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# Serum Neurofilament Light and Multiple Sclerosis Progression Independent of Acute Inflammation

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## Introduction

Efforts to explore the utility of neurofilament light (NfL) as a biomarker associated with disability progression in multiple sclerosis (MS) have accelerated in recent years in the absence of pharmacodynamic or treatment response markers for clinical trials or patient care.[1] The International Progressive MS Alliance stated in 2020 that serum NfL (sNfL) measurements may serve as a useful biomarker associated with progressive MS, although further work is needed to define the relative contributions of inflammatory activity and neurodegeneration to longitudinal changes in disability and sNfL.[2] Using data from a large clinical trial of patients with secondary progressive MS (a phase 3, randomized, double-blind, placebo-controlled trial exploring the effect of natalizumab on disease progression in participants with Secondary Progressive Multiple Sclerosis [ASCEND in SPMS]; NCT01416181), we investigated whether sNfL could be used as a dynamic biomarker associated with progressive MS disease course. That is, we investigated whether longitudinal changes in sNfL concentration were associated with disability progression measures in the absence of relapses and magnetic resonance imaging (MRI) evidence of inflammatory activity.

## Methods

Approval for the study protocol of the clinical trial used in this cohort study, including any amendments, was granted by each center's ethics committee. Ethical approval of the parent randomized controlled trial (ASCEND) extended to this study. All patients provided written informed consent to participate in the ASCEND study, which included consenting to future use of their study data for medical and pharmaceutical research, such as this post hoc analysis. Generalized estimating equations were used for partial correlations between changes in sNfL concentration and disability assessment scores (Expanded Disability Status Scale [EDSS; range, 1-10], timed 25-foot walk [T25FW] in seconds, and 9-hole peg test [9HPT] in seconds). Associations between changes in sNfL concentration and composite confirmed disability progression (CDP) measured by EDSS, T25FW, or 9HPT at week 48 or week 96 were estimated by logistic regression. Analyses were restricted to the noninflammatory population, defined as individuals with no MRI activity or clinical relapses at baseline or during the study. Details of the clinical trial, statistical methods, and distribution of sNfL concentrations are in eMethods in the Supplement. Analyses were performed in R statistical software version 4.0.4 (R Project for Statistical Computing) with extension packages tidyverse and geepack. The significance level was set at .05. Significance for partial correlations was determined by whether 95% CIs crossed 0. Significance for logistic regression was determined by whether 95% CIs crossed 1. Hypothesis tests were 2-sided. Data were analyzed from January through April 2021.

## Results

Among 751 participants in the ASCEND intention-to-treat population with sNfL data, 214 individuals in the natalizumab group and 103 individuals in the placebo group had no inflammatory activity at baseline or throughout the study. Among these 317 patients, mean (SD) baseline age was 49.3 (6.3) years,

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and there were 206 (65.0%) women. Median (IQR) EDSS score at baseline was 6 (5-6.5). There was no correlation at most time points for most assessments between changes in sNfL concentration and current or future changes in EDSS, T25FW, or 9HPT in either treatment group (**Table 1**). For example, of 24 partial correlations performed, 2 were significant, for 9HPT dominant hand in the natalizumab group (−0.10 [95% CI, −0.20 to −0.002]) and nondominant hand in the placebo group (0.12; [95% CI, 0.002 to 0.24]) between baseline and week 48, in opposite directions (Table 1).

When stratifying individuals by CDP status, sNfL concentration changes were not independently associated with current CDP (ie, same epoch) or CDP over the subsequent 48 weeks (**Table 2**). The absence of association between change in sNfL and composite CDP (ie, EDSS, T25FW,

**Table 1. Estimated Partial Correlation Between Percentage Change in sNfL and DAS**

| DAS | Estimated partial correlation (95% CI)[a,b,c] | | | Participants, No. |
|---|---|---|---|---|
| | sNfL vs DAS, BL to wk 48 | sNfL BL to wk 48 vs DAS wk 48-96 | sNfL vs DAS, wk 48-96 | |
| **Natalizumab** | | | | |
| EDSS | 0.03 (−0.07 to 0.13) | 0.02 (−0.08 to 0.12) | −0.12 (−0.26 to 0.02) | 176 |
| T25FW | 0.02 (−0.08 to 0.12) | 0.03 (−0.07 to 0.13) | 0.07 (−0.01 to 0.15) | 173 |
| 9HPT | | | | |
| Dominant hand | −0.10 (−0.20 to −0.002) | 0.09 (−0.03 to 0.21) | −0.01 (−0.17 to 0.15) | 174 |
| Nondominant hand | 0.03 (−0.13 to 0.19) | 0.00 (−0.10 to 0.10) | −0.08 (−0.18 to 0.02) | 174 |
| **Placebo** | | | | |
| EDSS | −0.03 (−0.17 to 0.11) | −0.04 (−0.14 to 0.06) | −0.02 (−0.12 to 0.08) | 82 |
| T25FW | −0.14 (−0.32 to 0.04) | 0.05 (−0.07 to 0.17) | −0.05 (−0.19 to 0.09) | 82 |
| 9HPT | | | | |
| Dominant hand | 0.16 (−0.13 to 0.45) | −0.03 (−0.13 to 0.07) | −0.05 (−0.21 to 0.11) | 81 |
| Nondominant hand | 0.12 (0.002 to 0.24) | −0.11 (−0.27 to 0.05) | −0.03 (−0.19 to 0.13) | 81 |

Abbreviations: 9HPT, 9-hole peg test; BL, baseline; DAS, disability assessment score; EDSS, Expanded Disability Status Scale; sNfL, serum neurofilament light; T25FW, timed 25-foot walk.

[a] sNfL change is analyzed as a continuous scale.

[b] Adjusted for sex, baseline age, baseline sNfL, and baseline DAS.

[c] In the absence of inflammatory activity.

**Table 2. Odds Ratio of Composite-Confirmed Disability Progression per 10% Increase in sNfL**

| Confirmed disability progression epoch | OR (95% CI)[a,b,c] | | | Participants, No.[d] |
|---|---|---|---|---|
| | sNfL BL to wk 48 | sNfL wk 48 to wk 96 | sNfL BL to wk 96 | |
| **Natalizumab** | | | | |
| BL to week 48 | 1.02 (0.86-1.21) | NA | NA | 208 |
| Week 48 to week 96 | 1.05 (0.80-1.38) | NA | NA | 128[e] |
| Week 48 to week 96 | NA | 1.27 (0.87-1.84) | NA | 113[e] |
| BL to week 96 | 0.93 (0.73-1.18) | 1.11 (0.86-1.41) | NA | 177 |
| BL to week 96 | NA | NA | 1.00 (0.83-1.21) | 178 |
| **Placebo** | | | | |
| BL to week 48 | 0.90 (0.64-1.27) | NA | NA | 98 |
| Week 48 to week 96 | 1.27 (0.65-2.47) | NA | NA | 56[e] |
| Week 48 to week 96 | NA | 0.91 (0.41-2.02) | NA | 48[e] |
| BL to week 96 | 0.84 (0.55-1.29) | 0.96 (0.67-1.36) | NA | 84 |
| BL to week 96 | NA | NA | 0.94 (0.68-1.29) | 86 |

Abbreviations: BL, baseline; NA, not applicable; OR, odds ratio; sNfL, serum neurofilament light.

[a] In the absence of inflammatory activity.

[b] Adjusted for sex and baseline age.

[c] sNfL change is analyzed as a continuous scale, and the OR is for change in odds of progression with respect to every 10% increase in log2-transformed values of sNfL. Median (IQR) sNfL concentrations were 9.55 (7.42-13.88) pg/mL at baseline, 9.83 (7.77-12.23) pg/mL at week 48, and 9.88 (7.98-13.32) pg/mL at week 96 in the natalizumab group and 10.38 (8.28-13.20) pg/mL at baseline, 10.88 (8.20-14.10) pg/mL at week 48, and 11.78 (8.40-14.31) pg/mL at week 96 in the placebo group.

[d] All analyses were performed based on data availability.

[e] Participants whose progression was confirmed up to week 48 were excluded from the analysis when the outcome was progression confirmed after week 48 up to week 96. Only participants who did not progress between baseline and week 96 and participants whose progression was confirmed after week 48 up to week 96 were included.

and 9HPT) was consistent across treatment groups and time epochs (ie, baseline to week 48, week 48 to week 96, and baseline to week 96) (Table 2).

## Discussion

This cohort study found that change in sNfL concentration was not a biomarker associated with disability progression or associated with future disability progression in progressive MS. Although the dynamics of sNfL have been explored previously in populations with progressive MS, including the population from ASCEND,[3] progression was not addressed independently of inflammatory activity, to our knowledge. These results are consistent with recent findings in relapsing-remitting MS in a large cohort of patients treated with natalizumab in which sNfL similarly was not associated with progression independent of clinical or MRI signs of acute inflammatory disease activity.[4]

There are several reasons why changes in sNfL may not capture the dynamics of MS disability progression in the absence of acute inflammation in MS. First, disease progression in MS may not be directly related to loss of neurons but instead mediated by a failure of effective remyelination, astrocytic proliferation, or chronic active demyelination related to smoldering inflammation,[5] leading to slowly expanding lesions (or possibly a combination of all these mechanisms). Second, if disability progression in MS is the result of a slow, cumulative, and relatively constant rate of neurodegeneration, dynamic changes in concentrations of sNfL, which has a relatively short turnover and degradation time, may not be expected to be sensitive to this chronic pathology or standard clinical disability scores that are commonly used to capture this biology.[6] The relatively low and constant sNfL concentrations observed in this cohort throughout the study support all of these hypotheses.

This study has several limitations, including that it was restricted to participants with SPMS. The smaller sample size in the placebo population could have underpowered the analysis in this group. Furthermore, sNfL measurements were performed at only 3 times over 96 weeks, which prevented modeling the association of sNfL with longer-term progression.

Based on evidence from patients with SPMS without acute inflammation, sNfL did not appear to be a dynamic biomarker associated with disability progression in progressive MS. Our findings may have implications for the utility of sNfL measurements as surrogate biomarkers in the relatively short-duration setting of MS clinical trials; further work is required to explore their broader context of use when applied to progression independent of acute inflammation across all forms of MS.

ARTICLE INFORMATION

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**Author Contributions:** Dr Jiang had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

*Concept and design:* Gafson, Jiang, Shen, Kapoor, Zetterberg, Belachew.

*Acquisition, analysis, or interpretation of data:* All authors.

*Drafting of the manuscript:* Gafson, Jiang, Kapoor.

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*Statistical analysis:* Gafson, Jiang, Shen.

*Supervision:* Gafson, Shen, Belachew.

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## SUPPLEMENT.

**eMethods.**
**eReferences.**