Retinal thickness measured with optical coherence tomography and risk of disability worsening in multiple sclerosis: a cohort study

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Summary

Background Most patients with multiple sclerosis without previous optic neuritis have thinner retinal layers than healthy controls. We assessed the role of peripapillary retinal nerve fibre layer (pRNFL) thickness and macular volume in eyes with no history of optic neuritis as a biomarker of disability worsening in a cohort of patients with multiple sclerosis who had at least one eye without optic neuritis available.

Methods In this multicentre, cohort study, we collected data about patients (age ≥16 years old) with clinically isolated syndrome, relapsing-remitting multiple sclerosis, and progressive multiple sclerosis. Patients were recruited from centres in Spain, Italy, France, Germany, Czech Republic, Netherlands, Canada, and the USA, with the first cohort starting in 2008 and the latest cohort starting in 2013. We assessed disability worsening using the Expanded Disability Status Scale (EDSS). The pRNFL thickness and macular volume were assessed once at study entry (baseline) by optical coherence tomography (OCT) and was calculated as the mean value of both eyes without optic neuritis for patients without a history of optic neuritis or the value of the non-optic neuritis eye for patients with previous unilateral optic neuritis. Researchers who did the OCT at baseline were masked to EDSS results and the researchers assessing disability with EDSS were masked to OCT results. We estimated the association of pRNFL thickness or macular volume at baseline in eyes without optic neuritis with the risk of subsequent disability worsening by use of proportional hazards models that included OCT metrics and age, disease duration, disability, presence of previous unilateral optic neuritis, and use of disease-modifying therapies as covariates.

Findings 879 patients with clinically isolated syndrome (n=74), relapsing-remitting multiple sclerosis (n=664), or progressive multiple sclerosis (n=141) were included in the primary analyses. Disability worsening occurred in 252 (29%) of 879 patients with multiple sclerosis after a median follow-up of 2·0 years (range 0·5–5 years). Patients with a pRNFL of less than or equal to 87 μm or less than or equal to 88 μm (measured with Spectralis or Cirrus OCT devices) had double the risk of disability worsening at any time after the first and up to the third years of follow-up (hazard ratio 2·06, 95% CI 1·36–3·11; p=0·001), and the risk was increased by nearly four times after the third and up to the fifth years of follow-up (3·81, 1·63–8·91; p=0·002). We did not identify meaningful associations for macular volume.

Interpretation Our results provide evidence of the usefulness of monitoring pRNFL thickness by OCT for prediction of the risk of disability worsening with time in patients with multiple sclerosis.

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Introduction

Multiple sclerosis has an unpredictable course, which makes accurate prognosis and personalised treatment difficult.[1] Development of imaging biomarkers for prediction of the clinical course of multiple sclerosis and future disability would improve clinical management and might inform the selection of patients for enrolment in randomised controlled trials of neuroprotective or regenerative drugs.[2]

Most of the retinas of patients with multiple sclerosis show inflammatory and neurodegenerative signs.[3] Optical coherence tomography (OCT) is a well tolerated, reproducible, and high-resolution imaging method for the assessment of retinal integrity. Peripapillary retinal nerve fibre layer (pRNFL) thickness and macular volume are the most reported indicators to measure retinal atrophy on OCT. Macular volume is used to ascertain retinal ganglion cell integrity because nearly half of all ganglion cell bodies are located in the macula. Since 2010, the use of segmentation algorithms have enabled quantification of macular retinal ganglion cell layer thickness.

In a study of spectral-domain OCT, the retinas of eyes without previous optic neuritis (non-optic neuritis eyes) had thinner pRNFL and retinal ganglion cell layers in most patients with different phenotypes of multiple

Research in context

Evidence before this study

We searched PubMed for articles about retinal atrophy assessed with optical coherence tomography (OCT) and disability worsening or progression in multiple sclerosis published before Oct 15, 2015, using the terms "optical coherence tomography AND multiple sclerosis" and "retinal nerve fibre layer thickness AND multiple sclerosis". We identified several studies addressing the association between disability in patients with multiple sclerosis and retinal nerve fibre layer thickness, macular volume, or both assessed by OCT. However, most data available have been collected with time-domain OCT, which is less accurate and reproducible than spectral-domain OCT, in cross-sectional designs. Most previous studies were included in a systematic review and meta-analysis, which showed inverse correlations (r=–0·3 to –0·7) between Expanded Disability Status Scale (EDSS) and retinal nerve fibre layer thickness in 67% of the included studies. Nevertheless, there is still a need for definitive longitudinal evidence showing that changes in retinal architecture are valid and useful surrogate endpoints for disability worsening in multiple sclerosis.

Added value of this study

Our results suggest that the thickness of the peripapillary retinal nerve fibre layer (pRNFL) is a marker of increased risk of disability worsening, as measured with EDSS. This multicentre, collaborative initiative to assess the value of this novel biomarker for risk of disability worsening in the short and medium terms in patients with multiple sclerosis provides evidence of the usefulness of OCT imaging to monitor the course of multiple sclerosis.

Implications of all the available evidence

Our results support the use of baseline pRNFL thickness as a predictor of disability worsening in multiple sclerosis. Our findings might help neurologists to monitor the disease in clinical settings and, more importantly, to help drive treatment decisions based on a marker of neuro-axonal damage. Quantification of pRNFL thickness will identify patients at high risk of disability worsening, and use of the cutoffs identified will help with recruitment of the most informative population for randomised controlled trials testing neuroprotective or regenerative drugs, thereby reducing sample sizes and associated costs.

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sclerosis than did those of healthy controls;[4,5] similar findings were reported even for patients with benign multiple sclerosis.[5] Because these OCT variables have been reported to be associated with multiple sclerosis disability[6,7] and brain atrophy,[6,8] they might be suitable imaging surrogates for the prediction of the course of multiple sclerosis. So far, most data have been collected by use of time-domain OCT, which is less accurate and reproducible than the new spectral-domain devices,[9] and were gathered in cross-sectional or short-term studies.[10]

Because of an unmet need for biomarkers for the prediction of the course of multiple sclerosis, the aim in this study was to assess whether a single measurement of pRNFL thickness or macular volume with OCT in eyes without optic neuritis can be used as a biomarker of the risk of disability worsening in patients with multiple sclerosis.

Methods

Study design and participants

We did a multicentre cohort study using de-identified data from the International Multiple Sclerosis Visual System Consortium (IMSVISUAL) database. Patients were recruited from centres in Spain (Barcelona, Madrid, and Zaragoza), Italy (Milan), France (Lille), Germany (Düsseldorf and Berlin), the Czech Republic (Prague), the Netherlands (Amsterdam), Canada (Calgary), and the USA (San Francisco, Dallas, Baltimore, New York, and Philadelphia). Briefly, data from reported and ongoing cohort studies at multiple sclerosis centres were stored in the IMSVISUAL repository. The fist cohort started in 2008 and the latest cohort started in 2013. The raw dataset is available from IMSVISUAL on request. All cohort studies with de-identified data in the IMSVISUAL repository were approved by their institutional review boards. All patients provided written informed consent for participation in their respective studies.

In this cohort study, we enrolled patients aged 16 years or older who had a clinically isolated syndrome or multiple sclerosis as per the revised 2010 McDonald Criteria,[11] including relapsing-remitting, secondary progressive, and primary progressive subtypes of multiple sclerosis. Another inclusion criterion was the availability of longitudinal information about the patients' neurological disability.

In patients with multiple sclerosis, retinal atrophy after acute optic neuritis is often more pronounced than in the absence of optic neuritis.[10,12] For this reason, we excluded eyes with previous optic neuritis from the analysis, and selected patients with at least one eye without optic neuritis. Other exclusion criteria were previous diagnoses of ophthalmological, neurological, or drug-related causes of vision loss or retinal damage not attributable to multiple sclerosis.[8,13]

Procedures

We collected baseline data for the following clinical variables: age at inclusion, sex, disease duration, multiple sclerosis subtype, disability measures, previous history of optic neuritis, and use of disease-modifying therapies. Neurological disability was assessed with the Expanded Disability Status Scale (EDSS).[14] Data for the Multiple Sclerosis Functional Composite (MSFC)[15] were available for a subset of participants (197), and these data were used as an additional measurement of disability. A history of optic neuritis was ascertained from the participants' medical records, as previously described.[16] The same team

For the **IMSVISUAL website** see http://www.imsvisual.org

of EDSS-certified neurologists examined the patients during follow-up at each participating centre. Researchers who did the OCT at baseline were masked to EDSS results and the researchers assessing disability with EDSS were masked to OCT results.

We defined disability worsening as a documented increase in neurological disability (≥1 point increase in the EDSS score, or ≥0·5 point increase for patients with a baseline score of ≥5·5, confirmed in a second visit 3–6 months later) as a consequence of relapses or progressive disease.[17] EDSS assessment was always done in the absence of acute relapses. MSFC worsening was defined as a worsening of at least 20% in any of three components: timed 25 ft walk, timed nine-hole peg test, and the 3 s version of the paced auditory serial addition test.[18] We established whether the patient showed disability worsening (yes or no) using the baseline and the follow-up EDSS and, if available, MSFC assessments. If the patient showed disability worsening during the study, his or her follow-up period in this study corresponded to the first timepoint when documented disability worsening was noted. If the patient did not show disability worsening during the entire study, his or her follow-up period corresponded to the last follow-up available in the repository.

pRNFL thickness and macular volume (6 mm ring area) were evaluated once at study entry (baseline visit) by use of spectral-domain OCT with Spectralis (Heidelberg Engineering, Heidelberg, Germany) or Cirrus (Carl Zeiss, Dublin, CA, USA), the instruments that patients are commonly assessed with. For patients without a history of optic neuritis, pRNFL thickness and macular volume were calculated as the means of the values for both eyes, and for patients with a history of unilateral optic neuritis only the values for the eyes without optic neuritis were used in the analyses.

To identify cases of subclinical optic neuritis, we used a previously described[13] OCT approach based on interocular asymmetry in pRNFL thickness and macular volume (appendix). Eight patients (six assessed with Spectralis and two with Cirrus) without optic neuritis in either eye met the interocular asymmetry criterion to be considered as probably having subclinical optic neuritis and ten patients (seven assessed with Spectralis and three with Cirrus) met the criterion for macular volume. For these patients, we included only the eye with the highest values for pRNFL thickness and macular volume.

See Online for appendix

Statistical analysis

We described baseline features of the study population and the distribution of events (disability worsening) with respect to key demographic and clinical characteristics using absolute and relative frequencies for categorical variables. For quantitative variables, we used medians and IQR to describe baseline features of the study population and the 20th, 40th, 60th, and 80th percentiles to describe the distribution of events.

In view of the different periods of follow-up for patients in the study, we fitted proportional hazards models to measure the pRNFL thickness and macular volume of eyes without optic neuritis as markers of the risk of disability worsening. First, we evaluated the associations between OCT-derived metrics and disability worsening, including continuous variables of pRNFL thickness and macular volume in the models. These models quantified the effect (increase or decrease) on the risk of disability worsening associated with each unit of change (1 μm for pRNFL thickness and 1 mm³ for macular volume). Results from previous studies assessing the value of OCT-derived metrics for the prediction of visual disability suggested a non-linear relation between retinal atrophy and clinical outcomes.[12,19] Thus, we used tertile-based categories of pRNFL thickness and macular volume, and this approach allowed us to pool data for both Cirrus and Spectralis devices and to assess the non-linear association between retinal integrity and disability worsening. We chose pRNFL thickness as the main outcome measure because it has a better signal-to-noise ratio than macular volume. We compared the risks associated with being in the lowest tertile (pRNFL ≤87 μm) and intermediate tertile (pRNFL >87–97 for Spectralis and >87–98 μm for Cirrus) versus being in the highest tertile (pRNFL >97 μm for Spectralis and >98 μm for Cirrus). We used proportional hazards models with only OCT measures to calculate unadjusted hazard ratios (HRs) for pRNFL thickness and macular volume. We generated Kaplan-Meier plots of cumulative incidence of disability worsening during follow-up according to the three groups of pRNFL thickness in the entire study population and in multiple sclerosis subgroups.[20]

We compared baseline features of patients who had worsening of disability with those who remained stable at the end of follow-up. We did these bivariate analyses with Fisher's exact test for categorical data and the Mann-Whitney *U* test for quantitative variables. We used proportional hazards models to assess the univariate effect of each of the baseline features on the risk of disability worsening. We fitted proportional hazards models including the covariates age, disease duration, previous unilateral optic neuritis, baseline EDSS, and use of disease-modifying therapies to estimate the adjusted HRs for OCT measures for the entire study population and for the multiple sclerosis subgroups. If data were missing for any baseline characteristics significantly associated with the risk of disability worsening, we would fit proportional hazards models in the group of participants with complete information for these baseline features. We did sensitivity analyses with different cutoffs for the clinical outcome as well as another accepted criterion (a difference of pRNFL thickness values of more than 20% between the two eyes) to identify subclinical episodes of optic neuritis.

A key assumption in these models is proportional hazards. We used time-dependent covariates, including

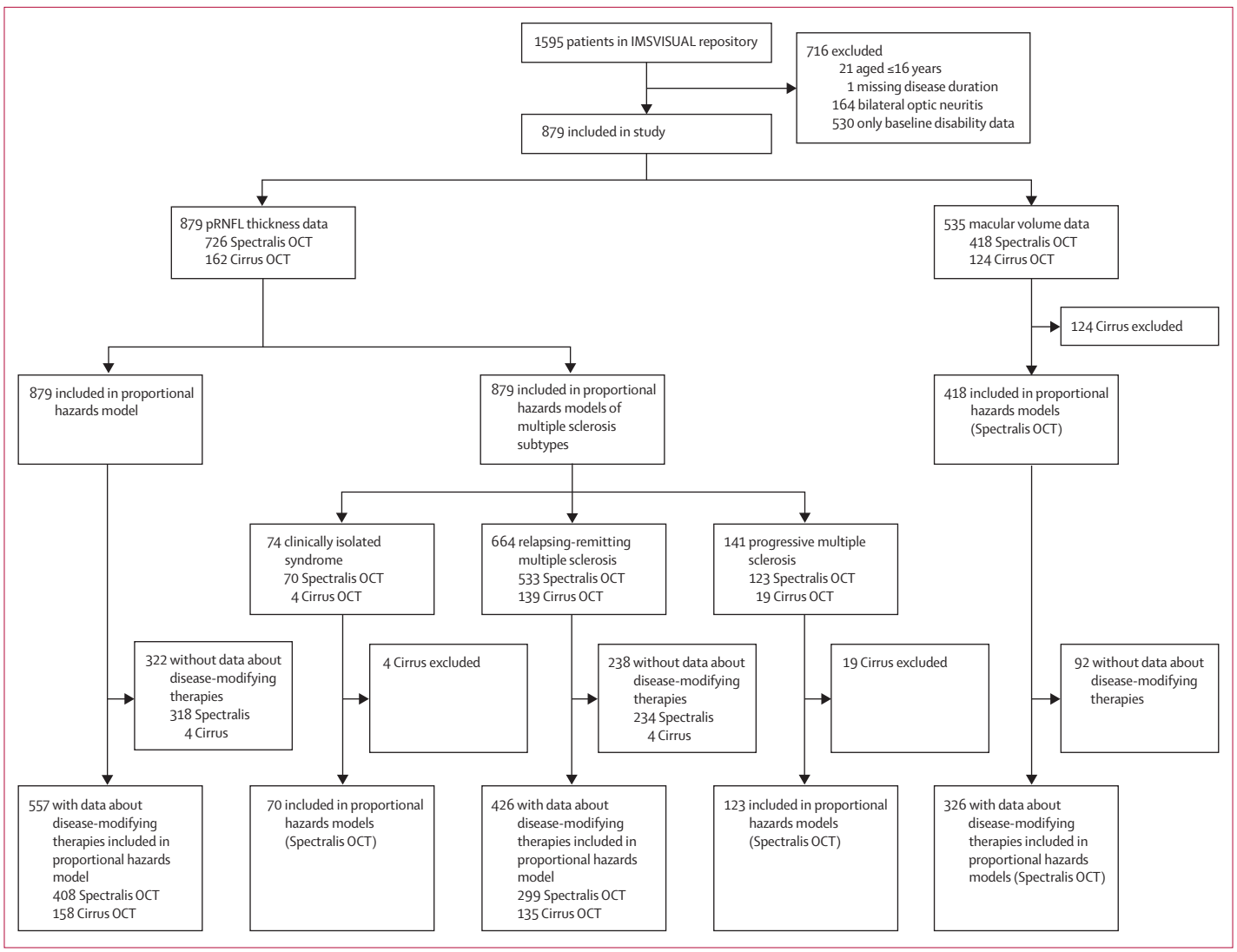

Figure 1: **Study profile**
pRNFL=peripapillary retinal nerve fibre layer. OCT=optical coherence tomography.

interaction terms with time, to assess proportionality for OCT metrics and each covariate included in the proportional hazards models (age, previous unilateral optic neuritis, disease duration, baseline EDSS, and use of disease-modifying therapies). We evaluated linear interaction (covariate×time), logarithmic interactions (covariate×ln[time] and covariate×$\log_{10}$[time]), and the effect of these covariates during the first year of follow-up (covariate×time ≤1·00 year), from the first to the third years (covariate×time >1·00 and ≤3·00), and from the third to the fifth years (covariate×time >3·00 and ≤5·00). Baseline EDSS (models including total study population), disease duration, and presence of previous unilateral optic neuritis (models including progressive multiple sclerosis subgroup) showed non-proportionality for the analyses of pRNFL thickness. Thus, we extended the proportional hazards models by adding the interaction between the non-proportional covariate and the time of observation as another covariate. Patients who had disability worsening within a given follow-up period were excluded from the analyses of subsequent follow-up because we only included patients who were free of the event of interest to assess the risk of disability worsening in the next follow-up. Patients who did not present with the event at a given follow-up were included for the analyses of the next follow-up only if we had available information about their disability status during the next follow-up; otherwise, they were censored. We used the likelihood ratio test and Harrell's *C* statistic to evaluate the goodness of fit of the proportional hazards models.[21] The accuracy and significance of the coefficients estimated by use of the proportional hazards methods become unreliable when the number of events per

variable is fewer than ten.[22,23] Thus, we used this cutoff as a reference and only ran proportional hazards models with events per variable of at least ten. Two-tailed p values of less than 0·05 were deemed significant. Analyses were done with the SPSS statistical package (version 20.0). Harrell's *C* statistic was estimated with SAS (version 9.4).

Role of funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

	Study population (n=879)
Sex, female	584 (66%)
Age (years)	40·6 (32·5–48·7)
Disease duration (years)	6·5 (2·7–13·4)
Multiple sclerosis type	
Clinically isolated syndrome	74 (8%)
Relapsing-remitting multiple sclerosis	664 (76%)
Secondary progressive multiple sclerosis	83 (9%)
Primary progressive multiple sclerosis	58 (7%)
Optic neuritis status	
Previous optic neuritis	281 (32%)
No previous optic neuritis	598 (68%)
Disease-modifying therapies (n=557)	
None	150 (17%)
Interferon beta 1b, subcutaneous	61 (7%)
Interferon beta 1a, subcutaneous	81 (9%)
Interferon beta 1a, intramuscular	98 (11%)
Glatiramer acetate	81 (9%)
Fingolimod	14 (2%)
Natalizumab	45 (5%)
Other*	27 (3%)
Expanded Disability Status Scale (EDSS)	2·0 (1·5–3·5)
Multiple Sclerosis Functional Composite (MSFC)	
PASAT (0–60; n=197)	49 (36–55)
Non-dominant hand (s; n=195)	21·2 (19·0–23·6)
Dominant hand (s; n=195)	19·9 (17·9–23·8)
Timed 25 ft walk (s; n=191)	4·6 (3·9–5·7)
Peripapillary retinal nerve fibre layer (pRNFL) thickness (μm)	
Spectralis OCT (n=726)	92·1 (84·0–99·7)
Cirrus OCT (n=162)	91·6 (84·5–100·4)
Macular volume (mm^3)	
Spectralis OCT (n=418)	8·5 (8·2–8·8)
Cirrus OCT (n=124)	10·0 (9·8–10·4)

Data are median (IQR) or n (%). *Cyclophosphamide (n=5), mitoxantrone (n=10), azathioprine (n=5), diazoxide (n=2), flupirtine (n=1), and unknown but with the patient reported as under therapy (n=4). OCT=optical coherence tomography. PASAT=Paced Auditory Serial Addition Test.

Table 1: **Baseline demographics and clinical characteristics**

Results

In the IMSVISUAL database, information on longitudinal disability and OCT data from at least one eye without optic neuritis were available for 879 of 1595 patients with multiple sclerosis, and these patients were eligible for inclusion in our cohort study (figure 1). The study population had mainly mild to moderate disability, with a median EDSS score of 2·0 (table 1). Information about immunomodulatory therapies was available for 557 participants. 535 (61%) of 879 patients had macular volume values available.

We identified 252 (29%) of 879 patients with disability worsening after the follow-up. The study follow-up ranged from 0·5 years to 5 years (median 2·0 years, IQR 1·3–3·0). Table 2 shows the baseline characteristics of patients with disability worsening and those with stable multiple sclerosis after follow-up. Patients who had disability worsening were older, were more likely to have progressive multiple sclerosis, had higher baseline EDSS, and had longer disease duration at baseline. Moreover, the prevalence of unilateral optic neuritis before study enrolment was higher for patients who remained stable during follow-up. Patients who had disability worsening less frequently received disease-modifying therapies than did those who had stable disease, but the difference was not significant. We also analysed the frequency of events by key demographic and clinical characteristics (appendix p 1) and ran univariate proportional hazards models to assess the effect of these baseline characteristics on disability worsening (appendix p 1). The results of the univariate analyses showed that age, disease duration, baseline disability, and the presence of the progressive multiple sclerosis phenotype were associated with the risk of disability worsening, whereas receiving disease-modifying therapies and a history of unilateral optic neuritis before study enrolment were inversely related to the risk of disability worsening during the follow-up.

We used separate proportional hazards models for baseline pRNFL thickness measured with Spectralis and Cirrus OCT because differences between the segmentation algorithms[24] prevent the comparison of quantification with the two devices (726 patients assessed with Spectralis, 162 with Cirrus OCT, and nine patients were assessed with both devices). The unadjusted HRs were 0·98 (95% CI 0·97–0·99, p<0·0001) for Spectralis and 0·99 (0·97–1·01, p=0·210) for Cirrus. These results suggested that higher pRNFL thickness was associated with reduced likelihood of disability worsening.

Then, we assessed the risk associated with the different categories of pRNFL thickness. Patients in the lowest pRNFL thickness category at baseline had increased risk of disability worsening (unadjusted HR 1·65, 95% 1·23–2·21, p=0·001) compared with those in the highest category, whereas no difference existed between patients in the intermediate and highest categories (figure 2). The unadjusted analysis showed that

cumulative probability of being free of disability worsening was lowest in the lowest pRNFL group and did not differ between the intermediate and highest categories (appendix p 3).

We used proportional hazards models with age, previous unilateral optic neuritis, disease duration, and baseline EDSS as covariates to evaluate pRNFL thickness as a predictor of disability worsening. The adjusted HRs for pRNFL thicknesses were 0·98 (95% CI 0·97–0·99, p=0·006) with Spectralis OCT and 0·98 (0·96–1·00, p=0·102) with Cirrus OCT (table 3). When we also included the use of disease-modifying therapies as a covariate, the risk of disability worsening associated with pRNFL thickness increased slightly (HR 0·97, 95% CI 0·95–0·99, p=0·001) for Spectralis OCT. The proportional hazards model was not tested for Cirrus OCT because the number of events per variable with disease-modifying therapies as a covariate was fewer than ten (9·57).

The results of the adjusted analysis showed that patients in the lowest tertile of pRNFL thickness had increased risk of disability worsening (HR 1·75, 95% CI 1·19–2·59, p=0·005; table 3) compared with those in the highest tertile. The risk of disability worsening did not differ between patients in the intermediate and highest categories (table 3). We therefore merged the intermediate and highest categories, and estimated the risk associated with pRNFL thickness in the lowest category by comparison with this new aggregate category. Patients who had pRNFL of less than or equal to 87 µm (Cirrus OCT) and less than or equal to 88 µm (Spectralis OCT) had almost twice the risk of worsening in the 5 years of follow-up compared with patients in the higher aggregate category (HR 1·96, 95% CI 1·39–2·76, p=0·00013; table 3).

The risk of disability worsening was increased at any time after the first year until the fourth year of follow-up (HR 2·02, 95% CI 1·03–3·93, p=0·040) for patients with macular volume of less than or equal to 8·7 mm³ on the Spectralis device (appendix pp 2–3).

The association between the risk of disability worsening and baseline pRNFL thickness was not significant for the first year (table 3). Since sample size and HR were similar for years 2 and 3 and for years 4 and 5, we merged these timepoints. Therefore, for the exploratory analyses, we provided estimations from baseline to the first year, from the first to third years, and from the third to fifth years. Patients with pRNFL of less than or equal to 87 µm (Cirrus OCT) or less than or equal to 88 µm (Spectralis OCT) had double the risk of disability worsening at any time after the first year and until the third year of follow-up (table 3). This risk increased almost four times after the third year and up to the fifth year of follow-up (table 3).

74 patients had clinically isolated syndrome (four assessed with Cirrus OCT and 70 assessed with Spectralis OCT; figure 1). We assessed the association between disability worsening and pRNFL thickness in eyes without optic neuritis only in the group with Spectralis OCT data because the small sample size of the group assessed with Cirrus OCT prevented further analysis. We did not detect any associations between pRNFL thickness and risk of disability worsening in the subgroup of patients with clinically isolated syndrome (appendix p 4).

664 patients had relapsing-remitting multiple sclerosis, of whom 171 (26%) had disability worsening during follow-up. Patients with relapsing-remitting multiple sclerosis and pRNFL thickness of less than or equal to 88 µm had increased risk of disability worsening during follow-up (appendix p 5). We estimated the adjusted HR for pRNFL thickness in eyes without optic neuritis and risk of disability worsening in the 426 patients with relapsing-remitting multiple sclerosis for whom we had information about the use of disease-modifying therapies (appendix p 6). Patients with relapsing-remitting multiple sclerosis and pRNFL

	Stable disease (n=627)	Disability worsening (n=252)	p value
Sex, female	420 (67%)	164 (65%)	0·636
Age (years)	39·6 (31·9–47·9)	43·3 (33·6–50·2)	0·001
Disease duration (years)	6·0 (2·3–12·8)	7·37 (3·3–15·0)	0·008
EDSS score	2·0 (1·5–3·0)	2·5 (1·5–4·0)	0·002
Progressive multiple sclerosis	75 (12%)	66 (26%)	<0·0001
Received disease-modifying therapies (n=557)	299 (75%)	108 (68%)	0·091
Previous optic neuritis	223 (36%)	58 (23%)	0·0003

Data are median (IQR) or n (%) unless otherwise specified. For comparisons, we used Fisher's exact test for categorical data and the Mann-Whitney *U* test for quantitative variables. EDSS=Expanded Disability Status Scale.

Table 2: **Comparison of baseline characteristics between patients with disability worsening and those with stable multiple sclerosis**

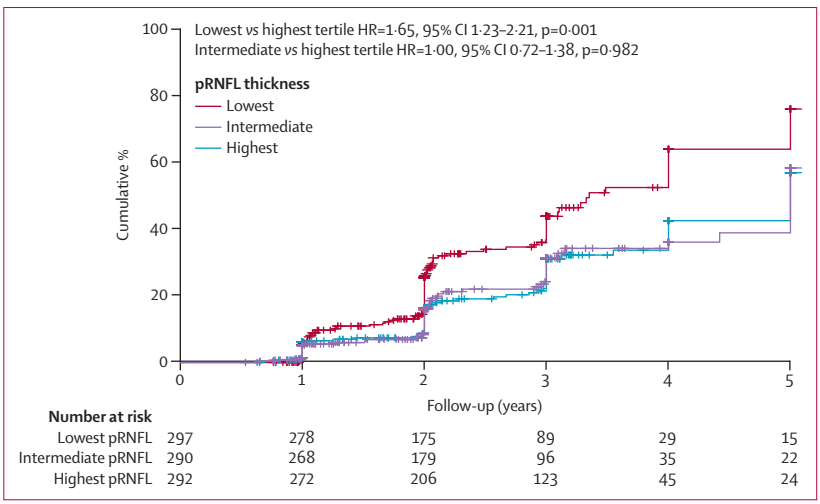

Figure 2: **Cumulative percentage of disability worsening in the entire population with multiple sclerosis according to pRNFL thickness in eyes without optic neuritis**

Numbers at risk are the number of patients at risk of disability worsening just before the selected timepoints. The difference in the number of patients between one timepoint and the next is the sum of the number of events and number of censored patients. HR=hazard ratio. pRNFL=peripapillary retinal nerve fibre layer. pRNFL thicknesses are lowest (≤87 µm), intermediate (>87–97 µm [Spectralis OCT] and >87–98 µm [Cirrus OCT]), and highest tertiles (>97 µm [Spectralis OCT] and >98 µm [Cirrus OCT]).

	n/N	Hazard ratio (95% CI)	p value	Harrell's C (95% CI)
Proportional hazards models 1*				
pRNFL (Spectralis OCT)	187/726	0·98 (0·97–0·99)	0·006	0·56 (0·43–0·68)
pRNFL (Cirrus OCT)	67/162	0·98 (0·96–1·00)	0·102	..
pRNFL 87–97 µm (Spectralis OCT) or 87–98 µm (Cirrus OCT) *vs* >97 µm (Spectralis OCT) or >98 µm (Cirrus OCT)	252/879	0·97 (0·70–1·34)	0·839	0·58 (0·42–0·73)
pRNFL ≤87 µm (both OCT devices) *vs* >97 µm (Spectralis OCT) or >98 µm (Cirrus OCT)	252/879	1·49 (1·08–2·04)	0·015	0·58 (0·42–0·73)
pRNFL ≤87 µm (both OCT devices) *vs* >87 µm (both OCT devices)	252/879	1·51 (1·16–1·98)	0·003	0·57 (0·41–0·72)
Follow-up ≤1 year	46/879	0·76 (0·40–1·42)	0·387	0·57 (0·41–0·73)
Follow-up >1 year and ≤3 years	163/708	1·65 (1·19–2·28)	0·003	0·57 (0·41–0·73)
Follow-up >3 years and ≤5 years	43/171	2·28 (1·22–4·25)	0·010	0·57 (0·41–0·73)
Proportional hazards models 2†				
pRNFL (Spectralis OCT)	94/408	0·97 (0·95–0·99)	0·001	0·55 (0·39–0·70)
pRNFL (Cirrus OCT)‡	..	..	..	..
pRNFL 87–98 µm (Cirrus OCT) or 88–98 µm (Spectralis OCT) *vs* >98 µm (both OCT devices)	159/557	0·78 (0·51–1·20)	0·265	0·58 (0·42–0·73)
pRNFL (µm) ≤87 µm (Cirrus) or ≤88 µm (Spectralis) *vs* >98 µm (both OCT devices)	159/557	1·75 (1·19–2·59)	0·005	0·58 (0·42–0·73)
pRNFL (µm) ≤87 µm (Cirrus OCT) or ≤88 µm (Spectralis OCT) *vs* >87 µm (Cirrus OCT) or >88 µm (Spectralis OCT)	159/557	1·96 (1·39–2·76)	0·00013	0·57 (0·41–0·72)
Follow-up ≤1 year	31/557	1·06 (0·51–2·23)	0·873	0·57 (0·41–0·73)
Follow-up >1 year and ≤3 years	99/422	2·06 (1·36–3·11)	0·001	0·57 (0·41–0·73)
Follow-up >3 years and ≤5 years	29/104	3·81 (1·63–8·91)	0·002	0·57 (0·41–0·73)

n is number of events and N is number of patients at risk in the analyses. Nine patients had data from both Spectralis and Cirrus devices so the sum of n and N from Spectralis OCT and Cirrus OCT were 254 and 888, respectively, instead of 252 and 879 for the proportional hazards models. All proportional hazard models were also fitted in a stepwise fashion including all covariates except pRNFL thickness in a first model and adding pRNFL thickness in a second model. The likelihood ratio tests for all proportional hazard models were significant, with the exception of the model including only data from Cirrus ,for which Harrell's C statistic was not estimated. pRNFL=peripapillary retinal nerve fibre layer. OCT=optical coherence tomography. EDSS=Expanded Disability Status Scale. *Models include variables for age (years), disease duration (years), unilateral optic neuritis (yes *vs* no), baseline EDSS, time (linear) × EDSS, and pRNFL thickness (continuous or tertiles). †Models include variables for age (years), disease duration (years), unilateral optic neuritis (yes *vs* no), baseline EDSS, time (linear) × EDSS, use of disease-modifying therapies (yes *vs* no) and pRNFL thickness (continuous or tertiles). ‡Model not presented because the number of events per variable was less than ten (9·57).

Table 3: **Proportional hazards models for baseline pRNFL thickness as a predictor of disability worsening**

of less than or equal to 88 µm in eyes without optic neuritis had a 90% increase in the adjusted risk of disability worsening compared with patients with a thicker pRNFL than these cutoffs (appendix p 6). Patients with relapsing-remitting multiple sclerosis with pRNFL less than or equal to 88 µm had twice the risk of disability worsening at any time after the first year and until the third year, and the risk was increased by almost three times from the third year to the end of follow-up (appendix p 6).

83 patients had secondary progressive multiple sclerosis and 58 had primary progressive multiple sclerosis. Because of the small sample sizes, we merged both subtypes so that we had a group of 141 patients with progressive multiple sclerosis (123 assessed with Spectralis OCT, 19 assessed with Cirrus OCT, and one patient assessed with both). 66 (47%) of 141 patients with progressive multiple sclerosis had disability worsening during follow-up (60 [49%] of 122 patients with Spectralis OCT and six [32%] of 19 patients with Cirrus OCT). Since so few patients were imaged with Cirrus, we estimated the association between disability worsening and pRNFL thickness in eyes without optic neuritis only in patients with progressive multiple sclerosis who had data from Spectralis. Patients with progressive multiple sclerosis with pRNFL thickness of less than or equal to 79 µm (Spectralis OCT) had a non-significantly increased unadjusted risk of disability worsening during follow-up compared with patients with higher pRNFL thickness (appendix pp 7–8). We did not detect significant differences in baseline features between patients with progressive multiple sclerosis whose disability worsened and those who remained stable (appendix p 8). In the adjusted analysis, patients with progressive multiple sclerosis and pRNFL of less than or equal to 79 µm (Spectralis OCT) at baseline had 82% increased risk of disability worsening during the follow-up compared with those with pRNFL thicker than this cutoff (appendix p 8). We did not have enough events per variable to evaluate risk in the different periods of follow-up in the subgroup of patients with progressive multiple sclerosis (appendix p 7–8).

We did a sensitivity analysis using an increase of at least 1·5 points in the EDSS score as the criterion for disability worsening for patients with baseline EDSS scores of 0 and another using a definition of subclinical optic neuritis based on the interocular asymmetry of pRNFL thickness of more than 20%.[16] Our results were

similar to those of the main analyses (appendix pp 8–9). pRNFL thickness in eyes without optic neuritis might be reduced in patients with a previous history of optic neuritis due to retrograde axonal degeneration of optic nerve fibres because both optic nerves are connected by the optic chiasm. We evaluated the association between pRNFL thickness and risk of disability worsening separately for patients with and without previous unilateral optic neuritis. Patients in the lowest category who had previous unilateral optic neuritis had increased risk of disability worsening compared with patients who had previous optic neuritis and belonged to the highest category. Patients in the lowest category who did not have previous unilateral optic neuritis also had increased risk of disability worsening during follow-up compared with patients without previous unilateral optic neuritis who were in the highest category. The risk was higher for patients with previous optic neuritis (appendix pp 9–10) but the term included to evaluate the interaction (OCT×previous optic neuritis) was not significant (p=0·444) in the proportional hazards models.

The main result of this study was that patients with multiple sclerosis and pRNFL thickness of less than or equal to 87 µm on Cirrus OCT and less than or equal to 88 µm on Spectralis OCT had roughly twice the risk of disability worsening (HR=1·96, 95% CI 1·39–2·76); p=0·00013; table 3) during the follow-up compared with patients with thicker pRNFL during the 5 years of follow-up. We estimated that the post-hoc statistical power for this result was 82% under the following assumptions: event rates of 25·08% in the group of patients with multiple sclerosis and pRNFL thickness of greater than 87 µm (Cirrus OCT) or 88 µm (Spectralis OCT) and 35·69% in patients with pRNFL thickness of less than or equal to 87 µm (Cirrus OCT) or 88 µm (Spectralis OCT) during 5 years of follow-up; a sample size of 366 patients with pRNFL thickness of greater than 87 µm (Cirrus OCT) or 88 µm (Spectralis OCT) and a sample size of 191 patients with pRNFL thickness of less than or equal to 87 µm (Cirrus OCT) or 88 µm (Spectralis OCT) (ratio 2:1); an α error of 0·05; and two-tailed tests for survival analyses.

Discussion

In this multicentre longitudinal study, our results showed that pRNFL thickness measured by OCT at a single timepoint during the course of the disease can be used as a marker of subsequent worsening of neurological disability in multiple sclerosis during 1 year and 5 years of follow-up. The key result of this study is that patients with multiple sclerosis and pRNFL thickness of up to 87 µm (Cirrus OCT) or 88 µm (Spectralis OCT) in eyes without optic neuritis had roughly twice the risk of disability worsening during follow-up compared with patients with thicker pRNFL. This risk was independent of other factors known to be associated with disability worsening, including age, disease duration, baseline level of disability (EDSS), and the use of disease-modifying therapies. These results are the first step towards validation of OCT of pRNFL as an imaging marker to monitor disability worsening in multiple sclerosis.

Retinas of patients with multiple sclerosis show neurodegenerative changes such as axonal loss and shrinkage of neuronal soma shrinking.[3] Whether such ongoing or relapsing damage is attributable to retrograde axonal degeneration due to subclinical optic neuritis or microscopic optic nerve inflammation,[7] trans-synaptic degeneration, primary retina neurodegeneration, or systemic effects of inflammation remains unclear.[13,25]

Our finding that patients with a given level of axonal damage, as shown by pRNFL thickness, are more likely to have disability worsening lends supports to the notion of a threshold in CNS damage, after which further damage translates to increasing clinical disability. The mechanisms underlying disability worsening in multiple sclerosis are not well understood but probably involve acute damage during relapses or chronic CNS inflammation (eg, microglial activation) and degenerative processes (eg, neuro-axonal degeneration, trans-synaptic degeneration, myelin loss).[13,26,27] Consequently, as suggested by our results, monitoring pRNFL thickness in the retina can be useful to monitor risk of disability worsening in multiple sclerosis.

In this study, we did not assess pRNFL thinning during follow-up and for this reason we cannot infer whether pRNFL thickness decreases with time. Serbecic and colleagues[28] reported that pRNFL measurements in patients with multiple sclerosis were unchanged compared with baseline during 2 years of follow-up. By contrast, Ratchford and colleagues[7] noted that the rate of ganglion cell-inner plexiform layer thinning was faster for patients with active multiple sclerosis than for those with stable disease during a 2-year follow-up.[7] The findings of these studies suggest that pRNFL changes might be too subtle to be detected with existing OCT systems during short follow-up and might require longer observation. Some authors[10] have recommended observation for at least 2 years for patients with multiple sclerosis without optic neuritis.[10] However, the dynamics of retinal thinning in multiple sclerosis are not well established so it is not clear how many OCT investigations per year are appropriate to measure true disease-related pRNFL thinning.

Loss of brain volume measured with MRI is probably the most commonly used measure of neurodegeneration in multiple sclerosis.[29] However, monitoring of neurodegeneration and disability worsening on MRI has methodological and biological limitations. OCT is technically easier than MRI, is accessible in many ophthalmological centres, and provides results for an outcome such as pRNFL thickness, which is amenable to use in an outpatient clinic setting as an objective measure of risk of disability worsening to support the therapeutic decision-making process.[16,30] Spectral-domain OCT with predefined scan protocols has shown

very good reliability in repeated measures.[31] Also, pRNFL thickness is a specific measure of axonal loss that is not directly affected by inflammation (except during acute optic neuritis) and is less affected by astrogliosis than is MRI brain volume loss. Therefore, we propose OCT as an imaging approach for multiple sclerosis that would complement MRI.

The absence of biological specificity of neuronal layer thinning in the retina might explain the lack of consistent association of macular volume with disability worsening in our study. Retinas of patients also show other changes such as axonal loss, neuronal soma shrinkage, synaptic loss, activation of microglia, and astrocyte proliferation.[3] Thinning of the ganglion cell-inner plexiform layer has been reported to be associated with disability[7] and brain atrophy.[8] Thickening or thinning of the macular inner nuclear layer has been noted in multiple sclerosis.[32,33] Furthermore, during acute optic neuritis, the outer retinal layers have consistently increased in thickness,[12,34] possibly because of the presence of cytotoxic oedema.[34] Thus, changes in macular volume might be more complex and difficult to interpret than previously thought with several mechanisms, such as neuronal damage and glial activation, being involved in the final measured magnitude. Future studies applying retinal segmentation techniques might be able to more specifically parse layers with consideration for neuronal damage glial activation and other mentioned mechanisms.

A notable finding of our study is the effect of drugs for multiple sclerosis in the risk of disability worsening defined by pRNFL. Disease-modifying therapies reduce the frequency of relapses and new inflammatory MRI lesions. Hence, these therapies might reduce acute axonal transection. Although, it is unclear whether these treatments can also prevent disability worsening due to diffuse damage, untreated patients had increased risk of disability worsening based on our results, which was independent of the other variables included in the analyses such as pRNFL category, disease duration, age, previous optic neuritis and baseline EDSS score.

Our study has several strengths, including the large sample size, with a post-hoc statistical power of 82% for the main finding, clinical follow-up of up to 5 years, and validation with data from many centres and for different multiple sclerosis phenotypes. The same team of EDSS-certified neurologists in each participating centre examined the patients during follow-up and researchers doing OCT at baseline were not aware of EDSS scores, and the researchers assessing EDSS scores were masked to OCT results. However, the study has some limitations. First, the predictive value of our models were low (Harrell's *C* values close to 0·5), especially since some studies[35] suggested that Harrell's *C* statistics might lead to overoptimistic estimates of goodness-of-fit of proportional hazards models. However, the main objective of the study was not to construct a fully predictive model, but to assess whether measurement of pRNFL thickness with OCT can improve estimation of the risk of disability worsening in multiple sclerosis. The results of the likelihood ratio tests done in the stepwise fashion of proportional hazards models showed that the addition of pRNFL thickness improved the predictive value of the models. Nevertheless, future models that include OCT measures and other data such as MRI or neurophysiological information might have improved predictive value. The sample size and therefore the number of events in the subgroup of patients with progressive multiple sclerosis were small and these results would need further evaluation in studies with larger sample sizes. Our results cannot be applied to patients with multiple sclerosis who have had bilateral optic neuritis. Additionally, our study did not include children with multiple sclerosis and, for this reason, additional studies will be needed for this population. We did not use a central OCT reading centre to assess the quality of the OCT data, but the investigators involved were from groups with extensive experience of high quality OCT research. The IMSVISUAL repository did not include MRI data, so we could not compare pRNFL thickness by OCT with brain atrophy by MRI for the prediction of disability worsening. The analysis only included patients with eyes without previous optic neuritis. To identify subclinical optic neuritis, we used two previously described approaches[13,16] that are based on interocular asymmetry in the pRNFL thickness and macular volume as measured by OCT.[13,16] However, the IMSVISUAL database did not include neurophysiological data that would be useful to improve the identification of subclinical optic neuritis.

Our results support measuring of pRNFL thickness as a biomarker for disability worsening in multiple sclerosis that might help treatment decisions in clinical settings. Our results would also be useful for investigators doing randomised controlled trials. These studies need to enrol patients who are at high risk of presenting the event of interest. Although patients might fulfil inclusion criteria, some might have a low risk of disability worsening (event of interest). The inclusion of such patients might decrease the power of a randomised controlled trial testing a neuroprotective drug because the efficacy of the drug might not be captured because of insufficient numbers of events. Patients at high risk of disability worsening are the most informative population for such trials because their inclusion can help to maximise the effect size. Thus, quantification of pRNFL thickness by OCT in patients with multiple sclerosis and without optic neuritis will help to identify the most appropriate patients to be enrolled in a randomised controlled trial, reducing the sample size and the associated costs.

Contributors

All authors contributed to the recruitment of patients, clinical and OCT data acquisition, review of the database, and review of the results and manuscript. EHM-L and PVi designed the study, did the analyses, and wrote the draft of the manuscript.

Declaration of interests

EHM-L is a researcher in the OCTIMS study, an observational study (that involves no specific drugs) to validate OCT as a biomarker for multiple sclerosis, sponsored by Novartis. SA is a researcher in OCTIMS study. SSa has received consulting fees from Medical Logix for the development of CME programs in neurology, consulting fees from Axon Advisors LLC, Educational Grant Support from Novartis & Teva Neurosciences, speaking honoraria from the National Association of Managed Care Physicians, Advanced Studies in Medicine, and the Family Medicine Foundation of West Virginia, has served on a scientific advisory board for Biogen-Idec and Genzyme, and is a researcher in OCTIMS study. JLP has received speaker and consulting fees from Biogen, Novartis, and Teva and is a researcher in OCTIMS study funded by Novartis and ARPEGIO from Teva. TO has received speaker fees from TEVA and Bayer and is a researcher in OCTIMS study. AUB is founder and holds stock options of Motognosis; is named as co-inventor on several patent applications unrelated to this study; has received research grants, speaker honoraria, or consulting fees from Biogen, Teva, Novartis, and Bayer; and is a researcher in OCTIMS study. IG reports personal fees from Novartis, Biogen, Genzyme, and from Merck-Serono. OO reports a grant for research from Novartis; grants and personal fees from Biogen-Idec, funding for travel from Biogen-Idec, Genzyme-Sanofi, Merck-Serono, Novartis, and Teva Pharmaceutical Industries; and is a researcher in OCTIMS study. PA has received speaker honoraria, consulting fees and travel support from Biogen, Novartis, TEVA, IPSEN, Merz, Esai, and GlaxoSmithKline and research grants from Biogen, Merz, and Novartis, and is a researcher in the PASSOS and ARPEGIO trials aiming to validate OCT as biomarker of multiple sclerosis and sponsored by Novartis and TEVA, respectively. SL has received speaker fees from Genzyme, Biogen, and Heidelberg Engineering and has participated in the OCTIMS and the PASSOS studies, sponsored by Novartis. LiJB received research support from the Stichting MS Research The Netherlands and Novartis and is a researcher in OCTIMS study. JLF has served on scientific advisory boards for and received travel funding for these activities and honoraria from Biogen Idec, Merck Serono, Sanofi-Aventis, Genzyme, Teva, Novartis, and Takeda and has received speaker honoraria from Biogen Idec and Merck Serono. BS-D is a researcher in OCTIMS study. AS has received compensation for consulting services and speaking from Bayer-Schering, Merck-Serono, Biogen-Idec, Sanofi-Aventis, Teva, and Novartis and is a researcher in the OCTIMS study. RB has served as a consultant for Biogen, Novartis, Genzyme, and Genentech. AK has received grants from the NMSS and Novartis, and personal fees from Biogen Idec. AP has received consulting fees form Novartis and research support from the Stichting MS Research The Netherlands, and is a researcher in OCTIMS study and performs QC reading for the PASSOS study by Novartis. SSc has received research grants from Biogen, Bayer Healthcare, and Genzyme, and consulting and speaking fees from Bayer Healthcare, Biogen, Merck Serono, Novartis, TEVA and Genzyme/Sanofi; sits on the Steering Committees of the OCTiMS, BENEFIT, REFINE, and EMPIRE studies; and has participated in the OCTIMS and PASSOS studies (both studies sponsored by Novartis). OA has received research support from the German Research Council (DFG), the German Ministry of Education and Research (BMBF-EDEN), Bayer, Biogen Idec, Merck Serono, Novartis, and Teva and serves on an advisory board for MedImmune; is a researcher in PASSOS and ARPEGIO aiming to validate OCT as biomarker of multiple sclerosis and sponsored by Novartis and TEVA, respectively; and is an academic editor for *PLoS One*. PVe has received honoraria and consulting fees from Biogen, Genzyme-Sanofi, Bayer, Novartis, Teva, Merck-Serono, GlaxoSmithKline, and Almirall; research support from Biogen, Bayer, Novartis, and Merck-Serono; and is co-chairman of the scientific advisory board of the OCTIMS study. CO-G has received honoraria and consulting fees from Biogen, Genzyme-Sanofi, Bayer, Novartis, Teva, and Merck-Serono and is an investigator in OCTIMS. GC has received personal compensation and research funding from Teva, Sanofi, Genzyme, Merck Serono, Biogen, Bayer, Serono Symposia International Foundation, Excemed, Roche, Almirall, Chugai, Receptos, and Forward Pharma. 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EH has received clinical trials advisory board membership and speaker's honoraria from Biogen, Merck Serono, Novartis, Sanofi Genzyme, Alkermes, Actelion, and Teva; and is a researcher in OCTIMS study. TF has received speaker and consulting fees from Novartis, Genzyme, and Acorda. EF has received speaker and consulting fees from Novartis, Genzyme, Acorda, and TEVA. LaJB has received consulting fees from Biogen and Genzyme and has received research funding from Biogen. AJG has received personal fees from Inception Sciences and Mylan Pharmaceuticals and grants or awards from Novartis; has served on endpoint adjudication committees for Biogen and MedImmune; serves on trial steering committees for Novartis and the scientific advisory board for Bionure and Inception 5; and is a researcher in OCTIMS study. PAC has received consulting fees from Vertex, Abbvie, and Merck and has received research funding from Biogen, Novartis, and MedImmune; and is co-chairman of the scientific advisory board of the OCTIMS study. PVi has received consultancy fees from Heidelberg Engineering regarding the clinical applications of OCT; serves as academic editor of Current Treatment Options in Neurology, Neurology & Therapy, Multiple Sclerosis & Demyelinating Disorders, *PLOS One*, and MS in focus; is founder and hold stocks in Bionure and Spire Bioventures; has received consultancy fees from Roche, Novartis, Health Engineering, and stock options from Mint-Labs; has received unrestricted grants from Genzyme, Roche and Novartis; and is a researcher in OCTIMS study. JAW, LEP, SG, A-KM, WC, CF, JR, CC, IZ, MA, FC, and EG-M declare no competing interests.

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