



## RESEARCH ARTICLE

# Retinal measurements predict 10-year disability in multiple sclerosis

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## Introduction

Multiple sclerosis (MS) is an immune-mediated demyelinating disease of the central nervous system (CNS), in which neurodegeneration is the principal substrate of disability.<sup>1,2</sup> Easily obtainable biomarkers that predict MS disease course represent a major unmet need. The anterior visual pathway is recognized as an important site for MS-related inflammation and neuroaxonal degeneration.

## Abstract

**Objective:** Optical coherence tomography (OCT)-derived measures of the retina correlate with disability and cortical gray matter atrophy in multiple sclerosis (MS); however, whether such measures predict long-term disability is unknown. We evaluated whether a single OCT and visual function assessment predict the disability status 10 years later. **Methods:** Between 2006 and 2008, 172 people with MS underwent Stratus time domain-OCT imaging [160 with measurement of total macular volume (TMV)] and high and low-contrast letter acuity (LCLA) testing ( $n = 150$ ; 87%). All participants had Expanded Disability Status Scale (EDSS) assessments at baseline and at 10-year follow-up. We applied generalized linear regression models to assess associations between baseline TMV, peripapillary retinal nerve fiber layer (pRNFL) thickness, and LCLA with 10-year EDSS scores (linear) and with clinically significant EDSS worsening (binary), adjusting for age, sex, optic neuritis history, and baseline disability status. **Results:** In multivariable models, lower baseline TMV was associated with higher 10-year EDSS scores (mean increase in EDSS of 0.75 per 1 mm<sup>3</sup> loss in TMV (mean difference = 0.75; 95% CI: 0.11–1.39;  $P = 0.02$ ). In analyses using tertiles, individuals in the lowest tertile of baseline TMV had an average 0.86 higher EDSS scores at 10 years (mean difference = 0.86; 95% CI: 0.23–1.48) and had over 3.5-fold increased odds of clinically significant EDSS worsening relative to those in the highest tertile of baseline TMV (OR: 3.58; 95% CI: 1.30–9.82;  $P_{\text{trend}} = 0.008$ ). pRNFL and LCLA predicted the 10-year EDSS scores only in univariate models. **Interpretation:** Lower baseline TMV measured by OCT significantly predicts higher disability at 10 years, even after accounting for baseline disability status.

Approximately 50% of people with MS experience an episode of clinical optic neuritis (ON) at some point in their disease course.<sup>3</sup> Moreover, postmortem studies of MS eyes demonstrate retinal ganglion cell loss, optic nerve gliosis, and atrophy in the vast majority of MS eyes, in addition to signs of inflammation, even in late-stage disease.<sup>4,5</sup> Given the virtually ubiquitous involvement of the anterior visual pathway in MS, the retina may therefore represent an opportune and accessible site

for quantifying and tracking neurodegeneration in people with MS.

Optical coherence tomography (OCT) is a rapid, non-invasive imaging tool that uses near-infrared light to quantify retinal layers and may serve as an *in vivo* biomarker of the effects of MS pathology.<sup>6</sup> Reductions of peripapillary retinal nerve fiber layer (pRNFL) thickness, composite ganglion cell and inner plexiform layer (GCIP) thickness and macular volume have been robustly demonstrated in people with MS (both with and without a history of ON) relative to healthy controls in cross-sectional analyses.<sup>7–10</sup> Longitudinal studies have demonstrated that retinal atrophy in MS eyes is progressive and correlates with brain atrophy,<sup>11,12</sup> clinical disability,<sup>11–14</sup> and quantitative spinal cord measures,<sup>15</sup> suggesting that retinal layer atrophy may represent a *bona fide* marker of global CNS neurodegeneration. The macula may be a retinal region of particular interest in assessing burden of neurodegeneration, as patients with predominant macular thinning display higher levels of disability.<sup>16</sup> Furthermore, GCIP makes up a substantial proportion of the macula and GCIP thinning correlates most strongly with brain atrophy.<sup>11</sup> A recent study by Martinez-Lapiscina *et al.* has shown that baseline retinal layer measurements may predict disability worsening over the following 5 years.<sup>17</sup> However, it has not been demonstrated to date whether OCT measurements predict disability at 10 years; a more crucial and representative period of ascertainment.

Measures of visual function are other potential attractive biomarkers to clinicians as testing can be easily performed in routine clinical practice. Low-contrast letter acuity (LCLA) is sensitive and reliable for detecting visual dysfunction in people with MS<sup>18</sup> and correlates with disability and quality-of-life scores.<sup>19,20</sup> LCLA has been included as an exploratory outcome measure in a number of MS clinical trials, but the utility of visual function measures for predicting long-term disability in people with MS has remained largely unexplored.

The objective of this study was to examine whether clinic-based measurement of retinal layer thicknesses and visual function at a single time-point may help predict disability 10 years later in people with MS.

## Methods

### Patients and clinical data

Participants were recruited by convenience sampling from the Johns Hopkins MS Center for an ongoing longitudinal cohort study. Of this cohort, 172 participants underwent a baseline third-generation Stratus time domain-OCT (TD-OCT) scan [pRNFL was measured in all participants; however, total macular volume (TMV)

was only measured in 160 of 172 participants] between 2006 and 2008 (after 2008, almost all OCT scans at our site were performed using newer spectral domain-OCT (SD-OCT)). Expanded Disability Status Scale (EDSS) evaluations were performed at baseline and at least 8 years later (median follow-up time: 9.5 years). EDSS raters were blinded to the patient's MS subtype, prior EDSS scores and OCT measurements. Baseline binocular 100% high-contrast visual acuity (VA), as well as 2.5% and 1.25% LCLA were performed in 150 of these participants. MS diagnosis was confirmed by the treating neurologist based on the 2005 McDonald criteria,<sup>21</sup> and patients were classified as having either relapsing-remitting MS (RRMS), primary-progressive MS (PPMS), or secondary-progressive MS (SPMS). Patients with comorbid neurological or ophthalmological disorders, refractive errors of  $\pm 6$  diopters, diabetes, poorly controlled hypertension, ON less than six months prior to the baseline OCT, and/or history of ocular surgery or trauma were not enrolled. Baseline clinical and demographic characteristics, including EDSS scores, were recorded. Johns Hopkins University Institutional Review Board approval was obtained for all study protocols, and written informed consent was obtained from all participants.

### Optical coherence tomography

All OCT scans were performed without pupillary dilation by experienced technicians, as described in detail elsewhere.<sup>10,16</sup> Scans were performed on Stratus TD-OCT (model 3000, software version 4.0; Carl Zeiss Meditec, Dublin, CA). pRNFL thickness was measured in all patients using the fast pRNFL thickness protocol, consisting of three consecutive 3.4  $\mu\text{m}$ -diameter circular scans (256 A-scans/B-scan) centered on the optic disc. Although not performed at our site from the outset of first utilizing Stratus TD-OCT, TMV was measured in 160 (93%) of these patients following the implementation of the fast macular thickness protocol on the Stratus TD-OCT at our center; demographic characteristics were similar between patients with/without TMV measurements. This protocol used six 6- $\mu\text{m}$  long intersecting radial lines centered on the fovea (128 A-scans/B-scan). Optic disc and macular scans with an image quality signal strength less than 7 (on a scale of 1–10) or with artifact, including motion artifact, were excluded from analyses.

### Visual function testing

Standardized visual function testing was performed with retro-illuminated eye charts in a darkened room prior to OCT examination in 150 patients (87%). Hundred percent high-contrast visual acuity (Early Treatment of

Diabetic Retinopathy Study (ETDRS) charts at 4 m) and 2.5% and 1.25% low-contrast letter acuities (Sloan letter charts at 2 m) were measured. All testing were performed monocularly and binocularly. The results were recorded as the number of letters that was correctly identified (maximum of 70 letters per chart; 14 lines with 5 letters per line). Participants were allowed to attempt the letters on a line if they correctly identified three or more letters on the previous line. Participants used their habitual glasses/contact lenses.

## Statistical analyses

Statistical analyses were completed using Stata version 13 (StataCorp, College Station, TX). Initial analyses compared demographic and clinical characteristics including age, sex, race, disease duration, MS subtype, and optic neuritis history across tertiles of baseline TMV. Our primary analyses assessed the association between mean TMV and pRNFL (as the average of both eyes) with 10-year EDSS scores, adjusting for baseline EDSS score. We chose the approach of adjusting our regression model for baseline EDSS score (rather than just examining change in EDSS as the primary outcome measure) as changes in EDSS over time may be related to some degree to the point of the EDSS scale at which a patient starts (e.g., a person with a lower baseline EDSS may be more likely to experience a sustained change in EDSS after 10 years). The composite measure of both eyes was applied because our primary outcome measure (EDSS) is a global measure; additionally, this approach may reduce the impact of prior ON on analyses. We performed a series of sensitivity analyses to ensure the robustness of this approach; these included: (1) modeling anterior visual pathway measures as the lowest measure from either eye (rather than the average), (2) excluding individuals with history of ON, and (3) excluding individuals with inter-eye differences of  $\geq 5 \mu\text{m}$  in pRNFL thickness (suggestive of either possible unilateral ON or unilateral predominant optic neuropathy). We considered OCT and visual function measures as continuous terms, as well as categorizing them into tertiles within regression models to investigate their association with 10-year EDSS scores, while adjusting for age, sex, history of ON, and baseline EDSS. In similarly adjusted models, we tested for trends across tertiles by modeling the individual's tertile ranking as a continuous covariate; *P*-values associated with the continuous covariate representing the individual's tertile ranking denote the test for linear trend. To assess the association between anterior visual pathway measures and risk of clinically significant EDSS worsening, we used adjusted logistic regression models, where clinically significant EDSS worsening was defined as an increase of  $\geq 2.0$  if

baseline EDSS was  $< 6.0$ , or an increase of  $\geq 1.0$  if baseline EDSS was  $\geq 6.0$ . We chose these criteria for changes in EDSS, which are more stringent than those used in contemporary clinical trials for MS,<sup>22</sup> because 10-year EDSS was measured at a single time-point (i.e., EDSS assessment was not repeated at 3 or 6 months to confirm if disability progression was "sustained"). Other sensitivity analyses were restricted to Caucasian patients only (as African Americans typically have thicker pRNFL than Caucasians),<sup>23</sup> patients with RRMS at baseline, or those with relatively low baseline disability (EDSS  $\leq 3$ ). Type I error for significance was defined as  $\alpha = 0.05$ .

## Results

### Characteristics of study population

One hundred and seventy-two people with MS underwent OCT imaging (at baseline, 151 [88%] had RRMS, 14 [8%] SPMS, and 7 [4%] PPMS, Table 1), of whom 45 (26%) experienced clinically significant worsening in EDSS scores over the 10-year period. In terms of disease modifying therapies (DMTs), 44 patients (26%) at baseline were not receiving any treatment, as compared to 24 patients (14%) at 10-year follow-up (36 initially untreated patients had commenced DMT, while 16 initially treated patients were no longer on treatment). TMV was available in 160 (93%) patients, of whom 42 (26%) experienced clinically significant worsening in EDSS scores over the 10-year period. At baseline, individuals in the lowest tertile of TMV ( $< 6.34 \text{ mm}^3$ ) were more likely to have longer disease duration (21.8 [9.3] vs. 14.3 [5.5] years), a positive history of ON (57.9% vs. 24.1%) and higher baseline EDSS scores (3.3[2.0] vs. 1.9[1.5]) than those in the highest tertile (Table 2). As expected, individuals in the lowest tertile of TMV also had lower pRNFL (83.1 [12.6] vs. 102.7 [11.8]  $\mu\text{m}$ ) and poorer baseline visual function (bilateral [OU] letter acuity at 1.25%: 15.9 [11.7] vs. 26.8 [8.4] letters). Average follow-up time was similar across tertiles of TMV (9.5 [0.6] vs. 9.5 [0.6]).

### Association between baseline anterior visual pathway measures and 10-year disability

After accounting for age, sex, history of ON, and baseline EDSS score, lower baseline TMV was associated with higher 10-year EDSS score (mean difference in EDSS per  $1 \text{ mm}^3$  loss in TMV: 0.75; 85% CI:  $-1.39$  to  $0.11$ ; *P* = 0.02). In similarly adjusted models, individuals in the lowest tertile of TMV had an average 0.86 (Fig. 1A; 95% CI: 0.23–1.48; *P* for linear trend across tertiles = 0.008) higher EDSS scores at 10-years and were at an over 3.5-

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

	RRMS	SPMS	PPMS	P-value
Number of patients (%)	151 (87.8)	14 (8.1)	7 (4.1)	<0.0001
Age at first OCT; mean, years (SD)	39.4 (9.9)	51.7 (5.8)	49.9 (8.3)	<b>&lt;0.0001</b> <sup>1</sup>
Females; <i>n</i> (%)	120 (79.5)	10 (71.4)	4 (57.1)	0.22 <sup>2</sup>
Race; <i>n</i> (%)				
Caucasian	122 (80.8)	14 (100.0)	6 (85.7)	0.74 <sup>2</sup>
African American	21 (13.9)	0 (0.0)	1 (14.3)	
Hispanic	3 (2.0)	0 (0.0)	0 (0.0)	
Asian-Pacific Islander	1 (0.7)	0 (0.0)	0 (0.0)	
Other	4 (2.7)	0 (0.0)	0 (0.0)	
Disease Duration; mean, years (SD)	15.9 (6.7)	30.4 (9.0)	16.1 (3.9)	<b>&lt;0.0001</b> <sup>1</sup>
Optic neuritis history; <i>n</i> (%)	70 (46.4)	6 (42.9)	0 (0.0)	0.11 <sup>2</sup>
Follow-up duration; mean, years (SD)	9.4 (0.7)	9.5 (0.7)	9.5 (0.5)	0.90 <sup>1</sup>
Baseline EDSS; mean (SD)	2.0 (1.4)	5.5 (1.9)	4.8 (1.5)	<b>&lt;0.0001</b> <sup>2</sup>

RRMS, relapsing remitting multiple sclerosis; SPMS, secondary progressive multiple sclerosis; PPMS, primary progressive multiple sclerosis; OCT, Optical coherence tomography; EDSS, Expanded Disability Status Scale.

<sup>1</sup>Determined by Kruskal–wallis equality-of-population rank test.

<sup>2</sup>Determined by Fisher's exact test.

fold increased odds of clinically significant EDSS worsening relative to individuals in the highest tertile of baseline TMV (OR for EDSS worsening: 3.58; 95% CI: 1.30–9.82; *P* for linear trend across tertiles = 0.008; Fig. 1B). These findings were consistent and qualitatively stronger in analyses restricted to baseline RRMS patients (*n* = 140) and those with EDSS ≤ 3.0 (*n* = 114) suggesting a potential added predictive utility of TMV in patients earlier in disease course or those at lower levels of disability (Fig. 1C–F). Among patients with baseline RRMS, individuals in the lowest tertile of TMV were at an over 5-fold increased odds of clinically significant EDSS worsening relative to individuals in the highest tertile of baseline TMV (OR for EDSS worsening: 5.20; 95% CI: 1.52–17.82; *P* for linear trend across tertiles = 0.002; Fig. 1D). Similarly, among patients with baseline EDSS scores ≤ 3.0, individuals in the lowest tertile of TMV were at an nearly 7-fold increased odds of clinically significant EDSS worsening relative to individuals in the highest tertile of baseline TMV (OR for EDSS worsening: 6.84; 95% CI: 1.62–29.73; *P* for linear trend across tertiles = 0.008; Fig. 1F).

Results showed similar trends although altered levels of statistical significance in sensitivity analyses assessing the relationship between TMV and EDSS scores at 10 years, in analyses (1) excluding individuals with a history of ON (*n* = 86; mean difference in EDSS per 1 mm<sup>3</sup> loss in TMV: 0.79; 85% CI: −0.02 to 1.60; *P* = 0.06), (2) excluding individuals with an inter-eye difference ≥ 5 μm (*n* = 81; mean difference in EDSS per 1 mm<sup>3</sup> loss in TMV: 0.75; 85% CI: −0.14 to 1.64; *P* = 0.10), and (3) using the lowest unilateral TMV measures, rather than the average TMV from both eyes, (mean difference in EDSS per 1 mm<sup>3</sup> loss in TMV: 0.53; 85% CI: −0.02 to

1.39; *P* = 0.10). Results were also relatively similar when restricting to Caucasian individuals only (*n* = 132; mean difference in EDSS per 1 mm<sup>3</sup> loss in TMV: 0.79; 85% CI: −0.14–1.44; *P* = 0.06). They were also similar in models additionally adjusted for disease duration; individuals in the lowest tertile of TMV had on average 0.73 (95% CI: 0.10–1.37; *P* = 0.03) higher EDSS scores at 10-years and were at an over 3-fold increased odds of clinically significant EDSS worsening relative to individuals in the highest tertile of baseline TMV (OR for EDSS worsening: 3.06; 95% CI: 1.07–8.75; *P* = 0.04).

In secondary analyses assessing the association between pRNFL and contrast letter acuity and 10-year EDSS scores, neither baseline pRNFL nor contrast letter acuity predicted 10-year outcomes in models adjusted for age, sex, history of ON and baseline disability in either primary nor any of the sensitivity analyses (Table 3).

## Discussion

In this longitudinal study we demonstrate that lower TMV identified on OCT at a single time-point is associated with higher EDSS score 10 years later in people with MS, and greater risk of clinically meaningful EDSS worsening over the same period. As neuroaxonal degeneration is now recognized to be the primary driver of disability in MS,<sup>1,2,11,24</sup> our findings support growing evidence that neurodegeneration within the retina in MS is a meaningful biomarker of global neurodegeneration occurring as part of the disease process.<sup>11,14</sup> The data provide further support for the utility of OCT technology, not only for tracking/monitoring MS over time, but also for potentially helping to predict the clinical course.

**Table 2.** Clinical and demographic characteristics of included study participants across tertiles of baseline TMV.

	Tertile of Baseline TMV <sup>1</sup> (Range [mm <sup>3</sup> ])			P-value <sup>2</sup>
	Highest (6.69–7.71)	Middle (6.35–6.69)	Lowest (5.37–6.34)	
N	54	53	53	
Mean of tertile (SD)	6.94 (0.21)	6.53 (0.11)	6.06 (0.22)	
Clinical and demographic characteristics				
Mean age at first OCT, years (SD)	40.75 (10.64)	47.71 (10.74)	39.45 (10.18)	0.54
Females; n (%)	43 (79.6)	40 (75.5)	40 (75.5)	0.87
Race				
Caucasian; n (%)	44 (81.5)	48 (90.6)	40 (75.5)	0.17
African-American; n (%)	6 (11.1)	3 (5.7)	11 (20.8)	
Other; n (%)	4 (7.4)	2 (3.8)	2 (3.8)	
Follow-up duration; median, IQR	9.54 (9.04–10.00)	9.37 (8.80–9.90)	9.52 (9.02–9.89)	0.69
Baseline MS characteristics				
Disease subtype at baseline				
RRMS; n (%)	47 (87.0)	47 (88.7)	46 (86.8)	0.21
SPMS; n (%)	2 (3.7)	5 (9.4)	6 (11.3)	
PPMS; n (%)	5 (9.3)	1 (1.9)	1 (1.9)	
Disease duration; mean, years (SD)	15.06 (6.56)	16.57 (7.95)	19.19 (8.46)	0.02
Optic neuritis history; n (%)	15 (27.8)	27 (50.9)	30 (58.5)	0.002
Baseline EDSS; median (IQR)	2 (1.25–3.25)	2 (1.5–3.25)	2.25 (1.5–3.5)	0.32
pRNFL thickness, $\mu\text{m}$ , mean (SD)	102.65 (11.72)	90.04 (12.44)	83.06 (12.55)	<0.0001
OU letter acuity, contrast 100%, mean (SD)	63.78 (5.94)	62.73 (5.10)	61.88 (7.08)	0.32
OU letter acuity, contrast 2.5%, mean (SD)	38.11 (7.01)	35.29 (9.83)	30.51 (11.35)	0.0007
OU letter acuity, contrast 1.25%, mean (SD)	26.78 (8.37)	22.10 (10.23)	15.90 (11.66)	<0.0001
10-year follow-up MS characteristics				
10-year EDSS; median (IQR)	2.0 (1.5–3.38)	2.0 (1.5–4.0)	3.5 (2.0–6.0)	0.01
Meaningful EDSS progression <sup>3</sup> ; n (%)	10 (18.5)	7 (12.2)	25 (47.2)	0.0001
10-year disease subtype				
RRMS; n (%)	41 (75.9)	42 (79.3)	37 (69.8)	0.26
Progressive MS; n (%)	11 (24.1)	11 (20.7)	16 (30.2)	
Change in subtype; n (%)	5 (9.3)	5 (9.4)	9 (17.0)	
OU letter acuity, contrast 100%, mean (SD)	61.51 (6.17)	58.49 (7.39)	58.71 (7.25)	0.08
OU letter acuity, contrast 2.5%, mean (SD)	36.91 (8.56)	31.36 (11.02)	28.23 (11.33)	0.0009
OU letter acuity, contrast 1.25%, mean (SD)	27.47 (9.02)	22.56 (11.30)	16.90 (11.90)	0.0001

<sup>1</sup>Values represent the average of total macular volume (TMV) for both eyes.

<sup>2</sup>P-values are derived from univariate generalized linear regression models assessing the association between characteristic in question and TMV tertile.

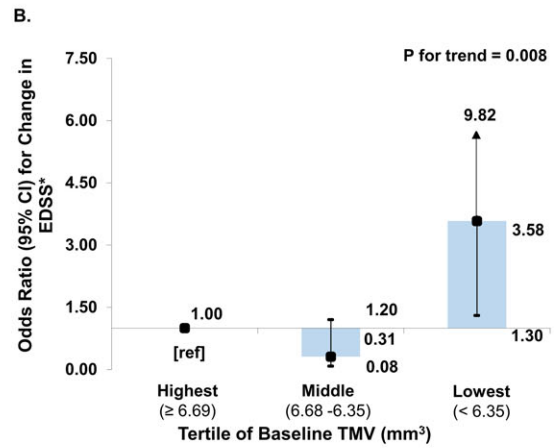
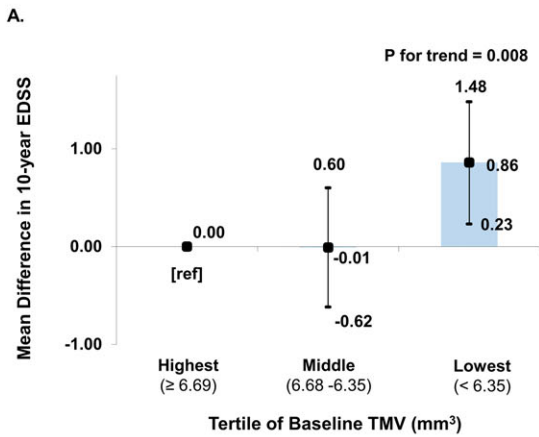
<sup>3</sup>Meaningful EDSS progression was defined as an increase of at least 2 points if baseline EDSS scores <6 and an increase of 1 point if baseline EDSS  $\geq$  6

Multiple studies have reported correlations between retinal layer measurements and disability measures in MS. Petzold et al. summarized available evidence in 2010 and identified that lower pRNFL thickness was associated with higher EDSS scores in numerous studies, including work using TD-OCT technology.<sup>7</sup> Cross-sectional studies using newer SD-OCT technology have confirmed this association (showing stronger correlations than TD-OCT) and have also demonstrated negative correlations between GCIP thickness and EDSS scores.<sup>12,25,26</sup> Correlations between rates of pRNFL and GCIP thinning with disability accumulation in MS have also been reported.<sup>13,14</sup> However, there is a paucity of studies examining the

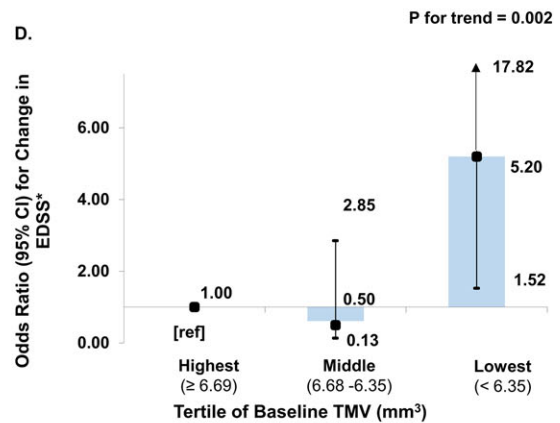
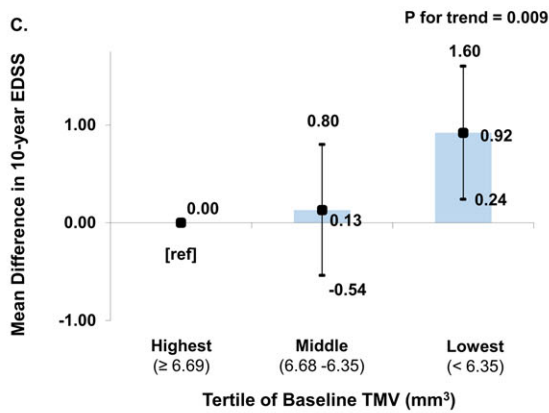
utility of a single OCT scan for predicting longer-term disability in MS.

The most important finding of our study was that a single OCT measurement might predict disease course over the next 10 years, as the lowest tertile of TMV is associated with higher likelihood of clinically meaningful EDSS progression, even after accounting for baseline disability status. Martinez-Lapiscina et al. reported a similar association between low pRNFL thickness and risk of disability progression over a shorter period; in that study people with MS with pRNFL less than  $\sim 88 \mu\text{m}$  had double the risk of disability worsening 1–3 years later, and almost fourfold increased risk of disability worsening 3–

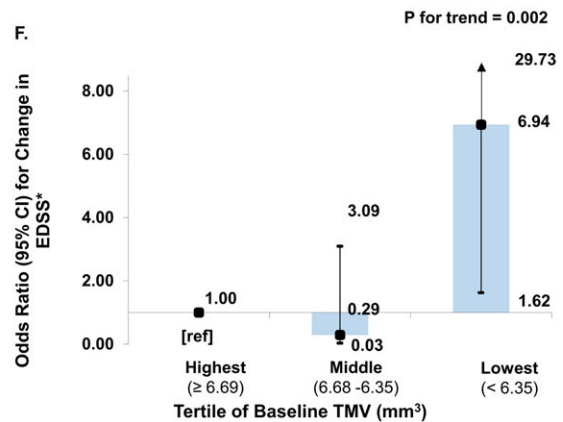
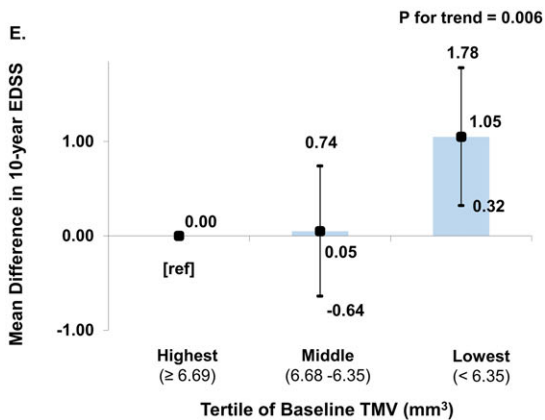
**All Patients**  
(n = 160)



**Baseline RRMS Only**  
(n = 140)



**Baseline EDSS ≤ 3**  
(n = 122)



**Figure 1.** Association between tertiles of baseline TMV and 10-year EDSS Scores. (A) Mean difference in 10-year EDSS scores across tertiles of baseline TMV. Analyses are adjusted for age, sex, history of optic neuritis, and baseline EDSS scores. (B) Values displayed are odds ratios (OR; 95% CI) for meaningful change in EDSS associated with baseline tertiles of TMV thickness. OR are additionally adjusted for age, sex, history of ON and baseline EDSS scores. Tests for linear trend across tertiles were conducted by modeling the individual's tertile ranking as a continuous covariate in a similarly adjusted regression models. We defined a clinically meaningful change in EDSS as a 2.0 point increase if baseline score <6.0 or a 1.0 increase if baseline score  $\geq$ 6.0. Of the 160, 42 (26%) experienced a meaningful change in EDSS. (C) Multivariable adjusted mean difference in 10-year EDSS scores across tertiles of baseline TMV among patients with RRMS at baseline. (D) Multivariable adjusted OR (95% CI) for meaningful change in EDSS associated with baseline tertiles of TMV among patients with RRMS at baseline. (E) Multivariable adjusted mean difference in 10-year EDSS scores across tertiles of baseline TMV among patients with EDSS scores  $\leq$ 3.0 at baseline. (F) Multivariable adjusted OR (95% CI) for meaningful change in EDSS associated with baseline tertiles of TMV among patients with EDSS scores  $\leq$ 3.0 at baseline. TMV, total macular volume; EDSS, Expanded Disability Status Scale; RRMS, relapsing-remitting MS.

5 years later.<sup>17</sup> These findings raise the question of how OCT measurements at a single time-point can predict disability progression. We propose that people with MS with significant retinal atrophy have de facto evidence of global CNS neurodegeneration. This may lead to a “thresholding effect” where further neuroaxonal loss is more likely to produce meaningful disability progression. Furthermore, microscopic evidence of neurodegeneration may help to define subsets of patients in whom there is increased tissue susceptibility to future similar pathology. MRI markers of neurodegeneration in MS also support this hypothesis, as reduced deep gray matter volumes at baseline are predictive of future EDSS progression.<sup>27</sup>

Noteworthy from the current study is that lower TMV at baseline predicted disability at 10 years, while pRNFL thickness after adjusting for baseline disability did not.

TMV has been previously reported to correlate with disability status in MS;<sup>28</sup> however, correlations between pRNFL or GCIP thickness and current disability in MS have been reported with greater frequency.<sup>7,12–14,25,26</sup> As our study used TD-OCT we could not separately examine the GCIP (segmentation of which requires higher-resolution OCT technology), the retinal layer that makes up a substantial proportion of TMV and is now recognized as the most reliable retinal marker of neurodegeneration in MS, as it correlates strongly with brain atrophy.<sup>11</sup> It is possible that the predictive value of TMV we have identified is driven by GCIP thickness, and further research using SD-OCT technology will allow testing of this hypothesis. Furthermore, pRNFL measurements are susceptible to confounding influences from subclinical inflammation of the optic nerve,<sup>29</sup> and error from mis-

**Table 3.** Other baseline visual pathway measure and 10-year EDSS.

	Median (IQR) EDSS at baseline	Median (IQR) EDSS at 10-year Follow-up	Mean difference (95% CI) in 10-year EDSS Adjusted for age, sex and ON history + baseline disability
pRNFL thickness <sup>1</sup>			
$\geq$ 98.8 $\mu$ m	1.5 (1.0–2.6)	2.0 (1.5–3.0)	0.00 [ref]
98.7–88.0 $\mu$ m	2.0 (1.5–2.5)	2.0 (1.5–4.0)	0.16 (–0.47 to 0.79)
<88.0 $\mu$ m	3.0 (1.5–4.5)	3.5 (2.0–6.5)	0.34 (–0.34 to 1.02)
<i>P</i> for trend			0.33
OU letter acuity, 1.25% contrast			
$\geq$ 28	1.5 (1.0–2.0)	2.0 (1.5–3.0)	0.00 [ref]
27–19	2.0 (1.5–3.0)	2.5 (1.5–4.0)	–0.23 (–0.86 to 0.38)
<19	2.5 (1.9–4.0)	3.5 (1.9–6.0)	0.01 (–0.63 to 0.66)
<i>P</i> for trend			0.99
OU letter acuity, 2.5% contrast			
$\geq$ 28	1.5 (1.0–2.0)	2.0 (1.5–3.0)	0.00 [ref]
27–19	2.0 (1.5–2.5)	2.5 (1.5–2.9)	–0.43 (–1.04 to 0.18)
<19	3.0 (1.5–4.0)	3.5 (2.1–6.0)	0.31 (–0.31 to 0.93)
<i>P</i> for trend			0.35
OU letter acuity, 100% contrast			
$\geq$ 67	2.0 (1.5–3.4)	2.0 (1.0–2.6)	0.00 [ref]
66–60	2.0 (1.5–4.4)	1.8 (1.5–2.6)	0.07 (–0.54 to 0.68)
<60	3.0 (1.8–5.0)	2.5 (1.5–3.5)	0.09 (–0.55 to 0.74)
<i>P</i> for trend			0.78

<sup>1</sup>Values represent the average of pRNFL thickness for both eyes.

centering of image acquisition over the optic nerve head with TD-OCT<sup>30</sup> (a limitation subsequently addressed by eye-tracking technology implemented in SD-OCT technology).<sup>31</sup> Ethnicity is also an important consideration in analyses of pRNFL. African Americans typically have thicker pRNFL,<sup>23</sup> though we did observe consistent results when restricting to Caucasians only, suggesting race/ethnicity may not be a primary driver of our results. In addition, biological differences in the pRNFL and TMV may explain their varying associations with disability. The macula is a region enriched for neuronal cell bodies so it is possible that measuring reductions in macular volume is a better indicator of irreversible pathology and therefore cumulative disability. This is consistent with our prior publication which described a subset of MS patients with predominant macular thinning on OCT who demonstrated higher disability scores compared to patients without this finding.<sup>16</sup> Collectively, studies suggest that the macula may be a particularly informative retinal region for assessing neurodegeneration in people with MS. Nevertheless, a single pRNFL measurement correlated strongly with measures of disability at each timepoint. Thus, pRNFL thickness still provides a valuable, quick assessment of a patient's current disability status or accrual of disability-associated neurodegeneration, which may be a surrogate of EDSS scores. Similar to pRNFL, in our study LCLA was significantly associated with baseline EDSS score but was not associated with 10-year EDSS (when adjusted for baseline) and clinically meaningful EDSS worsening. Other studies have also reported a disconnect between the functional measures of LCLA and EDSS,<sup>32</sup> and associations between poorer LCLA and elements of disability not captured by the EDSS, such as fatigue.<sup>33</sup> Together, these studies imply that LCLA may not be a strong predictor of future disability as measured by EDSS, but further work could examine the predictive value of LCLA for future cognitive, fatigue, or quality-of-life outcomes.

Interestingly, low TMV showed the strongest predictive value for clinically meaningful EDSS deterioration over the following 10 years in patients with RRMS and patients with low disability levels (EDSS score  $\leq 3.0$ ). This suggests that retinal thinning in these subgroups of patients is a more compelling marker of poor prognosis than retinal thinning in patients with advanced disease (in whom retinal layer thicknesses are already expected to be lower).<sup>26</sup> However, it is also important to consider confounding influences on this finding, specifically the ordinal nature of the EDSS scale and the nonlinear natural history of MS, with disability progression occurring faster in patients starting at a low disability baseline compared with patients who already have substantial disability.<sup>34</sup> This may result in a weaker association between

retinal measurements and future disability outcomes in patients with progressive disease. Future larger studies could separately assess the predictive utility of OCT measurements in people with progressive MS as compared to RRMS.

A history of clinical ON should be carefully considered in studies of OCT measurements in people with MS, as it is associated with a substantial, significant, and persistent reduction in both macular and peripapillary OCT measurements.<sup>7,8</sup> Prior ON has been demonstrated to weaken the relationship between OCT measurements and brain (particularly gray matter) volume<sup>35</sup> or atrophy.<sup>11</sup> While lower pRNFL or lower GCIP have shown relationships with higher levels of disability in people with MS overall, the impact of prior ON on these relationships has not been fully elucidated, particularly in the case of macular measures. Some studies have only identified a correlation between pRNFL and EDSS in patients without a history of ON,<sup>36,37</sup> whereas another study suggested that GCIP actually correlated better with EDSS in patients with a history of ON.<sup>26</sup> A number of different methodological approaches can be applied to account for ON history, such as the exclusion of ON eyes,<sup>17,38</sup> or applying a cutoff of an inter-eye pRNFL difference of 5–6 microns to identify and exclude eyes with optic nerve lesions.<sup>39</sup> Our sensitivity analysis did not identify different results when applying these alternative approaches. This may be explained by the combined limitations of both TD-OCT measurements and EDSS scoring, or alternatively could suggest that ON history causes less interference with relationships between OCT measurements and longer-term prognosis.

Predicting future disability outcomes in patients with MS remains a major challenge due to clinical heterogeneity, a long disease course and confounding from therapy effects. OCT measures likely represent one of multiple clinical, imaging, and biochemical biomarkers that help to predict medium to long-term outcomes. MRI is a cornerstone of disease evaluation in patients with MS; however, conventional measures alone such as T2 lesion load are weak predictors of future disability accumulation,<sup>40–42</sup> with lesion location likely acting as an important modifier.<sup>43</sup> More advanced measures including brain volume, substructure volumes (especially thalamic volume), or cortical lesions (seen on 7T imaging) may be stronger predictors of future disability, but technical challenges and costs limit the application of these techniques at an individual level.<sup>40</sup> Current disability and early disability progression using sensitive outcomes such as MSFC-15 are additional predictors of future disability.<sup>44</sup> Furthermore, neurofilament light chain measured in the CSF or serum is an exciting new biomarker in patients with MS,<sup>45,46</sup> and early studies suggest that baseline levels may



be predictive of medium to long-term outcomes.<sup>47</sup> Each of these individual biomarkers may inform about a certain aspect of the MS disease process so there is a trend to looking at several biomarkers together to provide clinically applicable prognostic information for individuals with MS.

The strengths of our study include a long follow-up period of a relatively large cohort of people with MS. Our study population was heterogeneous with varying disease subtypes and disease duration and may be representative of a typical MS population. However, our study has a number of limitations. We used TD-OCT imaging, which compared to newer SD-OCT technology has inherent limitations including inability to segment macular layers, greater motion artifact, lower depth resolution and mis-centering errors in image acquisition.<sup>30,48</sup> SD-OCT measurements show better reproducibility than TD-OCT<sup>49–51</sup> and stronger correlations with disability in people with MS.<sup>12,36</sup> However, despite the limitations, Stratus TD-OCT measures of TMV and pRNFL in people with MS actually correlate strongly with Cirrus SD-OCT measures.<sup>52</sup> Another inherent limitation of OCT analysis in people with MS is that patients with very high levels of physical disability, severe fatigue or poor visual acuity may not be able to complete this test, or may be less likely to attend clinic and consent to study inclusion, although in our experience patients who do not participate for these reasons represent only a small percentage of people with MS. Secondly, EDSS scores were our primary outcome measure of disability. While EDSS remains the most widely utilized measure of disability in MS, it has well-described weaknesses including its sensitivity to change, reliability, and focus on ambulatory disability. In addition, baseline and 10-year EDSS were assessed at a single time-point in our study, rather than the approach adopted in most clinical trials where EDSS scoring is repeated 3 or 6 months. Our approach may reduce the reliability of EDSS measures, but on the other hand we suggest that blinding of EDSS raters means that it is unlikely either baseline or 10-year EDSS were consistently under-rated or over-rated in individuals. Nevertheless, we chose a stringent definition of clinically meaningful EDSS change to help mitigate these concerns. Finally, while our study is relatively large considering the long follow-up period, evaluating the performance of OCT or visual function measures within subgroups (e.g., race, sex, or disease modifying therapy status) was not feasible given the small numbers within many of those groups. Furthermore, we opted for a limited approach in our evaluation of disease characteristics (we did not examine radiological parameters, timing of clinical relapses or disability progression before or during the follow-up period), again due to the heterogeneity of our cohort. Further work in a

larger and more homogeneous cohort could examine the predictive value of retinal measurements when additional potentially prognostic clinical and radiological characteristics are also considered.

## Conclusion

We have demonstrated that lower TMV in people with MS correlates with higher disability at 10 years, and predicts clinically significant EDSS worsening over the same period. People with MS exhibiting evidence of structural damage to the visual pathway have a greater propensity to accumulate disability. Our findings add to the growing body of evidence suggesting that OCT derived retinal measures are powerful surrogates of global neurodegeneration in people with MS. Measurement of these easily and rapidly acquired inexpensive biomarkers will be of significant value in both clinical and research settings amidst the impending era of potentially neuroprotective and even neurorestorative treatments for people with MS. Further investigation focusing on longer-term predictive value of newer SD-OCT technology is likely to be very informative, with particular interest in GCIP thickness, which shows strong associations with gray matter atrophy in the brain.

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## Author contributions

A.R.,S.S.,P.A.C. contributed to conception and design of the study, acquisition and analysis of data and drafting a significant portion of the manuscript and figures. O.C.M., K.F. contributed to conception and design of the study, analysis of data and drafting a significant portion of the manuscript and figures. J.B., E.G.L., J.R.,E.S.S., S.B.S.M., J.N.,N.G.C contributed to conception and design of the study, acquisition and analysis of data. S.D.N., E.M.,D.R. contributed to conception and design of the study and drafting a significant portion of the manuscript and figures. L.B.,E.F.,T.F.,C.C. contributed to conception and design of the study.

## Conflicts of Interest

A.R., O.C.M., K.F., E.G.L., J.R., E.M., E.S.S., S.B.S.M., J.N., N.G.C., D.R., and C.C. has nothing to report. S.D.N. has received consultant fees for scientific advisory boards from Biogen, Genentech, Celgene, EMD Serono and has received research funding from Biogen, Novartis, and Genentech (paid directly to institution). L.J.B. has received consulting fees from Biogen. E.F. has received speaker and consulting fees from Genzyme, Acorda, Novartis, and TEVA. T.F. has received speaker and consulting fees from Acorda, Genzyme, and Novartis. S.S. has received consulting fees from Medical Logix for the development of CME programs in neurology, consulting fees from Axon Advisors LLC and served on scientific advisory boards for Biogen-Idec, Genzyme, Genentech Corporation & Novartis. He receives research support from Genentech Corporation and Biogen Idec, and received support from the Race to Erase MS foundation. P.A.C. has received personal honorariums for consulting from Biogen and Disarm Therapeutics. He is PI on research grants to Johns Hopkins from MedImmune, Annexon, Biogen, and Genzyme.

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