Retinal thickness measured by optical coherence tomography and risk of disability worsening in multiple sclerosis

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Abstract

Background: Most of Multiple Sclerosis (MS) patients without previous optic neuritis (ON) show thinner retinal measurements compared to healthy controls. We aimed to evaluate the role of peripapillary retinal nerve fiber layer (pRNFL) thickness or macular volume (MV) in non-ON eyes as a biomarker of disability worsening in a cohort of MS patients with at least one non-ON eye available.

Methods: In this multicenter longitudinal study, we enrolled 879 patients (739 relapsing-remitting and 141 progressive patients). Disability worsening was assessed using the expanded disability status scale (EDSS). pRNFL thickness and MV were assessed by optical coherence tomography (OCT) and calculated as the mean value of both non-ON eyes for patients without previous ON or the fellow eye's value for those with previous unilateral ON. We estimated the association between pRNFL thickness and MV in non-ON eyes, and the risk of disability worsening using proportional hazards models including OCT metrics and age, disease duration, disability, presence of previous unilateral ON and use of disease modifying therapies as covariates.

Findings: Disability worsening was noted in 252 out of 879 (29%) patients after a mean follow-up of 2.32 years of follow-up. MS patients with a pRNFL≤92-93µm in non-ON eyes were associated with a 58% increased risk of disability worsening as compared to patients with pRNFL>92-93µm, at any time after the first year of ascertainment in this cohort [HR=1.58 95% CI (1.13-2.21); p=0.007]. Further, a pRNFL ≤87-88µm doubled the risk of disability worsening at any time during the second and third year of follow-up [HR=2.06 95%CI (1.36-3.11); p=0.001], and increased the risk by four-fold after 4-5 years of follow-up [HR=3.81 95% CI (1.63-8.91) p=0.002]. We did not identify meaningful associations for disability worsening with MV.

Interpretation: Our results provide compelling evidence of the utility of monitoring pRNFL thickness by OCT for predicting the likelihood of disability worsening in MS patients over time.

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Putting research into context

Evidence before this study

We searched Pubmed for articles on retinal atrophy by optical coherence tomography and disability worsening/progression in multiple sclerosis published before 15 October 2015 using the following terms: "optical coherence tomography AND Multiple Sclerosis" and "retinal nerve fiber layer thickness AND Multiple Sclerosis". We identified several studies addressing the association between disability in patients with multiple sclerosis and retinal nerve fiber layer thickness or/and macular volume assessed by optical coherence tomography (OCT). However, most of the data available to date have been collected using time-domain OCT (which is less accurate and reproducible as compared to the spectral domain OCT devices) in cross-sectional designs. Most of these previous studies were included in a systematic review This meta-analysis found inverse correlations (r=-0.3 to r=-0.7) between expanded disability status scale (EDSS) and retinal nerve fiber layer thickness in 67% of the included studies. Nevertheless, definitive longitudinal evidence linking changes in retinal architecture as a valid and useful surrogate end-point to disability worsening in MS is still lacking.

Added value of this study

Herein, we report, using multicenter longitudinal cohorts of 879 MS patients that the thickness of the peripapillary retinal nerve fiber layer (pRNFL) is a marker of risk of increased disability, as measured by the validated expanded disability status scale (EDSS). This multicenter, collaborative initiative to assess the value of this novel biomarker to inform about risk of disability worsening in short and medium terms in patients with multiple sclerosis provides evidences of the usefulness of OCT imaging for monitoring MS course.

Implications of all the available evidence

Our results support the use of pRNFL thickness as a marker of disability worsening in multiple sclerosis. Our findings might help neurologist to monitor of the disease in clinical settings and more importantly to help drive treatment decisions based on a marker of neuro-axonal damage. Quantification of pRNFL will identify patients at high risk of disability worsening, using the cut-offs identified, that will be most informative population to be recruited for randomized clinical trials testing neuroprotective or regenerative drugs, thereby reducing sample size and associated costs.

INTRODUCTION

Multiple sclerosis (MS) is a disease with an unpredictable course, which makes it very difficult to provide accurate prognostic information on an individual patient basis, representing a challenge for developing personalized medicine[1]. Developing an imaging biomarker for MS, in order to predict the clinical course and future disability will improve the clinical management, as well as may be useful for targeting the most appropriate patients to be enrolled in randomized clinical trials (RCT) testing neuroprotective or regenerative drugs[2].

The retina of patients with MS displays inflammatory and neurodegenerative findings[3]. Optical coherence tomography (OCT) is a well-tolerated, accurate and high-resolution imaging modality to evaluate retinal integrity. Peripapillary retinal nerve fiber layer (pRNFL) thickness and macular volume (MV) have been the most reported OCT measures to evaluate the construct of retinal atrophy. MV is used to ascertain retinal ganglion cell integrity, because most of them are located in the macula. Since 2010, macular retinal ganglion cell layer thickness is also quantified based on novel segmentation algorithms.

Studies using spectral-domain OCT have so far revealed that the retina in eyes without previous optic neuritis (non-ON eyes) shows thinner pRNFL and retinal ganglion cell layer thickness in most of the patients compared to healthy controls, and this was observed in all MS phenotypes including benign MS[4-6]. All of these OCT parameters have been associated with MS disability[7,8] and brain atrophy[7,9] and for this reason its application offers the promise of being used as an imaging surrogate of the MS disease course.

Nevertheless, up till now, most of the data have been collected using time-domain OCT (which is less accurate and reproducible than the new spectral domain devices[10]) in cross-sectional or short-term studies[11]. A definitive longitudinal evidence linking changes in retinal architecture as a valid and useful surrogate end-point of disability worsening in MS is a high research priority[12].

Based on the unmet need of biomarkers for predicting the course of MS, the aim of this study was to evaluate if a single assessment of the pRNFL thickness or MV by OCT in non-ON eyes can be used as a biomarker of the risk of disability worsening in a large multicenter cohort of MS patients with a longitudinal ascertainment of up to 5 years.

METHODS

Study design

The IMSVISUAL (http://www.imsvisual.org) database is composed of longitudinal cohorts either already published or being collected as part of ongoing prospective studies. All MS patients gave written informed consent and all of the prospective studies whose de-identified data were in the IMSVISUAL repository have been approved by their respective Institutional Review Boards. We followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement for reporting the data of our observational study.

Study population

In this large, multicenter, longitudinal cohort study, we enrolled patients (age > 16 years old) with clinically isolated syndrome (CIS) or MS according to the revised 2010 McDonald Criteria[13], including relapsing remitting (RRMS), secondary progressive (SPMS) and primary progressive (PPMS) subtypes. Patients from the database were only included in the analysis if longitudinal information regarding neurological disability from baseline to up to 5 years of follow-up was available

Retinal atrophy after acute ON is often more pronounced than the retinal thinning observed in the absence of ON in MS[11,14]. For this reason eyes with previous ON were excluded form the analysis. Consequently, we only selected patients with at least one non-ON eye available. Previous diagnoses of ophthalmological, neurological or drug-related causes of vision loss or retinal damage not attributable to MS are exclusion criteria for IMSVISUAL repository entry as described elsewere[9,15].

The IMSVISUAL database included 1,043 out of 1,595 patients with available information on longitudinal disability; 879 out of 1,043 patients had OCT data from at least one non-ON eye (**Figure 1)**.

Collection of demographics and clinical data

We collected a set of clinical variables at baseline, including age at inclusion, sex, disease duration, MS subtype, disability measures, previous history of ON, and use of disease modifying therapies (DMTs). Neurological disability was evaluated by the expanded disability status scale (EDSS). Multiple sclerosis functional composite (MSFC) was available only for a sub-set of participants (n=197), and was used as an additional outcome measurement of disability. The presence of prior ON was assessed in the subjects' medical record, as previously described[16]. The same neurologist and ophthalmologist in each centre examined the patients during the follow-up in the study.

Definition of the end-point: disability worsening

Disability worsening (confirmed accumulation of disability) was defined following recent criteria as a documented increase in neurologic disability (at least one-point increase in the EDSS score, or half-point increase for patients with a baseline score ≥5.5, confirmed in a second visit 3 to 6 months apart) as a result of relapses or progressive disease[12]. EDSS assessment was always performed in the absence of acute relapses. MSFC worsening was defined as having maintained worsening ≥ 20% by any of three components[17]. Disability worsening (yes/no) was estimated once for each patient at any time of follow-up and with either EDSS or MSFC.

Definition of the OCT measurements in non-ON eyes

pRNFL thickness and MV (6 mm ring area) were evaluated once at baseline visit by using spectral-domain OCT, either Spectralis® (Heidelberg Engineering, Germany) or Cirrus™ (Carl Zeiss, Dublin, US). The pRNFL thickness and MV were calculated as the mean values of both non-ON eyes, except for patients with a previous history of unilateral ON, of whom only the fellow non-ON eye's values entered the analyses.

In order to identify the cases of subclinical ON, we used a previously described approach based on the interocular asymmetry in the pRNFL thickness and MV by OCT[15] (see supplementary results for details). Only eight patients (6 imaged with Spectralis® and 2 with Cirrus™) with both eyes tagged as non-ON eye met this criterion for pRNFL thickness and 10 patients (7 imaged with Spectralis® and 3 with Cirrus™), for MV. For these patients, we included only the eye with the highest pRNFL thickness and MV value.

Statistical analysis

We described baseline features of the study population and the distribution of events (disability worsening) by key demographic and clinical characteristics using absolute and relative frequencies for categorical variables and medians, P25, P75 and interquartile ranges for quantitative variables for describing baseline features of the study population and P20, P40, P60, P80 for describing distribution of events.

Considering the different periods of follow-up for patients in the study, we fitted proportional hazards models (PHMs) to evaluate pRNFL thickness and MV in non-ON eyes as markers of the risk of

disability worsening. First, we evaluated these OCT measures quantitatively and then, we used median and tertiles to categorize pRNFL thickness and MV in two and three equally sized groups as an approach to merge data from both devices and to assess the non-linear relationship between retinal integrity and disability worsening. We defined pRNFL as the primary end-point of the study because it has better signal to noise ration than MV (see discussion for biological basis). We ran PHMs including only OCT measures in the models to provide unadjusted HRs for pRNFL thickness and MV. We estimated the Kaplan-Meier plots of cumulative incidence of disability worsening during the follow-up in the two groups (split by median) and three groups (split by tertiles) for the primary end-point for the study in the total population and for MS subgroups[18].

We compared baseline features between the patients who developed disability worsening and the patients who remained stable at the end of the follow-up. We performed these bivariate analyses by using Fisher's exact test for categorical data and Mann-Whitney-U test for quantitative variables. We ran PHMs to assess the univariate impact of each one of these features in the risk of disability worsening. We ran PHMs including all these covariates to estimate the adjusted HR for OCT measures for the study population and for the subgroups.

A key assumption in these models is proportional hazards. We used time dependent covariates, including interaction terms with time, to assess proportionality for each covariate included in the model, namely age, previous ON, disease duration, EDSS at baseline, use of DMTs and OCT metrics in PHMs. We evaluated linear interaction [covariate*Time], logarithmic interactions [covariate*LN (Time) and covariate*LG10 (Time)] and also we evaluated the effect of these covariates during the first year of follow-up [covariate*Time≤1.00], from first to third year [covariate *Time>1.00 and ≤3.00] and finally from third to fifth year [covariate *Time>3.00 and ≤5.00]. Baseline EDSS (models including total study population), disease duration and presence of previous ON (models including progressive MS (PMS) subgroup) showed non-proportionality for the analyses of pRNFL thickness. Median MV also displayed non-proportionality. Thus, we extended the PHM by adding the interaction between the non-proportional covariate and the time of observation also as covariate. A patient who displayed disability worsening within a given follow-up period was excluded for the analyses of subsequent follow-up periods because we only included patients who were free of the event of interest to evaluate the risk of disability worsening in the next follow-up period. A patient who did not present the event at a given follow-up period was included for the analyses of the next follow-up period only if we had available information

about his/her disability status during the next follow-up period; otherwise, he/she was censured. We used the likelihood ratio test and Harrell´s C statistics to evaluate the goodness-of-fit of PHMs. The accuracy and significance of the coefficients estimated by the proportional hazards methods would become untrustworthy when the number of events per variable (EVP) is lower than ten[19,20]. Thus, we considered this cut-off point as a reference and we only ran PHMs with EVP≥10. Two-tailed p-values <0.05 were considered statistically significant. Analyses were performed with the Statistical Package IBM-SPSS (SPSS Inc, Chicago, IL, USA) software version 20.0 for Mac. Harrell´s C statistic estimations were performed with SAS version 9.4.

Role of funding source

The funding agencies had no role in the design nor conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript. The raw IMSVISUAL dataset is available at the IMSVISUAL website (http://www.imsvisual.org) for registered researchers upon request. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Baseline characteristics of study population

The study population included 879 MS patients (**Figure 1, Table 1**). The cohort included mainly a mildly to moderately disabled population, with a median EDSS of 2.0. Information about therapies was available for 557 participants. Only 533 patients out of 879 had MV values available.

Patient's characteristics categorized by disability worsening

We identified 252 out of 879 (29%) patients who displayed disability worsening after the follow-up. The study follow-up period ranged from 1 to 5 years (mean 2.32 years and median 2.00 years). **Table 2** showed the comparison of baseline characteristics between patients with disability worsening and those with stable MS after follow-up. Patients who developed disability worsening were older, suffered more frequently PMS and had longer disease duration at baseline. Moreover, the prevalence of unilateral ON prior to study enrollment was higher for patients who remained stable during follow-up. Patients who showed disability worsening less frequently received DMTs. **Table S1** displayed the frequency of events

by key demographic and clinical characteristics**.** We also ran univariate proportional hazards models to assess the impact of these baseline features in disability worsening (**Table S2).** These results showed that age, disease duration, baseline disability and the presence of PMS phenotype were directly associated with the risk of disability worsening, whereas receiving DMTs and the presence of unilateral ON prior to study enrollment were inversely related with the risk of disability worsening during the follow-up.

Baseline pRNFL thickness in non-ON eyes predicts risk of disability worsening

Unadjusted analyses

We ran separate PHMs for Spectralis® and Cirrus™ as due to differences between the segmentation algorithms, pRNFL thickness quantification is directly not comparable between the two devices[21] (Spectralis® n=726, Cirrus™ n=162 and 9 patients had studies with both devices). The unadjusted hazard ratios (HR) in favor of disability worsening among patients with greater baseline pRNFL thickness was HR=0.98 [95%CI (0.97-0.99); p<0.001] for Spectralis® and HR=0.99 [95% CI (0.97-1.01); p=0.210] for Cirrus™. These results suggested that higher pRNFL thickness (lower retina atrophy) was associated with lower likelihood of disability worsening.

Previous studies suggested non-linear relationship between retinal atrophy and clinical outcomes based in the presence of thresholds for predicting visual disability[14,22]. Thus, we used median and tertiles to categorize pRNFL thickness in two and three equally sized groups and also this approach allowed us to pool data from both devices. Patients who had pRNFL thickness ≤92μm at baseline (median) had a 51% greater risk of disability worsening [HR=1.51 95%CI (1.17-1.93); p=0.001] compared to patients with thicker pRNFL thickness. Next, we compared the risk associated to being allocated in the lower tertile [pRNFL≤87μm] and intermediate tertile [pRNFL=87-98μm] versus being included into the higher tertile [pRNFL>97-98μm]. We found that patients in the lower tertile at baseline demonstrated increased risk of disability worsening [HR=1.65 95% (1.23-2.21); p=0.001] compared to those in the higher tertile, while there were no differences between patients of middle and higher tertile (**Figure 2).**

Adjusted analyses

We ran PHM including age, previous ON, disease duration, EDSS to evaluate the role of pRNFL thickness as a predictor of disability worsening.

The adjusted HR for pRNFL thicknesses were HR=0.98 [95%CI (0.97-0.99); p=0.006] for Spectralis® and HR=0.98 [95% CI (0.96-1.00); p=0.102] for Cirrus™. When we included also use of DMTs as a covariate, the risk of disability worsening associated to pRNFL thinning increased slightly (HR=0.97 [95% CI (0.95-0.99); p=0.001] for Spectralis® device. The Cirrus™ PHM is not shown because the number of EVP including DMTs as covariate was lower than 10 (EVP=9.57).

Patients who had a pRNFL≤ 92^{C}-93^{S}µm showed a 58% increase in the risk of disability worsening [HR=1.58; 95%CI (1.13-2.21); p=0.007] when compared to patients with a thicker pRNFL. Again, we found that patients in the lower pRNFL thickness tertile displayed increased risk of disability worsening [HR=1.75 95% CI (1.19-2.59); p=0.005] compared to those in the higher tertile. There were no differences between patients in the intermediate vs. higher tertiles. Thus, we merged the intermediate and higher tertiles, and thereby estimated the risk associated to pRNFL thickness in the lower tertile, as compared to this new aggregate category. Patients who displayed pRNFL≤87^{C}-88^{S}µm doubled the risk of worsening in the following years compared to patients with greater pRNFL [HR=1.96 95%CI (1.39-2.76); p<0.001]. All these PHMs were statistically significant (**Table 3**).

Regarding MV, we only found increased risk of disability worsening at any time after the first year follow-up until the fourth [HR: 2.02 95%CI (1.03-3.93); p=0.040] for those patients with MV≤8.73mm^3 (**see supplementary results and Table S3**).

Disability worsening prediction by pRNFL depends on follow-up time

The association of the risk of disability worsening with baseline pRNFL for the first year was non-significant. Considering than sample size and effects were similar for year 2 and 3 as well as for years 4 and 5, we merged these time points. Therefore, as exploratory analyses, we provided estimations from baseline to first year; from first to third year and from third to fifth year. Patients with pRNFL ≤87^{C}/88^{S}µm doubled the risk of disability worsening [HR=2.06 95% CI (1.36-3.11); p=0.001] at any time after the first year and until third year of follow-up. This risk almost increased four-fold [HR=3.81 95% CI (1.63-8.91); p=0.002] after the third year and until the fifth year of follow-up (**Table 3**).

Subgroup analyses: risk of disability worsening in CIS, RRMS and PMS

There were 74 patients in the CIS subgroup (Cirrus™ n=4 and Spectralis® n=70). We evaluated the association between disability worsening and pRNFL thickness in non-ON eyes only in the group

with data from Spectralis® (because the small sample size of Cirrus prevented further analysis). We did not find associations between pRNFL thickness and risk of disability worsening in the CIS subgroup (**supplementary figure S3**).

There were 664 RRMS patients of whom 171 patients (25.7%) presented disability worsening during the follow-up. RRMS patients with pRNFL thickness $\leq 92^{C}/93^{S}$µm at baseline and specially those with pRNFL thickness ≤88µm displayed increased risk of disability worsening during follow-up (**supplementary figure S4**). We estimated adjusted HR for pRNFL thickness in non-ON eyes and risk of disability worsening in the group of 426 RRMS for whom we had information about use of DMTs (**supplementary Table S4.1**). RRMS Patients with pRNFL≤88µm in non-ON eyes (lower tertile) displayed a 90% increase in the risk of disability worsening [HR=1.90; 95%CI (1.27-2.83); p=0.002] compared to patients with a thicker pRNFL than these cut-offs in PHMs including the following covariates: age, disease duration, previous ON, baseline EDSS, use of DMTs and pRNFL thickness. RRMS patients with pRNFL≤88µm had twice the risk [HR=2.17 95% CI (1.34-3.51); p=0.002] of disability worsening at any time after the first year and until the third year, and the risk was multiplied by almost three [HR=2.77 95% CI (1.12-6.85); p=0.028] thereafter until the end of follow-up in our study (**supplementary results and Table S4.2).**

There were 83 SPMS and 58 PPMS patients. Considering the sample size, we merged both phenotypes in a group of PMS of 141 patients (Spectralis® n=123, Cirrus™ n=19, and 1 patient had data from both OCT). && out of 141 PMS patients (46.8%) had disability worsening during the follow-up (Spectralis®: 60 out of 122 patients (48.7%) and Cirrus™: 6 out of 19 patients (31.5%)). Considering the small sample size of patients imaged with Cirrus™, we estimated the association between disability worsening and RNFL thickness in non-ON eyes only in PMS patients with data only from Spectralis®. We did not find statistically significant differences in the baseline features between PMS with disability worsening and those who remained stable (**Table S5**). PMS patients with pRNFL$\leq 79^{S}$µm at baseline had increased risk of disability worsening during the follow-up [HR=1.82 95% CI (1.06-3.11): p=0.030] compared to those with pRNFL thicker than this cut-off in a PHM including age, disease duration and previous ON as covariates. DMTs have not showed relevant benefit to counteract risk of disability worsening in MS patients with progressive phenotype. Therefore, we did not consider this variable in our PHMs for PMS patients. We did not have enough EPV to evaluate risk of disability worsening in the different periods of follow-up in the PMS subgroup (**supplementary results**).

Sensitivity analyses

Finally, we performed sensitivity analysis using an increase ≥1.5 point in the EDSS score as criteria for disability worsening for patients with baseline EDSS=0 and another definition of subclinical ON based in the interocular asymmetry of RNFL thickness >20%[16], founding similar results (**supplementary Table S6 and Table S7)**. pRNFL thickness in non-ON eyes may be reduced in patients with previous history of ON due to retrograde axonal degeneration of optic nerve fibers since both nerves are connected by the chiasm. We evaluated the association between pRNFL thickness and risk of disability worsening separately for patients with and without previous unilateral ON. Patients in the lower tertile for both groups displayed an increased risk of disability worsening during the follow-up although the risk seems to be higher for patients with previous ON (**supplementary T**able S8).

Power calculation for key findings

The key result of this study is that MS patients with pRNFL thickness in non-ON eyes ≤87-88μm had twice the risk of disability worsening [HR=1.96 95%CI (1.39-2.76); p<0.001] during the follow-up. We calculated that the statistical power for this result was 82% under the following assumptions: (1) event rates of 25.08% in the group of MS patients with pRNFL thickness >87-88μm and 35.69% in the group of MS patients with pRNFL thickness ≤87-88μm in a 5-year follow-up period; (2) sample size for the group of MS patients with pRNFL thickness >87-88μm equal to 366 and sample size for the group of MS patients with pRNFL thickness ≤87-88μm equal to 191 [ratio 2]; (3) an alpha error of 0.05 and (2) two-tailed test for survival analyses.

DISCUSSION

In this multicenter longitudinal study we have demonstrated that pRNFL thickness measured by OCT, can be used as a marker of worsening of neurological disability over a time horizon of between one and five years of follow-up. The key result of this study is that MS patients with pRNFL thickness in non-ON eyes ≤87-88μm had twice the risk of disability worsening during the follow-up. This risk was independent of other factors known to be associated with disability worsening including age, disease duration, baseline level of disability (EDSS) and use of DMTs. These results constitute the first step to validate the use of OCT as an imaging marker for monitoring MS.

The retina of patients with MS displays neurodegenerative changes such as axonal loss, neuronal soma shrinking[3]. Whether such continuously ongoing or relapsing damage is attributable to retrograde axonal degeneration due to subclinical ON or microscopic optic nerve inflammation[8], trans-synaptic degeneration, primary retina neurodegeneration or systemic effects of inflammation remains unclear[15,23]. Our findings, that patients with a given level of axonal damage, as revealed by pRNFL thickness, are more likely to become disabled supports the concepts of a threshold in CNS damage; after which, further damage translates to a greater clinical disability. The mechanisms underlying disability worsening in MS are not well known but must likely involve acute damage during relapses or chronic CNS inflammation (e.g. microglia activation) as well as degenerative processes (neuro-axonal degeneration, transynaptic degeneration, myelin loss)[24,25]. Consequently, as shown in our study, monitoring pRNFL thickness in the retina is useful to monitor risk of disability worsening in MS.

In this study, we did not evaluate pRNFL thinning over the follow-up and for this reason we cannot contribute to whether RNFL thickness decrease overtime. Serbecic N et al have found found that pRNFL measurements in MS patient were unchanged compared to baseline[6]. By contrast, Ratchford JN et al found that the rate of ganglion cell plus inner plexiform layers thinning in MS was faster for patients with active disease in a cohort of MS patients followed over a two year period[8]. These studies suggested that pRNFL changes might be too subtle to be detected with current OCT systems for short follow-up periods and might require longer observation periods. Some authors recommended an observation period of at least two years for MS patients without ON[11]. However, the dynamics of retinal thinning in MS is not well established so far so it is not clear how many OCT investigations per year are appropriate to evaluate pRNFL thinning and monitor MS course.

Brain volume loss by MRI is probably the most commonly used measure of neurodegeneration in MS[26]. However, monitoring neurodegeneration and disability worsening by MRI has methodological and biological limitations. In contrast, OCT is technically easier, accessible in many ophthalmologic centers and provides an immediate bedside-like outcome such as pRNFL thickness, amenable to be used in an outpatient clinic setting as an objective measure of risk of progression supporting the therapeutic decision-making process[16,27]. Spectral-domain OCT with predefined scans protocols has shown very good reliability of repeated measures. Finally, pRNFL thickness is a specific measure of axonal loss not affected directly by inflammation (except during acute ON) and less affected by astrogliosis compared to

MRI brain volume loss. Therefore, we propose OCT as an imaging marker for MS that would complement MRI evaluation.

The lack of biological specificity of neuronal layer thinning in the retina might explain the lack of consistent association of MV with disability worsening in our study. The retina of patients also show different changes such as axonal loss, neuronal soma shrinking, synaptic loss, microglia activation and astrocyte proliferation[3]. The ganglion cell complex thinning has been associated with disability[7,8] and brain atrophy[9]. Macular inner nuclear layer thinning or thickening has been reported in MS[28,29]. Also, during acute ON, outer retinal layers have consistently been observed to show an increase in thickness[14,30], may be due to presence of cytotoxic edema[30]. Thus, MV thickness changes may be much more complex and difficult to interpret with several mechanisms being involved in the final disposition of its measured magnitude. Future studies applying retinal segmentation may be able to more specifically parse layers with glial activation.

A striking finding of our study is the effect of the use of MS drugs in the risk of disability worsening defined by pRNFL. DMTs reduce the frequency of relapses and new inflammatory MRI lesions. As a consequence, it may reduce acute axonal transection. Although, it is unclear if these treatments may prevent disability worsening due to diffuse damage, untreated relapsing MS patients displayed an independent increased risk of disability worsening according to our results.

Our study has several strengths, including the large sample size with statistical power of 82% for the main findings, clinical follow-up of up to 5 years and the validation with data from multicenter source as well as for different MS phenotypes. However, the study has also some limitations as well. For example, our results cannot be applied to MS patients who have experienced bilateral ON. The sample size and therefore, the number of events in the progressive MS subgroup was small and these results in progressive MS would need further evaluation in studies with larger sample sizes. Also, our study has not included patients with pediatric MS and for this reason additional studies will be required for this population. This study has not used a central OCT reading centre to evaluate the quality of the OCT data, but we included patients from groups with extensive expertise on high-quality OCT research. The IMSVISUAL repository did not include MRI data, so we could not evaluate the role of pRNFL thickness in comparison to brain atrophy by MRI for predicting disability worsening. The analysis was based on eyes without previous ON. In order to identify subclinical ON, we used two previously described approaches based on the interocular asymmetry in the pRNFL thickness and MV by OCT[15,16]. However,

IMSVISUAL database did not include neurophysiological data that would be useful to improve the identification of subclinical ON.

Our results support pRNFL thickness measures as a biomarker of disability worsening in MS that may help drive treatment decisions in clinical settings. Additionally, our results would be useful for RCTs. RCT needs to enroll patients that are at high risk of presenting the event of interest. Patients may fulfill inclusion criteria but some may have low risk of disability worsening (main event). Their inclusion may decrease power of a RCT testing a neuroprotective drug because it may not capture the efficacy of the drug because lack of events. Patients at high risk of developing disability worsening constitute the most informative population for these RCT because they maximize the effect size. Thus, quantification of pRNFL thickness by optical coherence tomography in non-ON eyes of MS patients and the proposed cut-offs will help to identify the most appropriate patients to be enrolled in a RCT; thus reducing the number of non-informative patients and the associated costs. Future multicenter prospective studies may solve some of these limitations and provide definitive validation of these findings.

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Author's contribution

All authors have contributed to patient's recruitment, clinical and OCT data acquisition, review of the database and review of the results and the manuscript. EHML and PV designed the study, performed the analysis and wrote the draft of the manuscript.

Declaration of interests

Elena H Martinez-Lapiscina is a researcher in OCTIMS study, an observational study for validating OCT as a biomarker for MS sponsored by Novartis (this study is not involving any specific drug).

Sam Arnow is a researcher in OCTIMS study.

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Jana Lizrova Preiningerova has received speaker and consulting fees from Biogen, Novartis and Teva. She is a researcher in OCTIMS study. She is supported by National grant IGA MZ ČR NT/13239.

Timm Oberwahrenbrock has received speaker fees from TEVA. He is a researcher in OCTIMS study.

Alexander U. Brandt is founder and holds stock options of Motognosis; and is named as co-inventor on several patent applications unrelated to this study. He has received research grants, speaker honoraria or consulting fees from Biogen, Teva, Novartis and Bayer. He is a researcher in OCTIMS study.

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Simone Guerrieri has nothing to disclose

Ines González has nothing to disclose

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Irati Zubizarreta has no conflict of interest

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Patrick Vermersch has received honoraria and consulting fees from Biogen, Genzyme-Sanofi, Bayer, Novartis, Teva, Merck-Serono, GSK and Almirall. Research supports from Biogen, Bayer, Novartis and Merck-Serono. He is co-chairman of the scientific advisory board of the OCTIMS study.

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Peter A Calabresi has received consulting fees from Vertex, Abbvie, and Merck and has received research funding from Biogen, Novartis and MedImmune. He is co-chairman of the scientific advisory board of OCTIMS study.

Pablo Villoslada has received consultancy fees from Heidelberg Engineering regarding the clinical applications of OCT. He serves as academic editor of Current Treatment Options in Neurology, Neurology & Therapy, Multiple Sclerosis & Demyelinating Disorders, PloSONE and MS in focus. He is founder and hold stocks in Bionure Inc and Spire Bioventures Inc. He has received consultancy fees from Roche, Novartis, Health Engineering and stock options from Mint-Labs. He has received unrestricted grants from Genzyme, Roche and Novartis. He is a researcher in OCTIMS study.

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Table 1: Baseline demographics and clinical characteristics

	Study population (N=879)
Sex, female n (%)	584 (66)
Age (years)	40.6 [32.5-48.7; 16.2]
Disease duration (years)	6.5 [2.7-13.4; 10.7]
Multiple sclerosis type	
CIS, n (%)	74 (8)
RRMS, n (%)	664 (76)
SPMS, n (%)	83 (9)
PPMS, n (%)	58 (7)
Optic neuritis status	
Previous ON, n (%)	281 (32)
No previous ON, n (%)	598 (68)
Treatment (n: 557)	
None, n (%)	150 (17)
Interferon B 1b sc, n (%)	61 (7)
Interferon B 1a sc, n (%)	81 (9)
Interferon B 1a im, n (%)	98 (11)
Glatiramer Acetate, n (%)	81 (9)
Fingolimod, n (%)	14 (2)
Natalizumab, n (%)	45 (5)
Others[1], n (%)	27 (3)
Expanded disability status scale (EDSS)	2 [1.5-3.5; 2]
Multiple sclerosis functional composite (MSFC)	
PASAT (0-60) (n: 197)	49 [36-55; 19]
Non-Dominant hand (seconds) (n: 195)	21.2 [19.0-23.6; 4.6]
Dominant hand (seconds) (n: 195)	19.9 [17.9-23.8; 5.9]
Timed 25-Foot Walk (seconds) (n: 191)	4.6 [3.9-5.7; 1.8]
Peripapillary retinal nerve fiber layer (RNFL) thickness	

(µm)	
Spectralis® (n: 726)	92.1 [84.0-99.7; 15.7]
Cirrus™ (n: 162)	91.6 [84.5-100.4; 15.9]
Macular volume (mm^3)	
Spectralis® (n: 418)	8.5 [8.2-8.8; 0.6]
Cirrus™ (n: 124)	10.0 [9.8-10.4; 0.6]

Data represents median [P25-P75; interquartile range] unless otherwise indicated.

[1]Cyclophosphamide (n=5), Mitoxantrone (n=10), Azathioprine (n=5), Diazoxide (n=2), Flupirtine (n=1) and unknown therapy but reported the patient as under therapy (n=4).

Table 2 Comparison of baseline characteristics between patients with disability worsening and those with stable MS in the study population

	Stable (n: 627)	**Disability Worsening** (n: 252)	**P value**
Sex, female n (%)	420 (67)	164 (65)	0.636
Age (years)	39.6 [31.9 -47.9; 16.0]	43.3 [33.6-50.2; 16.6]	0.001
Disease duration (years)	6.0 [2.3-12.8; 10.5]	7.37 [3.3-15.0; 11.7]	0.008
Baseline EDSS	2.0 [1.5-3; 1.5]	2.5 [1.5-4; 2.5]	0.002
Progressive MS n (%)	75 (12)	66 (26)	<0.001
DMTs, treated n (%) (n: 557)	299 (75)	108 (68)	0.091
Previous ON status, (yes)	223 (36)	58 (23)	<0.001

EDSS: expanded disability status scale; MS: multiple sclerosis; DMTs: disease modifying therapies; ON: optic neuritis.

Data represents median [P25-P75; interquartile range] unless otherwise indicated.

Fisher's exact test for categorical data and U-Mann Whitney for quantitative variables

Table 3. Proportional hazards models for baseline pRNFL thickness in non-ON eyes as predictor of disability worsening in MS.

Proportional hazards models[1]	n /N	HR (95% IC)	P value	C of Harrell (95% IC)
$pRNFL^S$ (µm)	187/726	0.98 (0.97-0.99)	0.006	0.56 (0.43-0.68)
$pRNFL^C$ (µm)	67/162	0.98 (0.96-1.00)	0.102	---
pRNFL (µm) ≤ 92 vs. >92	252/879	1.39 (1.07-1.81)	0.014	0.57 (0.41-0.72)
Follow-up ≤1 year	46/879	1.02 (0.56-1.83)	0.959	0.58 (0.42-0.73)
Follow-up >1 and ≤3 years	163/708	1.44 (1.04-1.93)	0.026	
Follow-up >3 and ≤5 years	43/171	1.70 (0.92-3.14)	0.090	
pRNFL (µm) 87-97^S/98^C vs. >97^S/98^C	252/ 879	0.97 (0.70-1.34)	0.839	0.58 (0.42-0.73)
pRNFL (µm) ≤ 87 vs. >97^S/98^C		1.49 (1.08-2.04)	0.015	
pRNFL (µm) ≤ 87 vs. >87	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 and ≤3 years	163/708	1.65 (1.19-2.28)	0.003	
Follow-up >3 and ≤5 years	43/171	2.28 (1.22-4.25)	0.010	
Proportional hazards models[2]	n /N	HR (95% IC)	P value	C of Harrell (95% IC)
$pRNFL^S$ (µm)	94/408	0.97 (0.95-0.99)	0.001	0.55 (0.39-0.70)
$pRNFL^C$ (µm)[3]	------	-------	------	------
pRNFL (µm) ≤ 92^C/93^S vs. >92^C/93^S	159/557	1.58 (1.13-2.21)	0.007	0.57 (0.41-0.72)
Follow-up ≤1 year	31/557	1.36 (0.66-2.81)	0.405	0.58 (0.42-0.73)
Follow-up >1 and ≤3 years	99/422	1.59 (1.06-2.40)	0.025	
Follow-up >3 and ≤5 years	29/104	1.85 (0.83-4.14)	0.134	
pRNFL (µm) 87^C/88^S-98 vs. >98	159/557	0.78 (0.51-1.20)	0.265	0.58 (0.42-0.73)
pRNFL (µm) ≤87^C/88^S vs. >98		1.75 (1.19-2.59)	0.005	
pRNFL (µm) ≤87^C/88^S vs. >87^C/88^S	159/557	1.96 (1.39-2.76)	<0.001	0.57 (0.41-0.72)

Follow-up ≤1 year	31/557	1.06 (0.51-2.23)	0.873	
Follow-up >1 and ≤3 years	99/422	2.06 (1.36-3.11)	0.001	0.57 (0.41-0.73)
Follow-up >3 and ≤5 years	29/104	3.81 (1.63-8.91)	0.002	

n represents the number of events; N is the number of patients at risk entering in the analyses. There were 9 patients with data from both devices. Thus, the sum of n and N from Spectralis® OCT and [C] Cirrus™ OCT were 254 and 888, respectively instead of 252 and 879 for PHMs[1] and 161 and 566 instead of 159 and 557 for PHMs[2]

[S] Spectralis® OCT; [C] Cirrus™ OCT; pRNFL: peripapillary retinal nerve fiber layer. [1]Results represent hazard ratios (HR) and 95% confidence intervals (CI) from proportional hazards models including the following variables: age (years), disease duration (years), unilateral optic neuritis (yes/no), baseline EDSS, Time (linear)*EDSS and pRNFL thickness. pRNFL[S] median thickness: 92µm; pRNFL[S] thicknesses: lower tertile: <87µm; intermediate tertile: 87-97 µm and higher tertile > 97µm. pRNFL[C] median thickness: 92µm; pRNFL[C] thicknesses: lower tertile: <87µm; intermediate tertile: 87-98µm and higher tertile > 98µm.

[2]Results represent hazard ratios (HR) and 95% confidence intervals (CI) from proportional hazards models including the following variables: age (years), disease duration (years), unilateral optic neuritis (yes/no), baseline EDSS, Time (linear)*EDSS, use of DMTs (yes/no) and pRNFL thickness. pRNFL[S] median thickness: 93µm. pRNFL[S] thicknesses: lower tertile: <88µm; intermediate tertile: 88-98µm and higher tertile > 98µm. pRNFL[C] median thickness: 92µm; pRNFL[C] thicknesses: lower tertile: <87 µm; intermediate tertile: 87-98µm and higher tertile > 98µm.

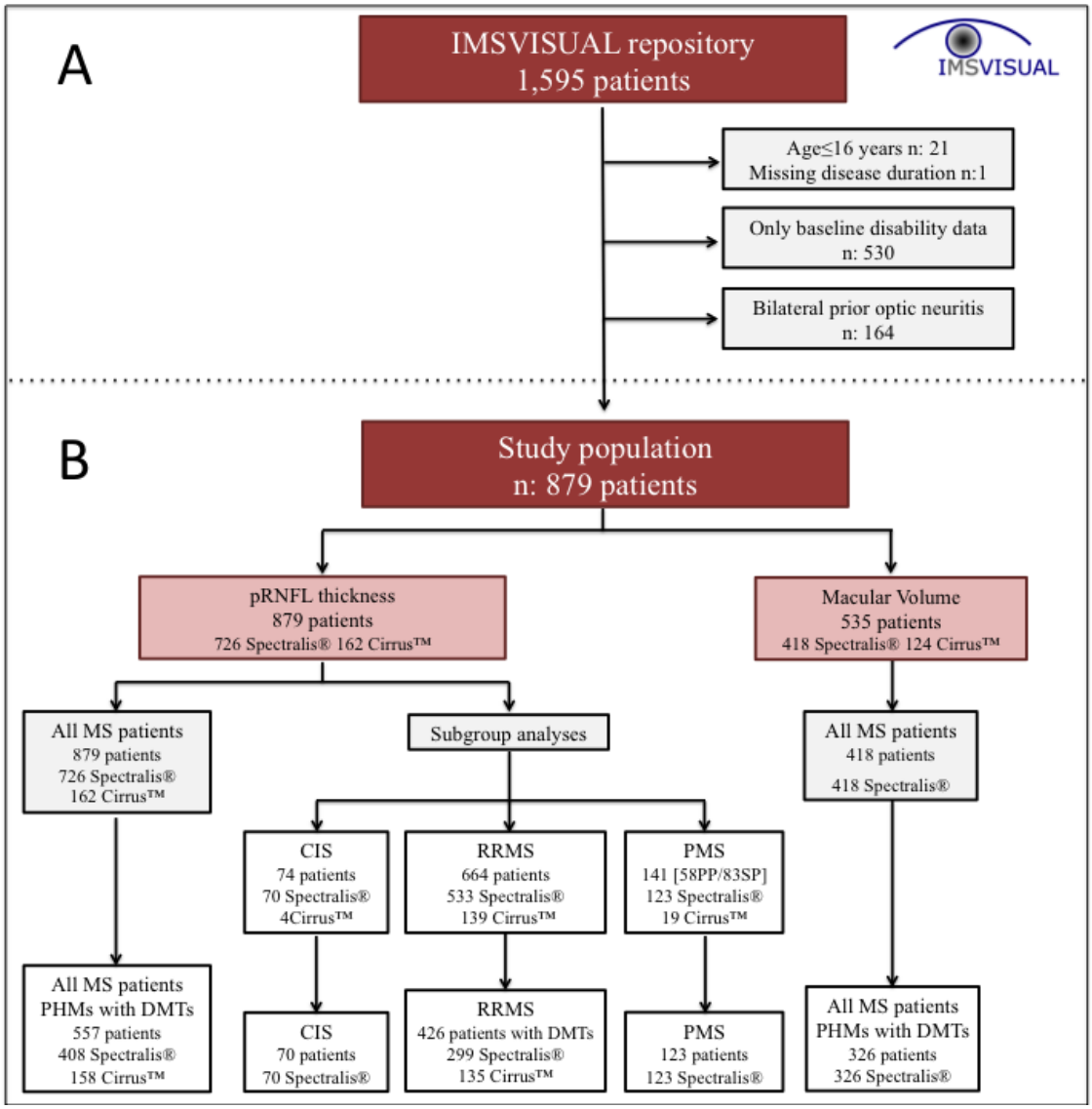
[3] This model was not presented because the number of events per variable was lower than ten (EVP=9.57).

All proportional hazard models were statistically significant with the exception of the PHM including only data from Cirrus™ OCT. Therefore; Harrell C´s statistics was not estimated. All proportional hazard models were also fitted in a stepwise fashion including all covariates in a first model [age, disease duration, unilateral optic neuritis, baseline EDSS and use of DMTs if applicable] and pRNFL thickness in a second model. The likelihood ratio test showed the addition of pRNFL thickness improved the models.

Figure 1. Flow chart of participants through the IMSVISUAL repository and this study. A) displays the assessment of the inclusion/exclusion criteria to be enrolled in the study; B) flow chart of participants through the study in the different proportional hazard models for the total population and subgroups analyses.

Figure 2. Kaplan-Meier plots of cumulative incidence of disability worsening in the complete MS population. A) cumulative percentage of patients with disability worsening during the follow-up in the two groups split by median of pRNFL thickness in non-ON eyes; B) cumulative percentage of patients with disability worsening during the follow-up in the three groups split by the tertiles of pRNFL thickness in non-ON eyes. Number at risk: number of patients at risk just before the selected time-points, namely baseline, zero, one, two, three, four and five years of follow-up. HR: hazard ratio; CI: confidence interval; RNFL: retinal nerve fiber layer.

Figure 1


A
IMSVISUAL repository
1,595 patients
IMSVISUAL
Age≤16 years n: 21
Missing disease duration n:1
Only baseline disability data
n: 530
Bilateral prior optic neuritis
n: 164
B
Study population
n: 879 patients
pRNFL thickness
879 patients
726 Spectralis® 162 Cirrus™
Macular Volume
535 patients
418 Spectralis® 124 Cirrus™
All MS patients
879 patients
726 Spectralis®
162 Cirrus™
Subgroup analyses
All MS patients
418 patients
418 Spectralis®
CIS
74 patients
70 Spectralis®
4Cirrus™
RRMS
664 patients
533 Spectralis®
139 Cirrus™
PMS
141 [58PP/83SP]
123 Spectralis®
19 Cirrus™
All MS patients
PHMs with DMTs
557 patients
408 Spectralis®
158 Cirrus™
CIS
70 patients
70 Spectralis®
RRMS
426 patients with DMTs
299 Spectralis®
135 Cirrus™
PMS
123 patients
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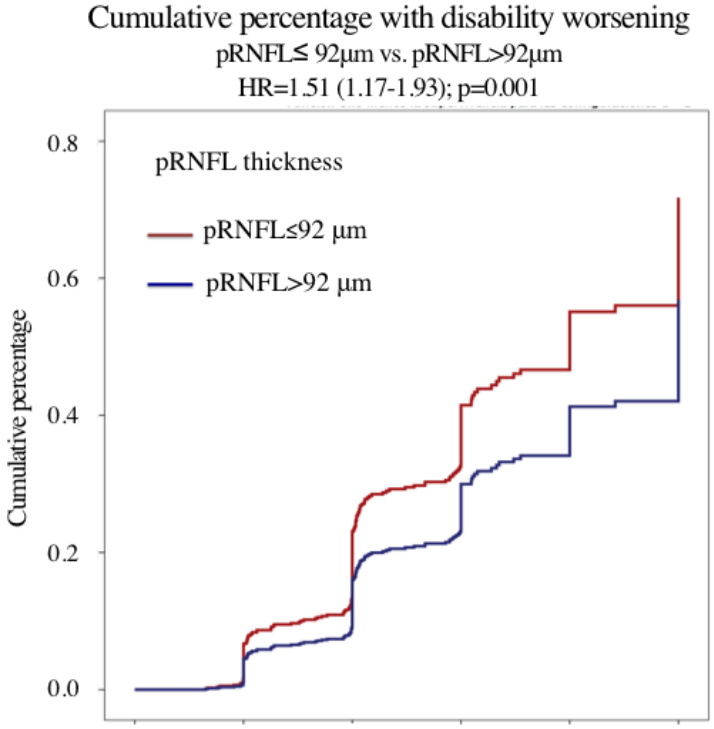
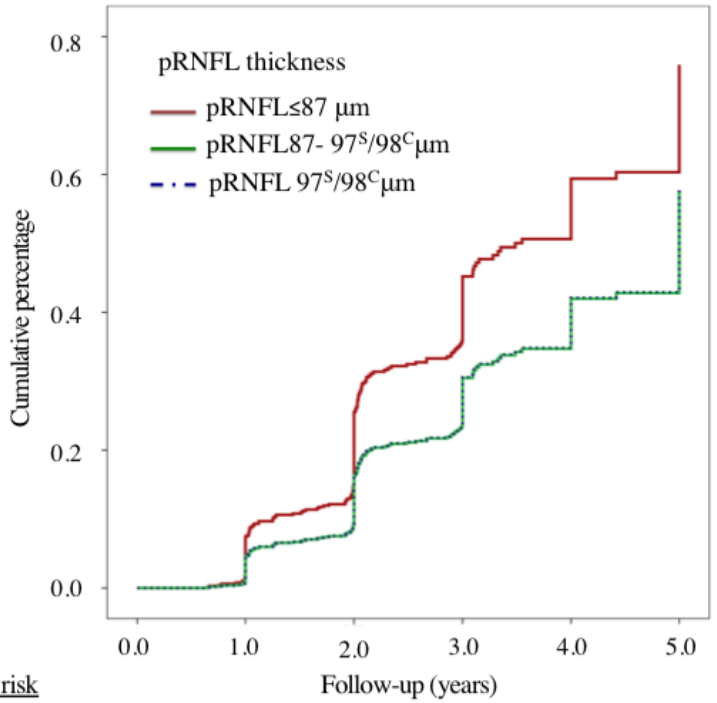
All MS patients
PHMs with DMTs
326 patients
326 Spectralis®

A



Number at risk						
pRNFL≤92µm	438	406	256	131	44	23
pRNFL>92µm	441	412	304	177	65	38

B



Number at risk						
pRNFL≤87µm	297	278	175	89	29	15
pRNFL 87-97S/98Cµm	290	268	179	96	35	22
pRNFL>97S/98Cµm	292	272	206	123	45	24