Temporal Relationship Between Serum Neurofilament Light Chain and Radiologic Disease Activity in Patients With Multiple Sclerosis

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Abstract

Background and Objectives

Serum neurofilament light chain (sNfL) levels correlate with multiple sclerosis (MS) disease activity, but the dynamics of this correlation are unknown. We evaluated the relationship between sNfL levels and radiologic MS disease activity through monthly assessments during the 24-week natalizumab treatment interruption period in RESTORE (NCT01071083).

Methods

In the RESTORE trial, participants with relapsing forms of MS who had received natalizumab for ≥12 months were randomized to either continue or stop natalizumab and followed with MRI and blood draws every 4 weeks to week 28 and again at week 52 The sNfL was measured, and its dynamics were correlated with the development of gadolinium-enhancing (Gd+) lesions. Log-linear trend in sNfL levels were modeled longitudinally using generalized estimating equations with robust variance estimator from baseline to week 28.

Results

Of 175 patients enrolled in RESTORE, 166 had serum samples for analysis. Participants with Gd+ lesions were younger (37.7 vs 43.1, $p = 0.001$) and had lower Expanded Disability Status Scale scores at baseline (2.7 vs 3.4, $p = 0.017$) than participants without Gd+ lesions. sNfL levels increased in participants with Gd+ lesions (n = 65) compared with those without (n = 101, mean change from baseline to maximum sNfL value, 12.1 vs 3.2 pg/mL, respectively; $p = 0.003$). As the number of Gd+ lesions increased, peak median sNfL change also increased by 1.4, 3.0, 4.3, and 19.6 pg/mL in the Gd+ lesion groups of 1 (n = 12), 2–3 (n = 18), 4–9 (n = 21), and ≥10 (n = 14) lesions, respectively. However, 46 of 65 (71%) participants with Gd+ lesions did not increase above the 95th percentile threshold of the group without Gd+ lesions. The initial increase of sNfL typically trailed the first observation of Gd+ lesions, and the peak increase in sNfL was a median [interquartile range] of 8 [0, 12] weeks after the first appearance of the Gd+ lesion.

Discussion

Although sNfL correlated with the presence of Gd+ lesions, most participants with Gd+ lesions did not have elevations in sNfL levels. These observations have implications for the use and interpretation of sNfL as a biomarker for monitoring MS disease activity in controlled trials and clinical practice.

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Introduction

MRI is a sensitive measure of focal CNS inflammation that has been validated in international consensus guidelines as a useful prognostic tool in multiple sclerosis (MS).[1,2] However, MRI is expensive and cumbersome, reducing its typical use to intervals of 6 months or longer.[3] Hence, a blood-based biomarker that identifies patients with inflammatory activity could help monitor disease activity more frequently and assess the need for a change in treatment or other management.

Neurofilaments are a structural protein specific to neurons that comprise 85% of the CNS cytoskeleton proteins.[4] When released by injured neurons, neurofilaments pass into the CSF and eventually the serum.[5] Neurofilaments are very stable, which makes them an attractive fluid-based biomarker to measure injury in many neurologic conditions.[5] In patients with MS, increased serum neurofilament light chain (sNfL) levels correlate with MS disease activity,[6-9] may predict worse long-term clinical and MRI outcomes,[9] and are reduced by disease-modifying treatments.[7,8,10] However, the temporal dynamics of sNfL, particularly their relationship with fluctuations in disease activity measured on MRI, remain unknown.

The RESTORE trial (NCT01071083)[11] was an exploratory, randomized, partially placebo-controlled study with the objective of exploring the course of MS disease activity and the effects of pharmacokinetic and pharmacodynamic parameters in participants undergoing interruption of natalizumab therapy for up to 24 weeks compared with those remaining on natalizumab.[11] The unique study design of RESTORE involved monthly serum sampling and MRI and afforded the opportunity to correlate MRI disease activity during the planned 24-week natalizumab dosage interruption and blood-based biomarkers such as sNfL.[12-15]

Understanding the temporal relationship between sNfL levels and gadolinium-enhancing (Gd+) lesions provides insight into the interaction between inflammation and neuroaxonal damage, as measured by MRI and sNfL, and the relative time course of new lesions on MRI and changes in sNfL levels. Therefore, this analysis will inform how sNfL might be used as a biomarker for monitoring disease activity in patients with MS.

Methods

Study Design and Participants

We conducted a post hoc analysis on a subset of participants with MS enrolled in RESTORE[11] (NCT01071083) who had serum samples available for sNfL measurements at 4-week time intervals corresponding to MRI acquisition. The RESTORE study design and results were reported previously.[11] Briefly, RESTORE enrolled patients aged 18–60 years with relapsing forms of MS who had been treated with natalizumab for ≥12 months before randomization and without relapses during those 12 months. Participants were excluded if, during screening, the presence of Gd+ lesions and antinatalizumab antibodies were detected, if they had a history of significant infectious illness or of significant disease other than MS, and if they were unable to undergo monthly MRI scans for 6 months.

Enrolled participants had received natalizumab (Tysabri) for ≥1 year and had no relapses in the previous year. Participants were randomized (1:1:2) to continue natalizumab, switch to placebo, or switch to other treatments (intramuscular interferon-β-1a, glatiramer acetate), or intravenous methylprednisolone [MP] 1 g every 4 weeks starting 12 weeks after randomization) for 24 weeks and then switch back to natalizumab for the follow-up period (weeks 28–52). In the other-therapies group, patients and their neurologist selected the immunomodulatory therapy on an individual basis. When deemed beneficial, MP was administered once every 4 weeks starting at week 12 of the randomized treatment period. Participants could receive a 3–5-day course of high-dose corticosteroids per local standard of care and/or restart natalizumab before week 28 if they had a protocol-defined clinical relapse or MRI disease activity per the rescue criteria and thus would immediately enter the follow-up period.

MRI was performed every 4 weeks during the randomized treatment period starting at week 0, at the time of suspected relapse, and at final visit. Some samples had missing data, but most participants had sNfL data for at least 7 of the 9 time points. MRIs were acquired using standardized protocol T2 and T1 sequences with and without Gd, as described previously.[11] The number and volume of Gd+ lesions were assessed at each time point by the Central MRI Reading Center (NeuroRx Research, Montreal, Canada).

Standard Protocol Approvals, Registrations, and Patient Consents

Each site's institutional review board reviewed and approved the study protocol and amendments, and all participants provided written informed consent. The study was performed in accordance with the Declaration of Helsinki and International Conference on Harmonization Guideline on Good Clinical Practice and is registered with ClinicalTrials.gov, NCT01071083.

sNfL Assay

A sensitive single-molecule array assay (SIMOA NF-light Advantage Kit; Quanterix, Lexington, MA) was used to measure sNfL levels in sera. Detailed methodology was previously described.[7] The assay was analytically validated for the

fit-for-purpose sNfL evaluation. The measurements were performed in one round of experiments using a single batch of reagents. At baseline and every 4 weeks to week 28 (or at the time of rescue) and week 52, sNfL levels were measured.

Data Analysis

Descriptive statistics of sNfL levels and percentage change in sNfL from baseline were presented. Median percentage change in sNfL levels, stratified by Gd+ lesion number, was compared using the Wilcoxon rank-sum test. To evaluate the magnitude of sNfL elevations, participants who did not develop Gd+ lesions were used to calculate a 95th percentile threshold for sNfL levels and percentage change from baseline. This 95th percentile threshold was used to derive sensitivity and specificity for elevations in sNfL to predict the presence of Gd+ lesions.

As a sensitivity analysis, age adjustment was conducted as previously described (Harp et al. 2022).

Log-linear trends in sNfL levels were modeled longitudinally using generalized estimating equations (GEEs) with robust variance estimator from baseline to week 28 with the goal of identifying which predictors were most influential on sNfL. The following covariates were included: age, sex, race, body mass index, cumulative Gd+ lesion count, baseline Expanded Disability Status Scale (EDSS) score, years since diagnosis, Gd+ lesion volume, baseline creatinine clearance, relapse occurrence during the study, DMT, and 4-week visit intervals. Interactions with time were assessed but ultimately were left out of the analysis because the added complexity did not affect the overall findings. The standardized beta coefficients were also evaluated to assess the impact of each variable in terms of SD on the dependent variable. The estimate from the standardized betas translates to the impact on sNfL for every 1 SD increase for the predictor. Of the patients who experience GD+ lesions during their randomized treatment period, missing sNfL measurements comprised 9.2%, 5.4%, and 12.5% of the data during the scheduled visit immediately preceding, during, and immediately following the identification of the incident GD+ lesion, respectively, and missingness was assumed to be missing at random. GraphPad Prism 7 software (GraphPad Software, Inc.) was used for graphical representation and basic statistical results.

Data Availability

Anonymized participant data collected during the trial will be shared with qualified scientific researchers who provide a methodologically sound proposal. Proposals should be submitted through Vivli (vivli.org). To gain access, data requestors will need to sign a data sharing agreement. Data are made available for 1 year on a secure platform.

Results

Participants

Of the 175 participants randomized into the RESTORE trial, 166 had longitudinal sNfL measurements that included a baseline value and were included in this analysis. sNfL data were available for 166 participants at baseline, 130 at week 4, 125 at week 8, 153 at week 12, 141 at week 16, 104 at week 20, 100 at week 24, 152 at week 28, and 151 at week 52.

Table 1 Patient Demographic and Clinical Characteristics

| Characteristic, mean (SD) unless otherwise stated | Stratified by Gd+ lesion during follow-up | | | Total |
| | Gd+ Neg. | Gd+ Pos. | | |
	n = 101	n = 65	p Value	N = 166
Female (%)	81 (80.2)	47 (72.3)	0.321	128 (77.1)
Age, y, mean ± SD	43.1 ± 9.8	37.7 ± 9.2	0.001	41.0 ± 9.9
Race, White, %	92 (91.1)	59 (90.8)	0.861	151 (91.0)
EDSS score at baseline				
Mean ± SD	3.4 ± 1.8	2.7 ± 1.5	0.017	3.1 ± 1.7
Median [IQR]	3.5 [2.0, 4.0]	2.8 [1.5, 3.5]	0.024	3.0 [2.0, 4.0]
Disease duration, y, mean ± SD	9.8 ± 6.3	8.5 ± 5.4	0.187	9.3 ± 5.96
No. of cumulative Gd+ lesions during follow-up, mean ± SD	0.0[a]	6.86 ± 9.4	<0.001	2.69 ± 6.8
Weeks until NfL peak after Gd+ lesion				
Mean ± SD	—	8.9 ± 14.3	—	—
Median [IQR]	—	8 [0, 12]	—	—
Baseline creatinine clearance, μmol/L, mean ± SD	69.0 ± 11.9	69.8 ± 12.3	—	69.3 ± 12.0

Abbreviations: EDSS = Expanded Disability Status Scale; Gd+ = gadolinium-enhancing; IQR = interquartile range.
[a] Exclusion criteria of the RESTORE trial included any evidence of Gd-enhancement at screening.

Baseline demographic and clinical characteristics of participants are presented in Table 1. Participants who did not (n = 101) and did (n = 65) develop Gd+ lesions during the study had a mean age of 43.1 ± 9.8 and 37.7 ± 9.2 years ($p = 0.001$) and disease duration of 9.8 ± 6.3 or 8.5 ± 5.4 years ($p = 0.187$), respectively. Before beginning natalizumab, 41.6% of those who did not and 41.5 who did develop Gd+ lesions during the study were considered to have high disease activity. Participants in both groups were moderately disabled at study baseline, as evidenced by a median [interquartile range] EDSS score of 3.5 [2.0, 4.0] in the group who did not and 2.8 [1.5, 3.5] in those who did develop Gd+ lesions ($p = 0.024$).

Relationship Between sNfL Levels and Gd+ Lesion Development

The development of Gd+ lesions varied between assigned treatment cohorts. For those who discontinued natalizumab (n = 121), 56 (46.3%) remained free of Gd+ lesions and 65 (53.7%) developed 1 or more Gd+ lesions. None of the 45 participants who continued natalizumab developed Gd+ lesions. Therefore, in the current analysis, the group without Gd+ lesions consisted of 45 participants who continued on natalizumab and 56 who discontinued natalizumab (n = 101).

Longitudinal data for sNfL levels were examined for their relation to Gd+ lesions, irrespective of randomized treatment assignment. In the participants who developed Gd+ lesions (n = 65), the mean ± SD sNfL levels were 8.9 ± 3.9 pg/mL at baseline and increased to 20.0 ± 30.5 pg/mL at week 28 (mean difference with 95% confidence interval [CI]: 10.7 (2.77–18.58), Student t-test $p = 0.009$; Figure 1B and eTable 1). For participants who did not develop Gd+ lesions (n = 101), sNfL levels remained low, with a mean ± SD sNfL level at baseline of 9.7 ± 5.0 pg/mL ($p = 0.267$) and at 28 weeks of 10.2 ± 7.9 pg/mL ($p = 0.004$; Figure 1A and eTable 1). Compared with those without Gd+ lesions, participants with Gd+ lesions had significantly higher sNfL mean levels at 28 weeks, which correlated to ~9 weeks after the first Gd+ lesion (20.0 ± 30.5 vs 10.2 ± 7.9 pg/mL; $p = 0.004$; eTable 1 and Figure 1, A and B). Figure 1, B and D plot sNfL from the time of first Gd+ lesion development for each participant. The mean ± SD percentage increase in sNfL from baseline to week 28 in participants who developed Gd+ lesions was 126.6% ± 232.2% vs 8.9% ± 46.2% ($p < 0.001$) in the participants who did not develop Gd+ lesions (Figure 1, C and D, eTable 1).

Among participants who did not develop Gd+ lesions, the 95th percentile threshold for sNfL levels and percentage

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Figure 1 Timecourse of sNfL Levels According to Gd+ Lesions Status
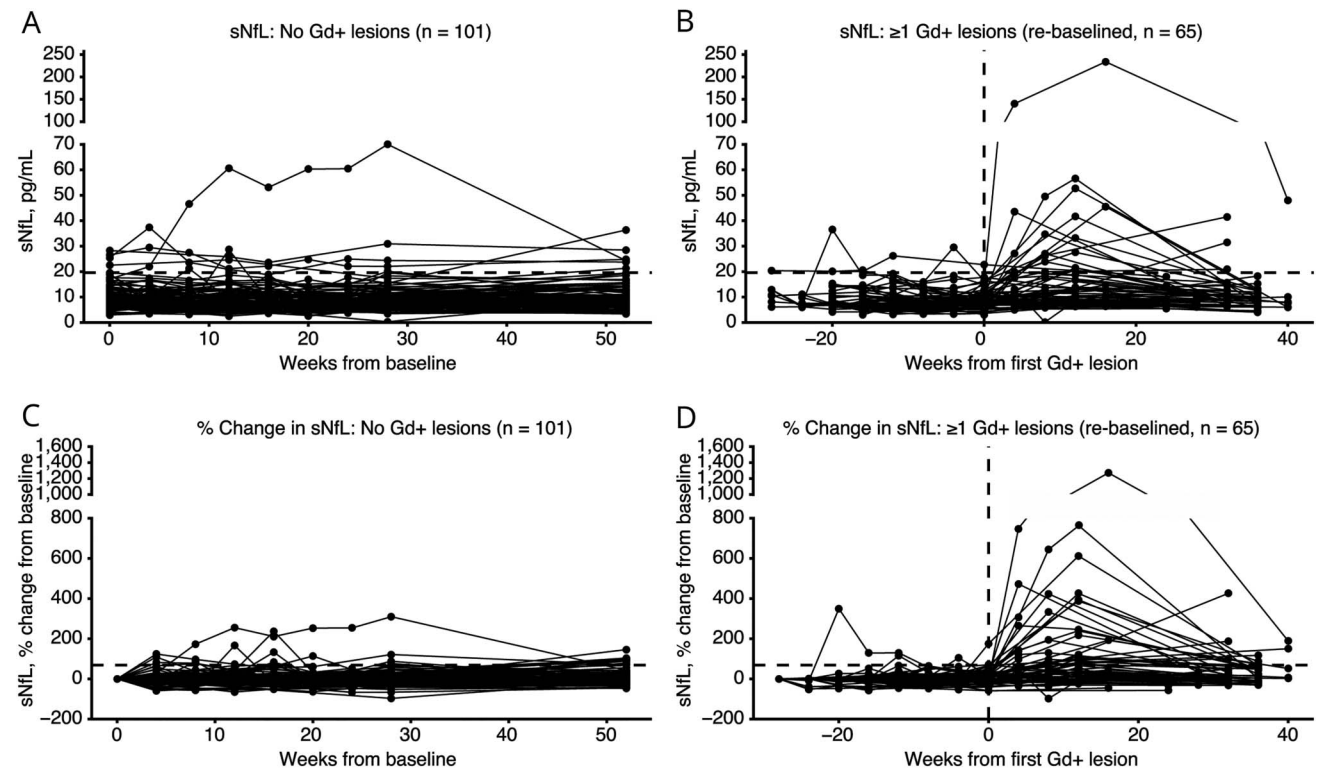
Individual subject sNfL levels over time among subjects without Gd+ lesions (absolute values A, percentage changes C) and with Gd+ lesions (absolute values B, percentage changes D). Horizontal dotted line in A and B represents 19.6 pg/mL change in sNfL from baseline. Horizontal dotted line in C and D represents the 68.8% change in sNfL from baseline. Vertical dotted line in B and D represents time of first detected Gd+ lesion (week 0). Gd+ = gadolinium-enhancing; sNfL = serum neurofilament light chain.

change from baseline were 19.6 pg/mL and 68.8% increase from baseline. There were 5 participants who never developed Gd+ lesions and who had sNfL levels above the 95th percentile. Most were among the oldest participants in the study (ages 28, 48, 51, 58, and 59 years) and had high EDSS scores at baseline (4.0, 5.0, 6.0, 7.0, and 7.0); 4 of the 5 had disability progression without relapses during the study and 1 had a clinical relapse and spinal stenosis. None other of these 5 participants had adverse events that would be expected to increase sNfL levels (i.e., stroke, traumatic brain injury, seizure). Using these cutoffs, peak sNfL >19.6 pg/mL yielded 27.9% sensitivity and 91.8% specificity; peak sNfL 68.9% above baseline sNfL yielded 42.6% sensitivity and 82.7% specificity (Table 2).

Changes in sNfL Levels and Association With the Number of Gd+ Lesions

The change between baseline and peak sNfL increased among participants who developed a greater number of Gd+ lesions (Table 3). The mean sNfL percentage change increased by 48.4% (1 new Gd+ lesion, Figure 2A); 92.2% (2–3 new Gd+ lesions, Figure 2B); 103.8% (4–9 new Gd+ lesions, Figure 2C); and 371.1% (≥10 new Gd+ lesions, Figure 2D). Any increases in sNfL levels followed the initial appearance of Gd+ lesions in 80% of patients; 13 patients (20%) reached peak sNfL before the first Gd+ lesion was identified. When looking at the 95th percentile threshold, only 13.8% (n = 4/65) participants crossed the 19.6 pg/mL sNfL threshold before the presence of their first Gd+ lesion and 23.1% (n = 15/65) breached the 19.6 pg/mL sNfL threshold after their first Gd+ lesion. Within the participants who developed Gd+ lesions, 70.8% (n = 46/65) never breached the 19.6 pg/mL sNfL threshold and 10.7% (n = 7/65) did not have sNfL greater than their baseline levels. Peak sNfL was delayed by an average of 8.7 ± 14.4 weeks after first Gd+ lesion identification (Figure 1, B and D). In 9.2% (6/65) of participants with Gd+ lesions, sNfL levels were not available at week 52.

Sensitivity analysis after adjusting for age did not significantly alter the findings (eFigure 1). Age-adjusted sNfL >10.6 pg/mL yielded 36.1% sensitivity and 91.8% specificity (eTable 2). Similarly, the age-adjusted maximum sNfL increase in participants by maximum number of Gd+ lesions changed little (eTable 3). Since age-adjustment changes the 95th percentile threshold and individual values equally, age adjustment did not change the percent elevations shown in Table 2 and Figure 1.

To explore the relationship between sNfL, baseline demographics, disease characteristics, and MRI findings, sNfL was longitudinally modeled using GEE. Models were generated for the overall and Gd+ populations with consistent findings. For participants who developed Gd+ lesions (n = 65), the number of cumulative Gd+ lesions and baseline EDSS were the only significant predictors for sNfL (eTable 4). This translated into an average of 4.2 and 6.3 percent increase in sNfL for each additional Gd+ lesion and level of EDSS worsening,

Table 2 Sensitivity and Specificity of sNfL Elevations Above the 95th Percentile Thresholds

	Gd+ lesion occurrence		
	Gd+	No Gd+	
Peak sNfL			
Above 19.6 pg/mL	17	8	PPV: 67.5%
Below 19.6 pg/mL	44	90	NPV: 67.7%
	Sensitivity: 27.9%	Specificity: 91.8%	
Peak sNfL % change			
Above 68.8%	26	17	PPV: 59.9%
Below 68.8%	35	81	NPV: 70.4%
	Sensitivity: 42.6%	Specificity: 82.7%	

Sensitivity: the proportion of people with Gd+ who were above the specified sNfL threshold compared with the total number of people who developed Gd+.
Specificity: the proportion of people with no Gd+ who were below the specified threshold of sNfL compared with the total number of people with no Gd+.
PPV: positive predictive value or probability that an elevation in sNfL above the identified threshold will have Gd+ lesions.
NPV: negative predictive value or the probability that the absence of an elevation in sNfL above the identified threshold will have Gd+ lesions.
Gd+ = gadolinium-enhancing; sNfL = serum neurofilament light.

respectively ($p < 0.001$). GEE modeling did not find DMT treatment to affect the relationship.

Discussion

There is a need for standardized fluid biomarkers that can inform the clinical practice of neurology. Serum concentrations of NfL have emerged as a promising biomarker for acute axonal injury and neurologic disease activity. As a potential measure of disease activity, sNfL could lend insight into the presence and evolution of neuroinflammatory diseases when clinical presentations of disease activity are less sensitive to tissue injury. In this study, we explored the association between sNfL levels and development of Gd+ lesions on brain MRI to better understand how sNfL might reflect the dynamics of MS focal inflammatory disease activity. As expected, average sNfL levels increased among participants who developed Gd+ lesions compared with those who did not. In general, a high number of Gd+ lesions were associated with high sNfL levels. However, increased sNfL was not consistently observed with Gd+ lesions, and indeed was particularly uncommon in patients with only a few Gd+ lesions. Even with very high Gd+ lesion levels, many participants showed no increase in sNfL that was greater than their baseline level, which may explain why Gd+ lesion volume was not a significant factor when modeling increased sNfL. In addition, large variability in sNfL increases were observed among participants, even those with similar numbers of Gd+ lesions,

Table 3 Maximum sNfL Increase in Participants by Maximum Number of Gd+ Lesions

	Maximum sNfL change for each group		Maximum No. of Gd+ lesions			
Gd+ lesions	0	>0	1	2–3	4–9	≥10
	n = 101	n = 65	n = 12	n = 18	n = 21	n = 14
Mean sNfL ±SD	3.2 ± 6.6	12.1 ± 28.7	3.9 ± 8.4	5.6 ± 7.8	5.6 ± 7.8	36.0 ± 56.5
p-value[a]	−	0.003	0.89	0.58	0.32	<0.001
Median sNfL [IQR]	2.1 [0.5, 4.1]	4.10 [1.6, 12.2]	1.4 [0.4, 4.9]	3.0 [0.9, 8.6]	4.3 [2.7, 7.2]	19.5 [8.3, 35.8]
p-value[b]	−	<0.001	0.94	0.31	0.003	<0.001
Participants with a peak sNfL ≥95th percentile of 19.6 pg/mL, n (%)	11 (10.9)	19 (29.2)	2 (16.7)	4 (22.2)	3 (14.3)	10 (71.4)
Participants with no sNfL peak > baseline, n (%)	18 (17.8)	7 (10.7)	3 (25.0)	3 (16.7)	1 (5.0)	0 (0.0)
	Maximum % sNfL change for each group		**Maximum No. of Gd+ lesions**			
Gd+ lesions	0	>0	1	2–3	4–9	≥10
	n = 101	n = 65	n = 15	n = 25	n = 16	n = 9
Mean ± SD	38.2 ± 54.6	145.1 ± 223.1	48.4 ± 100.5	92.2 ± 130.3	103.8 ± 127.7	371.1 ± 355.2
p-value[a]	−	<0.001	0.79	0.10	0.04	<0.001
Median [IQR]	22.8 [5.8, 66.9]	63.9 [18.2, 190.0]	20.0 [5.3, 64.6]	36.7 [7.6, 127.7]	68.2 [31.2, 100.7]	239.6 [117.5, 423.6]
p-value[b]	−	<0.001	0.73	0.28	0.003	<0.001
Participants with % change in sNfL ≥95th percentile of +68.8%, n (%)	25 (24.8)	29 (44.6)	2 (16.7)	6 (33.3)	10 (47.6)	11 (78.6)

Abbreviations: Gd+ = gadolinium-enhancing; IQR = interquartile range; sNfL = serum neurofilament light chain.
[a] *t*-test *p*-value; *p*-value indicates comparison with Gd+ = 0 group.
[b] Wilcoxon rank sum test, *p*-value indicates comparison with Gd+ = 0 group.

suggesting that there may be differing levels of tissue injury associated with each Gd+ lesion.

In general, we did not see the first increase in sNfL until after initial development of Gd+ lesions and observed a peak increase in sNfL an average of 9 weeks after the first Gd+ lesion was seen. It appears to take at least several weeks for NfL to start being released from CNS tissue with inflammatory injury and become measurable in the peripheral blood. This delay is important for clinicians to recognize when interpreting sNfL measures in clinical practice. sNfL levels remained elevated after resolution of Gd+ lesions in many participants. The time course of sNfL resolution is difficult to assess in this study because most participants had a gap in sNfL sampling between 12–16 weeks after Gd+ lesion development and the final study visit. sNfL levels remained low and stable in participants who continued on natalizumab, as well as in participants who discontinued natalizumab and did not develop Gd+ lesions. This finding is in agreement with results from a large clinical trial in secondary progressive MS, where natalizumab was shown to reduce sNfL concentrations compared with placebo, with or without evidence of acute inflammatory disease activity.[16] A more recent study,[17] using data from the same clinical trial, concluded that sNfL was not associated with disability progression in the absence of

relapses and MRI evidence of inflammatory disease activity. Taken together, these observations suggest that sNfL could be used as a biomarker for inflammatory disease activity, but the relation to disability progression independent of relapses or new lesion formation is still controversial.

The large majority (80%) of sNfL elevations followed the appearance of the first Gd+ lesions, which led us to find sNfL a relatively specific biomarker of MRI disease activity in MS (82%–91.9% specificity), but somewhat low sensitivity (27.9%–42.6% sensitive). Analyses of age-adjusted sNfL levels yielded similar results. Through closer disease activity monitoring, sNfL might still contribute to improving outcomes in MS. Serial imaging for clinically stable patients is costly and often impractical; sNfL could be a marker of subclinical disease activity both in clinical trials that may require frequent imaging, such as phase 2 studies in relapsing MS or in routine clinical practice. However, our study highlights the limitations of sNfL for monitoring disease activity: Its elevation was delayed weeks to several months after Gd+ development and was, in this 28-week study, an insensitive marker of the acute focal inflammation observed on MRI. Another caveat is that elevated levels of sNfL could be attributed to non-MS–related complications.[18]

Figure 2 Timecourse of Changes in sNfL Levels According to Number of Gd+ Lesions
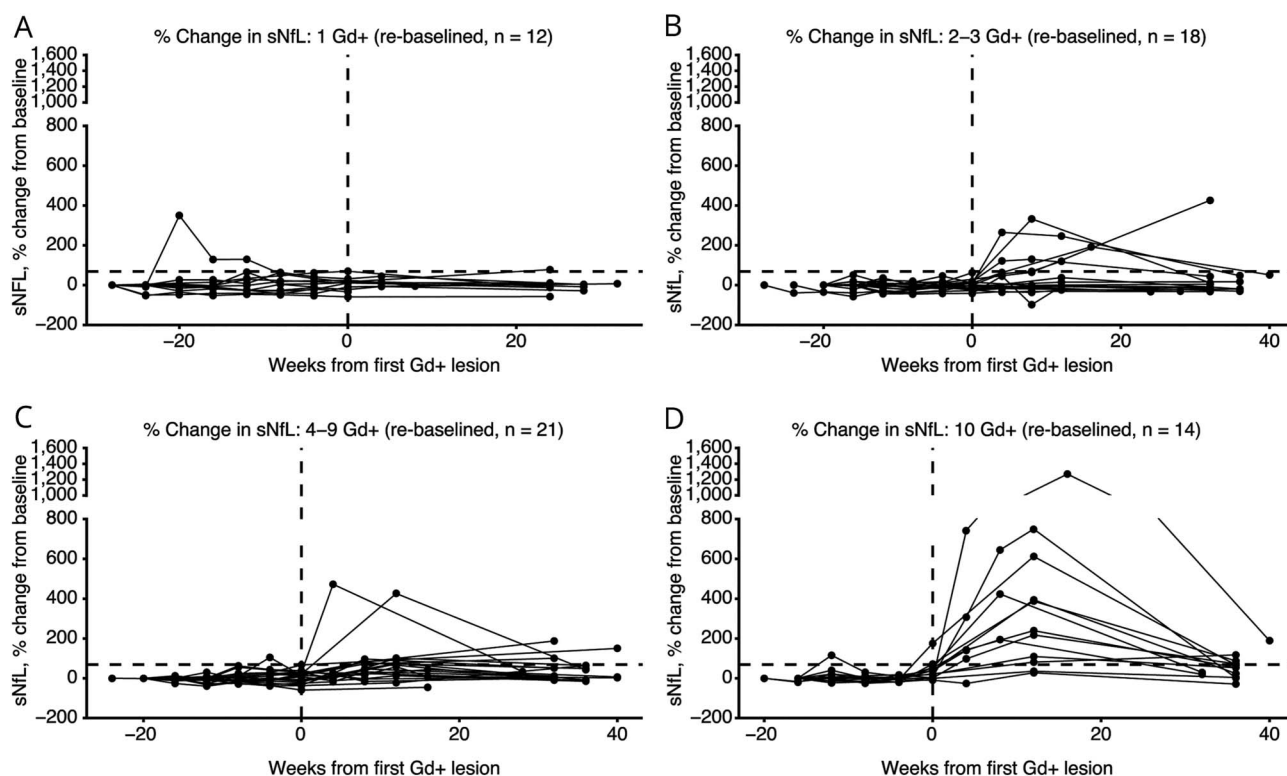
Proportional change in individual subject sNfL levels over time according to the number of Gd+ lesions observed in each subject. Vertical dotted line represents time of first detected Gd+ lesion; horizontal dotted line represents the 68.8% change in sNfL from baseline. Gd+ = gadolinium-enhancing; sNfL = serum neurofilament light chain.

This study has limitations. As a post hoc analysis, these results should be interpreted as hypothesis-generating rather than definitive. Multiple Gd+ lesions in the same patient, sometimes developing sequentially over time and each with a potentially different contribution of sNfL, were combined within the same patient to a single sNfL value at each time point. Thus, the contribution of each Gd+ lesion to sNfL levels could not be determined. Furthermore, some Gd+ lesions may have been short-lived and not detected within 4-week MRI intervals. Since Gd+ lesions did not appear until week 12 or later of the 28-week treatment interruption period, there was limited time to explore subsequent sNfL pharmacokinetics beyond 12–16 weeks after Gd+ lesion development. The sNfL peak and subsequent resolution might have occurred at time points later than this study's follow-up or at times with missing samples. While missingness was assumed to be random, we do not have evidence for this. Since our analysis focused on the peak sNfL observed, missing sNfL measurements would, at worst, render our study conservative by underrepresenting of the true peak in sNfL.

In conclusion, approximately two-thirds of participants with the onset of new Gd+ lesions (46/65) did not have sNfL levels that crossed the 95th percentile threshold, and most

sNfL elevations were after the initial appearance of Gd+ lesions. Although monthly paired MRI and serum sampling demonstrated an association between sNfL and MRI as indicators of disease activity, sNfL did not correlate closely with onset of new Gd+ lesions. These attributes need to be recognized when considering implementation of sNfL as a biomarker for MS disease activity and individual management of patients with MS.

Disclosure

R.J. Fox receives consulting fees from AB Science, Biogen, Bristol Myers Squibb, EMD Serono, Genentech, Genzyme,

Publication History

Appendix Authors

Name	Location	Contribution
Robert J. Fox, MD	Mellen Center for Multiple Sclerosis, Neurological Institute, Cleveland Clinic, OH	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
Bruce A.C. Cree, MD, PhD, MAS	Weill Institute for Neurosciences, Department of Neurology, University of California San Francisco	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
Jérôme de Sèze, MD	Department of Neurology, Hôpital Civil, Strasbourg, France	Drafting/revision of the manuscript for content, including medical writing for content
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Appendix (continued)

Name	Location	Contribution
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