

Serum NfL levels in the first five years predict 10-year thalamic fraction in patients with MS

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Abstract

Background: Serum neurofilament light chain (sNfL) levels are associated with relapses, MRI lesions, and brain volume in multiple sclerosis (MS).

Objective: To explore the value of early serum neurofilament light (sNfL) measures in prognosticating 10-year regional brain volumes in MS.

Methods: Patients with MS enrolled in the Comprehensive Longitudinal Investigations in MS at Brigham and Women's Hospital (CLIMB) study within five years of disease onset who had annual blood samples from years 1–10 (n = 91) were studied. sNfL was measured with a single molecule array (SIMOA) assay. We quantified global cortical thickness and normalized deep gray matter (DGM) volumes (fractions of the thalamus, caudate, putamen, and globus pallidus) from high-resolution 3 T MRI at 10 years. Correlations between yearly sNfL levels and 10-year MRI outcomes were assessed using linear regression models.

Results: sNfL levels from years 1 and 2 were associated with 10-year thalamus fraction. Early sNfL levels were not associated with 10-year putamen, globus pallidus or caudate fractions. At 10 years, cortical thickness was not associated with early sNfL levels, but was weakly correlated with total DGM fraction.

Conclusions: Early sNfL levels correlate with 10-year thalamic volume, supporting its role as a prognostic biomarker in MS.

Keywords: serum neurofilament light (sNfL), thalamus, caudate, biomarker, deep gray matter

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Introduction

Multiple sclerosis (MS) is an autoimmune demyelinating disorder of the central nervous system (CNS),^{1,2} Progressive disability due to axonal and neuronal loss, resulting from cumulative inflammatory damage is a prominent feature of the disease.³

Whole-brain and regional atrophy are the result of axonal and neuronal damage, and correlate with disability and disease progression in MS and specific clinical and pathological endpoints.^{4–6} Thalamic atrophy in MS occurs early in the disease course and is linked to disability and cognitive impairment.^{4,5} Specific regions of the gray matter (GM),

can now be measured efficiently from MRI including cortical areas and the deep gray matter (DGM) structures.

Predictive biomarkers of future atrophy and axonal loss are needed for prognostication. One promising biomarker in MS is neurofilament light chain (NfL), which is a structural component of axons. NfL levels have been shown to correlate with acute CNS inflammation in patients with MS,^{7–11} and serum NfL levels (sNfL) can be reliably measured with single-molecule array (SIMOA) assays.^{11,12} sNfL has been shown to correlate with current brain atrophy and several studies have reported that

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baseline sNfL levels can be used to predict subsequent brain volumes.^{8,13–16} Two recent reports have demonstrated an association between CSF NfL and sNfL levels and gray matter (GM) volume.^{17,18}

Our prior work showed that early sNfL levels were prognostic of 10 year whole brain atrophy and T2 lesion volumes,¹⁶ and that sNfL is closely associated with new gadolinium enhancing lesions.^{19,20} We hypothesized that early serum NfL levels is associated with long-term gray matter volumes, supporting the view that axonal damage due to acute new lesion formation in the early phase of disease has long-term consequences. Our study goal was to evaluate the longitudinal association between sNfL and gray matter volumes at 10 years. We used log-transformed yearly sNfL levels to prognosticate 10-year cortical and DGM volumes.

Methods

Subjects

We included patients with MS enrolled in the Comprehensive Longitudinal Investigation of MS at the Brigham and Women's Hospital (CLIMB, www.climbstudy.org).²¹ The CLIMB study has enrolled over 2100 patients since 2000. Patients are followed up with biannual standardized clinical exams and annual MRI scans. Patients also provide biosamples, including blood samples annually. The patients included in this study were diagnosed with MS according to the 2010 McDonald criteria, had their first blood sample within five years of their first symptom, had at least 8/10 annual blood draws from first sample collection to year 10, provided consent for sample sharing, and were part of the quality of life (QOL) subgroup of the CLIMB study. We used the year 10 3 T MRI for all analysis. Further details on this patient population are reported in a prior study.¹⁶

Standard protocol approvals, registrations, and patient consents

Institutional Review Board approval was granted by the Partners Human Research Committee, and participants provided written informed consent for participation.

SNFL measurements

Serum samples were collected at the time of the annual CLIMB visit and were stored at -80°C following standardized procedures. Serum samples were shipped on dry ice from Boston, MA to Basel, Switzerland in a temperature-controlled container.

There, sNfL levels were measured with a SIMOA assay as described in our prior work.¹⁰ Inter-assay coefficients of variation (CV) for three native control serum samples were 10.8%, 8.3%, and 5.7% with averaged concentrations of 9.2 pg/mL, 24.4 pg/mL, and 101.4 pg/mL, respectively. The mean intra-assay CV of duplicated determinations for concentration was 5.1%. Repeat measurements were performed for samples with intra-assay CV above 20%. Four samples showed sNfL levels below the lower limit of quantification (i.e. < 1.3 pg/mL). These values were extrapolated from the standard curve, further information can be found in prior work by the same group.¹⁶ We transformed the sNfL levels by changing 0 to 1, for observations with 0 values. The sNfL values were further log transformed for individual year regression analysis.

MRI acquisition and processing

Brain MRI acquisition was performed at 3 T on a consistent platform for all subjects (Siemens Skyra), using a 20-channel head coil, at Brigham and Women's Hospital (BWH). This included a sagittal 3D T1-weighted gradient echo series covering the whole brain (TE/TR = 2.96/2300 ms, TI = 900 ms, flip angle = 9 deg), with 1 mm³ isotropic voxel sizes.²² All MRI scans were analysed in the Laboratory for Neuroimaging Research at BWH. We have previously reported on whole brain atrophy and T2 hyperintense lesion results in these patients.²² The current study was focused on regional GM volumes. MRI analysis was blinded to clinical and sNfL information.

Deep gray matter volumes and fractions:

MRI scans were analysed by a fully automated pipeline (FSL-FIRST, v.5.0, <https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/>) to derive volumes of the caudate, putamen, globus pallidus, and thalamus. Regional brain fractions were derived to normalize the raw volumes by calculating the ratio between each subject's structure volume and that subject's intracranial volume. The intracranial volume was obtained from an automated pipeline as previously described.²² This normalization step was performed to account for differences in head size across individuals.⁵

Cortical thickness:

In order to assess the relationship of sNfL levels with cortical pathology, MRI scans were analysed using a fully automated pipeline (FreeSurfer, v.6.0.0, <https://surfer.nmr.mgh.harvard.edu/>) to comprehensively derive average cortical thickness in 34 regions in each hemisphere. The average of these results across

Table 1. Population demographics.

Characteristic	Feature	N
Patients with MS		91
Race (N, %)	Black	2 (2.19%)
	More than one race	1 (1%)
	Unknown/not reported	2 (2.1%)
	White	86 (94.50%)
Sex (N, %)	Female	67 (73%)
	Male	24 (27%)
Age at the first sample (mean, SD) years		37.2 ± 9.06
Age at first symptom (mean, SD) years		35.6 ± 9.02
Disease duration at first visit (mean, SD) years		1.56 ± 1.05
Year 1 medications (62)	Untreated	26 (41.9%)
	Avonex	14 (22.5%)
	Copaxone	11 (17.7%)
	Betaseron	5 (8%)
	Rebif	5 (8%)
Legend: MS = multiple sclerosis; N = patient count; SD = standard deviation; Disease duration is defined as time from MS diagnosis.		

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the 68 regions was calculated to represent the global cortical thickness a primary outcome measure. The individual cortical regions were also compared to NfL levels, with no significant relationships noted; these regional data are not shown to limit the number of comparisons as these did not represent pre-planned primary outcomes.

Statistical analysis

We assessed the relationship of individual log transformed sNfL values taken at years 1–10 with 10-year DGM fractions and cortical thickness using simple linear regression analysis. We conducted both univariate and multivariate linear regression analyses to assess the association between baseline annual sNfL levels as the main predictor and 10-year total DGM, regional DGM nuclei fractions (thalamus, caudate, putamen and globus pallidus), and global cortical thickness as the outcomes. The multivariate linear regression model included age at first symptom, disease duration, sex, medication status, changes in brain volume in presence of gad lesions and T2 lesion, and total number of relapses for each year as the additional predictors. We adjusted the results from the all univariate and multivariate linear regression with $p.adjust$ function in R with the 'fdr' (Benjamini, Hochberg, and Yekutieli) method for the correction due to the multiple comparisons performed. Furthermore, we determined the additional variance in brain volumes explained by sNfL levels by comparing the reduced linear

regression model (without sNfL as a predictor) to the full linear regression model (with sNfL as a predictor).

Data availability statement

The datasets generated for the analysis of the study are available from the corresponding authors upon reasonable request to qualified investigators.

Results

Patients and sNfL characteristics

The patient cohort evaluated in this study was described in our previous publication and is outlined in Table 1.¹⁶ There was a majority (73%) female patients (89 females vs 33 males), and the age at first sNfL value was 37.95 ± 9.09 years (mean ± SD). All patients had a short disease duration from the first symptom at the first visit (1.61 ± 1.08 years). Most patients were treated with DMTs, including 66% of patients at the first sNfL measurement (year 1), which increased to 85% at year 2.

Association of sNfL levels with DGM fraction

We evaluated the relationship between log sNfL levels during years 1–10 and 10-year DGM. On adjusted univariate analysis, we found that sNfL levels for years 1, 2 and 5 were significantly associated with 10-year total DGM fraction. Years 2 and 5 were associated with total DGM in the multivariate analysis, however this significance was lost after

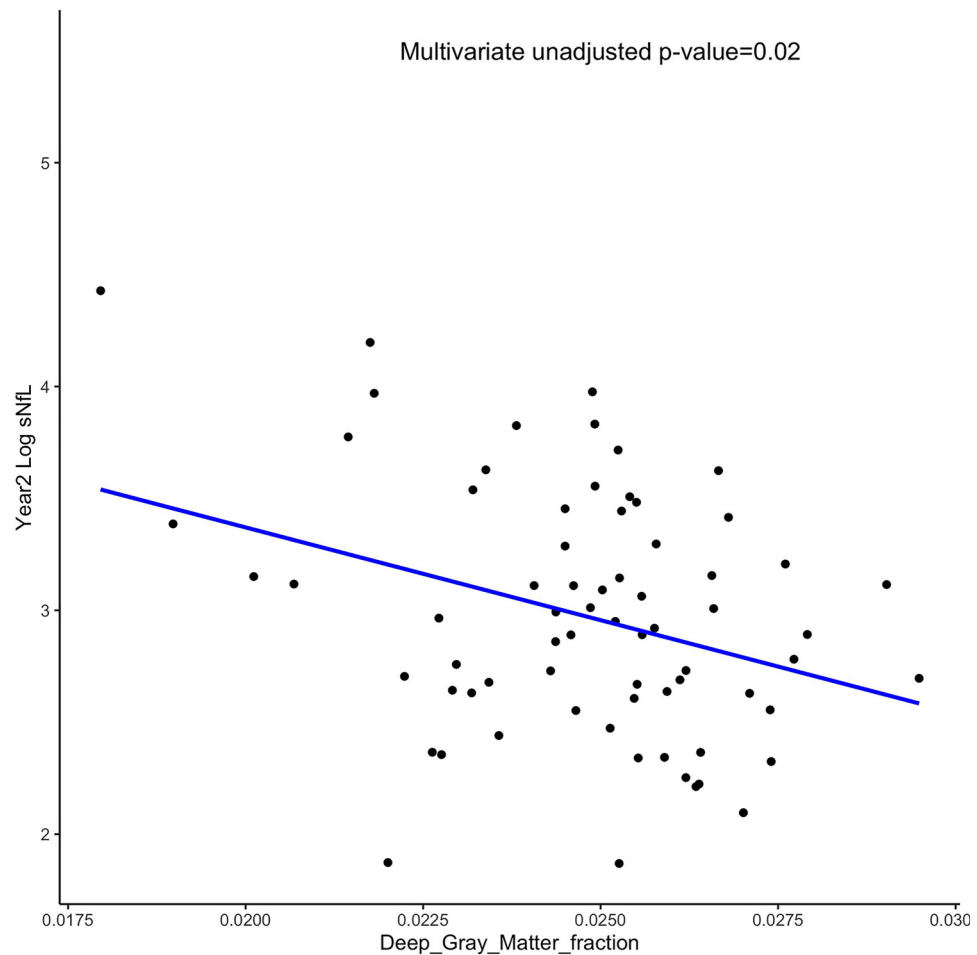


Figure 1. sNFL levels at year 2 correlate with 10-year deep gray matter fraction: regression analysis between year2 log sNFL values and total deep gray matter adjusted for age, sex, disease duration, medication, total number of relapses, changes in the brain due to presence of T2 lesion and GAD lesion showed a negative correlation (multivariate p-value 0.02, adjusted p-value 0.16).

adjusting for multiple testing correction. The greatest decrease in explained variance between the full and reduced model (without sNFL) was seen for the association in years 2 (14.2% vs 6.5%). Supplementary Table 1 shows both the adjusted univariate and multivariate association for yearly sNFL and 10-year DGM fractions. Figure 1 represent a scatter plot of year 2 log sNFL and 10-year DGM.

Association of sNFL levels with thalamic fraction

We next assessed the association between log sNFL levels during years 1–10 and specific DGM sub-structure fractions. Only years 1, 2 and 5 sNFL levels were associated with 10-year thalamic fraction in the adjusted univariate analysis. We found years 1 and 2 were associated in the multivariate analysis, however the significance was lost when adjusted for multiple testing correction. Year 2 presented the

greatest decrease in the variance (19.9% vs 10.4%) between the full and reduced model (without sNFL). Table 2 shows the both the adjusted univariate and multivariate association for yearly sNFL and 10-year thalamic fraction. Figure 2 represent a scatter plot of year 2 log sNFL and 10-year thalamic fraction.

There was no association between individual year sNFL levels and 10 year caudate, putamen or globus pallidus fractions on multivariate analysis.

Association of sNFL levels with cortical thickness

We assessed the association between yearly log sNFL levels from year 1 to any follow-up time point until year 10 and global cortical thickness. We did not find any associations in the multivariate analysis and after adjusting for multiple testing corrections.

Table 2. Association of yearly log transformed sNFL and thalamic fraction.

Year	N	Univariate			Multivariate			R ² Full	R ² Reduced	Adjusted	
		Estimate	95% CI	P-value	Adjusted	Estimate	95% CI				P-value
1	62	-4.5*10 ⁻⁴	-7.2*10 ⁻⁴ , -1.7*10 ⁻⁴	<0.05	<0.05	-3.1*10 ⁻⁴	-6.1*10 ⁻⁴ , -6.8*10 ⁻⁶	<0.05	25.5%	19.7%	0.17
2	70	-6.9*10 ⁻⁴	-1.2*10 ⁻³ , -2.7*10 ⁻⁴	<0.05	<0.05	-6.0*10 ⁻⁴	-1.0*10 ⁻³ , -1.5*10 ⁻⁴	<0.05	19.9%	10.4%	0.08
3	76	-3.2*10 ⁻⁴	-6.8*10 ⁻⁴ , 5.1*10 ⁻⁵	0.09	0.48	-3.3*10 ⁻⁴	-7.3*10 ⁻⁴ , 6.3*10 ⁻⁵	0.09	13.5%	9.9%	0.2
4	85	-1.8*10 ⁻⁴	-6.1*10 ⁻⁴ , 2.4*10 ⁻⁴	0.39	1	-1.3*10 ⁻⁴	5.7*10 ⁻⁴ , 2.9*10 ⁻⁴	0.52	13.2%	12.7%	0.74
5	78	-4.8*10 ⁻⁴	-8.2*10 ⁻⁴ , -1.4*10 ⁻⁴	<0.05	<0.05	-3.8*10 ⁻⁴	-7.7*10 ⁻⁴ , -2.8*10 ⁻⁶	0.051	13.2%	8.4%	0.17
6	71	-5.1*10 ⁻⁴	-1.1*10 ⁻⁴ , 4.2*10 ⁻⁵	0.06	0.48	-4.1*10 ⁻⁴	-9.9*10 ⁻⁴ , 1.6*10 ⁻⁴	0.16	13.5%	10.8%	0.27
7	81	-1.0*10 ⁻⁴	-5.2*10 ⁻⁴ , 3.1*10 ⁻⁴	0.61	1	-1.2*10 ⁻⁴	-5.8*10 ⁻⁴ , 3.4*10 ⁻⁴	0.59	6.8%	6.5%	0.74
8	80	-2.1*10 ⁻⁴	-8.5*10 ⁻⁴ , 4.2*10 ⁻⁴	0.5	1	-1.3*10 ⁻⁴	-8.0*10 ⁻⁴ , 5.3*10 ⁻⁴	0.69	12.2%	12%	0.76
9	70	-3.8*10 ⁻⁴	-8.0*10 ⁻⁴ , 3.4*10 ⁻⁵	0.07	0.48	-3.6*10 ⁻⁴	-8.1*10 ⁻⁴ , 7.3*10 ⁻⁵	0.1	14.8%	11%	0.2
10	75	-9.7*10 ⁻⁵	-4.7*10 ⁻⁴ , 2.7*10 ⁻⁴	0.6	1	-1.3*10 ⁻⁵	-4.2*10 ⁻⁴ , 4.0*10 ⁻⁴	0.94	5.4%	5.4%	0.94

Legend: 95% CI = 95% confidence interval; N = patient count; sNFL = serum neurofilament light chain;.

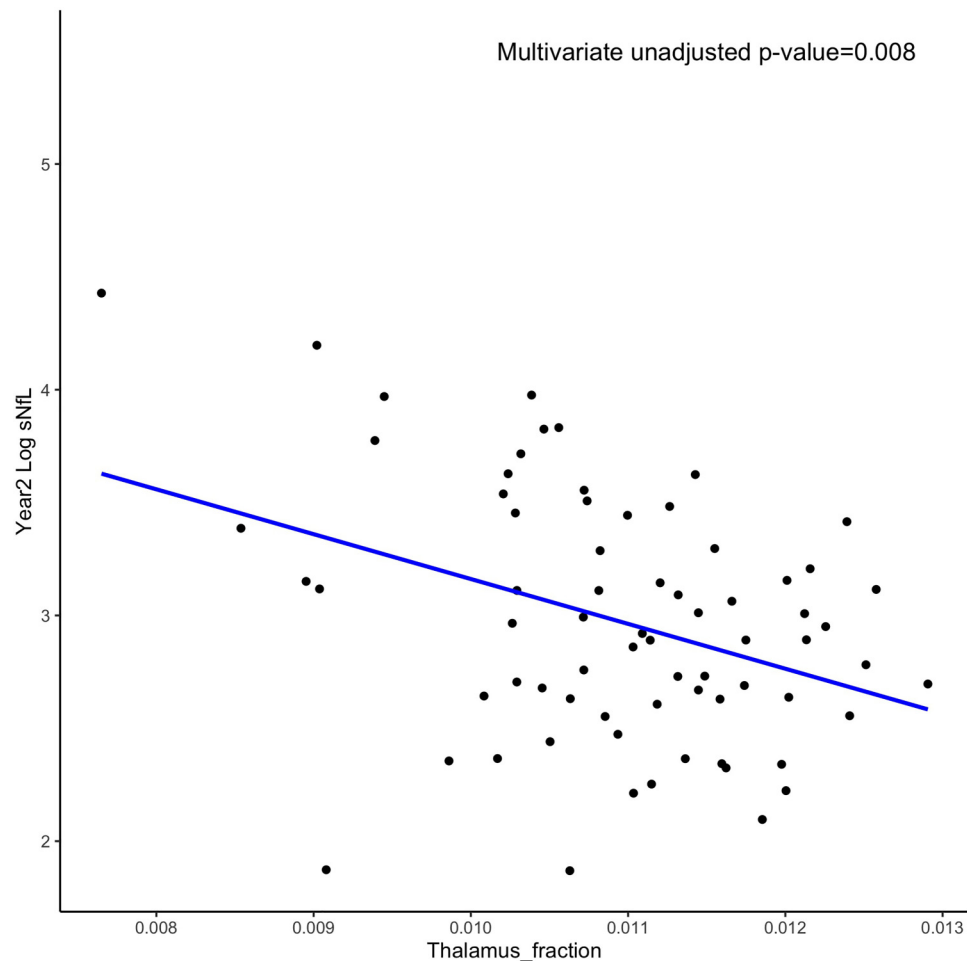


Figure 2. sNfL levels at year 2 correlate with 10-year thalamic fraction: regression analysis between year2 log sNfL values and thalamus fraction adjusted for age, sex, disease duration, medication, total number of relapses, changes in the brain due to presence of T2 lesion and GAD lesion showed a negative correlation (multivariate p-value 0.008, adjusted p-value 0.08).

Association of DGM fractions with cortical thickness

We assessed whether cortical atrophy was associated with DGM atrophy. Year 10 cortical thickness was significantly associated with year 10 total DGM fraction (0.32, $p = 0.001$). Year 10 cortical thickness was also significantly associated with Year 10 fractions of several DGM substructures, including the thalamus ($r = 0.28$, $p = 0.007$), caudate nucleus ($r = 0.29$, $p = 0.004$), and putamen ($r = 0.30$, $p = 0.003$). We did not find any significant association at year 10 between cortical thickness and year 10 globus pallidus fraction ($r = 0.12$, $p = 0.25$).

Discussion

In a prior study, we reported that the same patient cohort showed an association between averaged sNfL levels from the first five years of disease onset and 10-year whole-brain atrophy.¹⁶ In this study, we investigated the prognostic value of sNfL for

longterm gray matter volumetrics. Individual sNfL yearly levels during years 1 and 2 were associated with thalamic volumes in univariate analyses, but not in multivariate models which included clinical and MRI measures.

sNfL levels in MS best correlate with axonal damage associated with acute new Gd+ lesions and are weakly associated with new T2 lesion development on interval imaging. The clinical value of our results is the demonstration that early sNfL values may be able to prognosticate 10-year MRI and potentially clinical outcomes. The value of this approach will need to be validated across different therapies, including higher efficacy therapies that have been more recently introduced.

The thalamus is a region of significant interest in MS, as it is affected by disease onset^{23,24} and thalamic

atrophy is related to disability,²⁵ cognitive dysfunction,^{26–28} ambulation impairment²⁹ and fatigue.³⁰ Its role as a central relay station suggest its role as a sensitive barometer of inflammation and CNS damage occurring throughout the brain in MS.³¹ sNfL levels are strongly associated with neuro-axonal injury caused by new/enlarging T2 lesions and inflammatory gadolinium enhancing lesions,^{10,20} and our finding associating early NfL levels with thalamic volume suggests that early white matter inflammation contributes to thalamic atrophy. Consistent with this hypothesis, studies have shown a link between white matter damage and DGM atrophy in MS, suggesting that Wallerian degeneration plays a key role.^{32,33}

In contrast, the lack of association between sNfL and cortical thickness suggests that cortical volume may not be driven by the same mechanisms as thalamic volume. For example, cortical volume is not tightly linked to white matter lesion accrual, suggesting that Wallerian degeneration may not play a major role,³⁴ unlike our findings with the thalamus. A predominant driver of cortical volume may include direct damage from cortical lesions.^{35,36} Secondly, another direct effect of the disease that may impact the cortex, is leptomeningeal inflammation, part of the “surface-in” hypothesis of MS pathophysiology stemming from CSF and leptomeningeal inflammation overlying the cerebral cortex.^{37–39} Our findings might also reflect that cortical volume may not be common early in MS, as suggested by prior studies.⁴⁰ This could have limited power/effect sizes for identifying cortical atrophy relationships in the present study.

Our results show that sNfL can be useful in predicting future brain atrophy of key structures, although this association is not consistent across all brain regions. In addition to the aforementioned neuroanatomic correlations, these results may have important clinical associations. As sNfL is responsive to high-efficacy DMTs, it is conceivable that switching to a different DMT may lower sNfL levels and possibly affect brain volume.^{10,41–45}

Our results align with the findings of several prior studies, which showed an association between sNfL levels and brain volumes. A few studies have reported that baseline sNfL levels correlated with future whole-brain atrophy.^{13,15,16} Furthermore, two studies showed a correlation between sNfL levels and both global GM atrophy and DGM atrophy.^{17,18} In particular, Jakimovski et al. showed that sNfL levels correlated with the development of atrophy in

key DGM structures, including the thalamus and globus pallidus.¹⁷ Both studies show an association between sNfL levels and future GM, thalamic, and pallidal atrophy. Our study also confirms the lack of association between longitudinal cortical atrophy and baseline sNfL levels, which Jakimovski et al. also reported. Our study adds to the results of Jakimovski et al. in several ways. We provide a longer duration of follow-up and the addition of cortical thickness measurements.

The strengths of our study include the longitudinal design, annual sNfL measurements, and the implementation of a well-characterized patient population with clinical and MRI outcomes. Further, we used advanced MRI volumetric measurements from high-resolution 3 T images, which allowed us to assess key brain regions. To the best of our knowledge, this was the first study to assess the association of sNfL and cortical thickness in patients with MS. The main limitation of this study was the presence of a single MRI timepoint, i.e., year 10, and we thus could not assess longitudinal atrophy, but are only able to show associations with year 10 outcomes. Other limitations include a high proportion of patients treated with DMTs during the early stages of disease. Additionally, future studies will need to further explain the high variance in sNfL levels among patients with MS, which may depend on several factors, including age, clinical relapses, and gadolinium-enhancing lesions.^{19,20}

In conclusion, this study supports the role of sNfL as a predictive marker of DGM volumes, including important DGM structures such as the thalamus. These results may indicate additional ways to improve the way we quantify disability accrual in MS patients. Further, our findings support the presence of some divergence in factors leading to cortical vs. thalamic atrophy. Thalamic volume correlates with both cortical thickness and averaged sNfL levels, although sNfL levels do not correlate with cortical thickness. These results are encouraging because they may pave the way toward the implementation of objective biomarkers such as sNfL, which can provide objective correlates to disability progression. Future studies are needed to confirm these findings and to assess the role of high-efficacy DMTs in the association between sNfL and whole-brain and regional volumes.

Declaration of conflicting interests

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Supplemental material

Supplemental material for this article is available online.

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