Serum neurofilament light chain levels predict long-term disability progression in patients with progressive multiple sclerosis

Manuel Comabella[1], MD, PhD, Jaume Sastre-Garriga[1], MD, PhD, Pere Carbonell-Mirabent[1], PhD, Nicolás Fissolo[1], PhD, Carmen Tur[1], MD, PhD, Sunny Malhotra[1], PhD, Deborah Pareto[2], PhD, Francesc X Aymerich[2,3], PhD, Jordi Río[1], MD, PhD, Alex Rovira[2], MD, Mar Tintoré[1], MD, PhD, Xavier Montalban[1], MD, PhD.

Author affiliations:

[1]Servei de Neurologia-Neuroimmunologia. Centre d'Esclerosi Múltiple de Catalunya (Cemcat). Institut de Recerca Vall d'Hebron (VHIR). Hospital Universitari Vall d'Hebron. Universitat Autònoma de Barcelona. Barcelona, Spain. [2]Service of Neurology. Hospital Clinic and Institut d'Investigacions Biomèdiques August Pi Sunyer. University of Barcelona. Barcelona, Spain. [2]Section of Neuroradiology, Department of Radiology, Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain. [3]Department of Automatic Control (ESAII), Universitat Politècnica de Catalunya, Barcelona, Spain.

*Corresponding author:

Manuel Comabella; Unitat de Neuroimmunologia Clínica, CEM-Cat. Edif. EUI 2ª planta, Hospital Universitari Vall d'Hebron. Pg. Vall d'Hebron 119-129 08035 Barcelona, Spain. Phone: +34932746834, Fax: +34932746084.

e-mail: manuel.comabella@vhir.org

ABSTRACT

Objective: There is a lack of sensitive and specific biomarkers for use in progressive multiple sclerosis (MS). The study aimed to assess the potential of serum neurofilament light chain (sNfL) levels as biomarker of disability progression in patients with progressive MS.

Methods: We performed a prospective observational cohort study in 51 patients with progressive MS who participated in a 2-year phase II single-center, randomized, double-blind, placebo-controlled trial of interferon-beta. Mean (standard deviation) follow-up duration was 13.9 (6.2) years. Levels of sNfL were measured using a single molecule array immunoassay at baseline, 1, 2 and 6 years. Univariable and multivariable analyses were carried out to evaluate associations between sNfL levels and disability progression at short term (2 years), medium term (6 years), and long term (at the time of the last follow-up).

Results: A sNfL cut-off value of 10.2 pg/mL at baseline discriminated between long-term progressors and non-progressors with a 75% sensitivity and 67% specificity (adjusted odds ratio (OR): 7.8; 95%CI: (1.8, 46.4); p=0.01). Similar performance to discriminate between long-term progressors and non-progressors was observed using age/body mass index-adjusted sNfL Z-scores derived from a normative database of healthy controls. A cut-off increase of 5.1 pg/mL in sNfL levels between baseline and 6 years also discriminated between long-term progressors and non-progressors with a 71% sensitivity and 86% specificity (adjusted OR: 49.4; 95%CI: (4.4, $2x10^3$); p=0.008).

Conclusions: sNfL can be considered a prognostic biomarker of future long-term disability progression in patients with progressive MS. These data expand the little knowledge existing on the role of sNfL as long-term prognostic biomarker in patients with progressive MS.

What is already known on this topic.

In patients with relapsing multiple sclerosis, the prognostic role of neurofilament light chain as a biomarker of disease activity and treatment response has been thoroughly investigated. However, in patients with progressive multiple sclerosis, the prognostic role of neurofilament light chain as a biomarker of disability progression is more elusive.

What this study adds.

The study provides for the first time relevant information on the potential for the serum neurofilament light chain as a biomarker to predict long-term disability progression in patients with progressive multiple sclerosis.

How this study might affect research, practice or policy

The study provides the rationale for measuring serum levels of neurofilament light chain in patients with progressive multiple sclerosis in order to predict future disability progression. These patients may benefit from early introduction of therapies to slow disability progression.

INTRODUCTION

Neurofilaments are neuron-specific cytoskeletal proteins released into the extracellular space following neuronal damage.[1,2] In this context, concentrations of neurofilaments in the extracellular fluid, cerebrospinal fluid (CSF) and peripheral blood, can reflect the degree of neuroaxonal damage in pathological processes, although irrespective of its cause.[2,3] CSF and blood levels of the neurofilament light chain (NfL) have been reported to be increased in a wide range of neuroinflammatory and neurodegenerative disorders.[2,4] In multiple sclerosis (MS), NfL is considered a highly sensitive biomarker of neuronal injury.[2-4] In patients with relapsing MS, the prognostic role of NfL as a biomarker of disease activity and treatment response has been thoroughly investigated.[2-5] In this respect, CSF and/or blood NfL levels are increased during relapses and correlate with the number of T2 lesions and enhancing T1 lesions; they are risk factors for transition to clinically definite MS in patients with clinically and radiologically isolated syndromes; they predict future magnetic resonance imaging (MRI) lesion activity, brain and spinal cord volume loss, relapse rate, and worsening of Expanded Disability Status Scale (EDSS); and they are associated with clinical and MRI outcomes in patients receiving disease modifying therapies. In patients with progressive MS, the prognostic role of NfL as a biomarker of disability progression is more elusive.[6] In the present study, we aimed to evaluate the potential of serum NfL (sNfL) levels as a prognostic biomarker of short-, medium- and long-term disability progression in a cohort of patients with progressive MS.

METHODS

Patients

Fifty-one out of 73 patients with progressive MS who participated in a two-year phase II single-center, randomized, double-blind, placebo-controlled trial of interferon (IFN)β-1b from December 1998 to October 2001 were included in the study.[7] Selection of patients was performed based on availability of serum samples at trial baseline and during follow-up. The study was approved by the corresponding Hospital Ethics Committee (approval number: PR(AG)222/2014), and participants gave written informed consent.

Clinical assessments and definition of disability progression

Patients were followed every three months for two years during the trial and then every 6 months until the time of the last follow-up, and disability data were recorded using the EDSS. Short-term disability progression was defined as an increase of at least 1 point in the EDSS if baseline EDSS $\leq$5.0, and 0.5 points if baseline EDSS $\geq$5.5 during the two-year trial duration. Considering that the majority of patients would fulfill this progression criterion at medium and long term, to evaluate disability progression at these time points progression rates were calculated by dividing EDSS changes by the time on follow-up between trial baseline and 6 years (for medium term), and between trial baseline and the time of last visit (for long term). Then, medium- and long-term progressors were defined as those patients displaying progression rates above the 75th percentile of disability progression. For all disability progression measures, EDSS scores were confirmed at 6 months.

MRI assessments

Brain T1 and T2 lesion volumes (T1LV / T2LV), brain parenchymal fraction (BPF), and mean upper cervical cord area (MUCCA) were calculated as previously described[7,8] at trial baseline, and at 1, 2, and 6 years of follow-up. No gadolinium-enhanced scans were performed in the clinical trial.

sNfL determinations

Blood was collected by standard venipuncture and allowed to clot spontaneously for 30 minutes. Serum was obtained by centrifugation and stored frozen at -80ºC until used. None of the patients received treatment with corticosteroids in the two months before baseline sample collection. sNfL levels were measured at trial baseline (n=51), and at 1 year (n=51), 2 years (n=50), and 6 years (n=35) of follow-up using a commercially available immunoassay kit (Quanterix, cat#103186) run on the fully automated ultrasensitive Simoa HD-1 Analyzer (Quanterix). Samples were run in duplicate in accordance with manufacturers' instructions with appropriate standards and internal controls. The intra-assay and inter-assay coefficients of variation were 7% and 11% respectively. Median times (interquartile ranges [IQR]) between blood collection to determine sNfL levels and corresponding EDSS measurements to evaluate disability progression were 1 (1 - 2) days at baseline and 170 (1 - 192.3) days at 6 years. For 1 and 2 years, EDSS measurements and blood collection at 1 and 2 years were performed the same day.

Statistical methods

Descriptive analyses for progressive MS patients were performed in terms of demographics, clinical and radiological variables. Univariable and age-adjusted linear models were built to evaluate the association between baseline sNfL levels and demographics, clinical and radiological variables. In all regression analyses, the natural logarithm of the NfL levels was computed to meet the required normality assumption. Then, in order to improve the interpretation of the results, we back-transformed the regression estimates by exponentiating the regression parameters. Therefore, the back-transformed estimates are interpreted as the multiplicative effect of increasing by one unit the independent variable to the dependent variable. We then investigated the effect of IFNβ treatment on the EDSS and sNfL trajectories at short, medium and long term. We refer to the trajectories as the longitudinal measurements performed repeatedly on each patient throughout their follow-up. These associations were assessed by building univariable and multivariable (adjusted by age and sex) generalized estimating equations (GEE), for which the best correlation structure was chosen as per the rule of the lowest quasi-likelihood under the independence model criterion (QIC). In addition, linear mixed-effects models were also built as sensitivity analyses. In these models, internal validation as per bootstrapping strategy were also performed. Univariable logistic regressions were built to assess the ability of baseline sNfL levels to discriminate between progressors and non-progressors at short, medium and long term. The receiver operating characteristic (ROC) curve was built to retrieve the best sNfL cut-off and the area under the ROC curve (AUC), sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were obtained to evaluate model performance. The best sNfL cut-off values were defined as those that showed the minimum Euclidean distance to the perfect classifier performance (true positive and true negative rates equal to 1). As sensitivity analysis, multivariable logistic regressions

adjusted for sex and age were built to account for possible confounding bias and odds ratio (OR) were reported as a measure of the relative risk associated to the exposure. Next, age / body mass index (BMI)-adjusted sNfL Z-scores calculated from a normative database (NDB) with 4,532 samples of healthy controls were also evaluated as possible thresholds to discriminate between progressors and non-progressors.[9] In the NDB, the non-linear association between NfL and age as well as BMI was modelled by spline terms using a Generalized Additive Model for Location, Scale and Shape (GAMLSS) model.[10-12] The age / BMI-normalized sNfL Z-scores were derived from this statistical model for each data point with available NfL and age. The sNfL Z-score is a bias-corrected measure of disease activity and represents the number of standard deviations a given adjusted sNfL value from a MS patient is above / below the mean in samples of healthy controls. Changes in sNfL levels during the first 6 years were also investigated as predictors of long-term disability progression by building a univariable logistic regression. As previously performed, multivariable logistic regressions adjusted for age and sex were built and the OR were also reported. Least-square regressions were built to analyze the association between baseline sNfL levels and changes in radiological variables (adjusted for age and sex). Finally, concurrent associations between sNfL levels and radiological parameters and EDSS were assessed by building linear mixed-effects models to address for the repeated patient-correlated measures and adjusted by age, sex, disease duration, and treatment. P-values below 0.05 were considered statistically significant and all analyses were performed using R 3.6.0 (R Foundation for Statistical Computing).

RESULTS

Demographic, clinical and radiological characteristics of progressive MS patients at baseline.

Table 1 summarizes the demographic, clinical and MRI information of patients with progressive MS. At baseline, the median age of patients was 49.4 (IQR, 45.0 - 54.9) years, and 30 (58.8%) were women. Thirty-five patients were labeled as primary progressive MS[13] and 16 patients as transitional progressive MS.[14] Mean (standard deviation) disease duration was 10.5 (6.6) years and median EDSS 5.5 (IQR, 4.0 - 6.0). Baseline T2LV and T1LV were 12943.3 mm^3 (IQR, 67864.4 - 34245.0 mm^3) and 4200.0 mm^3 (IQR, 2271.7 - 13000.5 mm^3) respectively; and BPF and MUCCA were 74.0% (IQR, 69.8 - 76.7%) and 79.4 mm^2 (IQR, 73.4 - 83.0%) respectively. No statistically significant differences were observed between progressive MS patients included in the study and those who participated in the trial but were not included (online supplemental Table 1).[7]

Table 1. Demographic, clinical and radiological characteristics of patients with progressive multiple sclerosis.

Characteristics	Progressive multiple sclerosis patients
n	51
Age (years)[a]	49.4 (45.0 - 54.9)
Female / male (% women)	30 / 21 (58.8)
PP / PT (% PP)	35 / 16 (68.6)
Disease duration (years)*[b]	10.5 (6.6)
Follow-up time (years)*[c]	13.9 (6.2)
Placebo / treated patients (% treated)	22 / 29 (56.9)
EDSS at baseline	5.5 (4.0 - 6.0)
EDSS / PR at 2 years	6.0 (4.0 - 6.5) / 0.5 (0 - 1.0)
EDSS / PR at 6 years	6.5 (6.0 - 7.5) / 0.2 (0.2 - 0.4)
EDSS / PR at last visit	8.0 (6.5 - 8.6) / 0.2 (0.1 - 0.3)
T2LV (mm^3)	12943.3 (6786.4 - 34245.0)
T1LV (mm^3)	4200.0 (2271.7 - 13000.5)
BPF (%)	74.0 (69.8 - 76.7)
MUCCA (mm^2)	79.4 (73.4 - 83.0)
sNfL levels at baseline (pg/mL)	9.1 (7.5 - 13.7)
sNfL levels at 2 years (pg/mL)	10.3 (8.1 - 12.5)

sNfL levels at 6 years (pg/mL)	10.4 (8.7 - 15.2)

Data are expressed as median (interquartile range) unless otherwise stated. *Data are expressed as mean (standard deviation). [a]Refers to age at trial baseline. [b]Refers to the time between disease onset and trial baseline. [c]Refers to the time between trial onset and the time of last visit. EDSS: Expanded Disability Status Scale. PP / PT: refers to the number of patients with primary progressive and transitional progressive MS. BPF: brain parenchymal fraction. MUCCA: mean upper cervical cord area. PR: progression rates. sNfL: serum neurofilament levels. T1LV: T1 lesion volume. T2LV: T2 lesion volume.

Associations between sNfL and demographic, clinical, and MRI variables at baseline.

Median sNfL levels in progressive MS patients were 9.1 pg/mL (IQR, 7.5 - 13.7 pg/mL) at baseline (Table 1). As shown in Table 2, in univariable analysis baseline sNfL levels were associated with the quadratic form of age at sample collection (β: 1.48; 95% confidence interval (CI): (0.54, 2.41); p=0.002) but not with sex. Baseline sNfL levels were not associated with disease duration, clinical form, or EDSS. Regarding radiological variables, baseline sNfL levels were associated with T2LV (β: 1.01; 95%CI: (1.00, 1.01); p=0.003), T1LV (β: 1.02 95%CI: (1.00, 1.04); p=0.002), and BPF (β: 0.97; 95%CI: (0.94, 0.99); p=0.04) but not with MUCCA (the estimates for T2LV and T1LV refer to an increase of 1,000 units of the variable, respectively). In multivariable analyses after adjusting by age, associations were significant for T2LV and T1LV and remained at a trend-level for BPF (Table 2).

Table 2. Associations between sNfL levels and demographic, clinical and radiological variables at baseline.

Variables	**sNfL levels**	**Univariable**			**Multivariable**		
	(pg/mL)	**β**	**95% CI**	**P value**	**β**	**95% CI**	**P value**
Age	-	1.48*	0.54 - 2.44	**0.003**	-	-	-
Gender							
Male	8.6 (7.4 - 11.8)	-	-	-	-	-	-
Female	9.6 (7.6 - 14.1)	1.18	0.88 - 1.57	0.25	1.15	0.85 - 1.54	0.34
Clinical form							
PP	10.2 (7.8 - 14.5)	-	-	-	-	-	-
PT	8.1 (7.2 - 10.8)	0.84	0.61 - 1.14	0.26	0.85	0.62 - 1.16	0.30
Disease duration	-	0.99	0.97 - 1.01	0.66	0.99	0.96 - 1.01	0.37
EDSS	-	1.08	0.97 - 1.22	0.14	1.07	0.95 - 1.21	0.20
T2LV[1]	-	1.01	1.00 - 1.01	**0.003**	1.01	1.00 - 1.01	**0.004**
T1LV[1]	-	1.02	1.00 - 1.04	**0.002**	1.02	1.00 - 1.03	**0.005**
BPF	-	0.97	0.94 - 0.99	**0.04**	0.97	0.95 - 1.001	0.05
MUCCA	-	1.00	0.98 - 1.02	0.82	1.00	0.98 - 1.02	0.81

Abbreviations. PP: primary progressive MS. PT: transitional progressive MS. sNfL: serum neurofilament levels. T1LV: T1 lesion volume. T2LV: T2 lesion volume. BPF: brain parenchymal fraction. MUCCA: mean upper cervical cord area. sNfL levels are expressed as median (interquartile range). Number of samples available for all variables included in the table: 51. Multivariable analysis was adjusted by age. Significant p values are shown in bold. [1]For the sake of better interpretation, estimates refer to an increase of 1,000 units of the variable. [*]Estimation referring to the quadratic form of the variable and therefore it is not back-transformed due to the lack of interpretation as a multiplicative effect.

Association between IFNβ treatment and sNfL levels.

Twenty-nine (57%) patients were treated with IFNβ during the first two years of follow-up as part of the clinical trial (Table 1). Baseline demographic, clinical, and radiological characteristics were similar between treated and untreated patients (data not shown). Only 5 (9.8%) patients, four from the treated arm and one from the placebo arm, received immunomodulatory treatment with IFNβ after trial completion. The remaining progressive MS patients included in the study were untreated from trial completion to the time of last visit. In univariable analysis, IFNβ treatment did not modify EDSS trajectories at short term (β: 0.002; 95%CI: (-0.01, 0.01); p=0.73), medium term (β: -0.006; 95%CI: (-0.01, 0); p=0.33), or long term (β: -0.002; 95%CI: (-0.004, 0); p=0.40). Similar results were obtained in multivariable analysis after adjusting for age and sex (data not shown). IFNβ slightly decreased sNfL levels during the 2-year treatment period (β: -0.13; 95%CI: (-0.19, -0.07); p=0.02).

Associations between baseline sNfL levels and short-, medium- and long-term disability progression.

Associations with disability progression during follow-up were evaluated according to baseline sNfL levels measured in the progressive MS cohort and also based on the sNfL Z-scores derived from a NDB of healthy controls.

Patients were followed for a mean time of 13.9 (6.2) years, and median EDSS scores at 2 years, 6 years, and at the time of last visit were 6.0 (IQR, 4.0 - 6.5), 6.5 (IQR, 6.0 - 7.5), and 8.0 (IQR, 6.5 - 8.6) respectively (Table 1). Twenty-four (47.1%) patients were classified as short-term progressors. A total of 9 (24.3%) and 12 (25.0%) patients had progression rates above 0.40 and 0.27 (75th percentiles of progression rates) at medium and long term and were classified as medium-term and long-term progressors respectively. As illustrated in Figure 1, no significant differences were observed in baseline sNfL levels between progressors and non-progressors at short and medium term; likewise, baseline sNfL levels showed poor performance to discriminate between progressors and non-progressors at those time points (Figure 1). In contrast, baseline sNfL levels were significantly higher in patients classified as progressors at long term ($p=0.03$; Figure 1), and in the univariable analysis a sNfL concentration of 10.2 pg/mL was the best cut-off to discriminate between long-term progressors and non-progressors with a sensitivity of 75%, specificity of 67%, and PPV and NPV of 42.9% and 88.9% respectively. In the adjusted logistic regression, the presence of baseline sNfL above 10.2 pg/mL remained as a significant risk factor to predict long-term disability progression (OR: 7.8; 95%CI: (1.8, 46.4); $p= 0.01$).

As shown in Figure 2A, age / BMI-adjusted sNfL Z-scores were significantly higher in long-term progressors compared to non-progressors (p=0.007) whereas no significant differences were observed at short and medium term between both groups of patients. Figure 2B shows the AUC for predicting short-, medium-, and long-term disability progression according to different healthy control-based sNfL Z-scores. Progressive MS patients with baseline sNfL levels equal or above the 1 and 1.25 healthy control-based Z-scores were at higher risk for long-term disability progression with an AUC of 76.4%, sensitivity of 75%, specificity of 77.1%, PPV of 52.9% and NPV of 90.0% for a Z-score of 1, and AUC of 76.7%, sensitivity of 66.7%, specificity of 85.7%, PPV of 61.5% and NPV of 88.2% for a Z-score of 1.25 (Figure 2B). Performance of sNfL levels for predicting disability progression based on other Z-scores and time points was overall poor (Figure 2B).

Associations between changes in sNfL levels at medium term and long-term disability progression.

We next evaluated whether changes in sNfL levels between baseline and 6 years predicted long-term disability progression. As shown in Figure 3, changes in sNfL levels at medium term were significantly higher in MS patients classified as long-term progressors (p=0.03) compared to long-term non-progressors. In univariable analysis, an increase in sNfL levels between baseline and 6 years above 5.1 pg/mL was the best cut-off to discriminate between long-term progressors and non-progressors with a sensitivity of 71%, specificity of 86%, and PPV and NPV of 55.6% and 92.3%, respectively (Figure 3). In the adjusted logistic regression, changes in sNfL levels at medium term above 5.1 pg/mL remained as a significant risk factor to predict long-term

disability progression (OR: 49.4; 95%CI: (4.4, $2x10^3$); p=0.008), although with high variability.

Associations between baseline sNfL levels and MRI parameters during follow-up.

We next assessed whether baseline sNfL levels predicted changes in T1LV, T2LV, BPF and MUCCA between the baseline and 1, 2, and 6 years. As shown in Figure 4, baseline sNfL levels only correlated significantly with changes in T1LV at first year (β: -9.69; 95%CI: (-18.66, -0.73); p=0.03). Trends for significant correlations were also observed between baseline sNfL levels and changes at 2 years for T1LV (β: -10.52; 95%CI: (-21.64, 0.59); p=0.06) and T2LV (β: -10.38; 95%CI: (-21.24, 0.49); p=0.06) (Figure 4).

Concurrent associations between sNfL levels and clinical and radiological parameters across longitudinal determinations.

To evaluate whether sNfL levels have the potential to reflect the current conditions of the disease in progressive MS patients, all sNfL determinations performed at baseline, 1, 2, and 6 years were plotted against the simultaneous assessments of BPF, cervical cord area, T2LV, T1LV, and EDSS. As shown in Figure 5, multivariable analyses adjusted by age, sex, disease duration and treatment revealed significant correlations between sNfL levels and BPF (β: -0.021; 95%CI: (-0.035, -0.002); p=0.02), T2LV (β: $6.8x10^{-6}$; 95%CI: ($2.1x10^{-6}$, $1.1x10^{-5}$); p=0.008), T1LV (β: $1.7x10^{-5}$; 95%CI: ($5.4x10^{-6}$, $2.3x10^{-5}$); p=0.006), and EDSS (β: 0.08; 95%CI: (0.03, 0.13); p<0.001) but not for MUCCA.

DISCUSSION

A number of studies conducted in treated and untreated patients with relapsing forms of MS have shown an association between high CSF and sNfL levels and increased risk of disability worsening in the short and long term,[10,11,15-19] although some studies failed to find such association.[20-22] Interestingly, though conducted in patients with relapse-onset MS, in the study by Uphaus et al. sNfL levels at baseline predicted the relapse-free disability progression after a median follow-up of 6 years,[19] and in the study by Cantó et al. a steeper trajectory of sNfL levels was observed over time in long-term progressors.[22]

The role of CSF or blood NfL levels as a biomarker of disability progression in patients with progressive MS is even less defined, with the majority of studies showing negative results.[23-25] Furthermore, there are no studies evaluating the relationship between NfL levels and long-term disability progression in this group of patients. In the present study we aimed to fill this gap by examining the association between sNfL levels and development of disability progression at short, medium, and long term in a trial cohort of patients with progressive MS who were followed for a mean time of 14 years. Disability progression at short term was defined based on EDSS changes, whereas medium- and long-term disability progression was defined according to progression rates and subsequent selection of patients positioned above the 75th percentile of the disability progression distribution. Although 57% of patients received treatment with IFNβ during the 2-year trial duration, the vast majority of patients (90%) were untreated

after trial completion for the rest of the studied period of time. Moreover, in our study IFNβ had little effect on sNfL levels and did not influence EDSS trajectories at short, medium, or long term. Other studies have shown either a reduction in sNfL levels due to IFNβ treatment[15] or no significant effects.[16,26] As shown in previous studies,[2] baseline sNfL levels were influenced by age; however, baseline sNfL levels were not associated with sex, clinical form (whether patients had primary progressive or transitional progressive MS) or EDSS.

One of the main findings in our study was the potential for baseline sNfL levels to predict future disability progression. Interestingly, the association between baseline sNfL levels and disability progression was stronger as the period of time to evaluate disability progression increased, and became significant for the long-term assessment. In this context, high sNfL levels (above 10.2 pg/mL) at baseline in patients with progressive MS predicted long-term disability progression with very good specificity and acceptable sensitivity. Interestingly, similar results were obtained using age / BMI-adjusted sNfL Z-scores obtained from a NDB of a large number of healthy controls, particularly for progressive MS patients with baseline sNfL levels equal or above the 1 and 1.25 Z-scores of healthy controls. Not only individual baseline sNfL levels predicted long-term disability progression in patients with progressive MS, but also the changes in sNfL levels observed during the first 6 years of follow-up had great potential to predict disability progression in the long term, with specificity and sensitivity above 70%. These data indicate that baseline sNfL levels as well as medium-term changes in sNfL levels predict long-term disability progression in patients with progressive MS.

Regarding radiological variables, sNfL levels in patients with progressive MS only correlated with T1LV and T2LV at baseline, and slightly with T1LV changes during the first year. However, baseline sNfL levels were not associated with either brain volume or cervical cord area loss at baseline, or with brain volume or cervical cord area changes during follow-up. These data contrast with the numerous studies showing significant correlations between blood and CSF sNfL levels and brain volume loss in patients with relapsing MS,[11,15-18,22,27-31] although data in patients with progressive MS are scarce.[3] Noteworthy, when sNfL determinations across all time points were plotted together, sNfL levels significantly correlated with EDSS and with all radiological parameters except for the MUCCA. These findings indicate that sNfL levels provide a real-time picture of the neuroaxonal damage taking place in the CNS of patients with progressive MS at a particular time point. Building on our findings, higher sNfL levels, which are probably indicative of higher tissue destruction, will translate into irreversible disability in the long term in patients with progressive MS.

Finally, based on these results, it will also be interesting to explore the prognostic potential of other body fluid biomarkers such as chitinase 3-like 1 and 2,[32,33] and GFAP[34] on the long-term disability progression of patients with progressive MS.

In summary, sNfL levels can be considered a biomarker not only of concurrent neuroaxonal injury secondary to inflammation and/or neurodegeneration, but also of long-term disability progression in patients with progressive MS. However, despite the unique characteristics of the progressive MS cohort (unicentric and well-controlled setting), one limitation of study is sample size. In this regard, owing to the relatively low number of patients included the study, these findings need certainly to be confirmed

in similarly conducted independent studies of well-characterized unicentric cohorts of patients with progressive MS and long-term follow-up. Overall, these data will expand the little knowledge existing on the role of sNfL levels as long-term prognostic biomarker in patients with progressive MS.

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CONTRIBUTORS

M.C., J.S.G., and X.M. contributed to conception and design of the study. P.C.M. contributed to analysis of data. N.F., C.T., S.M., D.P., F.X.A., J.R., A.R., and M.T. contributed to acquisition of data. All coauthors contributed to editing and approval of the final draft.

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COMPETING INTERESTS

None declared.

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FIGURE LEGENDS

Figure 1. Performance of sNfL levels at baseline in patients with progressive MS to discriminate between progressors and non-progressors at short, medium, and long term. Number of patients in each category is shown in parentheses. Dashed lines in boxplots represent the best cut-offs of sNfL levels for each ROC curve. Significant p values are shown in bold. AUC: area under the ROC curve. IQR: interquartile range. Se: sensitivity. Sp: specificity. sNfL: serum neurofilament levels.

Figure 2. **Performance of sNfL levels at baseline to predict short-, medium-, and long-term disability progression based on the sNfL Z-scores derived from a NDB of healthy controls.** (A) Boxplots showing the distribution of age / BMI-adjusted healthy control-based sNfL Z-scores in short-, medium-, and long-term progressors and non-progressors. Dashed lines indicate the 1, 1.25, 1.5, and 1.75 sNfL Z-scores. P-values were obtained using a Wilcoxon rank sum aka Mann-Whitney test. (B) ROC curves according to the 1, 1.25, 1.5, and 1.75 sNfL Z-scores. AUC: area under the ROC curve. Se: sensitivity. Sp: specificity.

Figure 3. **Performance of change in sNfL levels at medium term to predict long-term disability progression.** Number of patients in each category is shown in parentheses. The dashed line in the boxplot represents the best cut-off change in sNfL

levels between baseline and 6 years. Medium-term change in sNfL estimate: odds ratio = 1.5 CI 95% (1.1, 2.2), p=0.025). AUC: area under the ROC curve. IQR: interquartile range. Se: sensitivity. Sp: specificity. sNfL: serum neurofilament levels.

Figure 4. **Concurrent associations between sNfL levels and EDSS scores and radiological parameters.** Each graph represents all sNfL determinations at baseline, 1 year, 2 years, and 6 years plotted against EDSS scores and radiological variables at the corresponding time points. Multivariable models were adjusted by age, sex, disease duration, and treatment. Significant p values are shown in bold. Log(sNfL): natural logarithm of the sNfL levels. BPF: brain parenchymal fraction. MUCCA: mean upper cervical cord area. T1LV: T1 lesion volume. T2LV: T2 lesion volume. LMM: linear mixed model.

Figure 5. **Associations between baseline sNfL levels and changes in radiological parameters at 1, 2, and 6 years.** Linear models were adjusted by age and sex. Significant p values are shown in bold. Log(sNfL): natural logarithm of the sNfL levels. BPF: brain parenchymal fraction. MUCCA: mean upper cervical cord area. T1LV: T1 lesion volume. T2LV: T2 lesion volume.