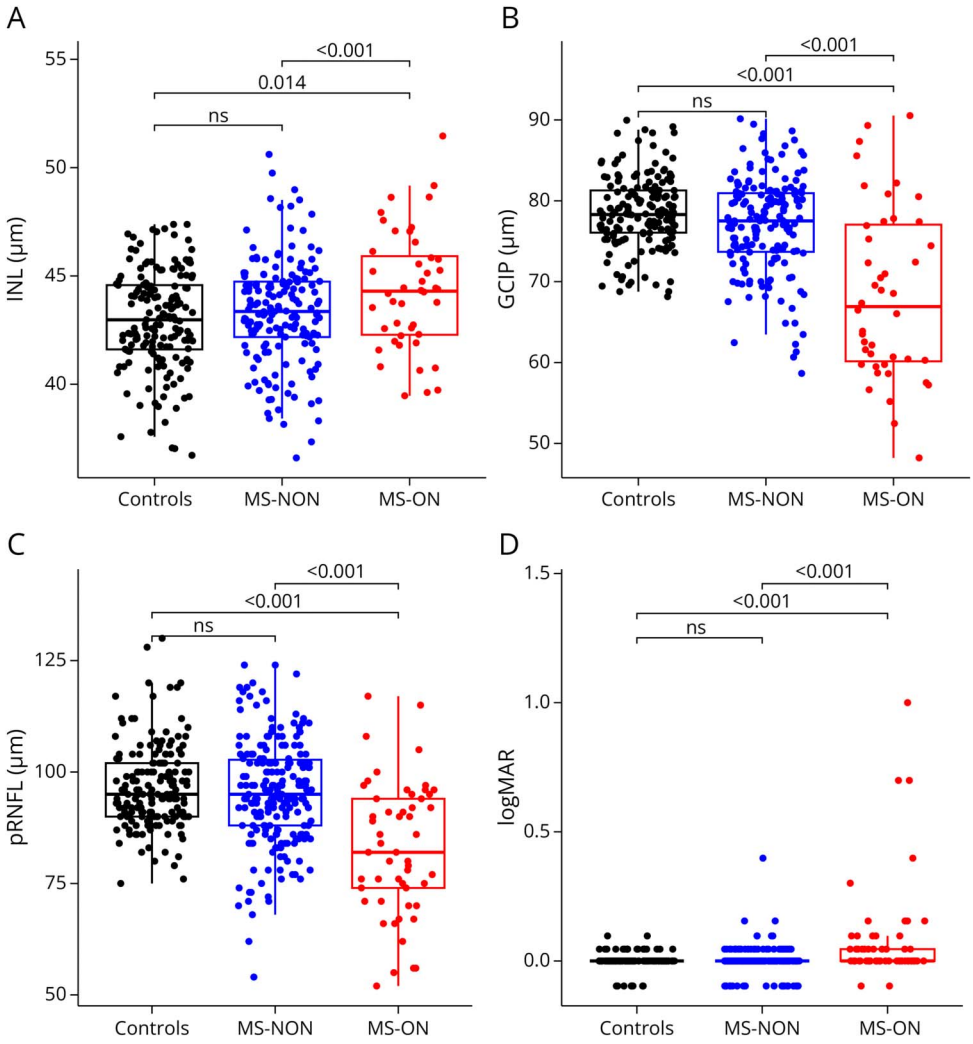
In this prospective single-center study investigating the retinal INL in 153 patients with POMS, we found that (1) INL thickness is increased in eyes after ON, (2) INL thickness is normal in eyes without previous ON, (3) INL thickness is not associated with visual function and overall clinical disability, disease duration, age, sex, or MRI parameters, and (4) only 1 eye (0.5%) showed signs of macular microcysts. We further confirm neuroaxonal damage measured as pRNFL and GCIP in eyes with prior ON, whereas pRNFL and GCIP in eyes without history of ON did not differ from healthy control (HC) eyes.

Table 2 Optic Neuritis and Nonoptic Neuritis Eyes

	ON	NON	Controls	ON vs controls		NON vs controls	
Eyes (n [%])	**61 (21%)**	**229 (79%)**	**184**	**Test statistic**	***p* Value**	**Test statistic**	***p* Value**
INL, μm (mean [SD])	44.31 (2.76)	43.32 (2.41)	42.96 (2.27)	B = 1.10, SE = 0.39	0.014	B = 0.27, SE = 0.35	0.716
pRNFL, μm (mean [SD])	83. (15)	95(12)	97 (9)	B = −16, SE = 2	<0.001	B = −2, SE = 2	0.403
GCIP, μm (mean [SD])	68.42 (10.52)	77.03 (5.97)	78.53 (4.42)	B = −11.03, SE = 1.09	<0.001	B = −1.60, SE = 0.87	0.163
BCVA, logMAR (mean [SD])	0.08 (0.19)	0.00 (0.05)	0.00 (0.03)	B = −0.08, SE = 0.01	<0.001	B ≈ 0.00, SE = 0.01	0.985

Abbreviations: B = coefficient from mixed linear model effect; BCVA = best corrected visual acuity; GCIP = ganglion cell and inner plexiform layer thickness; INL = inner nuclear layer thickness; NON = eyes without history of optic neuritis; ON = eyes with previous optic neuritis; pRNFL = peripapillary nerve fiber layer thickness; SE = standard error from mixed linear model effect; W = Wilcoxon rank-sum statistic.

Figure 2 Layer Thickness Differences and Visual Function Between Patients With MS and Controls Differences in Layer Thicknesses Between Controls (in Black) and POMS Patient Eyes Without History of ON (NON, in Blue) and With Prior ON (in Red) as Well as Visual Function

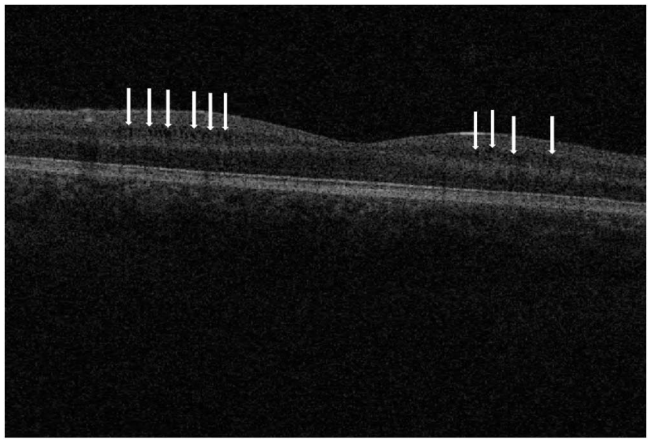


(A) INL, (B) GCIP, (C) pRNFL, and (D) BCVA. Coefficients (B), standard error (SE), and *p* values in (A–C) are from linear mixed models correcting for age and sex. Boxplots follow standard boxplot convention. BCVA = best corrected visual acuity; GCIP = ganglion cell and inner plexiform layer thickness; INL = inner nuclear layer thickness; MS = multiple sclerosis; NON = eyes without history of optic neuritis; ON = eyes with previous optic neuritis; POMS = pediatric onset multiple sclerosis.

Prominent neuronal loss in the INL in 40% of MS patient eyes has been reported in a large histopathologic study from the United Kingdom.[17] However, this does not directly correspond to INL thinning as measured by OCT. Other studies reported that INL thickening correlates with inflammatory disease activity and disability progression.[19] The current model for INL thickness changes in MS includes a thickness increase with inflammatory activity and a thickness decrease with neuronal atrophy. This has been supported by multiple studies investigating the INL in certain scenarios. For example, INL thickness has been reported to increase as a response to ON.[18,31,32] INL thickness may reduce with successful immunomodulatory therapy presumably as a sign of a normalizing inflammatory milieu.[23,24] Although the prospect of using INL thickness as a potential therapeutic response marker is intriguing, in practice, it is difficult to discriminate between atrophic and inflammatory changes of the INL.[33]

Our findings nicely fit to these changes reported in adult MS. Normal INL thickness in eyes without history of ON likely reflects the short duration and/or limited neurodegeneration in our cohort of children with POMS. Similarly, increased INL thickness in eyes with prior ON and a trending association with VEP p100 latency suggest a reactive increase to the inflammatory and demyelinating damage from ON. A rather local reaction of the INL to ON and optic nerve demyelination is also supported by the lack of any association of INL thickness with disease severity, and abnormal GCIP thickness in eyes without prior ON, suggesting little to no subclinical neurodegeneration in our patients. Although it is unlikely, based on these findings, that INL thickness may be a useful biomarker in children with POMS, this should be further investigated in a longitudinal study including correlation with MRI parameters. In this study, we could not find any correlation with T2 lesion count or lesion volume. The higher relapse rate in children and higher lesion load on MRI

Figure 3 OCT With Macular Microcysts



OCT B scan from a representative region showing macular microcysts in 1 eye of 1 patient with POMS. OCT = optical coherence tomography.

support that there is a high inflammatory component in the early stages of the disease compared with adult onset MS and later disease stages.[33] Against this background, increases in INL thickness might be associated with higher future disease activity, if an inflammatory rather than neurodegenerative aspect should also be confirmed longitudinally. Our findings are in contrast to a previous publication [13] that investigated INL thickness in POMS but found reduced INL in POMS independent of ON and NON eyes compared with controls in a study comprising 53 patients with POMS and 19 controls. Although demographic characteristics in theirs and our study are comparable, the reason for this discrepancy remains unclear. Furthermore, our study sample is considerably larger, and our results are in line with INL studies in adult patients with MS. Therefore, further independent investigation and confirmation of either results are warranted.

Only 1 eye of 1 patient showed signs of macular microcysts in the INL, suggesting that macular microcysts are exceedingly rare in eyes from children with POMS with no more than 0.5% frequency. Another study also reported 1 eye with macular microcysts in a study with 53 children with POMS.[13] In adult patients with MS, macular microcysts have been reported in approximately 5% of eyes.[18,20] However, macular microcysts are not specific for MS and occur in neuromyelitis optica spectrum disorders with even higher frequency[34] as well as in other optic neuropathies.[35] The current model suggests that macular microcysts form as an unspecific reaction to ganglion cell damage in the adjacent ganglion cell damage and represent an extreme form of INL thickening.[21,36] Indeed, the patient with signs of macular microcysts experienced previously a severe ON in this eye, indicated by very low GCIP and pRNFL thickness as chronic neuroaxonal damage from this ON.

We confirm in a large cohort that pRNFL and GCIP thickness are reduced in POMS patients with prior ON. In a study with 38 children with POMS and 15 healthy controls, a significant reduction of pRNFL thickness in patients of 83 ± 12 μm vs 107 ± 12 μm was reported[10,12,37].In a study comprising 14 MS and 15 controls, reduced pRNFL of 82.5 + 18.5 μm in patients vs 98.0 + 19.2 μm in healthy controls was reported.[11] In a study with 22 children with POMS and 29 controls, pRNFL in control eyes was 109 ± 9 μm, and highly significantly reduced in patient eyes with a history of ON to 86 ± 22 μm[14]. In the first study using intraretinal segmentation a cohort of 37 patients with demyelinating disorders was analyzed against 18 controls. Patients comprised other diseases than MS, only 16 of 37 patients were diagnosed with MS, and results may not be fully applicable. The authors found significant GCIP thickness reduction to 68.1 ± 2.6 μm in patients in eyes with a history of ON compared with controls (84.8 ± 1.2 μm). In a study investigating 53 children with POMS vs 19 controls POMS patients were found to have 26% lower ganglion cell layer volumes compared with control eyes.[13] In a study investigating 24 children with POMS and 10 controls, reduced GCIP thickness from 83 ± 6 μm in control eyes to 72 ± 9 μm in eyes from children with POMS and prior ON is reported[15]. In comparison with these studies, pRNFL reduction (B = −16 SE = 2 μm) and GCIP reduction (B = −11.03, SE = 1.1 μm) were notably smaller in our study. This may also explain discrepancies regarding pRNFL and GCIP loss in eyes without history of ON, which some studies reported[10,12,13] and others did not.[14,15] It is likely that this is caused by difference in disease severity between the cohorts and that severity of pRNFL and GCIP thickness loss in ON and NON eyes rather determined by disease duration and severity than principally different in POMS compared with adult MS.[38] However, other factors, i.e., race and ethnicity, may be relevant and need further investigation.

Our study on OCT in POMS is based on alarge cohort and is the first European study. A clear strength is the large sample size, compared with previous studies. Furthermore, our analysis includes its own control group on normally developing retina in healthy children and children with disorders not affecting the eye or optic nerve. Under physiologic conditions, the complete development of retinal layers takes until the 18th month of life, being completed earlier in the inner retinal layers than in the outer ones.[39] The developmental process of the fovea continues until the end of puberty.[40] The majority of studies agree that retinal development is independent of sex.[41-44] Several studies confirmed that pRNFL thickness does increase until the age of 6 months[41,45] but is stable afterward until the late 4th decade.[41-43,46-49] Most studies demonstrated that pRNFL is not dependent from sex.[41-44] One study found no relationship of ganglion cell layer (GCL) thickness with age in children of 0–5 years and no relationship of GCL with sex in 3–16-year-old White children,[41] but another one described that the GCL, ICLPC, and INL had significant higher values in boys than in girls in 5–15-year-old Europeans[50].

However, our study also has several limitations. As the pediatric MS center in Göttingen acts as a reference center for

Germany, patients may have a more severe disease course. Nevertheless, by not including children with a clinically isolated syndrome, and no cases of primary or secondary progressive disease, the cohort remains relatively homogenous. The racial and ethnic distribution is less diverse than e.g. in the United States and consists exclusively of White patients. Being cross-sectional, this study allows no conclusion on the predictive power of INL thickness for the clinical course. Here, further longitudinal studies are needed. The EDSS score as a marker for clinical disease course in children has its limitations as the majority of children recovers well from relapses, and EDSS therefore does not discriminate between a severe and a more benign disease course. For further longitudinal studies, EDSS change over time, relapse rate, and MRI activity would be necessary for evaluation of clinical course.

A further limitation is the composition of the control group. Only 41% are healthy, 59% presented for a medical issue that we have assigned to psychiatric, cardiologic, oncologic, neurologic, rheumatologic, and gastrointestinal disorders and type I diabetes. None of these children had any retinal disease. Statistically, we found no difference between patients in different diagnostic groups regarding the different intraretinal layers (data not shown). The group therefore seems to be suitable as a control group.

In conclusion, INL in POMS shows changes similar to what has been reported in adult MS. A lack of association with disease severity most likely reflects the early disease stage of patients with POMS, where neuroinflammation is predominant rather than neurodegeneration. Our study further confirms previous findings of reduced pRNFL and GCIP after a history of ON in a large cohort.

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Author Contributions

H. Hummel-Abmeier: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. S. Naxer: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. E.M. Kadas: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. H. Zimmermann: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data. B. Knaack: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. P. Huppke: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. A. Kowallick: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. K. Meier: major role in the acquisition of data. A.U. Brandt: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data. F. Paul: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. M. Schittkowski: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. F.C. Oertel: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. J. Gärtner: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data.

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