Assessing Neurofilaments as Biomarkers of Neuroprotection in Progressive Multiple Sclerosis

From the MS-STAT Randomized Controlled Trial

Thomas E. Williams, BA, MB BChir, MRCP, Katherine P. Holdsworth, MSc, Jennifer M. Nicholas, PhD, Arman Eshaghi, PhD, Theodora Katsanouli, MSc, Henrietta Wellington, PhD, FRCP, Amanda Heslegrave, PhD, Henrik Zetterberg, PhD, Chris Frost, PhD, and Jeremy Chataway, PhD, FRCP

Neurol Neuroimmunol Neuroinflam 2022;9:e1130. doi:10.1212/NXI.0000000000001130

Correspondence
Dr. Williams
thomas.e.williams@ucl.ac.uk

Abstract

Background and Objectives
Improved biomarkers of neuroprotective treatment are needed in progressive multiple sclerosis (PMS) to facilitate more efficient phase 2 trial design. The MS-STAT randomized controlled trial supported the neuroprotective potential of high-dose simvastatin in secondary progressive MS (SPMS). Here, we analyze serum from the MS-STAT trial to assess the extent to which neurofilament light (NfL) and neurofilament heavy (NfH), both promising biomarkers of neuroaxonal injury, may act as biomarkers of simvastatin treatment in SPMS.

Methods
The MS-STAT trial randomized patients to 80 mg simvastatin or placebo. Serum was analyzed for NfL and NfH using Simoa technology. We used linear mixed models to investigate the treatment effects of simvastatin compared with placebo on NfL and NfH. Additional models examined the relationships between neurofilaments and MRI and clinical measures of disease severity.

Results
A total of 140 patients with SPMS were included. There was no evidence for a simvastatin treatment effect on NfL or NfH: compared with placebo, NfL was 1.2% lower (95% CI 10.6% lower to 9.2% higher; \( p = 0.820 \)) and NfH was 0.4% lower (95% CI 18.4% lower to 21.6% higher; \( p = 0.969 \)) in the simvastatin treatment group. Secondary analyses suggested that higher NfL was associated with greater subsequent whole brain atrophy, higher T2 lesion volume, and more new/enlarging T2 lesions in the previous 12 months, as well as greater physical disability. There were no significant associations between NfH and MRI or clinical variables.

Discussion
We found no evidence of a simvastatin treatment effect on serum neurofilaments. While confirmation of the neuroprotective benefits of simvastatin is awaited from the ongoing phase 3 study (NCT03387670), our results suggest that treatments capable of slowing the rate of whole brain atrophy in SPMS, such as simvastatin, may act via mechanisms largely independent of neuroaxonal injury, as quantified by NfL. This has important implications for the design of future phase 2 clinical trials in PMS.

Trial Registration Information
MS-STAT: NCT00647348.
Classified Evidence
This study provides class I evidence that simvastatin treatment does not have a large impact on either serum NfL or NfH, as quantified in this study, in SPMS.

Methods

MS-STAT Trial and Sample Processing
The MS-STAT study protocol has been outlined previously. Briefly, patients with SPMS, aged 18–65 years with Expanded Disability Status Scale (EDSS) score 4.0–6.5, were eligible. Key exclusion criteria included primary progressive MS; relapse or steroid use within 3 months; and the use of immunosuppressive or disease-modifying therapy within the last 6 months. A total of 140 patients were randomized, 1:1, to simvastatin 80 mg or placebo. Baseline characteristics are shown in Table 1. Blood samples were acquired at baseline and months 6, 12, and 24. Serum was separated and stored at −80°C until time of analysis.

Neurofilament Quantification
NfL and NfH are components of a neurone-specific intermediate filament, differing in their C-terminal domain and phosphorylation state. Both are released following neuroaxonal injury into the CSF and blood, where they may be quantified. Serum NfL and NfH were measured by Simoa technology on a HD-1 analyzer, according to the manufacturer’s instructions (Quanterix, Billerica, MA). The Simoa NF-Light Advantage and Simoa pNF-heavy Discovery Kits (Quanterix) were used. Briefly, serum samples were thawed at 21°C, vortexed, and centrifuged at 10,000 RCF for 5 minutes at 21°C. On-board the HD-1, samples were diluted 1:4 with sample diluent and bound to paramagnetic beads coated with a capture antibody specific for human NfL or NfH. Antibody-coated beads were incubated with a biotinylated anti-NfL or anti-NfH detection antibodies, that in turn were labeled with a streptavidin-β-galactosidase complex. Following the addition of the β-galactosidase substrate resorufoxin β-n-galactopyranoside, a fluorescent signal proportional to the concentration of neurofilament present in the sample was generated in the antigen-containing microwells of the Simoa plates.

Duplicate measurements were taken of each sample. Sample concentrations were extrapolated from a standard curve, fitted using a 4-parameter logistic algorithm. The lower limit of quantification (LLoQ) for NfL is 0.174 pg/mL and for NfH is 2.88 pg/mL. Values below the LLoQ were assigned the value of half the LLoQ. The coefficient of variation (CoV) between sample replicates tends to be higher for lower value results. To avoid bias, all data were therefore included in the primary statistical analysis regardless of the CoV. Each assay was run in the same or consecutive batches by the same operator, who was blinded to treatment allocation.

MRI Processing
The imaging data have been previously published and were acquired as previously described. Briefly, 3D T1-weighted, double-echo proton density, and T2-weighted MRI was obtained at baseline, month 12, and month 25. Whole brain
atrophy was determined using the boundary shift integral method and expressed as percentage change in whole brain volume. T2 new/enlarging lesions were expressed as a count and T2 lesion volume (T2LV) in milliliters.

Statistical Analysis
The prespecified primary analysis was to examine the effect of simvastatin (80 mg) vs placebo on levels of serum NfL at 24 months. The primary analysis was conducted on the intention to treat population regardless of treatment adherence. An exploratory analysis was undertaken to examine the treatment effect using a per-protocol data set, which included patients who complied with treatment and completed follow-up to 25 months. Participants were considered compliant with treatment if they reported taking, on average, at least 90% of their tablets at the protocol dose of 2 tablets per day.

The prespecified secondary analysis examined the relationship between serum NfL and whole brain atrophy rate. Further analyses of the association of NfL with other MRI and clinical variables and all analyses of NfH data were exploratory. Neurofilament data were skewed, and hence, all analyses were

| Table 1 Characteristics of the MS-STAT Trial Cohort and Descriptive Statistics for Serum NfL and Serum NfH Across Time Points |
|---------------------------------------------------------------|
| **Baseline characteristics** | **Placebo (n = 70)** | **Simvastatin (n = 70)** | **All (N = 140)** |
| Sex, n (%), female | 48 (69) | 49 (70) | 97 (69) |
| Ethnicity, n (%), White | 63 (90) | 69 (99) | 132 (94) |
| Relapse in last 24 mo, n (%) | 18 (26) | 8 (11) | 26 (19) |
| Age, y, mean (SD) | 51.1 (6.8) | 51.5 (7.0) | 51.3 (6.9) |
| MS duration, y, mean (SD) | 20.3 (8.8) | 22.1 (8.3) | 21.2 (8.6) |
| SPMS duration, y, mean (SD) | 7.1 (4.8) | 7.3 (5.6) | 7.2 (5.2) |
| EDSS score, median (IQR) | 6 (5.5–6.5) | 6 (5.5–6.5) | 6 (5.5–6.5) |
| Previous use of interferon, n (%) | 12 (17) | 10 (14) | 22 (16) |
| **Serum NfL, pg/mL** | | | |
| Baseline, median (IQR) | 15.3 (10.2–22.2) | 13.9 (10.9–19.7) | 14.6 (10.8–20.2) |
| N | 63 | 65 | 128 |
| Month 6, median (IQR) | 14.8 (12.0–23.0) | 15.0 (11.5–21.8) | 14.8 (11.7–22.6) |
| N | 53 | 59 | 112 |
| Month 12, median (IQR) | 16.6 (12.1–22.3) | 16.8 (13.3–23.1) | 16.7 (12.8–22.9) |
| N | 53 | 59 | 112 |
| Month 24, median (IQR) | 16.9 (12.4–22.6) | 16.0 (11.7–21.4) | 16.0 (11.9–22.2) |
| N | 48 | 64 | 112 |
| **Serum NfH, pg/mL** | | | |
| Baseline, median (IQR) | 64.2 (24.0–136.0) | 67.4 (23.9–116.0) | 65.5 (24.0–118.5) |
| N | 63 | 62 | 125 |
| Month 6, median (IQR) | 71.4 (25.7–135.4) | 58.0 (22.7–112.1) | 62.5 (22.7–121.9) |
| N | 49 | 53 | 102 |
| Month 12, median (IQR) | 66.5 (22.9–135.5) | 67.2 (25.2–107.1) | 67.0 (25.2–113.6) |
| N | 52 | 57 | 109 |
| Month 24, median (IQR) | 71.9 (26.6–111.8) | 60.0 (27.0–125.6) | 69.7 (27.0–119.5) |
| N | 48 | 64 | 112 |

Abbreviations: EDSS = Expanded Disability Status Scale; IQR = interquartile range; MS = multiple sclerosis; NfH = neurofilament heavy; NfL = neurofilament light; SPMS = secondary progressive MS.
Table 2 Effect of High-Dose Simvastatin on Serum NFL and Serum NFH

|                        | Difference in geometric mean (%) | 95% CI               | p Value |
|------------------------|----------------------------------|----------------------|---------|
| Serum NFL (pg/mL)      |                                  |                      |         |
| Month 6                | −3.29                            | −16.2 to 11.6        | 0.646   |
| Month 12               | 5.35                             | −7.4 to 19.9         | 0.429   |
| Month 24               | −5.21                            | −17.4 to 8.8         | 0.448   |
| Mean treatment effect  | −1.16                            | −10.6 to 9.2         | 0.820   |
| Serum NFH (pg/mL)      |                                  |                      |         |
| Month 6                | −7.22                            | −27.9 to 19.4        | 0.560   |
| Month 12               | 4.74                             | −15.1 to 29.3        | 0.666   |
| Month 24               | 1.69                             | −18.4 to 26.7        | 0.881   |
| Mean treatment effect  | −0.39                            | −18.4 to 21.6        | 0.969   |

Abbreviations: EDSS = Expanded Disability Status Scale; IQR = interquartile range; NFH = neurofilament heavy; NFL = neurofilament light. 
*Percentage difference in geometric mean serum neurofilaments, simvastatin vs placebo, adjusted for age, sex, dichotomized EDSS scores (4.0–5.5, 6.0–6.5) and study site. Differences are shown for each follow-up visit and also the average treatment effect across all follow-up visits.

performed following log2 transformation. Clinical data included EDSS scores, 25-foot timed walk (25FW) expressed as speed (inverse of completion time in seconds), and 9-hole peg test (9HPT) expressed as a speed (1,000 × inverse of completion time in seconds). Analyses were conducted in Stata 15.1 or later.

Analysis of Simvastatin Treatment Effect on NFL and NFH

A mixed effect model was used to estimate the simvastatin treatment effect on serum neurofilaments at 6, 12, and 24 months, for NFL and NFH. A single model was used to estimate a separate treatment effect at each visit (6, 12, and 24 months) by including a categorical variable for visit and an interaction between visit and treatment group. Baseline neurofilament data were included as an additional end point, but with the treatment effect here constrained to be 0. This is essentially equivalent to adjusting for the baseline neurofilament level.16 The model included an unstructured residual covariance matrix for the residuals (hence allowing a different variance at each visit and different covariances between each pair of measurements on the same participant). The following baseline variables, which were used as minimization factors in the randomization, were adjusted for by including them and their interactions with visit as fixed effects: age, sex, dichotomized EDSS scores (4.0–5.5, 6.0–6.5), and study site. The mean of the estimated treatment effects across all follow-up visits is also presented. In addition, an exploratory analysis was conducted which adjusted for the minimization factors in the randomization and the following baseline variables: T2LV and number of relapses in the previous 24 months. In these models, we did not perform adjustments for covariates measured after randomization as they may be influenced by the treatment allocation and hence introduce bias into the analysis of treatment effect.17

Analysis Relating NFL and NFH to MRI and Clinical Variables

This analysis used data on baseline neurofilament levels and the rates of change in neurofilament levels during the trial as predictors of MRI and clinical outcomes. A 2-stage analysis was performed.

In the first stage, a summary measure for rate of change in NFL and NFH for each participant was calculated as the slope from a simple linear regression model relating each participant’s repeated measures of log NFL and separately log NFH, to time from baseline using ordinary least squares.18 In the second stage, each participant’s estimated rate of change in log NFL or log NFH was used as a predictor variable, along with baseline-centered log NFL or log NFH, in a series of separate models for MRI and clinical outcomes. For each outcome, a separate model was fitted for log NFL and log NFH.

The model for whole brain atrophy was an extension of a previously described linear mixed model for directly measured change between each pair of MRI visits (baseline to 12, baseline to 25, and 12–25 months) as the outcome.19 It included participant-level random slopes for time between scans and random effects for visit. All predictor variables were included as interactions with time between scans in order to model the associations with atrophy rate. The model included baseline neurofilaments and change in neurofilaments, as well as the following baseline variables: age, sex, MRI site, SPMS duration, relapse in the previous 24 months, previous use of interferon, and baseline treatments for fatigue, depression, neuropathic pain, spasticity, and bladder urgency.

The model for T2LV was a linear mixed model with T2LV at each MRI visit (baseline, 12 months, and 25 months) as the outcome. Analysis of 25FW and 9HPT used a linear mixed model with speed at each visit (baseline, 12 months, and 24 months) as the outcome. These models included participant level random slopes for change over time and random intercepts, allowing for correlation between these random effects. In each model, baseline log neurofilament was included as a predictor, along with its interaction with time since baseline. Analysis of T2LV additionally included change in log neurofilament and its interaction with time. The models for T2LV included the same variables that were adjusted for in the whole brain atrophy model on their own as well as an interaction with time. The models for clinical variables included these same adjustments, with the exception of MRI site. As T2LV and clinical variables violated normality assumptions, inference from these models is based on nonparametric bias-corrected and accelerated 95% and 99% CIs calculated from 10,000 bootstrap replications clustered on participant. p
values are therefore not calculated for these models, but the ranges in which they lie can be inferred from the CIs.

For the EDSS score, the linear mixed model did not converge. Instead, a linear regression model for score at each visit (baseline, 12 months, and 24 months) was used. To allow for the nonindependence of measures from the same participant, nonparametric bias-corrected and accelerated CIs clustered on participant were used as described above. The models for the EDSS score included the same adjustment variables as for the other clinical outcomes.

Although T2LV may reflect overall disease burden, the identification of active lesions (either gadolinium-enhancing lesions on a single scan or new/enlarging T2 lesions when comparing 2 time points) is the key MRI measure of ongoing neuroinflammation. The MS-STAT cohort did not include gadolinium-enhanced imaging, and new/enlarging T2 lesions cannot be determined at baseline. To further explore the known relationship between serum NfL and neuroinflammation in this SPMS cohort, we therefore performed additional exploratory linear regression modeling using month 24 log NfL as the dependent variable. This allowed inclusion of recent active lesions (new/enlarging T2 lesions during month 0 to month 12 and month 12 to month 25) and concurrent T2LV (month 25) as predictors. Models were fitted including each of the MRI variables on their own and then together in a mutually adjusted multivariable model. These models included the same covariates as the T2LV models.

**Standard Protocol Approvals, Registrations, and Patient Consents**

The study was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. The MS-STAT protocol was approved by each study site’s institutional review board and a national ethics committee; all patients gave written informed consent before entering the study; and ethical approval for the retrospective analysis of serum samples was received. The MS-STAT clinical trial identification number is NCT00647348.

**Data Availability**

Anonymized NfL and NfH data are provided as a supplementary data file (links.lww.com/NXI/A679).

**Results**

**NfL and NfH Data**

Data on NfL were available from at least 1 visit for 138 patients (69 in each treatment group), with 128 patients having NfL data from at least 1 follow-up visit (61 placebo; 67 simvastatin). For NfH, data were available from 137 patients (69 placebo; 68 simvastatin), with 127 patients having NfH data from at least 1 follow-up visit (60 placebo; 67 simvastatin). No NfL measures were below the LLoQ, and all sample replicates had a CoV <20%. For NfH, 8 samples (1.8%) were below the LLoQ, and 39 samples (8.6%) had a CoV >20%. At baseline, median NfL was 14.6 pg/mL (IQR 10.8–20.2 pg/mL), and median NfH was 65.5 pg/mL (IQR 24.0–118.5 pg/mL) (Table 1). Characteristics of the per-protocol dataset are included in eTable 1 (links.lww.com/NXI/A679).

**Analysis of Simvastatin Treatment Effect on NfL and NfH**

There was no evidence of a simvastatin treatment effect on either NfL or NfH at any time point (Table 2), with adjusted marginal mean NfL and NfH levels being similar in the 2 treatment groups at each follow-up visit (Figure 1). Taking the mean of the treatment effects across all follow-up time points, the geometric mean NfL was 1.2% lower in the simvastatin group than in the placebo group (95% CI 10.6% lower to 9.2% higher; p = 0.820), whereas the geometric mean NfH was 0.4% lower in the simvastatin group than on placebo.
The results from the exploratory per-protocol analysis were similar to those found for the intention-to-treat analysis, with no evidence of a simvastatin treatment effect on either NfL or NfH at any time point (eTable 2, links.lww.com/NXI/A679). Sensitivity analyses found that the results were not materially altered following the exclusion of 2 individuals with outlying neurofilament values. In addition, an exploratory analysis found that results were essentially unchanged with adjustment for baseline lesion volume and relapses within the last 24 months (eTable 3, links.lww.com/NXI/A679).

### Association of NfL and NfH With MRI Variables

The relationships between both NfL and NfH and each of whole brain atrophy and T2LV are shown in Table 3 and Figure 2. There was evidence for an association between higher baseline NfL and faster whole brain atrophy rate and between higher baseline NfL and greater T2LV: a twofold increase in baseline NfL was associated with a 0.21%/year increase in brain atrophy and with a 7.7 mL higher baseline T2LV. The rate of change in NfL was not associated with the rate of change in T2LV or the rate of brain atrophy. Patients with a greater increase in NfL from baseline to month 24, however, tended to have a higher T2LV at baseline and follow-up time points. As there was little effect of change in NfL on T2LV, the effect was similar across all visits: an increase of 1 extra doubling per year in NfL was associated with a 15.1 mL higher baseline T2LV and 15.9 mL higher month 25 T2LV. There was no evidence for an association between NfH and any MRI variables (Table 3; Figure 2).

### Table 3 Relationship Between Serum NfL, NfH, and Imaging Variables

| Parameter estimate | 95% CI | p Value |
|--------------------|-------|---------|
| **Whole brain atrophy** | | |
| Relationship with rate of whole brain atrophy (% per year) | | |
| **Baseline NfL (per doubling)** | 0.207 | 0.072 to 0.341 | 0.003 |
| **Rate of change in NfL (doublings per year*)** | 0.093 | −0.201 to 0.387 | 0.535 |
| **Baseline NfH (per doubling)** | 0.048 | −0.005 to 0.102 | 0.078 |
| **Rate of change in NfH (doublings per year*)** | 0.016 | −0.105 to 0.136 | 0.795 |
| **T2 lesion volume** | | |
| Relationship with baseline T2 lesion volume (mL) | | |
| **Baseline NfL (per doubling)** | 7.68 | 3.66 to 12.48 | <0.01 |
| **Rate of change in NfL (doublings per year*)** | 15.08 | 6.67 to 26.40 | <0.01 |
| **Baseline NfH (per doubling)** | 0.736 | −0.921 to 2.631 | >0.05 |
| **Rate of change in NfH (doublings per year*)** | 0.413 | −6.696 to 8.001 | >0.05 |
| Relationship with change in T2 lesion volume (mL/y) | | |
| **Baseline NfL (per doubling)** | 0.29 | −0.14 to 0.63 | >0.05 |
| **Rate of change in NfL (doublings per year*)** | 0.39 | −0.60 to 1.02 | >0.05 |
| **Baseline NfH (per doubling)** | 0.070 | −0.218 to 0.051 | >0.05 |
| **Rate of change in NfH (doublings per year*)** | 0.032 | −0.535 to 0.541 | >0.05 |

Abbreviations: NfH = neurofilament heavy; NfL = neurofilament light. The results of 4 separate models are presented, with whole brain atrophy or T2 lesion volume as the dependent variables and NfL or NfH data as the predictor variables. In all analyses, neurofilament data were log2 transformed. Results are from covariate adjusted models as indicated in the Methods section. For T2 lesion volume, the p value bounds (<0.05 and >0.01) can only be inferred from the 95% and 99% bias-corrected and accelerated cluster bootstrap (10,000 replications) CIs.

*A 1-unit increase in the number of doublings per year corresponds to a change from stable levels to a doubling per year or from doubling once every year to doubling every 6 months (2 doublings per year).*
predictors in the same mutually adjusted model, both con-
current T2LV and month 12–25 new/enlarging T2 lesions
remained independently associated with month 24 NfL,
whereas the association with new and enlarging lesions be-
tween baseline and month 12 was lost. In the final model,
month 24 NfL was increased by 5.7% for each new/enlarging
T2 lesion between months 12 and 25 and by 0.89% for each
milliliter increase in month 25 T2LV.

**Association of NfL and NfH With
Clinical Variables**

Higher baseline NfL was significantly associated with
higher baseline EDSS score, but not with the rate of change
in the EDSS score from baseline to 2 years (Table 5). Higher
baseline NfL was also associated with worse baseline
9HPT performance and with a greater rate of wors-
ening in the 25FW speed from baseline to 2 years. Baseline
NfH was not materially associated with any clinical vari-
able. Sensitivity analyses demonstrated that the results
were not materially changed following the exclusion of 2
neurofilament outliers.

**Classification of Evidence**

This study assessed the ability of serum NfL and NfH to act as
biomarkers of a neuroprotective treatment response with high-
dose simvastatin, compared with placebo, in patients with
SPMS. It provides Class I evidence that these biomarkers, as
quantified in this study, do not act as biomarkers of neuro-
protection with simvastatin.

**Discussion**

Our results demonstrate that despite simvastatin reducing the
annualized whole brain atrophy rate by 43% per year, compared
with placebo, we did not find evidence to support a simvastatin
treatment effect on serum NfL or NfH. We also replicate
previously observed findings, demonstrating that higher NfL is
associated with a greater subsequent rate of whole brain atro-
phy and that recent inflammatory activity (new/enlarging T2
lesions), as well as T2LV, is associated with higher NfL.

The existing literature suggests that in MS, the degree of
neuroaxonal injury reflected by serum NfL is predominantly

---

**Figure 2** Relationship Between Each of Baseline Serum NfL and NfH and Each of Whole Brain Atrophy Rate and Baseline T2 Lesion Volume

Points represent individual patient data. Whole brain atrophy rate is reported as yearly % change from baseline to month 25 and baseline T2 lesion volume in
milliliters. (A) Baseline NfL and baseline to month 25 whole brain atrophy rate. (B) Baseline NfH and baseline to month 25 whole brain atrophy rate. (C)
Baseline NfL and baseline T2 lesion volume. (D) Baseline NfH and baseline T2 lesion volume. NfH = neurofilament heavy; NfL = neurofilament light.
related to ongoing neuroinflammation. Such neuroinflammation may be detected by conventional MRI measures, such as recent T1 gadolinium-enhancing lesions or new T2 lesions, or by advanced MRI measures of chronic neuroinflammation, such as the identification of chronic active lesions via their paramagnetic rims.5,23 Our previous findings suggested that simvastatin treatment was not systemically immunomodulating in this cohort, hence providing 1 possible rational for the absence of a treatment effect on NfL.21

Many of the pathophysiologic mechanisms contributing to progressive MS ultimately converge on neuroaxonal damage, which may be reflected by increased NfL.23 The dissociation between the previously observed benefits of simvastatin (on whole brain atrophy and disability measures) and the absence of a treatment effect on NfL, however, does also highlight the potential importance of additional processes, independent of neuroaxonal injury, in the pathophysiology of SPMS. Comorbidities, particularly cardiovascular, are prevalent within the MS population and are known to have an impact on future disability and brain atrophy.24-26 MRI measures of brain atrophy have been validated against clinical treatment effects, long-term disability outcomes, and measures of neuroaxonal loss.27-30 Brain atrophy is, however, not specific to neuroaxonal injury and may be influenced by volume changes in other CNS tissue compartments. The mechanism of action of simvastatin in progressive MS is the subject of an ongoing mechanistic vascular perfusion study (OPT-MS, NCT03896217), and the ultimate confirmation of the efficacy of simvastatin in SPMS awaits the results of the ongoing phase 3 MS-STAT2 trial (NCT03387670). Our data therefore suggest that caution is required when considering NfL as an outcome measure for treatments in progressive MS if the mechanism of action is not known to directly affect neuroaxonal injury, such as with simvastatin.

Although our results are not necessarily generalizable to other neuroprotective treatments in PMS, they are supported by data from ibudilast. The SPRINT-MS study demonstrated a significant 48% reduction in the rate of brain atrophy in PMS with ibudilast compared with placebo.31 There was, however, no significant difference between the treatment groups in either serum or CSF NfL.9 Although ibudilast is likely to have pleotropic effects (such as modulation of CNS innate immunity through inhibition of phosphodiesterases, macrophage migration inhibitory factor, and Toll-like receptor 4), like simvastatin, it is not thought to be systemically immunomodulatory.52

NfL has shown utility as a biomarker of treatment with fingolimod, siponimod, natalizumab, and ocrelizumab in PMS cohorts.6-8 These treatments all share a predominantly immunomodulatory mechanism of action, and their ability to reduce NfL is therefore entirely in keeping with the known association between NfL, neuroaxonal injury, and markers of inflammatory activity in PMS.5 Supporting this, subgroup analyses suggest that there may be a greater treatment effect of natalizumab, siponimod, and ocrelizumab on NfL in patients with recent inflammatory activity.5-8

The estimated treatment effects of simvastatin on NfL and NIH were small and not statistically significant, with the 95% CI sufficiently narrow to exclude an important treatment effect in either direction. Exploratory analyses did find that the month 24 serum NfL level increased by 6% (95% CI 2% to 9%) for each new/enlarging T2 lesion in the preceding year and by 0.9% (95% CI 0.3% to 1.5%) for each milliliter increase in concurrent T2LV, further supporting the known relationship between NfL and neuroinflammation. The key question of this study, however, was to determine the extent to which serum neurofilaments may act as biomarkers of a neuroprotective treatment that does not appear to have direct effects on neuroinflammation, using simvastatin as our example. Indeed, NfL has shown utility as a biomarker of nonimmunomodulatory neurodegeneration and neuroprotection in other neurologic conditions.33,34 Our results, however, together with those from SPRINT-MS, suggest that either these treatments produce benefits on the rate of whole brain atrophy by mechanisms independent of neuroaxonal injury or that the degree of neuroprotection induced is insufficient to produce material

---

### Table 4 MRI Predictors of Month 24 Serum NfL

| Predictor variable | Separate model for each T2 lesion variable | Combined, mutually adjusted model |
|-------------------|--------------------------------------------|----------------------------------|
|                   | % Increase in 24 mo NfL per unit increase in predictor | % Increase in 24 mo NfL per unit increase in predictor |
|                   | 95% CI | p Value | 95% CI | p Value |
| Month 0–12 T2 new/enlarging lesions (count) | 3.23 | 0.29 to 6.26 | 0.031 | −0.15 | −3.08 to 2.86 | 0.919 |
| Month 12–25 T2 new/enlarging lesions (count) | 6.76 | 3.45 to 10.18 | <0.001 | 5.73 | 2.40 to 9.17 | 0.001 |
| Month 25 T2 lesion volume (mL) | 1.10 | 0.51 to 1.67 | <0.001 | 0.89 | 0.29 to 1.49 | 0.004 |

Abbreviation: NfL = neurofilament light.

Results are presented from the 3 separate models, and then from the combined linear regression model, with month 24 NfL out the outcome and T2 lesion variables as predictors. Results are from covariate adjusted models as indicated in the Methods section. As previously, NfL was log2 transformed. T2 new/enlarging lesions are reported as a count and T2 lesion volume in milliliters. Coefficients are expressed as % increase in 24 month NfL per unit increase in T2 lesion variables.

---

Neurology: Neuroimmunology & Neuroinflammation | Volume 9, Number 2 | March 2022 Neurology.org/NN
changes in serum NfL. We speculate that the latter may be due to the association between neuroinflammation and NfL persisting independent of such neuroprotective treatment. Future work should focus on replicating this NfL analysis in samples from the ongoing phase 3 MS-STAT2 clinical trial, once the effects of simvastatin on clinical disability progression are confirmed, and also on developing novel CNS biomarkers capable of capturing neuroprotective treatment effects independent of neuroinflammation.

In 2 studies assessing the neuroprotective potential of sodium channel blockade (with phenytoin in acute optic neuritis and lamotrigine in SPMS), NfH has shown promise as a biomarker of neuroprotective treatment.11,12 Our data, however, found no evidence of a simvastatin treatment effect on NfH or any associations of NfH with MRI or clinical measures of disease severity. Although previous studies have shown strong and consistent correlations between serum and CSF NfL,35 inconsistent results have been found for correlation between

| Table 5 Relationship Between Baseline Serum NfL and Serum NfH and Clinical Variables |
|-----------------------------------------------|----------|----------------|
| Parameter estimate | 95% CI | p Value |
| **EDSS score** |
| Relationship with baseline EDSS score |
| Predictor variable |
| Baseline NfL (per doubling) | 0.284 | 0.096 to 0.500 | <0.01 |
| Baseline NfH (per doubling) | -0.030 | -0.109 to 0.058 | >0.05 |
| Relationship with change in the EDSS score (units per year) |
| Predictor variable |
| Baseline NfL (per doubling) | 0.026 | -0.052 to 0.112 | >0.05 |
| Baseline NfH (per doubling) | 0.002 | -0.030 to 0.036 | >0.05 |
| **25FW** |
| Relationship with baseline 25FW (1/s) |
| Predictor variable |
| Baseline NfL (per doubling) | -0.159 | -0.390 to 0.056 | >0.05 |
| Baseline NfH (per doubling) | 0.055 | -0.044 to 0.147 | >0.05 |
| Relationship with change in 25FW (1/s per year) |
| Predictor variable |
| Baseline NfL (per doubling) | -0.183 | -0.312 to -0.055 | <0.01 |
| Baseline NfH (per doubling) | 0.028 | -0.041 to 0.081 | >0.05 |
| **9HPT** |
| Relationship with baseline 9HPT (1,000/s) |
| Predictor variable |
| Baseline NfL (per doubling) | -3.600 | -5.488 to -1.629 | <0.01 |
| Baseline NfH (per doubling) | -0.113 | -1.267 to 1.011 | >0.05 |
| Relationship with change in 9HPT (1,000/s per year) |
| Predictor variable |
| Baseline NfL (per doubling) | -0.046 | -0.904 to 0.858 | >0.05 |
| Baseline NfH (per doubling) | -0.168 | -0.524 to 0.162 | >0.05 |

Abbreviations: 9HPT = 9-hole peg test; 25FW = timed 25-foot walk; EDSS = Expanded Disability Status Scale; NfH = neurofilament heavy; NfL = neurofilament light.

The results of 6 separate models are presented, with EDSS, 25FW, or 9HPT as the dependent variables and NfL or NfH data as the predictor variables. EDSS score is reported as the score; both 9HPT and 25FW are reported as a speed (9HPT as 1,000 × s⁻¹ and 25FW as s⁻¹). In all analyses, neurofilament data were log₂ transformed. Results are from covariate-adjusted models as indicated in the Methods section. p Value bounds (<0.05 and <0.01) can only be inferred from the 95% and 99% bias-corrected and accelerated cluster bootstrap (10,000 replications) CIs.
serum and CSF NfH. This suggests that NfH instability between CSF and serum compartments may have limited its potential as a biomarker in our cohort. The Quanterix Simoa NF-Light Advantage assay has been widely used and validated against clinical and MRI outcomes. The Simoa pNF-heavy Discovery assay, however, has been less widely used. One study has used this assay to demonstrate modest associations between serum NfH and T2LV in a mixed MS cohort. Although Simoa digital ELISA platforms tend to improve sensitivity and accuracy over traditional ELISA techniques, the limited data from this NfH assay therefore suggest that the NfH data should be interpreted with caution.

In conclusion, our results show that despite simvastatin treatment being associated with a significant reduction in whole brain atrophy and benefits in secondary outcomes, our results are most compatible with no important effect on serum NfL or NfH in SPMS. Although higher NfL is associated with greater disease severity and faster progression, our results, together with those from ibudilast, suggest that candidate nonimmunomodulatory neuroprotective treatments in PMS may act via mechanisms independent of the main determinants of serum neurofilament concentrations. While confirmation of the neuroprotective efficacy of simvastatin in SPMS is awaited from the ongoing phase 3 study, our results, together with those of others, suggest that the utility of serum neurofilaments as biomarkers of treatment response in progressive MS may be limited to interventions that are either known to suppress acute or chronic neuroinflammatory activity or to otherwise directly affect neuroaxonal injury.

Study Funding
No targeted funding reported.

Disclosure
T.E. Williams has received honorarium for educational talks from Novartis and Merck. K.P. Holdsworth, J.M. Nicholas, A. Eshaghi, T. Katsanouli, H. Wellington, and A. Heslegrave report no disclosures relevant to the manuscript. H. Zetterberg has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pintec Therapeutics, Nervgen, AZTherapies, and CogRx; has given lectures in symposia sponsored by Cellecricton, Fuijerebio, Alzecure, and Biogen; and is a cofounder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. C. Frost reports no disclosures. J. Chataway has received support from the Efficacy and Mechanism Evaluation Programme and Health Technology Assessment Programme (NIHR); UK Multiple Sclerosis Society; the National Multiple Sclerosis Society; and the Rosetrees Trust. He is supported in part by the National Institute for Health Research, University College London Hospitals, Biomedical Research Centre, London, UK. He has been a local principal investigator for a trial in MS funded by the Canadian MS society; a local principal investigator for commercial trials funded by: Actelion, Biogen, Novartis and Roche; has received an investigator grant from Novartis; and has taken part in advisory boards/consultancy for Azadyne, Biogen, Celgene, Janssen, MedDay, Merck, NervGen, Novartis and Roche. Go to Neurology.org/NN for full disclosures.

Publication History
Received by Neurology: Neuroimmunology & Neuroinflammation June 16, 2021. Accepted in final form November 23, 2021.

Appendix Authors

| Name                  | Location                                      | Contribution                                                                 |
|-----------------------|-----------------------------------------------|------------------------------------------------------------------------------|
| Thomas E. Williams, BA MB BChir, MRCP | Queen Square Multiple Sclerosis Centre, UCL | Acquisition of patient samples, neurofilament laboratory assay, data analysis and interpretation, and drafting of the manuscript and revisions |
| Katherine P. Holdsworth, MSc | London School of Hygiene and Tropical Medicine, United Kingdom | Data analysis and interpretation and manuscript revisions                        |
| Jennifer M. Nicholas, PhD | London School of Hygiene and Tropical Medicine, United Kingdom | Data analysis and interpretation and manuscript revisions                        |
| Arman Eshaghi, PhD | Queen Square Multiple Sclerosis Centre, UCL | MRI analysis and manuscript revisions                                          |
| Theodora Katsanouli, MSc | London School of Hygiene and Tropical Medicine, United Kingdom | Data analysis and interpretation and manuscript revisions                        |
| Henrietta Wellington, PhD, FRCP | UK Dementia Research Institute at UCL | Supervision of neurofilament laboratory analysis and manuscript revisions |
| Amanda Heslegrave, PhD | UK Dementia Research Institute at UCL | Supervision of neurofilament laboratory analysis and manuscript revisions |
| Henrik Zetterberg, PhD | UK Dementia Research Institute at UCL | Oversight of neurofilament laboratory analysis, data analysis and interpretation, and manuscript revisions |
| Chris Frost, PhD | London School of Hygiene and Tropical Medicine, United Kingdom | Supervision of data analysis and interpretation and manuscript revisions |
| Jeremy Chataway, PhD, FRCP | Queen Square Multiple Sclerosis Centre, UCL | Chief investigator of the MS-STAT clinical trial, study design and conception, data interpretation, and manuscript revisions |

References
1. Lublin FD, Reingold SC, Cohen JA, et al. Defining the clinical course of multiple sclerosis. Neurology. 2014;83(3):278-286.
2. Chataway J, Schuerer N, Alsanousi A, et al. Effect of high-dose simvastatin on brain atrophy and disability in secondary progressive multiple sclerosis (MS-STAT): a randomised, placebo-controlled, phase 2 trial. Lancet. 2014; 383(9936):2213-2221.
3. Chan D, Binks S, Nicholas JM, et al. Effect of high-dose simvastatin on cognitive, neuropsychiatric, and health-related quality of life measures in secondary progressive multiple sclerosis: secondary analyses from the MS-STAT randomised, placebo-controlled trial. Lancet Neurol. 2017;16(8):591-600.
Assessing Neurofilaments as Biomarkers of Neuroprotection in Progressive Multiple Sclerosis: From the MS-STAT Randomized Controlled Trial
Thomas E. Williams, Katherine P. Holdsworth, Jennifer M. Nicholas, et al.

Neurol Neuroimmunol Neuroinflamm 2022;9;
DOI 10.1212/NXI.0000000000001130

This information is current as of January 14, 2022
### Updated Information & Services
including high resolution figures, can be found at:
http://nn.neurology.org/content/9/2/e1130.full.html

### References
This article cites 32 articles, 2 of which you can access for free at:
http://nn.neurology.org/content/9/2/e1130.full.html##ref-list-1

### Citations
This article has been cited by 1 HighWire-hosted articles:
http://nn.neurology.org/content/9/2/e1130.full.html##otherarticles

### Subspecialty Collections
This article, along with others on similar topics, appears in the following collection(s):
- **Class I**
  http://nn.neurology.org//cgi/collection/class_1
- **Clinical trials Randomized controlled (CONSORT agreement)**
  http://nn.neurology.org//cgi/collection/clinical_trials_randomized_controlled_consort_agreement
- **Multiple sclerosis**
  http://nn.neurology.org//cgi/collection/multiple_sclerosis

### Permissions & Licensing
Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at:
http://nn.neurology.org/misc/about.xhtml#permissions

### Reprints
Information about ordering reprints can be found online:
http://nn.neurology.org/misc/addir.xhtml#reprintsus