Supplementary material

Table S1: List of exclusion criteria for participants in the MS group and reference group (when applicable)

Exclusion criteria
1. HIV or any other known immunodeficiency syndrome (disease or drug-induced) 2. Any ophthalmologic reason for RNFL pathology other than MS, such as: a. optic neuropathy, active advanced glaucoma, injury of the optic nerve based on the ophthalmologist's clinical judgment or b. history of presence of severe myopia: i. in subjects who had not had refractive surgery, a refractive error of greater than 6.00 diopters ii. pathologic fundus changes of high myopia, such as retinal pigmentary atrophy, besides peripapillary atrophy (atrophy involving the macula) or a staphyloma iii. in subjects that had previous refractive surgery, an axial eye length of greater than 26 mm 3. Acute optic neuritis within the past 6 months before Baseline 4. Evidence of advanced, non-proliferative or proliferative diabetic retinopathy 5. Presence of retinal conditions associated with edema, subretinal fluid, cysts, etc. 6. History of a severe head trauma 7. Any of the following neurologic/psychiatric disorders: a. history of substance abuse (drug or alcohol) in the past five years or any other factor (i.e., serious psychiatric condition) that may interfere with the subject's ability to cooperate and comply with the study procedures b. specific MRI findings (tumor, subdural haematoma, post-contusional changes, territorial stroke, neurodegenerative disorders, aneurysm/arteriovenous malformation, evidence of past macroscopic haemorrhage, or other relevant MRI findings that would interfere with evaluation) c. progressive neurological disorder, other than MS, which may affect participation in the study 8. Concomitant use of drugs that may directly affect retinal structure and function (e.g.chronic systemic corticosteroids [>30 consecutive days; doses higher than Cushing threshold e.g. prednisone 7.5mg/d], intraocular anti-angiogenic drugs [ranibizumab, bevacizumab], intraocular steroids etc.) 9. Any medically unstable condition, progressive disease (other than MS) or other condition that would preclude reliable participation in the study as assessed by the investigator 10. Patients unable to undergo MRI scans including gadolinium enhancement: a. reduced renal clearance (eGFR <45 ml/min) b. history of severe hypersensitivity to gadolinium-DTPA c. claustrophobia that cannot be overcome otherwise 11. Patients who have received an investigational drug or therapy within 30 days or 5 half-lives, whichever is longer, of the baseline visit.

Note: While non-severe glaucoma was not part of the exclusion criteria, it should be noted that only 2 patients (0.6%) had glaucoma in the MS group and 1 (1.4%) in the reference group

Optical coherence tomography (OCT)

OCT scans in all participants were performed on Spectralis OCT1 machines (Heidelberg Engineering, Heidelberg, Germany), software version 1.6.2.0 or higher, with the eye tracking function enabled for best accuracy. Data were collected from a peripapillary ring scan (circular scan, 12 degrees diameter, Automatic Real-Time [ART] set at 100), a macular volume scan (centered on the fovea with 20×20 degrees, 25 vertical B-scans, ART 49, 240µm B-scan distance), the optic nerve head volume scan (centered on the optic nerve head with15×15 degrees, 73 vertical B-scans, ART >25, 60µm B-scan distance), and the papillomacular bundle volume scan (very dense, oblique volume scan oriented from optic nerve head to macula in a 7-degree angle to image the papillomacular bundle with 20×4 degrees, 105 B-scans, ART 9, 10µm B-scan distance). All OCT scans underwent quality control (QC) assessment by the central reading center (Vienna Reading Center [VRC]). Scans failing QC had to be repeated within a 7-day visit window. Supplementary Figure S1 gives an overview of the scan patterns and the evaluation fields for each pattern used in the analysis.

OCT scans were performed at baseline and at every visit (1 month, 6 months, and every 6 months thereafter) for both groups. Scans were sent to the VRC via a server. All operators at participating sites were certified by the VRC for the use of the correct scanning protocol, correct pseudonymization of study specific image labelling of patients and image transfer before patient inclusion according to standardized certification processes to obtain images for the above-described acquisitions. The baseline scan was used as the reference for alignment during acquisition of subsequent scans to assure that the scan was done on a matching area of retina for each scan.

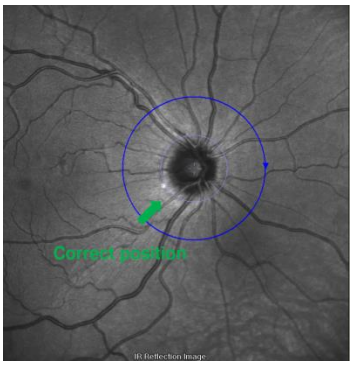
For the purposes of the short-term reproducibility assessment, the baseline and 1-month scans were used. For the primary endpoint assessment, the average pRNFL thickness was used. The key OCT parameters examined in this study were: the change in pRNFL thickness over time, including average thickness, papillomacular bundle thickness (i.e., fibers that serve the macula) and temporal quadrant thickness (both assessed independently), as well as the change from baseline to end-of-study in average mGCIPL thickness.

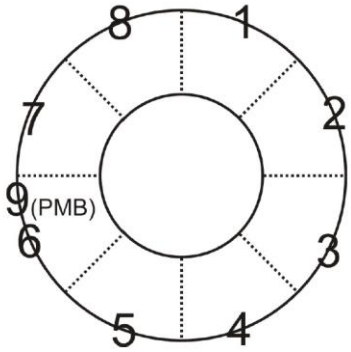
Automated segmentation was performed with the manufacturer's software (HEYEX version 1.9.10.0, Viewing Module version 6.0.9.0) and subsequently corrected by certified and trained graders of the VRC, supervised by a retina specialist, according to a predefined grading protocol. All graders were masked to the clinical status of the study participants (MS or healthy control group) and any other diagnostic information. Areas around the optic nerve head and

around vessels that can artificially increase pRNFL were excluded from the analysis on an a scan level. To reduce repeated measurement variability, the baseline scan was used as the reference scan for the placement of the scan on the same anatomical location of the retina for subsequent scans (registration). Axial thickness of the pRNFL and mGCIPL, including ganglion cell and inner plexiform layers, were taken for analyses. All manual pRNFL segmentation corrections were performed at each visit, whereas the mGCIPL was only manually corrected at the first and last visit for each participant. The pRNFL layer is bordered by the inner limiting membrane on top and the transition between pRNFL and ganglion cell layer at the bottom; the mGCIPL layer is bordered by the transition between the pRNFL and the ganglion cell layer on top and the border between inner plexiform layer and inner nuclear layer at the bottom. The averaged mGCIPL thickness in the 3-mm ring of the early treatment diabetic retinopathy study (ETDRS) grid (equals the 3-mm area excluding the 1-mm area) were used for mGCIPL assessment (refer to Figure S1, where the fields 1-8 in letter b) macular scan were averaged). Studies were performed and described in accordance to the QC criteria at the VRC, OSCAR-IB and the Advised Protocol for OCT Study Terminology and Elements (APOSTEL) criteria.[1, 2]

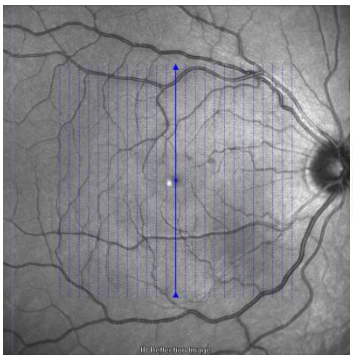
Figure S1: Different scan patterns used in the OCTiMS study for OCT imaging and respective evaluation areas for each scan. a) peripapillary retinal nerve fiber layer scan, b) macular volume scan (25 B scans), c) maculo-papillary bundle scan, d) disc volume scan

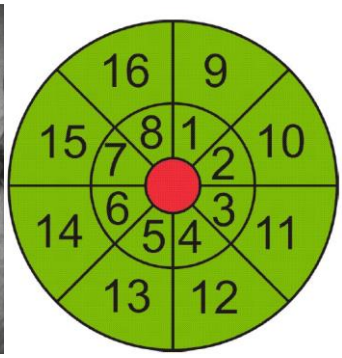
a)



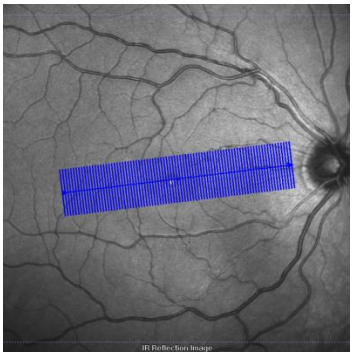


b)



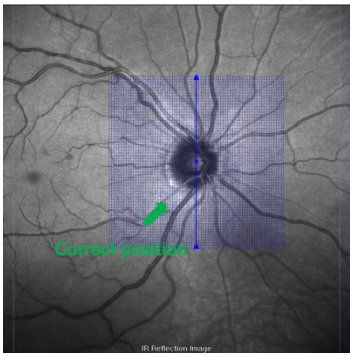


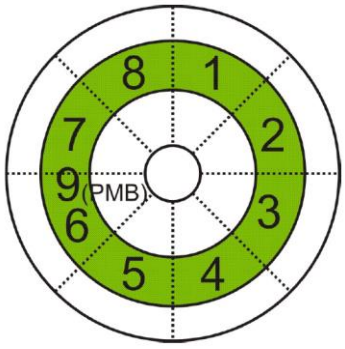
c)



05	**04**	**03**	**02**	**01**
10	**09**	**08**	**07**	**06**

d)





Correct position (green arrow), centered on optic nerve head

References

1. Cruz-Herranz A, Balk LJ, Oberwahrenbrock T, et al. The APOSTEL recommendations for reporting quantitative optical coherence tomography studies. Neurology. 2016 Jun 14;86(24):2303-9.

2. Schippling S, Balk LJ, Costello F, et al. Quality control for retinal OCT in multiple sclerosis: validation of the OSCAR-IB criteria. Multiple sclerosis (Houndmills, Basingstoke, England). 2015 Feb;21(2):163-70.