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Rate of Retinal Layer Thinning as a Biomarker for Conversion to Progressive Disease in Multiple Sclerosis

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Abstract

Background and Objectives

The diagnosis of secondary progressive multiple sclerosis (SPMS) is often delayed because of the lack of objective clinical tools, which increases the diagnostic uncertainty and hampers the therapeutic development in progressive multiple sclerosis (MS). Optical coherence tomography (OCT) has been proposed as a promising biomarker of progressive neurodegeneration. To explore longitudinal changes in the thicknesses of retinal layers on OCT in individuals with relapsing-remitting MS (RRMS) who converted to SPMS vs matched patients with RRMS who did not convert to SPMS. Our hypothesis is that the 2 cohorts exhibit different rates of retinal thinning.

Methods

From our prospective observational cohort of patients with MS at the American University of Beirut, we selected patients with RRMS who converted to SPMS during the observation period and patients with RRMS, matched by age, disease duration, and Expanded Disability Status Scale (EDSS) at the first visit. Baseline retinal measurements were obtained using spectral domain OCT, and all patients underwent clinical and OCT evaluation every 6–12 months on average throughout the study period (mean = 4 years). Mixed-effect regression models were used to assess the annualized rates of retinal changes and the differences between the 2 groups and between converters to SPMS before and after their conversion.

Results

A total of 61 participants were selected (21 SPMS and 40 RRMS). There were no differences in baseline characteristics and retinal measurements between the 2 groups. The annualized rates of thinning of all retinal layers, except for macular volume, were greater in converters before conversion compared with nonconverters by 112% for peripapillary retinal nerve fiber layer ($p = 0.008$), 344% for tRNFL ($p < 0.0001$), and 82% for cell-inner plexiform layer (GCIPL) ($p = 0.002$). When comparing the annualized rate of thinning for the same patients with SPMS before and after conversion, no significant differences were found except for tRNFL and GCIPL with slower thinning rates postconversion (46% and 68%, respectively).

Discussion

Patients who converted to SPMS exhibited faster retinal thinning as reflected on OCT. Longitudinal assessment of retinal thinning could confirm the transition to SPMS and help with the therapeutic decision making for patients with MS with clinical suspicion of disease progression.

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Glossary

AMIR = American University of Beirut MS Interdisciplinary Research; **EDSS** = Expanded Disability Status Scale; **GCIPL** = cell-inner plexiform layer; **CC** = corpus callosum; **DMT** = disease-modifying therapy; **FLAIR** = fluid-attenuated inversion recovery; **GM** = gray matter; **ICV** = intracranial volume; **IMSVISUAL** = International Multiple Sclerosis Visual System Consortium; **INL** = inner nuclear layer; **OCT** = optical coherence tomography; **pRNFL** = peripapillary retinal nerve fiber layer; **RRMS** = relapsing-remitting MS; **RNFL** = retinal nerve fiber layer; **SPMS** = secondary progressive MS; **SDMT** = symbol digit modality tests; **STROBE** = Strengthening the Reporting of Observational Studies in Epidemiology; **tRNFL** = temporal retinal nerve fiber layer; **WM** = white matter.

The progressive phase of multiple sclerosis (MS) is associated with gradual and irreversible accumulation of neurologic deficits.[1,2] During the early disease stages, the inflammatory demyelinating processes predominate, although insidious relapse-independent progression can be observed.[3] The diagnosis of secondary progressive MS (SPMS) is solely based on the judgment of the treating clinician, which delays the diagnosis for up to 3 years,[4,5] especially because no definitive diagnostic tool that clearly detects disease progression exists in clinical practice.[6,7] This diagnostic uncertainty is of great relevance today with the emergence of novel disease-modifying therapies (DMTs) that can slow disease progression if initiated early.[8,9]

Optical coherence tomography (OCT) is a noninvasive imaging technique depicting retinal topography that was recently introduced into the field of MS.[5,6] There is growing evidence of the beneficial use of the retina as a surrogate markers of CNS inflammation and degeneration in observational studies using OCT.[10] There are significant correlations between changes in thickness of the ganglion cell-inner plexiform layer (GCIPL) and the peripapillary retinal nerve fiber layer (pRNFL) with clinical and radiologic characteristics of MS, including physical disability measured by Expanded Disability Status Scale (EDSS), cognitive impairment,[11,12] and whole-brain atrophy.[13,42] Furthermore, cross-sectional OCT measurements are also useful as predictive biomarkers of disease activity and long-term disability worsening reflected by EDSS scores.[14-16] Nevertheless, the utility of OCT in routine clinical practice is still limited by the scarcity of longitudinal assessments of retinal changes correlated with disease progression because most studies on retinal changes are either cross-sectional or encompass a short follow-up period.[15-17] Faster retinal atrophy was recently reported in progressive MS independently of the effects of aging.[18] The aim of our study was to evaluate retinal OCT as a biomarker for the transition from relapsing-remitting MS (RRMS) to SPMS.

In this study, we evaluated the longitudinal retinal changes in patients with MS before and after the transition to SPMS. The primary objective of this study was to determine whether patients with SPMS exhibited different rates of retinal thinning before conversion compared with patients with RRMS who did not convert to SPMS during the study period. Secondary objectives included[1] determining whether the rate of retinal thinning in patients with MS who convert to SPMS is different before conversion to SPMS compared with after conversion and[2] evaluating the correlation between retinal changes on OCT and brain atrophy measures on MRI.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

American University of Beirut MS Interdisciplinary Research (AMIR) is a longitudinal prospective observational study, established in 2012, at the Nehme and Therese Tohme MS Center at the American University of Beirut Medical Center. All patients seen at the MS Center are offered enrollment in AMIR. Enrolled participants have provided written informed consent for participation as required and were followed up between October 2014 and October 2020. This study was approved by American University of Beirut's Institutional Review Board. This study is compliant with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines for data reporting.[19]

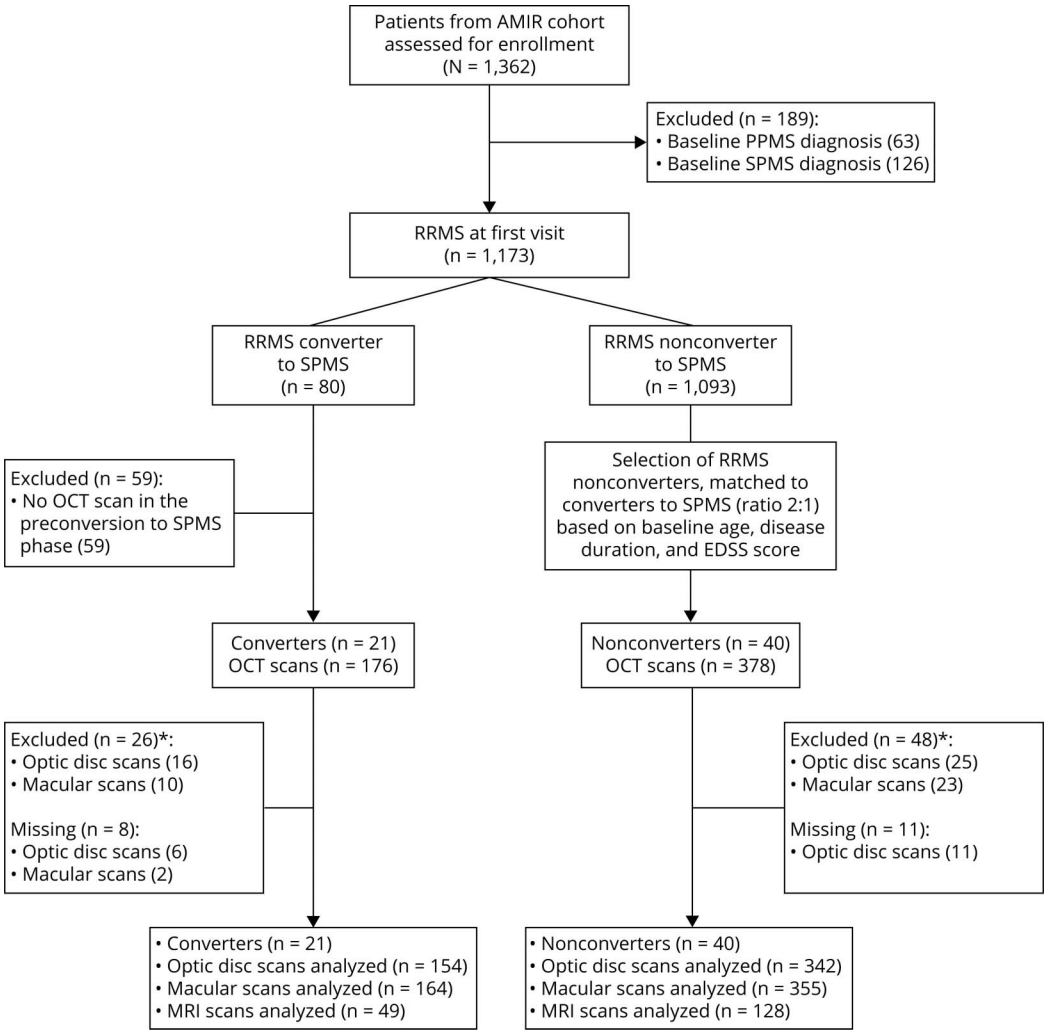
Study Participants

Longitudinal clinical data were collected using REDCap (Research Electronic Data Capture).[20] We identified patients with a diagnosis of RRMS who transitioned to SPMS during the follow-up period. Inclusion criteria were age 18 years or older, confirmed diagnosis of MS by the 2010 revision to the McDonald criteria,[21] minimum follow-up period of 12 months with at least 2 clinic visits, and the presence of at least 2 OCT scans recorded ≥6 months apart for at least 1 eye. Patients who already had a diagnosis of SPMS or primary progressive MS at enrollment into AMIR were excluded. Baseline visit was identified as the first visit with retinal OCT scanning. Participants with a baseline diagnosis of RRMS were categorized 2 groups.

Patients with RRMS patients who converted to SPMS as per the MS specialist (converters) and patients with RRMS who did not convert to SPMS (nonconverters). Converters were further assessed for eligibility; patients who did not have at least 1 OCT scan in the preconversion phase before clinically being labeled as SPMS were excluded. The nonconverters control patients were matched to converters on a 2:1 ratio by age (age 18–40, 41–49, and older than 50 years), disease duration (less than 8, 8–16, 17 or more years), and EDSS score (0–2, 2.5–3.5, 4, or more) at the first OCT scan visit (Figure).

Participants presented for clinical assessment every 6–12 months, with a median (range) time between visits of 6[5-13] months.

Figure Flow Chart Diagram of Study Participants



Demographic and clinical variables as well as retinal layer thicknesses were collected by trained clinicians from all patients and were updated at every visit. Study demographics included age, sex, and education and clinical variables such as the date of symptom onset, date of treatment onset, disease-modifying therapies (DMTs) including first-line medications (interferons, teriflunomide, and dimethyl fumarate) and second-line medications (fingolimod, natalizumab, rituximab, and ocrelizumab), number of relapses at baseline and during follow-up, history of optic neuritis (ON), MRI measurements, and clinical measures including symbol digit modality tests (SDMT), 9-hole peg test time (9-HPT), timed 25-foot walk test (25-FWT), and EDSS scores. We defined EDSS progression[6] as an increase by 1.5 points if the last EDSS score before conversion to SPMS was 0, an increase by 1 point if the EDSS score was between 1 and 5.5, or an increase by 0.5 points if the EDSS score was above 5.5. The EDSS changes were confirmed at 6 months. Eyes with optic neuritis less than 6 months from the first OCT scan were excluded and eyes with optic neuritis during follow-up. The primary outcome parameter was conversion to SPMS based on the treating clinician's impression of the clinical disease course of each patient and according to Lublin revision of the definition of progressive MS.[22]

Retinal OCT Scans

Retinal layers were measured using spectral domain OCT scans with the Cirrus high-definition OCT device (model 5000) with software version 10.5 (Carl Zeiss Meditec, Dublin, CA) and included total peripapillary retinal nerve fiber layer (pRNFL), temporal quadrant RNFL (tRNFL), macular GCIPL and inner nuclear layer (INL) (all in microns), and macular volume (MV) (in mm^3). Atrophy rates of each layer were estimated for each eye using all eligible OCT scans and presented as annualized atrophy rates (microns/year).[23]

Peripapillary data were retrieved with the Optic Disc Cube 200 × 200 protocol, and macular data were retrieved with the Macular Cube 512 × 128 protocol. Tracking software was used to confirm correct fixation. Retinal scans that did not fulfill the OSCAR-IB criteria[24] were excluded including scans with artefacts and scans with signal strength less than 6/10 (Figure 1).

OCT methods and results were reported in accordance with the consensus Advised Protocol for OCT Study Terminology and Elements recommendations.[25] Macular cube scans were further analyzed in a blinded fashion using segmentation software developed and validated at Johns Hopkins University by Lang et al.[26,27] Segmentation of the macular cube scans performed in 3-D yielded the thicknesses of GCIPL and INL.[26-28]

MRI Measurements

MRI acquisitions were performed in 2 centers; the first (Center A) equipped with a 3-T Philips Ingenia MR System and the second (Center B) with a 3-T Siemens Magnetom Verio MR System. A standardized conventional MR imaging protocol was used.[29,30]

An experienced operator (S.H.) first performed a quality control step on all acquired images to rule out and exclude any images with major artefacts that could implicate an error during the processing steps. A bias field correction of both the T1 and fluid-attenuated inversion recovery (FLAIR) images was applied using the N4-algorithm in 3D Slicer (slicer.org/). A whole-brain extraction using the Brain Extraction Tool of the FMRIB Software Library (FSL5.0) was then applied to measure the intracranial volume (ICV) for normalization purposes.[31] T2 lesions were then automatically segmented using the lesion growth algorithm of the Lesion Segmentation Toolbox 3.0.0 found in the Statistical Parametric Mapping SPM12 software.[32] The resulting lesions mask was used to perform lesion filling on both the T1 and FLAIR images. The segmentation tool of SPM12 was run on the previously processed T1 images using the default settings to automatically obtain the gray matter (GM), white matter (WM), and CSF volumes as described elsewhere.[33] The lesion filled T1-weighted images were then fed into FIRST which is a model-based segmentation/registration tool that uses manually segmented models to obtain subcortical GM volume measurements of the thalamus.[34] An atlas-based segmentation was performed to extract the corpus callosum (CC) volume. The Johns Hopkins University–International Consortium of Brain Mapping atlas was nonlinearly registered to each patient's T1 image using the NiftyReg software.[35] The resulting transformation was applied on the atlas labels mask from which the CC was extracted. All extracted masks were checked for accuracy, manually corrected if needed, and had their volumes extracted and normalized by the ICV.

Statistical Analyses

Analyses were performed using the statistical package for social sciences software (SPSS version 25 Armonk, NY: IBM Corp.) and STATA version 13 (StataCorp, College Station, TX).

We assessed the normality of demographic and clinical variables using the Shapiro-Wilk test. Bivariate analyses for continuous variables were performed using the Kruskal-Wallis test or independent *t* test for non-normal and normally distributed data, respectively. The χ^2 test was used for categorical variables to determine the differences between the study groups at baseline. Statistical significance at the bivariate level was depicted with a *p* value of less than 0.05.

Mixed-effects regression models were used to compare baseline differences in retinal measurements and their rates of annualized change between different groups. In all those models, age, EDSS, gap time (defined as the time between initiation of DMT and the date of the OCT scan), and number of relapses at baseline and during follow-up were used as continuous variables, while sex, disease duration, DMT at each visit (current DMT), and history of ON at baseline were used as categorical variables. Gap time was included to control for any confounding effects of the DMT on retinal thinning among patients considering that certain DMTs require more time to optimize their therapeutic effects than others.

All analyses of longitudinal changes were performed using mixed-effects models controlling for the relevant variables identified in bivariate analyses including age, sex, disease duration, current DMT, and history of ON as fixed effects with random intercepts for patients and eyes controlling for within-patient intereye correlations and random slope for the follow-up time. Annualized changes in retinal layer thicknesses (microns/year) in the preconversion phase of converters were compared with those of RRMS nonconverters and to those of the patients themselves after conversion to SPMS for the annualized retinal layer changes (microns/year) only. Missing values were excluded from the analyses. We also did sensitivity analyses on the annualized retinal changes excluding the patients with a history of ON from both cohorts (10 eyes from converters and 6 eyes from nonconverters).

p values in all mixed-effects regression models were Bonferroni adjusted for multiple comparisons to determine statistical significance (<0.01).

We finally explored the correlation between longitudinal retinal changes on OCT and brain volume changes on MRI among all patients during the entire observation period to ascertain how retinal OCT changes reflect overall brain MRI measurements. Annualized changes were computed for all OCT-derived and MRI-derived measures per participant and eye while controlling for intereye correlation, and correlation coefficients were obtained using generalized estimating regression models. No other clinical or demographic covariates were included in this exploratory analysis because the goal was to only to explore the clinical correlation between these 2 biomarkers. Correlations with *p* values <0.05 were considered statistically significant.

Data Availability

Anonymized data will be made available by request from the corresponding author.

Results

Study Population

Sixty-one participants were included in our study, 21 of whom were converters to SPMS and 40 were RRMS nonconverters matched by age, disease duration, and EDSS score at first OCT scan (Figure 1). The mean time of follow-up of all

participants with OCT scan was 4 years, and there was no difference between the 2 cohorts (p = 0.661). The median (range) number of visits was 3.[2-8] Our study included 41 female participants (67%), and the mean age at baseline was 41 years. There were no differences at baseline between the 2 groups in age (p = 0.767), disease duration (p = 0.171), EDSS score (p = 0.099), DMT (p = 0.4), and number of relapses (p = 0.211). However, the group of SPMS converters had a higher proportion of eyes with a history of ON (p = 0.033) at baseline compared with RRMS nonconverters (Table 1).

Baseline Retinal Layer Measurements

Baseline retinal layer thickness and comparisons between the 2 study groups are presented in Table 2. There were no differences in all retinal measurements at baseline including pRNFL (85.8 ± 7.1 μm vs 81.6 ± 6.4 μm, p = 0.093), tRNFL (48.6 ± 8.8 μm vs 48.7 ± 7.9 μm, p = 0.985), GCIPL (65.4 ± 5.7 μm vs 64.6 ± 5.1 μm, p = 0.683), and MV (9.56 ± 0.27 mm^3 vs 9.47 ± 0.25 mm^3, p = 0.345) between converters and nonconverters after controlling for age, sex, EDSS score, disease duration, DMT, history of ON, and gap time.

Table 1 Clinical Characteristics of Study Participants at Baseline

Clinical characteristics	Converters (n = 21) Eyes (n = 42)	Nonconverters (n = 40) Eyes (n = 80)	p Value[a]
Disease duration at first OCT in years, median (range)	7 (2–32)	8 (1–25)	0.171[b]
EDSS score, no. (%)			
≤1	1 (4.8%)	7 (17.5%)	
1.5–3.5	17 (81%)	32 (80%)	0.099[c]
≥4	3 (14.2%)	1 (2.5%)	
Age at first OCT visit in years, mean (SD)	41.71 (9.38)	41 (8.64)	0.767[d]
No. of relapses at first OCT scan			
≤2	7 (33.3%)	21 (52.5%)	0.211[c]
3–5	8 (38%)	14 (35%)	
≥6	6 (28.7%)	5 (12.5%)	
Total time of follow-up with OCT between first and last OCT scan in years, mean (SD)	3.96 (1.56)	4.12 (1.31)	0.661[d]
Eyes with chronic history of ON, no. (%)	10 (23.8%)	6 (7.5%)	0.033[a,c]
DMT no. (%)			
First-line DMT	10 (48%)	17 (43%)	0.4
Second-line DMT	9 (43%)	13 (33%)	

Abbreviations: DMT = disease-modifying therapy; OCT = optical coherence tomography; ON = optic neuritis.
[a] Statistically significant if p value < 0.05.
[b] Kruskal-Wallis test.
[c] Chi-square.
[d] Independent t test.

Longitudinal Changes in Retinal OCT Measurements

Longitudinally, over a mean (SD) follow-up time of 4.12 (1.31) years, both nonconverters and converters in the preconversion phase showed significant annualized reduction of pRNFL thickness compared with baseline; however, only converters exhibited significant annualized thinning of GCIPL (Table 3). Converters exhibited faster annualized mean reduction of most of the retinal layers than nonconverters: pRNFL (−1.1 ± 0.24 μm/y vs −0.52 ± 0.17 μm/y; difference = −0.58 ± 0.22 μm/y, p = 0.008), tRNFL (−2.62 ± 0.29 μm/y vs −0.59 ± 0.2 μm/y; difference = −2.03 ± 0.26 μm/y, p < 0.0001), and GCIPL (−1.11 ± 0.14 μm/y vs −0.19 ± 0.1 μm/y; difference = −0.91 ± 0.11 μm/y, p < 0.0001). Only converters had annualized decrease in MV thickness before conversion to SPMS of −0.04 ± 0.01 mm^3/y (p = 0.001), but the difference between groups for MV change did not reach statistical significance (p = 0.05). There were no significant differences in the annualized thinning of INL and outer nuclear layer between the 2 groups. Notably, gap time, EDSS score, and the number of relapses at baseline and during follow-up did not have any effect on the slopes of the annualized retinal changes. After excluding the eyes of patients with a history of ON in a sensitivity analysis (eTable 1, links.lww.com/NXI/A742), converters exhibited faster annualized GCIPL thinning compared with nonconverters (−0.88 ± 0.13 μm/y, p < 0.0001); however, there was no difference in the annualized pRNFL thinning between converters and nonconverters (p = 0.055).

Moreover, we evaluated the differences in the annualized changes of retinal measurements in converters before and after their conversion to SPMS (Table 4). Converters exhibited faster annualized tRNFL thinning before conversion compared with after conversion (−2.09 ± 0.35 μm/y vs −1.12 ± 0.39 μm/y; difference = 0.97 ± 0.26 μm/y, p < 0.0001). Similar observations were evident for GCIPL thinning in the period right before conversion to SPMS compared with after conversion (−1.02 ± 0.27 μm/y vs −0.33 ± 0.32 μm/y; difference = 0.69 ± 0.24 μm/y, p = 0.004). There were no significant differences in annualized thinning rates of pRNFL, INL, and MV between the preconversion and postconversion periods.

Longitudinal Changes in MRI Brain Volumes

In this subgroup analysis, we explored the annualized changes in different MRI measurements of 33 nonconverters and 18 converters who had available MRIs (Table 5). Both converters and nonconverters exhibited annualized decrease in their GM volume with faster annualized decrease in converters (−1.76 ± 0.34 mm^3/y vs −0.79 ± 0.09 mm^3/y; p = 0.005). In addition, both converters (p < 0.0001) and nonconverters (p = 0.005) showed annualized reduction in WM volume; however, there was no difference in the rates between the 2 groups (p = 0.08). Furthermore, both groups demonstrated an increase in CSF volume with faster annualized increase in converters (3.0 ± 0.6 mm^3/y vs 1.28 ± 0.15 mm^3/y; p = 0.003). Although there

Table 2 Baseline Retinal OCT Measurements of Participants by MS Subtypes

Variable	MS subtype (total n = 61; converters = 21, nonconverters = 40)		Difference between groups[a] *p* value
	Nonconverters (n = 40)	Converters (n = 21)	
Baseline pRNFL thickness (μm); mean (SD)	81.56 (6.40)	85.80 (7.13)	4.24 (2.53) 0.093
Baseline tRNFL thickness (μm); mean (SD)	48.68 (7.92)	48.62 (8.82)	−0.06 (3.12) 0.985
Baseline GCIPL thickness (μm); mean (SD)	64.58 (5.08)	65.41 (5.66)	0.83 (2.03) 0.683
Baseline INL thickness (μm); mean (SD)	46.42 (1.56)	47.02 (1.69)	0.6 (0.61) 0.321
Baseline MV (mm3); mean (SD)	9.47 (0.25)	9.56 (0.27)	0.09 (0.10) 0.345

Abbreviations: GCIPL = ganglion cell-inner plexiform layer; INL = inner nuclear layer; MV = macular volume, with random intercepts for patient and eye, and random slope for time of follow-up; pRNFL = peripapillary retinal nerve fiber layer; tRNFL = temporal retinal nerve fiber layer.
[a] Mixed-effects regression exploring differences in baseline retinal measurements between cohorts, controlling for age, sex, EDSS score, disease duration, current DMT (categorical), history of ON per eye, and gap time.

was an overall decrease in total thalamic ratio, it did not reach statistical significance.

Correlations Between Longitudinal OCT and MRI Measurements

To ascertain whether retinal OCT measures reflect brain atrophy, we explored the correlations between longitudinal retinal changes and changes in the volumes of different brain compartments on MRI among all study participants and during the entire observation period (Table 6). Longitudinally, annualized pRNFL thinning was correlated with annualized decrease in intracranial volume (r = 0.267, p = 0.012), WM volume (r = 0.376, $p < 0.0001$), GM volume (r = 0.223, p = 0.036), total thalamic volume (r = 0.433, $p < 0.0001$), CC volume (r = 0.253, p = 0.017), and with annualized increase in CSF volume (r = 0.285, p = 0.007). Moreover, annualized GCIPL atrophy was correlated with annualized changes in all MRI-derived brain compartments except for annualized decrease in intracranial volume (r = 0.039, p = 0.703). Finally, annualized INL atrophy was associated with annualized thalamic (r = 0.202, p = 0.049) and GM (r = 0.274, p = 0.007) atrophy.

Progression of Clinical Outcomes

We analyzed the clinical progression of participants over the follow-up period (eTable 2, links.lww.com/NXI/A742). Approximately 76% of the converters to SPMS experienced EDSS score progression as previously described while only 13% of the nonconverters experienced EDSS progression ($p < 0.0001$). Furthermore, a higher number of converters exhibited worsening

Table 3 Annualized Change of Retinal Layer Thicknesses in RRMS Converters to SPMS Before Conversion Compared With RRMS Nonconverters (Controls)

Annualized change, mean (SD) *p* value[a] (within-group testing for annualized change being different than 0)	MS subtype (total n = 61; converters = 21, nonconverters = 40)		Difference in annualized changes between groups[b] p value[a]
	Nonconverters (n = 40)	Converters (n = 21)	
pRNFL (μm/y)	−0.52 (0.17) 0.002[a]	−1.10 (0.24) $p< 0.0001$[a]	−0.58 (0.22) 0.008[a]
tRNFL (μm/y)	−0.59 (0.20) 0.004[a]	−2.62 (0.29) $p< 0.0001$[a]	−2.03 (0.26) $p< 0.0001$[a]
GCIPL (μm/y)	−0.19 (0.10) 0.057	−1.11 (0.14) $p< 0.0001$[a]	−0.91 (0.11) $p< 0.0001$[a]
INL (μm/y)	−0.08 (0.04) 0.051	−0.16 (0.07) 0.019	−0.08 (0.07) 0.227
MV (mm^3/y)	−0.02 (0.01) 0.012	−0.04 (0.01) 0.001[a]	−0.02 (0.01) 0.046

Abbreviations: GCIPL = ganglion cell-inner plexiform layer; INL = inner nuclear layer; MV = macular volume, with random intercepts for patient and eye, and random slope for time of follow-up; pRNFL = peripapillary retinal nerve fiber layer; tRNFL = temporal retinal nerve fiber layer.
[a] Statistically significant with a p value <0.01 after Bonferroni adjustment for multiple comparisons.
[b] Mixed-effects regression, controlling for age, sex, disease duration at first visit, history of ON, and current DMT, with random intercepts for patient and eye and random slope for time of follow-up.

Table 4 Annualized Change of Retinal Layer Thickness in Patients With RRMS Who Converted Into SPMS Before Their Conversion Compared With the Same Patients After Their Conversion

Annualized change, mean (SD)	MS subtype (converters n = 21)		
p value[a] (within-group testing for annualized change being different than 0)	Converters preconversion (n = 21)	Converters postconversion (n = 21)	Difference in annualized changes between groups[b] *p* value[a]
pRNFL (μm/y)	−1.07 (0.27) $p < 0.0001$[a]	−0.89 (0.32) 0.005[a]	0.18 (0.22) 0.403
tRNFL (μm/y)	−2.09 (0.35) $p < 0.0001$[a]	−1.12 (0.39) 0.004[a]	0.97 (0.26) $p < 0.0001$[a]
GCIPL (μm/y)	−1.02 (0.27) $p < 0.0001$[a]	−0.33 (0.32) 0.299	0.69 (0.24) 0.004[a]
INL (μm/y)	−0.13 (0.09) 0.129	−0.17 (0.11) 0.111	−0.04 (0.09) 0.69
MV (mm^3/y)	−0.04 (0.01) 0.003[a]	−0.01 (0.02) 0.420	0.03 (0.02) 0.123

Abbreviations: GCIPL = ganglion cell-inner plexiform layer; INL = inner nuclear layer; MV = macular volume, with random intercepts for patient and eye, and random slope for time of follow-up; pRNFL = peripapillary retinal nerve fiber layer; tRNFL = temporal retinal nerve fiber layer.
[a] Statistically significant with a *p* value <0.01 after Bonferroni adjustment for multiple comparisons.
[b] Mixed-effects regression, controlling for age, sex, disease duration at first visit, history of ON, and current DMT, with random intercepts for patient and eye and random slope for time of follow-up.

of the 25-foot walk test by 20% (57% of converters and 10% of nonconverters; $p < 0.0001$). However, there was no difference in the proportion of participants who experienced worsening of SDMT and 9-hole peg test by 20% or more between the 2 cohorts. In addition, patients in both cohorts developed new MRI lesions; however, there was no difference between the 2 groups (76% of converters and 75% of nonconverters; $p = 0.918$).

Discussion

We demonstrated in this study that patients with MS who had clinical progression exhibited faster retinal thinning in the period preceding clinical conversion to SPMS, with a mean follow-up period on OCT of 3 years before conversion, compared with patients who did not convert to SPMS. These results suggest that retinal OCT is a valuable biomarker of disease progression allowing earlier detection and subsequent intervention, especially with the increased use of novel DMTs in progressive patients with MS and the improved treatment outcomes when initiated early.

To the best of our knowledge, this study is the first to prospectively monitor retinal changes in the same patients with MS who converted from RRMS to SPMS before and after conversion. Changes in retinal layers of patients with MS have been studied for over a decade, and studies proved that both axonal degeneration and central neuronal atrophy begin early during the active inflammatory phase of MS.[36] A recently published systematic review and meta-analysis reported that the pRNFL atrophy rate is approximately 1 μm every 1–2 years, which is in line with our findings of pRNFL atrophy rates of −0.52 μm/y in RRMS nonconverters and −1.1 μm/y in converters to SPMS.[37]

Table 5 Annualized Change of MRI Measurements in RRMS Converters to SPMS Before Conversion Compared With RRMS Nonconverters (Healthy Controls)

Annualized change, mean (SD) *p* value[a] (within-group testing for annualized change being different than 0)	MS subtype (total n = 51; converters = 18, nonconverters = 33)		Difference in annualized changes between groups[b] *p* value[a]
	Nonconverters (n = 33) MRI scans (n = 128)	Converters (n = 18) MRI scans (n = 49)	
GM volume (mm^3/y)	−0.79 (0.09) $p < 0.0001$[a]	−1.76 (0.34) $p < 0.0001$[a]	−0.97 (0.34) 0.005[a]
WM volume (mm^3/y	−0.26 (0.07) $p < 0.0001$[a]	−0.71 (0.25) 0.005[a]	−0.45 (0.26) 0.08
Total thalamic ratio (mm^3/y)	−0.0004 (0.002) 0.8	−0.014 (0.006) 0.02	−0.013 (0.006) 0.028
CSF volume (mm^3/y)	1.28 (0.15) $p < 0.0001$[a]	3.0 (0.6) $p < 0.0001$[a]	1.71 (0.57) 0.003[a]

[a] Statistically significant with a *p* value <0.01 after Bonferroni adjustment for multiple comparisons.
[b] Mixed-effects regression, controlling for age, sex, disease duration at first visit, history of ON, and current DMT with random slope for time of follow-up.

Table 6 Correlation Coefficients[a] Between Rates of Change in MRI-Derived and OCT-Derived Measures Across Whole MS Cohort During the Entire Observation Period

Retinal layer thickness Annualized change	MRI-derived brain compartment volume annualized change					
	ICV	WM volume	CC volume	CSF volume	Thalamic volume	GM volume
pRNFL	0.267 (0.012)[b]	0.376 (<0.0001)[b]	0.253 (0.017)[b]	−0.285 (0.007)[b]	0.433 (<0.0001)[b]	0.223 (0.036)[b]
GCIPL	0.039 (0.703)	0.234 (0.022)[b]	0.211 (0.039)[b]	−0.206 (0.044)[b]	0.251 (0.014)[b]	0.246 (0.016)[b]
tRNFL	0.017 (0.871)	0.2 (0.06)	0.281 (0.008)[b]	−0.535 (<0.0001)[b]	0.194 (0.068)	0.419 (<0.0001)[b]
INL	−0.107 (0.298)	0.096 (0.355)	0.188 (0.068)	0.027 (0.793)	0.202 (0.049)[b]	0.274 (0.007)[b]
ONL	−0.014 (0.893)	0.236 (0.021)[b]	0.313 (0.002)[b]	0.004 (0.966)	0.331 (0.001)[b]	0.396 (<0.0001)[b]

Abbreviations: CC = corpus callosum; GCIPL = ganglion cell-inner plexiform layer; GM = gray matter; ICV = intracranial volume; INL = inner nuclear layer; ONL = outer nuclear layer; pRNFL = peripapillary retinal nerve fiber layer; tRNFL = temporal retinal nerve fiber layer
[a] Generalized estimating regression, controlling for intereye correlation.
[b] Statistically significant correlations with a p value <0.05.

Our findings support previous observation of greater retinal thinning in progressive patients compared with patients with RRMS and a greater loss of whole brain volume in the progressive phase of MS that correlates strongly with retinal thinning.[13,38-41] In a recent study conducted by the IMSVISUAL consortium, retinal atrophy did not only persist in progressive patients with MS but also occurred at faster rates compared with non-progressive patients with MS in all the measured retinal layers independently of age.[18] Other studies showed that reductions in retinal thickness measured at a single time point could predict long-term disability worsening and sustained cognitive impairment in patients with MS.[11-15,42] The strength of our study is the availability of longitudinal retinal measurements in the same patients before and after clinical confirmation of disease progression.

The results of our study showed that converters to SPMS experienced faster thinning of most retinal measurements immediately before converting to SPMS compared with nonconverters, including pRNFL (112% higher), tRNFL (344% higher), and GCIPL (82% higher).

Progressive patients in our cohort were deemed to be progressing based on the specialists' clinical judgment, which is the gold standard for SPMS diagnosis, and the objective findings of worsening EDSS scores and timed 25-FWT, described earlier in our results.[6] Therefore, faster retinal thinning in patients who converted to SPMS could reflect the clinical progression of MS evidenced by objective clinical worsening. It is worth noting that the difference in annualized pRNFL thinning between the groups was lost after excluding eyes with a history of ON, which could be explained by the fact that optic neuritis drives the axonal atrophy in the retina while preserving its neuronal component. This was reflected in our results where GCIPL thinning remained faster in converters than in nonconverters without a history of ON supporting the notion that GCIPL change is a substrate of neuronal degeneration and possibly disease progression.[13,18,42]

We also demonstrated that the annualized tRNFL thinning was particularly faster in converters than in nonconverters relative to other retinal layers, which aligns with a previous study that suggested changes in tRNFL thickness demonstrated better sensitivity in tracking progressive retinal atrophy longitudinally compared with pRNFL and GCIPL.[42] On the other hand, although our results showed overall decrease in INL thickness, there was no significant INL thinning in both cohorts and no difference in their rates during the study period, which does not align with the findings of a previous study that demonstrated evidence of faster INL atrophy in progressive patients with MS relative to patients with RRMS.[18] In addition, our results showed that retinal thinning in most layers continued after conversion to SPMS at a rate similar to that before conversion except for tRNFL and GCIPL that exhibited slower thinning rates after conversion to SPMS. Therefore, our findings suggest a plateau effect on the thinning rates of different retinal layers after MS progression. However, we do not know whether these rates would reach similar ceiling effects with longer follow-up periods after conversion to SPMS, especially because our observational period with OCT after conversion was relatively short. A study by Pietroboni et al. also showed a plateau in retinal atrophy, particularly in GCIPL. However, their cohort included patients with RRMS followed up over 12 months only, and the plateau effect was detected early during disease course and attributed to transient stabilization of retinal atrophy during the remission.[43]

A secondary objective of our study was to assess the biological validity of longitudinal retinal changes on OCT by evaluating the association between retinal and brain volume changes throughout the study period. The main findings in our study included a noticeable decrease in GM volume and an increase in CSF volume, both of which occurred at faster rates in converters to SPMS, supporting overall neurodegeneration in patients with MS and particularly in those with progressive MS.[44] We also demonstrated overall changes in the annualized WM volume and total thalamic ratio decrease over time that could support the increase in CSF volume and decrease in GM volume, respectively. As such, we provide evidence that retinal layer changes could potentially mirror brain atrophy and global neurodegeneration throughout the disease course

aligning with previous studies that proved strong correlations between retinal thinning and whole-brain atrophy.[13,45] In contrast to these studies, our results did not show a significant correlation between GCIPL thinning and whole-brain atrophy, likely because of the relatively small number of MRI readings that we were able to include in our analysis, and the fact that these were MRI scans performed routinely for clinical use and not primarily for research. Another possible explanation is that GCIPL atrophy aligns more with neuronal degeneration reflected by gray matter atrophy on MRI,[13] which is supported by the significant correlation between annualized GCIPL thinning and GM atrophy in our study. Interestingly, we demonstrated a significant correlation between annualized INL thinning and thalamic and GM volume decrease, suggesting INL atrophy as another marker of neuronal degeneration as previously suggested elsewhere.[43,46]

The major strength of this study is its prospective design tracking retinal changes before conversion to SPMS and after clinical confirmation of SPMS by the same MS specialists at a tertiary care center. It is worth noting that both cohorts were similarly treated with DMT subtypes at baseline and that we controlled for DMTs in our model to account for any shifts during follow-up that could influence the degree of neuroprotection. The importance of longitudinal assessments lies in their ability to detect the accelerated neurodegeneration in patients with RRMS early during SPMS transition and possibly before accumulating irreversible disabilities. Several studies showed that the diagnosis of SPMS in routine clinical practice faces a period of uncertainty extending over 2–3 years between clinical suspicion and clinical confirmation of SPMS because of the absence of a definitive diagnostic tool.[6-9] This diagnostic delay has deleterious effects on the treatment of progressive patients with MS, especially that several recent trials proved that novel DMTs could slow the progression of the disease and prevent irreversible worsening if initiated early during disease progression.[47,48] Therefore, there is a strong need for a reliable diagnostic tool of early MS progression in clinical settings, and retinal OCT could potentially address this gap in clinical grounds because it is a safe, noninvasive, and relatively inexpensive tool.[10]

One limitation of our study is the small sample size that did not allow for further interpretation of the retinal changes among the early vs late converters subgroups. Another limitation is the potential variability in the MRI measurements because the MRIs analyzed in this study were performed for clinical purposes, although MRIs included in this study were performed according to the standardized protocols enabling reliable post hoc processing and analysis. MRI scans performed at other centers because of logistical and financial constraints were thus excluded from the analyses.

In addition, the lack of randomization in our study cohort could have affected the analyses because the participants who were classified as patients with RRMS could also be transitioning into SPMS subclinically during the observation period. Nevertheless, our study is primarily a post hoc analysis after retrospective clinical confirmation of SPMS, which could justify the lack of randomization in the sample. Moreover, we did not have visual function tests, such as low-contrast visual acuity testing, to relate retinal changes and visual function in our study. Ideally, a larger cohort of newly diagnosed patients with RRMS would be prospectively followed clinically and with adequate OCT scanning to better track retinal changes over time in patients who are at risk of disease progression. As such, monitoring patients with MS by OCT scans over a longer duration before conversion to SPMS would allow us to better characterize the changes in slopes over time and explore whether there is an accelerated rate of thinning just preceding SPMS conversion or whether it is a constant phenomenon since MS onset in certain disease phenotypes.

Our data support the hypothesis that patients with RRMS who convert to SPMS exhibit accelerated retinal thinning compared with matched patients with RRMS who do not convert to SPMS. The incorporation of retinal OCT into routine clinical practice is crucial because early detection of accelerated retinal atrophy in patients with RRMS before advanced disease progression could facilitate the therapeutic decision making of clinicians to halt irreversible disability worsening. Future studies are needed to test the clinical and biological validity of OCT in the early diagnosis of SPMS by matching retinal atrophy on OCT with other clinical biomarkers including serum and CSF biomarkers and functional measures such as low-contrast visual acuity test outcomes.

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Continued

Appendix *(continued)*

Name	Location	Contribution
Hadi M. Sabbagh, MD	Nehme and Therese Tohme Multiple Sclerosis Center, American University of Beirut Medical Center, Beirut, Lebanon	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data; Analysis or interpretation of data
Nicole Bou Rjeily, MD	Nehme and Therese Tohme Multiple Sclerosis Center, American University of Beirut Medical Center, Beirut, Lebanon	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Salem Hannoun, PhD	Medical Imaging Sciences, Division of Health Professions, Faculty of Health Sciences, American University of Beirut, Beirut, Lebanon	Drafting/revision of the manuscript for content, including medical writing for content; Major role in the acquisition of data
Samia J. Khoury, MD	Nehme and Therese Tohme Multiple Sclerosis Center, American University of Beirut Medical Center, Beirut, Lebanon; Department of Neurology, American University of Beirut, Beirut, Lebanon	Drafting/revision of the manuscript for content, including medical writing for content; Study concept or design; Analysis or interpretation of data

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