Association of serum neurofilament light levels and disease progression in patients with relapsing remitting multiple sclerosis treated with natalizumab

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ABSTRACT

Objective

The objective of this study was to investigate the potential of serum neurofilament light (NfL) to reflect or predict progression mostly independent of acute inflammatory disease activity in patients with relapsing remitting multiple sclerosis (RRMS) treated with natalizumab.

Methods

Patients were selected from a prospective observational cohort study initiated in 2006 at the VU University Medical Center Amsterdam, The Netherlands, including patients with RRMS treated with natalizumab. Selection criteria included an age of 18 years or older and a minimum follow-up of 3 years from natalizumab initiation. Clinical and MRI assessments were performed on a yearly basis, and serum NfL was measured at 5 time-points during the follow-up, including on the day of natalizumab initiation (baseline), 3 months, 1 year and 2 years after natalizumab initiation, and on last follow-up visit. Using general linear regression models, we compared the longitudinal dynamics of NfL between patients with and without confirmed EDSS progression between year 1 visit and last follow-up, and between individuals with and without EDSS[+] progression, a composite endpoint including the EDSS, 9 hole peg test and timed 25 foot-walk.

Results

Eighty-nine natalizumab-treated patients with RRMS were included. Median follow-up time was 5.2 years (IQR 4.3-6.7, range 3.0-11.0) after natalizumab initiation, mean age at time of natalizumab initiation was 36.9 (SD: 8.5), and median disease duration was 7.4 years (IQR 3.8-12.1). Between year 1 and the last follow-up, 28/89 (31.5%) individuals showed confirmed EDSS progression. Data for the EDSS[+] endpoint was available for 73 out of the 89 patients and 35/73 (47.9%) showed confirmed EDSS[+] progression. We observed a significant reduction in NfL levels 3 months after natalizumab initiation,

which reached its nadir of close to 50% of baseline levels 1 year after treatment initiation. We found

no difference in the longitudinal dynamics of NfL in progressors versus non-progressors. NfL levels at

baseline and 1 year after natalizumab initiation did not predict progression at last follow-up.

Discussion

In our cohort of natalizumab-treated patients with RRMS, NfL fails to capture or predict progression

that occurs largely independently of clinical or radiological signs of acute focal inflammatory disease

activity. Additional biomarkers may thus be needed to monitor progression in these patients.

Classification of Evidence

This study provides Class II evidence that serum NfL levels are not associated with disease progression
in natalizumab-treated patients with RRMS.

ABBREVIATIONS

Multiple Sclerosis = MS

Relapsing remitting MS = RRMS

Secondary progressive MS = SPMS

Neurofilament light = NfL

Magnetic resonance imaging = MRI

Expanded Disability Status Scale = EDSS

Gadolinium enhancing lesions = GE lesions

INTRODUCTION

Multiple Sclerosis (MS) is a chronic inflammatory and degenerative disease of the central nervous system. After 10 to 15 years of disease evolution, progressive irreversible disability accumulates in a majority of patients largely independently of acute focal inflammatory disease activity, which includes relapses and new T2 or gadolinium enhancing (GE) MRI lesions. With the advent of highly effective disease modifying therapies (DMT) to treat relapsing-remitting multiple sclerosis (RRMS), acute focal inflammatory disease activity can be silenced in a significant majority of these patients.[1] Once considered a characteristic of secondary progressive MS (SPMS), evidence now indicates disability accrual can occur from disease onset, independently of acute focal inflammatory disease activity.[2,3] The uncoupling of these processes suggests the mechanisms underlying progression are, at least partly, independent of those causing relapse-related neuro-axonal damage. Treatments that significantly reduce the rate of disability progression are scarce yet critically needed, as progression contributes significantly to long-term disability. In order to evaluate the potential of novel therapies to reduce progression rate, biomarkers to quantify and/or predict this process are needed. Neurofilament light (NfL) is a biomarker of neuro-axonal damage.[4] Its levels increase in serum of patients with RRMS during relapses and concomitantly to the appearance of new T2 and/or GE lesions, returning to baseline within a couple of months of the acute event, and decrease following DMT initiation.[5] These data suggest NfL is a promising tool to monitor acute focal inflammatory disease activity in MS. In cross-sectional studies, NfL is associated with measures of disease severity such as the Expanded disability severity score (EDSS), and in longitudinal studies, high baseline NfL predicts EDSS worsening in the following year, or up to 15 years later in patients with clinically isolated syndrome.[6,7,8] These data suggest NfL holds potential for prediction of short- and long-term neurological disability.

We hypothesized that NfL levels increase over time in patients with disability progression, and can be used to monitor and predict progression that occurs largely independently of acute focal inflammatory disease activity. We tested this hypothesis by comparing the longitudinal trajectories of NfL in

natalizumab-treated patients with RRMS that either progressed or not over a period of at least 3 years.

We then evaluated the potential of NfL at time of natalizumab initiation or 1 year after treatment

initiation to predict progression during follow-up.

METHODS

Cohort

Patients were selected from the natalizumab pharmacovigilance study, an ongoing prospective observational cohort study initiated in 2006 at the VU University Medical Center Amsterdam, The Netherlands.[9] Selection criteria for the present study were the following: (1) an age of 18 years or older, and (2) a minimum follow-up of 3 years from natalizumab initiation. Clinical assessments were performed at initiation of natalizumab (baseline) and repeated every 12 months, and included relapse history, Expanded Disability Status Scale (EDSS) assessment by trained personnel, timed 25-foot walk test (T25W) and 9-hole peg test (9HPT) (Figure 1). The cohort was retrospectively divided into progressors and non-progressors, according to 2 outcomes: either the EDSS alone, or the EDSS+, a composite endpoint including the EDSS, the 9-HPT and the T25FW.[10] EDSS progression was assessed by comparing EDSS at last follow-up with EDSS at year 1. Year 1 and not baseline was used as a reference EDSS in order to reduce the impact of focal inflammatory disease activity occurring prior to natalizumab initiation, which may potentially affect EDSS at baseline. EDSS progressors (EP) were defined as having a sustained EDSS increase at both the last follow-up and the penultimate EDSS assessment, compared to year 1 EDSS, fulfilling the criteria of confirmed EDSS progression. The increase was at least 1.5 (if reference EDSS score=0), 1 (if reference EDSS score= 1 to 5.5), or 0.5 (if reference EDSS ≥ 6.0) (Figure 1). EDSS non-progressors (ENP) were defined as individuals not fulfilling the criteria of EP. EDSS+ progressors (E+P) were defined as having progression in one of the 3 components (EDSS, T25FW, and/or 9HPT), with a worsening of ≥20% in the T25FW or the 9-HPT at last follow-up, confirmed at the penultimate T25FW and 9-HPT assessment, or in the EDSS as outlined above. EDSS+ non-progressors (E+NP) were individuals not fulfilling the criteria for E+P. All patients gave written informed consent for the collection and use of medical data and biological fluids for research purposes. This study was in accordance with the ethical principles of the Declaration of Helsinki and received local ethics committee consent.

Serum NfL measurement

Blood was collected at baseline, after 3 months, 1 year, 2 years, and at last follow-up (Figure 1), i.e. the last available blood sample during natalizumab treatment before discontinuation or database closure via standard vena puncture, and centrifuged at 1800 *g* for 10 min at room temperature. Serum was aliquoted and stored at −80°C until analysis. NfL quantification was performed using an in-house developed Simoa assay.[11] The samples of each individual patient were analyzed within one run and the personnel performing the analyses was blinded for the clinical data.

Magnetic resonance imaging

MRI protocols included proton-density (PD)/T2-weighted and post-contrast T1-weighted images. Slice thickness was 3 mm with an in-plane resolution of 1mm2. Brain MRI scans were performed on a 1.5 Tesla or a 3.0 Tesla scanner in the VU University Medical Center Amsterdam. Image acquisition differed among patients (i.e. magnetic field strengths, pulse sequences, head coils and spatial resolution), which was taken into consideration by the raters in the radiological analyses. Nonetheless the MRI acquisition followed the Magnetic Resonance Imaging in Multiple Sclerosis (MAGNIMS) expert panel guidelines (Wattjes et al., 2015; Rovira et al., 2015). MRI scans were performed yearly and evaluated by experienced neuroradiologists for inflammatory activity, defined as new T2 lesions or GE (Figure 1).

Statistical analyses

Statistical data analysis was performed using SPSS for windows, version 22. Median comparisons were assessed using the Mann–Whitney U test. Proportion differences were assessed using the Chi-square test. Mean age differences were assessed using analysis of variance. To compare NfL levels between EP and ENP or and E⁺P and E⁺NP, age, gender, disease duration, relapse activity, and MRI disease activity-corrected univariate analyses of variance were performed on log-transformed NfL values. Binary logistic regression was used to identify predictors for clinical progression at last follow-up, with

EDSS progression or EDSS+ progression as dependent variables, and gender, age, disease duration, log transformed baseline and year 1 NfL as covariates. A p value<0.05 was considered statistically significant. The graphs in Figure 2 were constructed in GraphPad Prism version 7.02.

Standard Protocol Approvals, Registrations, and Patients Consents

This study received approval from the local ethics committee on human experimentation. All patients provided written informed consent.

Data availability statement

The raw data can be obtained upon reasonable request by contacting the corresponding author.

RESULTS

Patient characteristics

Eighty-nine natalizumab-treated patients with RRMS were selected, with a follow-up period of at least 3 years (median follow-up time of 5.2 years, IQR 4.3-6.7, range 3.0-11.0) after natalizumab initiation (Table 1). Data for the EDSS$^+$ endpoint was available for 73 out of the 89 patients (Table 1). Mean age of the entire cohort (n=89) at time of natalizumab treatment was 36.9 (SD: 8.5), and median disease duration at time of natalizumab initiation was 7.4 years (IQR 3.8-12.1) (Table 1). 14.6% patients had 1 relapse or more during the follow-up time excluding the first three months, and 10.1% patients had MRI disease activity during the follow-up time excluding the first year (Table 1). These numbers are in accordance with the high efficacy of natalizumab to prevent acute focal inflammatory disease activity.[12,13] Between year 1 and the last follow-up visit, 28/89 patients (31.5%) showed confirmed EDSS progression, and 35/73 (47.9%) showed confirmed EDSS$^+$ progression (Table 1). Accordingly, median EDSS at last follow-up was higher in EP versus ENP (5.8 (IQR 3.6-6.0) versus 3.5 (IQR 2.0-4.5), $p<10^{-5}$), and in E$^+$P (5.0 (3.5-6.0) versus E$^+$NP (3.5 (2.4-4.0), $p<10^{-5}$) (Table 1). At baseline, median age was higher in EP compared to ENP (40.0 versus 35.4, p=0.019), and in E$^+$P compared to E$^+$NP (39.5 versus 34.9, p=0.011) (Table 1). Median disease duration was longer in EP compared to ENP (8.2 (IQR 4.4-16.5), versus 6.9 (IQR 3.2-10.9), p=0.047), but not between E$^+$P and E$^+$NP (7.9 (IQR 4.3-15.7), versus 7.4 (4.4-11.9), p=0.480) (Table 1). The percentage of individuals with 1 relapse or more during the follow-up period excluding the first 3 months after natalizumab initiation was low and did not differ between EP and ENP, or between E$^+$P and E$^+$NP (Table 1). Similarly, the percentage of individuals with new T2/GE lesions during the follow-up period excluding the first year after natalizumab initiation did not differ significantly between EP and ENP, or between E$^+$P and E$^+$NP (Table 1).

Longitudinal dynamics of serum NfL levels after natalizumab treatment initiation

NfL was measured in serum sampled on the day of natalizumab initiation (baseline), 3 months, 1 year and 2 years after baseline, and on the last follow-up visit (Figure 1). Median NfL decreased significantly from 14.8 pg/ml at baseline to 11.1 pg/ml at 3 months, and reached its nadir of 7.9 pg/ml at year 1, remaining low thereafter (Table 1). Mean baseline and follow-up levels of NfL did not differ between EP and ENP (Table 1 and Figure 2A), and between E⁺P and E⁺NP (Table 1 and Figure 2B).

NfL as a predictor of future disability progression

NfL at baseline or at year 1 did not predict EDSS or EDSS⁺ progression at last follow-up visit, neither did gender, age at natalizumab onset, and disease duration (data not shown).

Sensitivity analysis

In this study, the follow-up time was heterogeneous, and patients with a longer follow-up period had a higher chance to progress than those with shorter follow-up periods, thereby introducing a possible classification bias. In order to assess the robustness of our findings, we performed a sensitivity analysis including only those patients who were followed for the same time period of 4 years. Confirmed EDSS and EDSS⁺ progression were assessed between year 1 and year 4 for all patients. We obtained results similar to those of the primary analysis, ie no difference in the longitudinal NfL dynamics between progressors and non-progressors (Table 2).

DISCUSSION

Highly effective therapies such as natalizumab have dramatically changed the short and possibly long-term neurological prognosis of MS patients.[14] These therapeutic breakthroughs have also revealed that disability worsening can occur in treated patients with RRMS, even in the absence of clinical and MRI signs of focal inflammatory disease activity.[3] While the evidence supporting serum NfL as a biomarker of neuro-axonal damage arising in the context of acute inflammatory disease activity is unequivoqual, its potential to capture disability progression is less clear.[15,8]

In this study, we take advantage of a cohort of natalizumab-treated patients with RRMS to study progression largely independent of acute focal inflammation, and how it reflects on serum NfL levels. We find that clinical and radiological acute inflammatory disease activity is abrogated in a majority of patients, in accordance with the high efficacy of this drug reported in clinical trials.[12,13] About 30% of the patients show confirmed EDSS progression during the follow-up time of the study, and about 45% confirmed EDSS+ progression. None of the patients fulfilled the criteria for transition towards secondary progressive MS during the follow-up period under natalizumab treatment.[16]

The percentage of individuals with relapses or new T2/GE lesions did not differ significantly between progressors and non-progressors, although small differences between the groups may have been missed due to the relative small size of the cohort. This supports the hypothesis that the mechanisms driving progression are distinct from those underlying acute focal inflammatory disease activity. We find that individuals who progressed either according to the confirmed EDSS or the confirmed EDSS+ outcome were slightly older and their disease duration at baseline was slightly longer compared to those who did not, suggesting an age and disease duration threshold before progression becomes clinically manifest.

We observe a reduction in NfL levels of almost 50% of baseline levels 1 year after natalizumab initiation, in accordance with other studies.[17,18] Further, we find that NfL remains low for the entire follow-up period under natalizumab treatment. We observe no differences in the longitudinal

dynamics of NfL levels between EP and ENP, and between E⁺P and E⁺NP, correcting for age, gender, disease duration, relapses, and MRI signs of acute focal inflammatory disease activity. Although the cohort size is relatively limited, the absence of even a trend towards significance suggests NfL does not capture progression occurring largely independently of relapse or MRI activity in natalizumab-treated patients.

Median follow-up time was slightly longer in EP and E⁺P compared to ENP and E⁺NP, and in order to evaluate the effect of a possible classification bias, we performed a sensitivity analysis with a fixed follow-up time of 4 years. We found similar results, suggesting the heterogeneity in follow-up periods does not introduce a large bias, although the cohort investigated in the sensitivity analysis was smaller than the initial cohort.

Few studies have investigated the potential of NfL to reflect disease progression or neurodegeneration in MS. Ibudilast, a molecule currently investigated as a treatment to slow progression in MS, is associated with a dose-dependent reduction in whole brain atrophy progression in patients with progressive MS.[19] In a recent study, it was reported that this reduction in brain atrophy is not reflected in NfL levels, as serum NfL levels did not differ between individuals with or without brain atrophy progression.[20] These data suggest NfL may not capture neurodegeneration, which is thought to underlie disability progression. However, in a phase 3 randomized controlled trial of natalizumab in SPMS, NfL levels at week 96 were higher in E⁺P versus E⁺NP.[21] In this poster, it is however not reported whether E⁺P and E⁺NP differed in terms of acute inflammatory disease activity, which may account, at least partially, for the differences in NfL levels.

NfL levels increase most substantially in neurological conditions characterized by a high rate of neuroaxonal loss, such as amyotrophic lateral sclerosis and stroke, while in conditions characterized by a lower yet sustained rate of neuroaxonal loss such as Alzheimer's disease, the increase in NfL levels is more subtle.[4] We may thus hypothesize that while a powerful tool to capture the massive increase in acute neuroaxonal damage that occurs over the relatively short time period of a relapse, NfL

probably lacks the sensitivity to reflect the lower rate of sustained neurodegenerative axonal damage that underlies progression in RRMS.

Our data do not support a prognostic value for baseline or year 1 NfL in terms of EDSS or EDSS[+] progression prediction at last follow-up, when focal acute inflammatory disease activity is largely suppressed. This finding suggests the prognostic value of NfL reported in other studies may rather be related to its ability to reflect acute neuroaxonal damage due to focal inflammatory disease activity than progression.[7,22–26.]

A limitation of our study is the use of EDSS worsening as clinical outcome measure of disability progression. Despite being the most widely used outcome measure for disability progression in MS, this metric has several limitations. First, it is based on neurological examination, which is intrinsically subjective, and EDSS scoring has been reported to have high intra- and inter-rater variability.[27] We mitigated measurement variability by having EDSS assessments made exclusively by trained medical personnel. Second, EDSS worsening occurs not only in the context of progression, but also transiently in the context of a relapse. To reduce the contribution of relapses to EDSS worsening, we used confirmed EDSS as an outcome. Confirmation of the EDSS was obtained at least one year apart, in order to reduce the likelihood of capturing events that would subsequently regress. Third, the EDSS may lack sensitivity to capture progression, especially in individuals with higher baseline EDSS score. To increase the sensitivity for identifying progression in SPMS, the EDSS[+] endpoint was developed, which includes measures of short-distance ambulatory function (T25W), and upper-extremity function (9HPT).[10] The EDSS[+] was reported to be more sensitive than the EDSS to detect progression in SPMS. [10] Although not validated as a measure of progression in RRMS, we reasoned that it is the rate rather than the nature of progression that differs between RRMS and SPMS, and the EDSS[+] may thus be an interesting alternative disability outcome measure in RRMS as well. The proportion of progressors according to the EDSS[+] outcome was higher compared proportion of progressors according to the EDSS outcome, suggesting a higher sensitivity for detection of progression in RRMS as well. Finally, the EDSS

score is non-linear, and the rate of EDSS progression varies as a function of the EDSS score at baseline.[28] We thus used a definition of EDSS worsening adjusted to baseline EDSS to lessen this limitation. Other limitations of the EDSS include an underrepresentation of cognitive function in disability scoring, which we did not address in this study.

In conclusion, using confirmed EDSS or EDSS⁺ worsening as clinical outcomes of disability progression, this study identifies progression in a significant proportion of patients with RRMS unmasked by the treatment with natalizumab, and reveals NfL trajectories do not vary between progressors and non-progressors, suggesting NfL may not be a well suited biomarker to monitor or predict this process.

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FIGURES AND TABLES

Figure 1

Title: Study set-up

Legend:

>EDSS, expanded disability status score ; 9HT, 9 hole peg test; T25W, timed 25 foot walk; NfL, neurofilament light. Blue arrows indicate when clinical assessment was performed with respect to natalizumab initiation (baseline). Green arrows indicate when MRI was performed with respect to natalizumab initiation (baseline). Red arrows indicate when NfL was measured with respect to natalizumab initiation (baseline).

Figure 2

Title: Longitudinal dynamics of neurofilament light

Legend:

>**A** Longitudinal dynamics of neurofilament light in EDSS progressors (red), non progressors (blue), and the entire cohort (black) over time.

>**B** Longitudinal dynamics of neurofilament light in EDSS+ progressors (red), non progressors (blue), and the entire cohort (black) over time. BL, baseline; NfL, neurofilament light; EDSS, expanded disability status score.

Table 1

Title: Participant characteristics

Legend:

Demographic and clinical characteristics of patients and neurofilament levels. EDSS, expanded disability status score; IQR, interquartile range; SD, standard deviation; mo, months.

	Total cohort EDSS outcome	EDSS non-progressors	EDSS progressors	p-value	Total cohort EDSS plus outcome	EDSS-plus non-progressors	EDSS-plus progressors	p-value
N (% of whole cohort)	89 (100)	61 (68.5)	28 (31.5)	-	73 (100)	38 (52.1)	35 (47.9)	-
% females	74.2	72.1	78.6	0.519	74.0	71.1	77.1	0.554
Mean age at baseline (SD) in years	36.9 (8.5)	35.4 (8.5)	40.0 (7.8)	0.019	37.1 (8.1)	34.9 (8.1)	39.5 (7.6)	0.011
Median disease duration at baseline (IQR) in years	7.4 (3.8-12.1)	6.9 (3.2-10.9)	8.2 (4.4-16.5)	0.047	7.6 (4.4-12.6)	7.4 (4.4-11.9)	7.9 (4.3-15.7)	0.480
Median follow-up time from baseline to last follow-up visit, (IQR) in years	5.2 (4.3-6.7)	5.0 (4.0-6.4)	5.8 (5.1-8.7)	0.004	5.2 (4.3-6.9)	5.0 (4.0-6.3)	5.4 (5.0-7.2)	0.043
% individuals with relapse during the follow-up time of the study, excluding the first 3 months after natalizumab initiation	14.6	16.4	10.7	0.481	16.4	21.1	11.4	0.268
% individuals with new T2 or GE lesions during the follow-up time of the study, excluding the first year after natalizumab initiation	10.1	9.8	10.7	0.898	9.6	10.5	8.6	0.777
Median EDSS at 12 mo follow-up (IQR)	3.5 (2.4-4.5)	3.0 (2.5-4.0)	3.8 (2.6-5.0)	0.390	3.5 (2.5-4.5)	3.0 (2.5-4.0)	3.5 (3.0-5.0)	0.095
Median EDSS at last follow-up (IQR)	4.0 (3.0-5.75)	3.5 (2.0-4.5)	5.8 (3.6-6.0)	$< 10^{-5}$	4.0 (3.0-5.8)	3.5 (2.4-4.0)	5.0 (3.5-6.0)	$< 10^{-5}$
Median sNfL at baseline (IQR) in pg/mL	14.8 (10.0-27.1)	15.2 (10.1-25.3)	14.0 (9.7-28.7)	0.912	15.6 (10.2-27.1)	16.3 (10.6-26.9)	14.2 (9.5-28.3)	0.719
Median sNfL at 3mo follow-up (IQR) in pg/mL	11.1 (8.4-16.0)	11.5 (5.8-16.5)	9.7 (7.6-13.1)	0.480	11.1 (8.4-15.6)	12.1 (10.0-17.4)	9.6 (7.4-12.9)	0.185
Median sNfL at 12 mo follow-up (IQR) in pg/mL	7.9 (5.9-11.0)	8.2 (5.8-10.8)	7.5 (6.0-11.9)	0.926	7.6 (5.9-10.5)	7.9 (5.9-9.9)	7.5 (5.9-11.0)	0.816
Median sNfL at 24 mo follow-up (IQR) in pg/mL	7.9 (5.7-10.5)	8.2 (5.6-10.5)	7.5 (5.8-11.2)	0.429	7.7 (5.7-10.2)	8.1 (5.5-10.2)	7.3 (5.7-10.4)	0.623
Median sNfL at last FU (IQR) in pg/mL	8.9 (5.6-11.3)	8.8 (5.5-11.6)	9.6 (6.7-11.1)	0.334	8.8 (5.8-11.3)	8.8. (5.5-11.7)	8.8 (6.7-10.9)	0.344

Table 2

Title: Participant characteristics of participants with a 4 year follow-up period

Legend:

Demographic and clinical characteristics of patients and neurofilament levels. EDSS, expanded disability status score; IQR, interquartile range; SD, standard deviation; mo, months.

	Total cohort EDSS outcome	EDSS non-progressors year1-year4	EDSS progressors year1-year4	p-value
N (% of whole cohort)	65	57	8	-
% females	72.3	70.2	87.5	0.305
Mean age at baseline (SD) in years	38.1 (8.3)	37.9 (8.4)	39.3 (7.9)	0.649
Median disease duration at baseline (IQR) in years	7.6 (4.3-12.1)	8.5 (4.3-12.6)	6.5 (3.1-9.8)	0.231
% individuals with relapse during the 5 years follow-up, excluding the first 3 months after natalizumab initiation	10.8	15.8	12.5	0.809
% individuals with new T2 or GE lesions during the 5 years follow-up, excluding the first year after natalizumab initiation	15.4	12.3	0.0	0.294
Median EDSS at year 1 follow-up (IQR)	3.5 (2.5-4.5)	3.5 (2.5-4.5)	3.5 (3.0-5.6)	0.755
Median EDSS at year 4 follow-up	4.0 (2.5-5.0)	3.5 (2.5-4.5)	5.3 (4.0-6.5)	0.025
Median sNfL at baseline (IQR) in pg/mL	13.9 (9.4-21.5)	14.2 (9.5-22.2)	12.4 (9.3-16.6)	0.771
Median sNfL at 3mo follow-up (IQR) in pg/mL	10.3 (5.8-13.0)	10.3 (7.8-14.4)	10.1 (7.1-12.8)	0.981
Median sNfL at 12 mo follow-up (IQR) in pg/mL	7.3 (5.8-10.1)	7.4 (5.8-10.1)	6.5 (5.3-10.8)	0.426
Median sNfL at 24 mo follow-up (IQR) in pg/mL	7.1 (5.3-10.1)	7.5 (5.3-10.5)	6.5 (4.8-9.1)	0.298