Optimal Inter-Eye Difference Thresholds by OCT in MS: An International Study

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ana.25462

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Running head: Optimal OCT Inter-Eye Difference Thresholds in MS

Statistical analysis conducted by Rachel Nolan, Dr. Laura Balcer, and Dr. Menging Liu

Key Words: optic neuritis (ON); multiple sclerosis (MS); optical coherence tomography (OCT)

Abstract

Objective: To determine the optimal thresholds for inter-eye differences in retinal nerve fiber and ganglion cell+inner plexiform layer thicknesses for identifying unilateral optic nerve lesions in multiple sclerosis.

Background: Current international diagnostic criteria for multiple sclerosis do not include the optic nerve as a lesion site despite frequent involvement. Optical coherence tomography detects retinal thinning associated with optic nerve lesions.

Methods: In this multi-center international study at 11 sites, optical coherence tomography was measured for patients and healthy controls as part of the International Multiple Sclerosis Visual System Consortium. High- and low-contrast acuity were also collected in a subset of participants. Presence of an optic nerve lesion for this study was defined as history of acute unilateral optic neuritis.

Results: Among patients (n=1,530), receiver operating characteristic curve analysis demonstrated an optimal peripapillary retinal nerve fiber layer inter-eye difference threshold of 5 microns and ganglion cell+inner plexiform layer threshold of 4 microns for identifying unilateral optic neuritis (n=477). Greater inter-eye differences in acuities were associated with greater inter-eye retinal layer thickness differences ($p \leq 0.001$).

Interpretation: Inter-eye differences of 5 microns for retinal nerve fiber layer and 4 microns for macular ganglion cell+inner plexiform layer are robust thresholds for identifying unilateral optic nerve lesions. These thresholds may be useful to establish the presence of asymptomatic and

symptomatic optic nerve lesions in multiple sclerosis and could be useful in a new version of the diagnostic criteria. Our findings lend further validation for utilizing the visual system in a multiple sclerosis clinical trial setting.

Introduction

Multiple sclerosis (MS) is an immune-mediated demyelinating disorder of the central nervous system (CNS) and is currently diagnosed using the recently revised 2017 McDonald criteria[1]. Neuro-axonal degeneration is a prominent feature in MS, which correlates with underlying disability[2-6] and mirrors retinal atrophy [7, 8] in patients both with and without a history of acute optic neuritis[9, 10]. Current diagnostic criteria for MS do not include the optic nerve as a lesion site based on imaging criteria, despite high prevalence (50%) of acute optic neuritis in MS and evidence of subclinical optic neuropathy in nearly all MS patients post-mortem [11-14].

Spectral-domain optical coherence tomography (SD-OCT) has emerged in the last 10 years as a sensitive and reliable method to measure retinal axonal and neuronal loss *in-vivo* manifesting as peripapillary retinal nerve fiber layer (pRNFL) and ganglion cell + inner plexiform layer (GCIPL) thinning. Demyelination and transection lead to retrograde degeneration of optic nerve axons over time, which can be detected by measuring the pRNFL and GCIPL by OCT [9, 15-17]. OCT is useful to monitor disease progression, and to detect both clinical and subclinical optic nerve degeneration [9, 18-22]. Loss of retinal axons (measured by pRNFL thickness on OCT) and retinal neurons (measured by GCIPL thickness on OCT) correlate with visual function, cognitive disability, reduced quality of life and brain atrophy [8-10, 16, 23-32].

Given the technical and resolution limitations inherent in orbital MRI[33], OCT is thus a promising technique to identify optic nerve lesions even among patients with no clinical history of acute optic neuritis and has shown high reliability in multicenter segmentation studies[34]. Previous

studies have suggested an optimal inter-eye difference of 5-6 microns in pRNFL thickness to be a robust determinant of prior ON in MS [35], and have shown that a 5% inter-eye difference in macular GCIPL thickness [36] is a useful diagnostic measure for MS-associated optic neuritis. In this large international collaborative study, we evaluated the diagnostic utility of various thresholds for inter-eye difference in pRNFL and GCIPL thickness for identifying a history of unilateral optic neuritis as the model optic nerve lesion in MS. The aim for determining these inter-eye difference thresholds is to establish criteria for defining the presence of a unilateral optic nerve lesion in patients with MS and those presenting with clinically isolated demyelinating syndromes (CIS).

Methods

Study participants: Participants were ≥18 years of age and were part of an ongoing international collaborative study of visual outcomes in MS in the United States, Canada, Europe, and the Middle East. Participating investigators were part of the International Multiple Sclerosis Visual System Consortium (IMSVISUAL), a collaborative organization designed to facilitate study of vision in MS (www.imsvisual.org). MS patients both with and without a history of acute optic neuritis were included. Healthy controls with no history of ocular or neurological disease were included in the study in order to identify the 95th and 99th percentile inter-eye OCT differences, and to compare the mean pRNFL and GCIPL thicknesses between MS patients and healthy controls. Subjects with ocular comorbidities other than correctable refractive error, such as

macular edema, epiretinal membrane, glaucoma, macular degeneration, and pathologic myopia, and those whose onset of acute optic neuritis was within the past three months, were excluded. Ocular pathologies were identified by manual review of the OCT images by trained technicians or clinicians, by patient self-report and by ophthalmic exams when available. Healthy control subjects with OCT values lower than feasible for a healthy control (<75 for pRNFL, <50 for GCIPL) were excluded. Acute optic neuritis was defined by a patient-reported exacerbation consistent with an inflammatory demyelinating event affecting the optic nerve; the diagnosis of acute optic neuritis was verified by medical record review. Patients with an uncertain history of optic neuritis (n=70) and with bilateral ON (n=129) were excluded from analysis assessing the effect of inter-eye differences on evaluating unilateral optic neuritis history but were included in analysis assessing the relationship of visual function to inter-eye OCT differences. Subjects were included in the progressive MS (PMS) group if they had secondary progressive, primary progressive, or progressive relapsing phenotypes of MS. All participants provided written informed consent and study protocols were approved by each respective institution's Institutional Review Board (IRB). Agreements for the sharing of de-identified data were also in place between each participating institution and the New York University School of Medicine.

Optical coherence tomography (OCT): Spectral-domain OCT imaging was performed for all participants by a trained technologist using either Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany) or Cirrus OCT (Carl Zeiss Meditec, Dublin, CA). On Spectralis OCT,

pRNFL thickness was measured using a 3.4° ring scan centered on the optic disc. The ONH Cube 200x200 scans were used to measure pRNFL thickness on Cirrus OCT. Macular volume scans encompassing a 6 mm area surrounding the fovea were performed on Spectralis OCT and automated macular volume cube 200x200 or 512x128 scans encompassing an area of 4x5 mm surrounding the fovea[37] were performed on Cirrus OCT to measure GCIPL thickness. Macular GCIPL thickness was measured as the sum of the ganglion cell layer plus inner plexiform layer thickness for both machines. Segmentation was performed at each center using the automated segmentation protocols inherent in the machines with manual correction. All scans were reviewed to meet quality control standards. For Spectralis OCT scans, the OSCAR-IB criteria [38] for quality control were followed. For Cirrus OCT, all scans that were not centered or segmented properly, had a signal strength <7, or any algorithm failure were excluded. As some sites did not have GCIPL measurements collected on every subject, only subjects with these measurements in both eyes were included in analysis assessing inter-eye GCIPL differences. OCT results are reported in alignment with the APOSTEL guidelines[39].

Visual assessments: Low-contrast Sloan letter charts (Precision Vision, LaSalle, IL) at both 2.5% and 1.25% contrast levels were used to measure low-contrast letter acuity (LCLA) both monocularly for each eye and binocularly with both eyes together. Charts were placed in a retro-illuminated light box. Scores were calculated as the numbers of letters out of 70 read correctly

per chart. Each chart has 14 lines with five letters per line that are standardized with equal difficulty per line and equal spacing between lines [40].

High-contrast visual acuity was measured using either the Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity charts (standard high-contrast visual acuity charts used in ophthalmology clinical trials) or Snellen acuity; all scores were converted to the ETDRS scale. Low- and high-contrast acuity were not performed at every site. Only subjects with these measurements available were included in analysis of visual assessments in comparison to inter-eye OCT differences.

Statistical methods: Mean values for inter-eye differences in pRNFL and for macular GCIPL thicknesses were calculated for all participants. As candidate markers for diagnostic marker of a history of unilateral optic neuritis, we used two different approaches. First, we estimated the absolute value of 95th and 99th percentiles for RNFL and GCIP thickness inter-eye differences in healthy control group. The 95th percentile value represents an estimate of the upper boundary of expected values for inter-eye differences in a healthy population. Second, within the MS cohort, we identified the cutoff value for inter-eye RNFL and GCIP thickness differences that would optimize both sensitivity and specificity for distinguishing MS patients with vs. without a history of acute unilateral optic neuritis using receiver operating characteristic (ROC) curve analysis and the 'rocmic' command. Rocmic estimates the minimally important change thresholds using three methods. For this analysis, we used the 45-degree tangent line intersection where sensitivity and specificity are closest together. The optimized cutoff value for inter-eye pRNFL and GCIPL

thickness differences to distinguish MSNON from MSON patients was determined visually and statistically. This model was originally developed using the data from one site (New York University (NYU)) based on a previously published paper [35]. Once we identified the 6 candidate markers, we quantified sensitivity, specificity and predictive values of these markers to distinguish MS patients who had a history of acute unilateral optic neuritis vs. those with no acute optic neuritis history using 2x2 table analyses and we calculated the likelihood of a history of acute unilateral optic neuritis using logistic regression adjusting for age. In order to avoid overestimation of p-values and confidence intervals, this analysis was performed on the validation dataset including the remaining 10-sites from the international study, excluding the data from NYU that the model was originally developed on.

As an additional outcome, within the MS cohort, the relation of inter-eye differences in RNFL and GCIPL thicknesses to scores for inter-eye differences in visual function tests, including high- and low-contrast acuity, was examined using linear regression models, accounting simultaneously for age. Comparisons in mean pRNFL and GCIPL thickness between MS patients and healthy controls, and those with a history of optic neuritis compared to those without, were calculated using generalized estimating equation (GEE) regression models accounting for within-patient, inter-eye correlations because both eyes of each patient were included in the model.

Finally, we performed four types of sensitivity analyses. First, the subjects in the original cohort upon which the statistical model was developed, which were the subjects recruited from NYU (n=303), were excluded for validation purposes, and the ROC curve analysis was performed on the cohort consisting of the remaining 10 sites (n=1,595). Second, we added disease duration, disease subtype, gender and site as covariates, in addition to age, to the models to assess for any differences in results. Third, we compared accuracy parameters for best diagnostic marker from the whole group and from stratified cohorts based on disease characteristics and different devices to evaluate robustness of the data and impact of technical variability. Comparison of the area under the curve (AUC) of the ROC curves was performed as a paired comparison accounting for inter-patient correlation, when data was available for comparisons within the same patients, using the 'roccomp' command in Stata. This command is modeled after DeLong et al.'s nonparametric approach to analyze the difference between curves accounting for the correlated nature of the data[41]. When two separate groups were compared, independent groups comparison was used to compare the AUC of the ROC curves. Fourth, as PMS patients may have longer disease duration and are more likely to have retrograde degeneration, analysis excluding these patients was performed.

Statistical significance was defined as $p<0.05$ and statistical analyses were performed using Stata 15.1 (Stata Corp, College Station, TX).

Results

Study Cohort

The subject inclusion process is demonstrated in **Figure 1.** The initial cohort included 2,021 patients: 13 were excluded for having acute ON, 12 were excluded for being under 18 years of age, 21 were excluded for having pRNFL measurements in only one eye, 67 were excluded for having a diagnosis other than MS (NMO, anti-MOG), and 3 controls were excluded for having pRNFL and GCIPL values lower than feasible for a healthy control (<75 for pRNFL, <50 for GCIPL). A total of 1,898 subjects had pRNFL measurements in both eyes and were included for analysis. Of those, 1,173 subjects had GCIPL measurements available, and only those with measurements for both eyes were included (n=1,166) in GCIPL inter-eye difference analyses. A total of 1, 616 subjects had monocular high-contrast vision assessed (881 had bilateral measurements) and 1, 107 had monocular low-contrast acuity assessed (879 had bilateral measurements). There were 1301 RRMS, 99 SPMS, 60 PPMS, 16 PRMS, 44 CIS, and 13 radiologically isolated syndrome (RIS) patients, as well as 6 with unknown subtypes.

Characteristics of the study cohort are shown in **Table 1**. The MS cohort was slightly older, and had a higher percentage of females ($p<0.0001$). The cohort is representative of what is expected for an MS cohort with expected differences between eyes affected and unaffected by prior episodes of ON. Patients with MS (n=1,530) compared to healthy controls (n=368) had reduced mean pRNFL (87.2 ± 15.3 microns vs. 97.4 ± 9.6 microns, $p\leq0.0001$, GEE models accounting

for age and within-patient, inter-eye correlations) and GCIPL thicknesses (70.0 ± 10.7 microns vs. 81.5 ± 7.4 microns, $p<0.0001$). MS patients with a history of unilateral acute optic neuritis compared to those without an optic neuritis history had thinner pRNFL (78.9 ± 15.1 microns vs. 89.3 ± 14.5 microns, $p<0.0001$, GEE models accounting for age and within-patient, inter-eye correlations) and GCIPL thicknesses (63.5 ± 10.3 microns vs. 72.4 ± 9.8 microns, $p<0.0001$). Average inter-eye pRNFL thickness differences (absolute values) were greater among MS patients than healthy controls (7.1 ± 8.5 vs. 2.8 ± 2.5 microns, $p<0.0001$, linear regression accounting for age) and among MS patients with history of optic neuritis compared to MS patients without an optic neuritis history (11.4 ± 10.7 vs. 4.6 ± 6.0 microns, $p<0.0001$). Similarly, inter-eye GCIPL thickness differences were greater in the MS cohort than healthy controls (5.4 ± 6.4 vs. 1.1 ± 1.2 microns, $p<0.0001$, linear regression accounting for age) and among MS patients with a history optic neuritis compared to those without (8.9 ± 7.8 vs. 3.1 ± 4.3 microns, $p<0.0001$) (**Table 1)**.

Inter-Eye Difference Thresholds

Among healthy controls, the 95th percentile value, or upper boundary of expected values, was 7 microns for pRNFL thickness inter-eye difference and 3 microns for GCIPL thickness inter-eye difference. The 99th percentile value was 12 microns for pRNFL thickness and 5 microns for GCIPL thickness. Within the MS cohort, receiver operating characteristic (ROC) curve analysis was performed and demonstrated an optimal inter-eye pRNFL difference threshold of 5 microns

(area under ROC curve=0.74) and inter-eye GCIPL difference threshold of 4 microns (area under ROC curve=0.77) for identifying patients with history of unilateral optic neuritis (n=477) (**Figure 2**, **Table 2**). **Table 3** displays sensitivity, specificity, positive predictive value and negative predictive value of the 6 candidate diagnostic markers based on two estimates (pRNFL and GCIPL) and two mentioned strategies. These data also confirmed (from the previously-published single-site study) [35] the 5-micron threshold for inter-eye difference in pRNFL thickness for detecting a unilateral optic neuritis. The relative risk for a patient with MS having an acute unilateral optic neuritis history vs. not having a history of acute optic neuritis was 2.6 (95% CI 2.2, 3.2, $p<0.0001$, chi-square test) for the 5-micron inter-eye difference threshold for pRNFL thickness and 3.1 (95% CI 2.5, 3.9, $p<0.0001$, chi-square test) for the 4-micron inter-eye difference threshold for GCIPL thickness (**Table 3)**. All six threshold values for OCT measures (95^{th} and 99^{th} percentiles for inter-eye differences for controls and ROC curve analysis for pRNFL and GCIPL inter-eye differences in MS) were able to significantly identify a history of unilateral optic neuritis in patients with MS ($p<0.0001$, logistic regression accounting for age).

Comparisons of ROC curve analysis models

Although both pRNFL and GCIPL were able to significantly identify a history of unilateral optic neuritis, comparison of the area under the curve (AUC) for subjects who had both pRNFL and GCIPL (n=779) inter-eye measurements showed GCIPL to be a better model (p=0.0005).

Comparing RRMS and CIS patients (combined because there were too few CIS subjects to analyze separately) with progressive MS patients (PMS) showed both pRNFL and GCIPL to be better models in CIS/RRMS than PMS. GCIPL remained significantly associated with a unilateral ON history in both CIS/RRMS (p<0.0001) and PMS (p=0.02); pRNFL remained significantly associated with a unilateral ON history in CIS/RRMS (p<0.0001), but not PMS (p=0.30).

Sensitivity Analyses

Both the sensitivity analysis excluding subjects in the original cohort upon which the statistical model was developed and the sensitivity analysis adding disease duration, disease subtype, gender and site as covariates in the statistical models produced similar results as the original model.

Inter-site/Inter-machine Comparability

To assess agreement between machines, a separate analysis comparing Spectralis and Cirrus OCT was performed **(Table 4)**. Mean thickness values for pRNFL and GCIPL were significantly different between machines; however the inter-eye differences were not. ROC curve analysis identified similar optimal thresholds and AUCs in both pRNFL and GCIPL inter-eye differences for identifying a history of unilateral optic neuritis in both Spectralis and Cirrus

OCT. Sensitivity analyses adding site as a covariate in the statistical models did not significantly change any results, which also suggests comparability between sites and thus machines.

Relapsing MS Cohort sensitivity analysis

Excluding subjects with progressive MS, there were 1, 169 subjects with RNFL measurements and 660 with GCIPL measurements remaining in this group with a similar disease duration of 5.8 years (0-42 range). The results were similar with an optimal RNFL threshold of 5-microns (ROC curve 0.7623) and optimal GCIPL threshold of 4-microns (ROC curve 0.7938).

Correlation of Inter-Eye Difference Thresholds with Visual Assessments

Greater inter-eye differences in high- and low-contrast acuity scores were associated with greater inter-eye pRNFL and GCIPL thickness differences in the MS cohort ($p \leq 0.001$, linear regression accounting for age) (**Figure 3)**. For every 1-micron increase in inter-eye GCIPL difference, inter-eye 2.5% LCLA difference increased by 0.64 letters, and inter-eye high-contrast visual acuity difference increased by 0.41 letters in the MS cohort. In the MS cohort, for every 1-micron increase in inter-eye pRNFL difference, inter-eye 2.5% LCLA difference increased by 0.47 letters, and inter-eye high-contrast visual acuity difference increased by 0.38 letters. A reduction of 7 letters in 2.5% low-contrast acuity is considered clinically significant[9, 40], and this translates to an 11-micron increase in GCIPL inter-eye difference and a 15-micron increase in pRNFL inter-eye difference. A 5-letter reduction in high-contrast acuity is considered clinically

significant and this translates to a 12-micron increase in GCIPL inter-eye difference and a 13-micron increase in pRNFL inter-eye difference.

Binocular summation, defined as a binocular acuity score of 7 letters or greater than the patient's better eye monocular acuity measurement [42], was associated with lower inter-eye pRNFL and GCIPL differences in MS subjects for both 2.5% and 1.25% LCLA scores ($p<0.01$). For each 1-micron increase in inter-eye pRNFL thickness difference, patients with MS had a 4% reduction in likelihood of binocular summation for 2.5% LCLA ($p=0.007$, logistic regression accounting for age); for each 1-micron increase in inter-eye GCIPL thickness difference, MS patients had an 8% reduction in likelihood of binocular summation for 2.5% LCLA ($p<0.0001$, logistic regression accounting for age).

Cohort of MS subjects with inter-eye OCT differences above the threshold and no reported history of optic neuritis

In the MS cohort, 541 subjects had an inter-eye difference in pRNFL greater than 5-microns, 301 (55%) of which reported a history of unilateral optic neuritis, while 240 (45%) did not. These 240 patients with pRNFL inter-eye difference above 5-microns and no reported history of ON represent 18% of the entire MS cohort (n=1331, excluding those with bilateral ON or history of ON that was not known). For those with GCIPL measurements available, 271 had an inter-eye difference above the 4-micron threshold, 178 (66%) of which reported a history of optic neuritis

while 93 (34%) reported no history of optic neuritis. These 93 patients with a GCIPL inter-eye difference above 4-microns and no reported history of optic neuritis represent 12% of the larger MS cohort that had GCIPL measurements available (n=779, excluding those with bilateral ON or unknown history of ON).

Of the 60 PPMS patients, 19 had an inter-eye difference above the 5-micron threshold for RNFL thickness, and 9 had an inter-eye difference above the 4-micron threshold.

The subgroup of subjects with pRNFL inter-eye difference above 5-microns and no reported history of ON (n=240) and those with a GCIPL inter-eye difference above 4-microns and no reported history of optic neuritis (n=93) were similar in age, EDSS, disease duration, and high- and low-contrast visual acuities as the overall MS cohort.

The 240 subjects with an inter-eye pRNFL difference above 5 microns and no history of optic neuritis demonstrated an increased difference in LCLA with an increase in inter-eye pRNFL difference (2.5% contrast, $p<0.0001$; 1.25% contrast, $p=0.032$), but not for high-contrast acuity ($p=0.115$).

The 93 subjects with an inter-eye GCIPL difference above 4 microns but no history of ON also demonstrated an increase in inter-eye low-contrast visual acuity with an increase of inter-eye

GCIPL difference (2.5% contrast, p=0.001; 1.25% contrast p=0.025). The association of inter-eye GCIPL and inter-eye high-contrast visual acuity trended towards significance at p=0.071.

In the overall MS cohort, the thinner eyes of subjects were significantly different from their contralateral eyes (with thicker OCT values) for high- and low-contrast visual acuities ($p<0.0001$), but in this smaller cohort, there was no significant difference except for trend to significance for 2.5% LCLA (p=0.056 pRNFL and p=0.061 GCIPL)

Discussion

Results of this study demonstrate that several thresholds for inter-eye pRNFL and GCIPL thickness difference are able to identify a clinical history of unilateral optic neuritis, with the ideal threshold at which sensitivity and specificity are optimized being 5-microns for pRNFL inter-eye difference thickness and 4-microns for GCIPL inter-eye difference thickness. Inter-eye differences in pRNFL and GCIPL correlate with inter-eye differences in visual assessments. For these reasons, these cut-offs are of clinical relevance and should be considered surrogates of the minimal clinically important difference (MCID) for clinical trials.

These results are consistent with preliminary data that suggested [35] that an inter-eye difference of 5 microns in pRNFL thickness is optimal for identifying a history of unilateral acute optic neuritis, and thus an optic nerve lesion in MS. Importantly, these findings are the results of an

international collaborative effort among sites whose methodologies may have differed slightly, yet yielded consistent results. Furthermore, given the strong associations of increased GCIPL inter-eye differences with reduced visual function observed in this cohort, our investigation emphasizes the importance of measuring GCIPL as a sensitive marker of an optic nerve lesion in MS. The inter-eye cutoff of 4 microns for GCIPL also clearly separated those with an optic nerve lesion from those without a history of acute optic neuritis.

The mean inter-eye pRNFL thickness difference in healthy controls was 2.8 ± 2.5 microns which correlates with previously published OCT intra-subject variability of 2-3 microns[35, 43]. Although the intra-subject variability for OCT is 2-3 microns, the threshold of 5 microns in pRNFL measurements stems from group data, rather than individual data. Since the 5-micron threshold is based on group data, this measurement is more precise than for individual measurements, and the 2-3 micron variability of individual measurements is not as relevant.

A previous study by Coric et al. of 296 patients showed that an inter-eye difference percentage of 5% in pRNFL, and even more so GCIPL thickness, is of diagnostic value for MS-associated optic neuritis [36]. The mean GCIPL thickness value for our MS cohort was 70.0 ± 10.7 µm. An inter-eye difference of 4 microns in GCIPL translates into a 5.7% inter-eye difference, which is similar to the previous study. The mean pRNFL thickness value for our MS cohort was 87.2 ± 15.3 µm, making the inter-eye difference of 5-microns, also 5.7%.

While both pRNFL and GCIPL inter-eye differences are associated with a history of unilateral optic neuritis, GCIPL performed better when directly comparing models. Additionally, specificity and positive predictive value were higher for GCIPL at the 4-micron threshold when compared to the 5-micron threshold for pRNFL, while sensitivity and negative predictive value remained similar. When increasing the threshold values, both pRNFL and GCIPL increase in specificity and decrease in sensitivity as would be expected. However, the increase in specificity with GCIPL thresholds is not accompanied by such a decrease in sensitivity as noted for pRNFL. Due to the known fluctuations (such as edema) in pRNFL, particularly earlier in the disease, GCIPL may be a more stable marker than pRNFL.

The presence of an optic nerve lesion, defined by clinical examination, MRI, OCT, or visual-evoked potential (VEP) was recently recommended for inclusion in the diagnostic criteria for MS as evidence of dissemination in space by an expert consensus of the Magnetic Resonance Imaging in Multiple Sclerosis (MAGNIMS) study group[44]. However, when the International Panel on Diagnosis of Multiple Sclerosis met in 2017 to revise the 2010 McDonald criteria for MS diagnosis, they believed that the data for incorporating the optic nerve as a lesion site were insufficient [1] and identified this as a high priority area for ongoing and future research.

Brownlee et al. retrospectively analyzed a cohort of 160 patients with CIS followed over an average of 15 years. They found that adding symptomatic optic nerve lesions on MRI as evidence of dissemination in space (DIS), when combined with evidence of dissemination in time (DIT), increased the sensitivity, accuracy, positive predictive value and negative predictive value of the revised 2017 McDonald criteria while specificity remained the same[45]. Adding asymptomatic optic nerve lesions did not change these values; however, there were only three subjects in the cohort with evidence of asymptomatic optic nerve lesions as measured by VEP. This sample size may not have been enough to detect an effect. Larger studies, including those utilizing OCT, are needed to evaluate the value of including asymptomatic optic nerve lesions in the diagnostic criteria for MS.

The diagnostic criteria for MS are weighted heavily on MRI results[46]. However, while MRI can often detect a symptomatic optic nerve lesion, MRI performs poorly in assessing asymptomatic optic nerve involvement[33, 47]. In a study by Sisto et al[33], MRI did not show any optic nerve lesions in either symptomatic or asymptomatic patients, while VEP was abnormal in 54.4% of the asymptomatic patients. It is possible that part of the abnormal VEPs could be due to posterior pathway pathology such as optic radiation lesions. There is large subclinical involvement of the entire visual pathway in MS, and the bilateral abnormal findings in VEP and MRI of non-ON patients in previous studies may also include cases of retrograde trans-synaptic degeneration or progressive axonal loss without focal lesion. Since we have included here the inter-eye

difference asymmetry instead of "abnormality," the possibility of posterior pathway disease is significantly reduced. OCT has an approximately 1000-fold greater resolution than MRI, and reliably detects asymptomatic optic nerve degeneration[9, 10]. Of course, other causes of pRNFL and GCIPL thinning as summarized in the OSCAR-IB criteria[38] need to be thoroughly excluded when considering inter-eye OCT differences as a diagnostic criterion for MS.

Of the entire MS cohort in our study (excluding those with bilateral ON or an unknown history of ON), 18% had no symptomatic history of optic neuritis and inter-eye differences above the 5-micron threshold value for pRNFL and 12% had no symptomatic history of optic neuritis and inter-eye differences above the 4-micron threshold value for GCIPL. Additionally, these subjects had increased differences in inter-eye visual acuities with increased inter-eye OCT differences, as well as worse 2.5% low-contrast acuity in the thinner (possibly subclinically affected) eyes, suggesting clinically relevant visual disability in these patients. Further, nearly 1/3 of the PPMS group, which by definition would not have had an episode of optic neuritis, had inter-eye pRNFL difference above the 5-micron threshold. These patients may be considered to have potential subclinical, asymptomatic optic nerve degeneration. Since retinal thinning peaks at 3-4 months after an acute optic neuritis, adding these OCT thresholds to the diagnostic criteria for MS could count not only as dissemination in space, but also dissemination in time for those presenting with an acute symptomatic event of the brain or spinal cord. For instance, a patient with a symptomatic internuclear ophthalmoplegia with associated MRI findings and an inter-eye

difference of 5 microns of RNFL or 4 microns of GCIPL would have lesions that are disseminated in time and space.

A limitation of this study is that the MS cohort is 7 years older than the control group, however age effects on OCT may be significant in decades but not for shorter periods of time[9] and age was adjusted for in all of the statistical models, so this age discrepancy would not be expected to affect the results significantly. Similarly, the MS cohort had more females, however, the sensitivity analysis adjusting for gender did not significantly change the results. Another limitation is that not all measures were available for all participants in the study, since not all sites collected GCIPL or visual acuity assessments. However, OCT measures were the main analysis of this paper, and though only 7 of the 11 sites collected GCIPL, a significant number of subjects (n=1, 173) did have this information available. The missing data was based on sites not collecting the data as opposed to disease severity or other sociodemographic factors. Subjects with missing GCIPL data were similar in age, gender, disease subtype, disease duration, mean pRNFL measurements and inter-eye OCT differences as those with GCIPL measurements available. We do not expect any bias to be introduced from not having this GCIPL information on all subjects, particularly since when we added site as a covariate it did not change the results significantly. Only 7 of the 11 sites performed low-contrast visual acuity assessments, however this would not be expected to affect the results of the main analysis for identifying thresholds for OCT inter-eye differences, since that analysis was not limited to those sites. The analysis

comparing inter-eye OCT differences to inter-eye visual acuities was only performed on those who had these measurements, however we would not expect any bias to be introduced from this since the missing data was not based on disease severity or other demographic factors. Subjects with missing visual acuity data were similar in age, gender, disease subtype, and inter-eye OCT differences as those with visual acuity measurements available. Those with missing low-contrast visual acuity data had slightly longer disease duration than those without (10.2 vs. 6.7 years), however the distribution of their disease subtypes was similar, as were the EDSS values, and their mean pRNFL and GCIPL values, rendering it highly unlikely that this subgroup had more severe optic nerve disease. Those with missing high-contrast acuity data were similar in all characteristics to those without high-contrast acuity data available.

There were 70 MS patients with missing ON history information. These subjects were similar in age, gender, disease subtype, disease duration, and inter-eye OCT differences to the MS cohort with ON history information, so we do not expect results to be biased from the missing ON data.

While pRNFL measurements are comparable in regard to the measured area (Spectralis measures a 3.4° ring scan whereas Cirrus computes the 3.4° ring scan from the ONH volume scan), the area of interest differs in the GCIPL measurements between machines. However, while the mean values varied between machines, our results showed remarkable agreement between the machines when measuring both the inter-eye pRNFL and GCIPL differences. Comparing within eyes of the same subject should overcome potential variability among OCT devices. Further,

ROC curve analysis when separating by machine produced similar threshold values for the 45-degree tangent line. Great agreement between different scanners has been shown in a study comparing cross-sectional or longitudinal OCT at the cohort level[48] and between automated segmentation protocols in a multicenter study[34]. A meta-analysis including 40 studies of OCT in MS showed that OCT is a robust, reproducible and accurate method for quantification of pRNFL and GCIPL[49]. Our study is the largest study to date of inter-eye OCT differences evaluated as diagnostic criterion for optic neuritis, including diverse populations across the US, Europe and Middle East. Our findings align with other studies evaluating the use of OCT in assessing history of optic neuritis and our results are robust to different methods.

In conclusion, a 5-micron difference in inter-eye pRNFL thickness and a 4-micron difference in GCIPL thickness were found to be robust thresholds for determining a history of acute unilateral optic neuritis within an MS cohort. These cut-offs may be used to establish the presence of asymptomatic and symptomatic optic nerve lesions in MS patients. Adopting the optic nerve as a fifth anatomical location to provide evidence of dissemination in space, utilizing established clear criteria such as OCT pRNFL and GCIPL inter-eye difference measurements, may strengthen future revisions of the McDonald criteria for MS diagnosis. Future studies, with sensitivity analyses that substitute the optic nerve for other lesion sites, are needed to evaluate the effect of adding OCT as an imaging criterion for optic nerve degeneration on the statistical measures of the McDonald criteria for diagnosis of MS.

Acknowledgements

LJB was funded by the National Multiple Sclerosis Society RG 4649A5/1. BH was supported by grants from the German Federal Ministry for Education and Research (BMBF), the Multiple MS EU consortium and the SFB CRC 128. JH is (partially) funded by the German Federal Ministry of Education and Research under 01ZZ1603[A-D] and 01ZZ1804[A-H] (DIFUTURE).

Author Contributions

Conception and design of the study: RCN, PAC, FP, AP, LB, AB, EHML, SS, PV, EMF, SLG, LJB

Acquisition, analysis & interpretation of data: RCN, OA, PAC, FP, AP, LB, AB, EHML, SS, PV, AAA, RB, EMF, TF, JH, BH, HJ, BK, TK, LL, AP, MP, HZ, SLG, LJB

Drafting the manuscript: RCN, SLG, LJB

Potential Conflicts of Interest

AP (Petzold) has no disclosures at the time of submission, however in 2019 will give a talk at a Heidelberg Engineering organized symposium, co-organize the joint National Dutch-UK Neuro-ophthalmology meeting in Amsterdam sponsored by Heidelberg Engineering, and will lease a

device from Zeiss acting as a consultant on the leasing contract. Heidelberg Engineering and Zeiss manufacture OCT machines.

PV has received consultancy fees from Heidelberg Engineering (2016) and was member of the scientific advisory committee of OCTIMS study sponsored by Novartis and his center participated in the study.

JH received speaker honoraria from Heidelberg Engineering.

The remaining authors reported no disclosures.

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Table 1: Demographics and clinical characteristics of study participants

		MS Cohort		
	Healthy Controls (n=368)	All MS Patients (n=1530)	MS Patients without ON (n=854)	MS Patients with Unilateral ON (n=477)[§]
Age, years, mean ± SD(range)	35.3 ± 10.8 (18.9 – 73.0)	42.0 ± 11.3 (18.0 – 73.0)	42.9 ± 11.5 (18.9 – 71.9)	40.6 ± 11.0 (18.0 – 73.0)
Sex, n (% female)	195 (53%)	1065 (70%)	584 (68%)	325 (68%)
Relapsing-remitting MS, n (%)	--	1301 (85%)	698 (82%)	424 (89%)
Disease duration, years, median (range)	--	6 (0-42)	6 (0-39)	6 (0-42)
EDSS Median (range)	--	1.5 (0-8)	1.5 (0-8)	1.5 (0-8)
History of acute unilateral ON, n (%)	--	477 (33%)	0 (0%)	477 (100%)
pRNFL thickness, microns, mean ± SD	97.4 ± 9.6	87.2 ± 15.3	90.5 ± 14.2	78.9 ± 15.1
Inter-eye pRNFL difference, microns, mean ± SD	2.8 ± 2.5	7.1 ± 8.5	4.6 ± 6.0	11.4 ± 10.7
GCIPL thickness, microns, mean ± SD	n=259 controls 81.5 ± 7.4	n=907 patients 70.0 ± 10.7	n=481 patients 72.4 ± 9.8	n=298 patients 63.5 ± 10.3
Inter-eye GCIPL difference, microns, mean ± SD	n=259 controls 1.1 ± 1.2	n=907 patients 5.4 ± 6.4	n=481 patients 3.1 ± 4.3	n=298 patients 8.9 ± 7.8

[§] There were 129 patients with bilateral optic neuritis, and 70 with unknown history of optic neuritis not included in the unilateral ON cohort.

Abbreviations: ON = optic neuritis, MS = multiple sclerosis, SD = standard deviation, EDSS = expanded disability status scale, pRNFL= peripapillary retinal nerve fiber layer, GCIPL = ganglion cell + inner plexiform layer

Table 2: ROC curve analysis results for identifying a history of unilateral optic neuritis by inter-eye OCT differences

	MS with History of Unilateral Optic Neuritis (n=477)	
OCT measure	Optimal Threshold‡	Area under ROC curve
Inter-eye pRNFL thickness difference	5.0	0.74
Inter-eye GCIPL thickness difference	4.0	0.77

‡ Tangent Line on the ROC curve where sensitivity and specificity are optimized when assessing inter-eye OCT differences in identifying a history of unilateral optic neuritis. These thresholds were initially identified in the one-site NYU cohort. The ROC curve analysis results presented here are from performing the model on all 11-sites.

Abbreviations: OCT = optical coherence tomography, ROC = receiver operative characteristic, pRNFL= peripapillary retinal nerve fiber layer, GCIPL = ganglion cell + inner plexiform layer

Table 3: Sensitivity and specificity analyses for 6 candidate markers*

	Sensitivity [a]	Specificity [b]	Positive Predictive Value [c]	Negative Predictive Value [d]	*p*-value ‡	Relative Risk of Acute ON History
Inter-eye difference in pRNFL thickness ≥ 5 microns #	71%	65%	53%	80%	*p*<0.0001	2.6 (95% CI 2.2, 3.2)
Inter-eye difference in pPRNFL thickness ≥ 7 microns †	59%	79%	60%	78%	*p*<0.0001	2.7 (95% CI 2.3, 3.1)
Inter-eye difference in pRNFL thickness ≥ 12 microns §	38%	92%	72%	73%	*p*<0.0001	2.7 (95% CI 2.3, 3.1)
Inter-eye difference in GCIPL thickness ≥ 3 microns †	78%	69%	60%	84%	*p*<0.0001	3.7 (95% CI 2.9, 4.8)
Inter-eye difference in GCIPL thickness ≥ 4 microns #	68%	77%	63%	80%	*p*<0.0001	3.1 (95% CI 2.5, 3.9)
Inter-eye difference in GCIPL thickness ≥ 5 microns §	61%	81%	66%	77%	*p*<0.0001	2.9 (95% CI 2.4, 3.5)

* Analysis was performed on the 10-site validation set, excluding the original data that the model was built on, in order to prevent overestimation of confidence intervals and p-values.

‡ P-value from chi-square test

ROC curve analysis optimal threshold value

† 95th percentile value (upper boundary of expected inter-eye difference) for healthy controls

§ 99th percentile value for healthy controls

[a] Sensitivity = probability of having an pRNFL inter-eye difference at or above the threshold value if the patient has a unilateral ON history

[b] Specificity = probability of having an pRNFL inter-eye difference below the threshold value if the patient does not have a unilateral ON history

[c] Positive Predictive Value = probability of having a history of acute unilateral ON if the pRNFL inter-eye difference is at or above the threshold value

[d] Negative Predictive Value = probability of not having a history of acute unilateral ON if the pRNFL inter-eye difference is below the threshold value

Abbreviations: ON = optic neuritis, pRNFL= retinal nerve fiber layer, GCIPL = ganglion cell + inner plexiform layer

Table 4: Comparison of Spectralis and Cirrus OCT machines for pRNFL and GCIPL mean thickness and inter-eye thickness difference measurements

	pRNFL			GCIPL		
OCT	Spectralis	Cirrus	p-value	Spectralis	Cirrus	p-value
Thickness, microns, mean ± SD	92.5 ± 15.0	86.0 ± 13.4	<0.0001 [a]	68.3 ± 10.6	74.4 ± 10.8	<0.0001 [a]
Inter-eye difference, microns, mean ± SD	6.2 ± 8.2	6.0 ± 7.1	0.27	4.5 ± 6.2	4.4 ± 5.9	0.53
Optimal Threshold [b]	5.0	6.0	-	3.7	4.0	-
Area under ROC Curve	0.78	0.69	0.24[c]	0.86	0.73	0.005 [d]

[a] t-test

[b] Tangent Line on the ROC curve where sensitivity and specificity are optimized when assessing inter-eye OCT differences in identifying a history of unilateral optic neuritis

[c] Paired comparison in subjects who had pRNFL measurements on both Cirrus and Spectralis machines, accounting for correlation.

[d] Independent groups comparison. No subjects had GCIPL measurements on both Cirrus and Spectralis machines.

Abbreviations: pRNFL= retinal nerve fiber layer, GCIPL = ganglion cell + inner plexiform layer, ROC = receiver operating characteristic

Figure 1. Patient Inclusion Diagram