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Le neurofilament à chaîne légère sérique comme biomarqueur fiable de l'amyloïdose héréditaire à transthyrétine – l'expérience d'un centre de référence Suisse

Loser Valentin

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Département des neurosciences cliniques
Service de neurologie

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préparée sous la direction de la Docteure Marie Théaudin

et présentée à la Faculté de biologie et de médecine de
l'Université de Lausanne pour l'obtention du grade de

DOCTEUR EN MEDECINE

par

Valentin LOSER

Médecin diplômé de la Confédération Suisse
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IMPRIMATUR

La Faculté de biologie et médecine de l'Université de Lausanne, sur proposition du jury, autorise l'impression de la thèse de doctorat rédigée par

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Le neurofilament à chaîne légère sérique comme biomarqueur fiable de l'amyloïdose héréditaire à transthyrétine – l'expérience d'un centre de référence Suisse

sans se prononcer sur les opinions exprimées dans cette thèse.

Directrice	Docteure Marie Théaudin
Expert interne	Docteur Pierre Monney
Vice-directeur de l'Ecole doctorale	Professeur John Prior

Lausanne, le 09.05.2023

pour Le Doyen
de la Faculté de Biologie et de Médecine

Monsieur le Professeur John Prior
Vice-Directeur de l'Ecole doctorale

Résumé

Contexte : L'amyloïdose héréditaire à transthyrétine (hATTR) est une maladie rare, se présentant par une polyneuropathie axonale longueur-dépendante autonome et sensitivomotrice évolutive et fatale si non traitée. Sur les dernières années, des traitements efficaces ont vu le jour, permettant de retarder voir de stopper la progression de la neuropathie. Ces traitements ne permettent pas de récupérer les déficits déjà établis ; l'introduction la plus précoce possible de ces thérapies est donc primordiale.

Enjeu : Le neurofilament à chaine légère (NfL) est une protéine exprimée exclusivement dans le cytosquelette des neurones et est relâché dans le milieu extracellulaire en cas de dommage neuronal. Son taux sérique (sNfL) a déjà été corrélé à la dégénérescence neuronale dans certaines maladies du système nerveux central (SNC) comme la sclérose en plaques et dans certaines polyneuropathies. Le but de cette étude est de déterminer si le sNfL est un biomarqueur fiable de la polyneuropathie liée à l'amyloïdose hATTR.

Méthodologie : Dans cette étude prospective monocentrique, nous avons inclus 20 patients avec amyloïdose hATTR génétiquement prouvée, dont 14 patients symptomatiques et 6 patients asymptomatiques. Les patients ont été évalués à deux reprises à une année d'intervalle. Ces deux évaluations comprennent ; un examen neurologique complet avec mesure de plusieurs scores cliniques de sévérité de la maladie, la réalisation d'un électroneuromyogramme (ENMG) et une mesure de la conductance cutanée électrochimique par Sudoscan, et le dosage du sNfL. Nous avons également comparé le taux de sNfL des 20 patients avec ceux d'une cohorte de 4532 contrôles sains dérivé d'une base de données de référence.

Résultats et discussion : Le taux de sNfL est 3.6 fois plus élevé chez les patients symptomatiques que chez les asymptomatiques. Il est également plus élevé chez les patients asymptomatiques en comparaison aux contrôles sains, avec un Z-score de 2.52. Il n'y a cependant pas de différence significative entre les contrôle sains et les patients asymptomatiques, suggérant bien une corrélation du sNfL avec l'apparition de la polyneuropathie. Le taux de sNfL est également corrélé à la sévérité de cette dernière, avec une corrélation avec la plupart des scores de sévérité clinique et électrophysiologique utilisés. Le suivi sur une année n'a pas permis de détecter de patients s'aggravant, en particulier de patients asymptomatiques devenant symptomatique. En conséquence, nous n'avons pas détecté de modification significative du taux de sNfL durant le suivi.

Conclusion et perspectives : Le sNfL est un biomarqueur fiable de la polyneuropathie associée à l'amyloïdose hATTR. Il reste encore à confirmer s'il est un marqueur précoce de l'apparition de la polyneuropathie. Il serait également intéressant de pouvoir déterminer un seuil de sNfL au-delà duquel la neuropathie est établie, mais ceci est rendu difficile par la dépendance de ce biomarqueur à d'autres variables, en particulier l'âge et l'indice de masse corporelle (IMC). L'utilisation de mesures de déviations (telle que le Z-score) par rapport à des cohortes ajustées à l'âge et à l'IMC, au lieu de valeurs absolues permettrait de pallier ce problème. Pour répondre à ces questions, d'ultérieures études longitudinales doivent être entreprises, avec une extension du suivi sur plusieurs années.

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RESEARCH REPORT

WILEY

Serum neurofilament light chain as a reliable biomarker of hereditary transthyretin-related amyloidosis—A Swiss reference center experience

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Abstract

Hereditary transthyretin-related (hATTR) amyloidosis is a rare disease, causing a disabling and life-threatening axonal length-dependent polyneuropathy. Monitoring of disease progression and treatment response is difficult. We aimed to determine if serum neurofilament light chain (sNfL) is a reliable and early biomarker of peripheral neuropathy in hATTR amyloidosis. We prospectively included 20 hATTR patients, 14 symptomatic and 6 asymptomatic. Patients were assessed at baseline and 1 year, including a full clinical examination with disease severity and functional scores, electrochemical skin conductance measurement with Sudoscan and nerve conduction studies, and sNfL level. hATTR patient sNfL were also compared with sNfL of 4532 healthy controls of a reference database by calculating age and BMI-adjusted Z scores. At baseline, median sNfL concentration was 3.6-fold higher in symptomatic than asymptomatic hATTR patients ($P = .003$), and this difference was also found in our under 60-years-old patients ($P = .003$). There was no significant difference of sNfL concentration between asymptomatic patients and healthy controls (Z-score of −0.29), but a significant difference between symptomatic patients and healthy controls (Z-score of 2.52). We found a significant correlation between sNfL levels and most clinical and electrophysiological disease severity scores, the strongest correlation being with the NIS score. sNfL seems to be a reliable biomarker of peripheral neuropathy severity in hATTR amyloidosis and can distinguish between asymptomatic and symptomatic patients. sNfL could also become a reliable biomarker to establish disease onset and treatment response.

KEYWORDS
amyloidosis, ATTR, biomarker, disease severity, neurofilament light chain

1 | INTRODUCTION

Hereditary ATTR amyloidosis (hATTR) is a rare disease with the autosomal dominant transmission, caused by a point mutation in the transthyretin (*TTR*) gene.[1] The *pVal50Met* mutation is the most frequently reported and is the cause of an endemic disease in northern Portugal, Sweden, and Japan. Sporadic cases were also reported.[2-4] In Switzerland, most cases are patients of Portuguese ancestry, and a few are late-onset non-endemic Swiss patients, whose clinical characteristics are similar to the French hATTR patients.[5] Typical hATTR

amyloidosis features are a disabling and life-threatening axonal length-dependent polyneuropathy, which usually starts with a small-fiber dysfunction in lower extremities, and then progresses to a sensory-motor and autonomic polyneuropathy, with prominent autonomic features.[1]

The first treatment to halt disease progression was liver transplantation (LT), which was first proposed 30 years ago.[6] In the last decade, several therapies have emerged, including TTR kinetic stabilizers (tafamidis, diflunisal), and more recently small interfering RNA (patisiran) and antisense oligonucleotide inhibitors (inotersen).[7-9] Those therapies can greatly modify the natural history of the disease and early treatment initiation is recommended[10] as they can delay disease progression, but do not allow a recover of existing deficits. Early diagnosis is therefore essential to allow for the most rapid introduction of treatment. Patient follow-up, especially assessment of treatment response, relies on several clinical, biological, and electrophysiological tests, which are not always easy to perform and interpret, due to significant inter- and intra-individual variability. Moreover, scores, scales, and electrophysiological measurements do not always show similar trends over time for the same patient. As there is no composite score usable in clinical practice there is a need for a simple biological biomarker that could reliably assess at the individual level disease onset and progression.

Neurofilament light chain (NfL) is a scaffolding protein exclusively expressed in neuronal cytoskeleton and is released into the extracellular space following neuronal damage. NfL levels in the serum are related to central nervous system (CNS) neuronal degeneration, as demonstrated for example, in multiple sclerosis.[11] Serum NfL (sNfL) has been shown to be a promising biomarker for polyneuropathy in several acquired and inherited diseases of the peripheral nervous system.[12,13] Four recent reports suggest that sNfL is increased in patients with hATTR amyloidosis and polyneuropathy and is correlated with disease severity.[14-17]

In this prospective study, we aimed to determine if sNfL is a reliable and early biomarker of peripheral neuropathy in hATTR amyloidosis.

2 | MATERIAL AND METHODS

2.1 | Study population

Patients were prospectively recruited between October 2019 and July 2021 from the neuromuscular unit of the Lausanne University Hospital, which is, together with Zurich University Hospital, a Swiss reference center for hATTR amyloidosis. Inclusion criteria were as follows: age ≥ 18-year-old, carrier of a pathogenic *TTR* mutation, symptomatic or not. Patients were considered asymptomatic when they had either no clinical symptoms or mild subjective isolated sensory symptoms not suggestive of hATTR and normal nerve conduction study (NCS) and electrochemical skin conductance (ESC) measured with Sudoscan. Patients with known acquired diseases or injuries of the CNS, including leptomeningeal amyloidosis or late-onset central hATTR symptoms were excluded from the study. All patients signed an informed consent form to participate in this prospective study, which was approved by the local institutional review board (CER-VD 2019-00301).

We also compared our patients' sNfL levels with those of a reference database of 4532 healthy controls (HC), aged 20–75 years. This database comes from participants from four European and US population-based studies.[18]

2.2 | Methods

Patients were prospectively assessed at baseline and at 1 year (*T1*). Clinical assessment at both time points included: demographics, family history of hATTR amyloidosis (only at baseline), body mass index (BMI), full clinical examination with calculation of disease severity scores and functional scores, ESC assessment, and NCS. Sensory and vegetative subjective manifestations were rated with Small fiber Neuropathy-Symptom Inventory Questionnaire (SFN-SIQ)[19] and Compound Autonomic Dysfunction Test (CADT).[20] Neuropathy-related deficits were rated with Neuropathy Impairment Score (NIS) for both upper and lower limbs,[21] grip strength measured with Martin vigorimeter in both hands. Functional outcome was assessed with the Polyneuropathy Disability Score (PND)[22] and familial amyloid polyneuropathy (FAP) stage,[23] Rasch-built Overall Disability Score (R-ODS),[24] and the Karnofsky Performance Status.[25] Quality of life was evaluated using the Norfolk Quality of Life in Diabetic Neuropathy (QOL-DN).[26] Clinical scores are further described in the appendix.

Electrodiagnostic studies included NCS performed on the right side of the body, with calculation of the motor and sensory composite sum scores, and measurement of ESC in hands and feet with Sudoscan. The motor sum score is a composite of the amplitude of ulnar and fibular nerve compound muscle action potential (CMAP) in millivolt. The sensory sum score is a composite of the amplitude of ulnar (orthodromic testing) and sural (antidromic testing) nerve sensory nerve action potential (SNAP) in microvolt.[27]

Significant worsening was defined as; an increase of >10 points in the NIS score and/or a > 50% decrease in amplitude of the motor and/or sensory sum score and/or a > 25% decrease in ESC.[27]

All serum samples, at baseline and *T1* were frozen immediately and stored at −20°C. Samples were coded and sent blinded for clinical details to the University Hospital of Basel for analysis of sNfL levels. sNfL concentrations were determined using the ultrasensitive single-molecule array assay (Simoa) analysis as previously described.[28,29] The mean intra-assay coefficient of variation of duplicates and the mean inter-assay coefficient of variation was <10%.

2.3 | Statistical analysis

For descriptive statistics, mean, SD (SD), median and interquartile range (IQR) were used. For univariate comparisons of data, we used the Mann-Whitney *U* test for unpaired data and the Wilcoxon signed

TABLE 1 Clinical and demographic characteristics of symptomatic and asymptomatic patients at baseline. Significant *P*-values are in bold

	Symptomatic (n = 14)	Asymptomatic (n = 6)	*P*-value
Age in years (mean, SD)	47.7 (12.4)	39.9 (10.0)	.201
BMI in kg/m2 (mean, SD)	25.9 (5.3)	24.9 (5.3)	.837
PND (median, IQR)	1 (1–2)	1 (0–1)	.056
FAP (median, IQR)	1 (1–1.25)	1 (0–1)	.092
SFNSIQ (median, IQR)	7 (5.25–8.75)	2.5 (1.25–3.75)	.020
CADT (median, IQR)	14 (11.5–16)	16 (14–16)	.418
RODS (median, IQR)	43 (31.75–46)	48 (45–48)	.044
Total NIS UL (mean, SD)	14.3 (20.0)	0.17 (0.4)	.017
Total NIS LL (mean, SD)	19.1 (20.4)	0.3 (0.8)	**.009**
Total NIS (mean, SD)	33.5 (39.5)	0.5 (1.2)	**.005**
ESC feet in µS (mean, SD) n = 19	36.9 (24.2)	76 (7.3)	**.003**
ESC hands in µS (mean, SD) n = 19	41.1 (26.7)	65 (5.8)	.104
NCS motor sum score in mV (mean, SD) n = 16	8.5 (6.5)	15.8 (2.4)	.071
NCS sensory sum score in uV (mean, SD) n = 14	7.7 (12.3)	28.5 (10.0)	**.048**

rank exact test for paired data. For comparison of our data with the reference database of healthy controls, we converted absolute sNfL values into Z scores, in order to correct for confounding factors, namely age and BMI.[18] Z score represents the number of SDs of our sample, compared to the mean of the healthy control group. In the reference database, age- and BMI-adjusted Z scores have a mean of 0 and SD of 1. Multivariable regression analysis was used to explore disease severity according to sNfL, age and a few other factors. Receiver operating characteristic (ROC) curves were drawn by plotting the true-positive fraction (sensitivity) against the false positive fraction (100% - specificity) for varying cut-off values. The best cut-off was selected based on the value that maximized sensitivity and specificity at the same time, using the Youden index. Correlation between sNfL concentration and different variables, including diseases severity scores, were analyzed using Spearman correlation coefficient *R*. In all cases, statistical significance was set at $P < .05$. GraphPad Prism version 9.3.1 was used to generate the graphics. Statistical analysis was performed using R version 4.1.0 and 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria).

3 | RESULTS

3.1 | Baseline characteristics of hATTR patients

We prospectively included 20 genetically confirmed hATTR patients, 14 females, and 6 males. Mean age at inclusion was 45.4-year-old (SD 12.1 years). Most patients (n = 15/20, 75%) were of Portuguese ancestry, four of Swiss ancestry and one Italian. The most frequent mutation was *p.Val50Met* (n = 17/20, 85%). There were 14 symptomatic patients and six asymptomatic carriers. Three symptomatic patients were also known for type two diabetes. Among the 14 symptomatic patients, eight had undergone liver transplantation, two were on tafamidis and four on patisiran, including two liver transplanted patients. During the one-year follow-up, two symptomatic untreated patients were started on patisiran. Symptomatic patients had a mild to moderate neuropathy, with a median PND score of 1 (range 0–5), a median FAP stage of 1 (range 0–3), a mean NIS score of 33.5 (SD 39.5, range 0–129.5), and a median R-ODS score of 43 (range 23–48).

Demographics and clinical characteristics of symptomatic and asymptomatic patients at baseline are summarized in Table 1. Table A1 (appendix) describes the demographics and clinical characteristics and sNfL levels of each hATTR patient.

Of note, patient 2 was considered as symptomatic but had no clinical or electrophysiological sign of neuropathy. Indeed, this patient had undergone a liver transplantation in 2002 in Portugal, on the basis of minimal sensory symptoms (paresthesia in the four extremities). On our neurological examination, the NIS score was 0, and there was no notable electrophysiological abnormality (Sudoscan and NCS were within the norm). Because of the previous liver transplantation, we could not consider this patient as asymptomatic.

3.2 | NfL levels are increased in hATTR symptomatic compared to asymptomatic patients

At baseline, symptomatic hATTR patients had median (IQR) sNfL concentration of 19.7 (13.7–52.8) pg/ml, 3.6-fold higher than asymptomatic hATTR patients whose median (IQR) sNfL level was 5.4 (4.3–7.7) pg/ml ($P = .003$, Figure 1A). Because the sNfL level increases with age, primarily in patients older than 60-year-old, we performed the same comparative analysis in our patients younger than 60-year-old (11 symptomatic and 6 asymptomatic). At baseline, symptomatic hATTR patients under 60-year-old had a 3.4-fold higher sNfL concentrations, with a median (IQR) of 18.4 (11.8–31.3) pg/ml, than

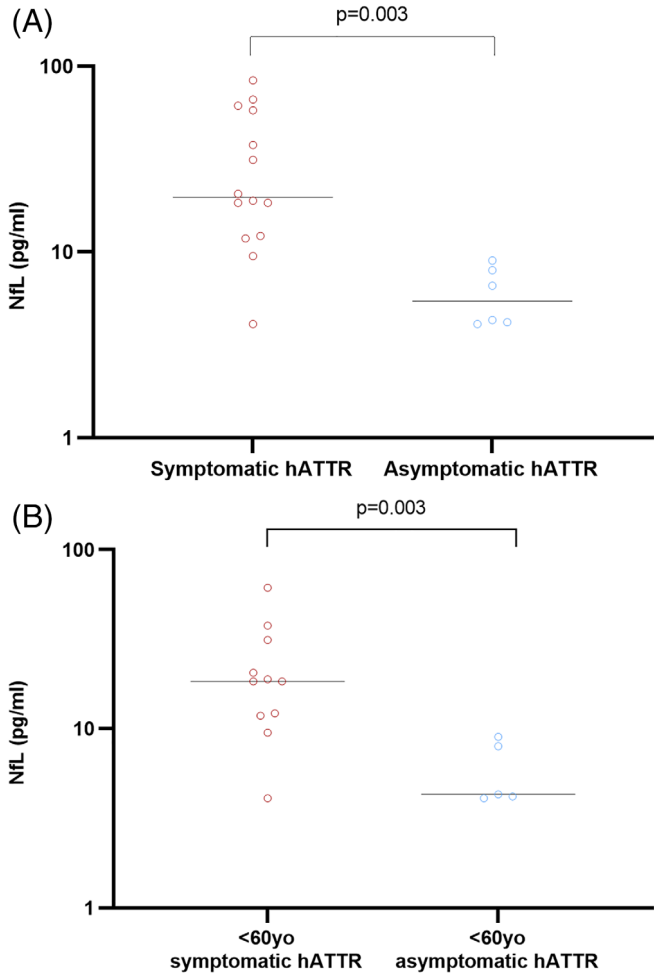

FIGURE 1 sNfL levels in symptomatic and asymptomatic patients at baseline. (A) sNfL levels in symptomatic and asymptomatic patients at baseline. The horizontal line represents the median. There is a significant difference between sNfL levels in both groups ($P = .003$). (B) sNfL levels in under 60-year-old symptomatic and asymptomatic patients. The horizontal line represent the median. There is significant difference between sNfL levels in both groups ($P = .003$)

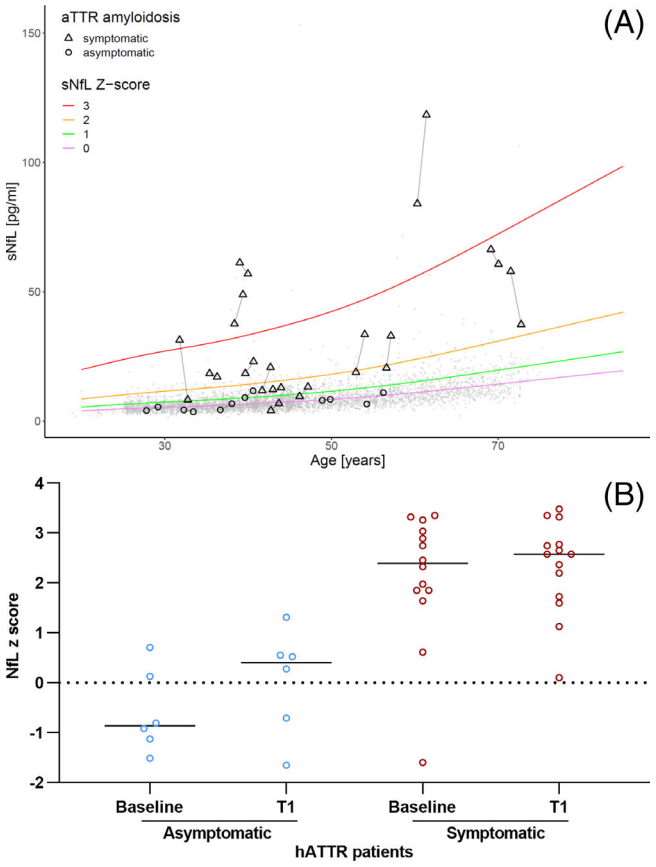

FIGURE 2 Comparison of sNfL levels of the 20 hATTR patients with a reference database of HC. (A) Individual values of all 4532 healthy controls are in small gray dots. Colored lines shows sNfL Z scores derived from the healthy controls data (z score 0 = purple line, z score 1 = green line, z score 2 = yellow line and z score 3 = red line). Individual hATTR patient data are displayed with baseline and *T1* data connected by a line, asymptomatic patients as circles and symptomatic patients as triangles.
(B) Representation of Z scores of asymptomatic and symptomatic patients at both baseline and *T1*. Healthy controls have a mean Z score of 0 (horizontal dashed line) and a SD of 1. The horizontal bold line represent the median. Asymptomatic hATTR subjects have a median (IQR) Z score of −0.86 (−1.22, 0.27) at baseline and 0.40 (−0.94, 0.74) at *T1*. Symptomatic hATTR patients have a median Z score of 3.39 (1.80, 3.09) at baseline and 2.58 (1.70, 2.91) at *T1*

asymptomatic hATTR patients, with a median (IQR) of 5.4 (4.2–8.3) pg/ml ($P = .003$, Figure 1B).

Receiver operating characteristics (ROC) curve analysis was used to assess sNfL capacity to discriminate asymptomatic to symptomatic transition. At baseline (Figure A1A, appendix), the area under the curve (AUC) comparing asymptomatic to symptomatic patients was 0.940 ($P = .002$). At both baseline and T1, sNfL level of 11.7 pg/mL discriminated these patients with a sensitivity of 85.7% and a specificity of 100% (Youden index of 0.86).

3.3 | NfL levels are increased in hATTR symptomatic patients compared to healthy controls (Figure 2A)

The median (IQR) sNfL levels in the 4532 HC was 7.3 (5.6–10.0) pg/ml.[18] The median (IQR) Z score of all hATTR patients, at both baseline and *T1*, was 1.85 (0.46, 2.75). There was no significant difference between asymptomatic patients, at both baseline and *T1*, and HC with a median (IQR) Z score of −0.29 (−0.97, 0.53), with a corresponding *P*-value of 0.77. There was however a significant difference between symptomatic patients, at both baseline and T1, and HC with a median (IQR) Z score of 2.52 (1.82, 2.93), with a corresponding *P*-value of .01. (Figure 2B).

ROC curve analysis was used to assess sNfL Z-score capacity to discriminate asymptomatic to symptomatic transition. At baseline (Figure A1B, appendix), the area under the curve (AUC) comparing asymptomatic to symptomatic patients was 0.917 ($P = .004$). At both baseline and *T1*, sNfL Z score of 1.45 discriminated these patients

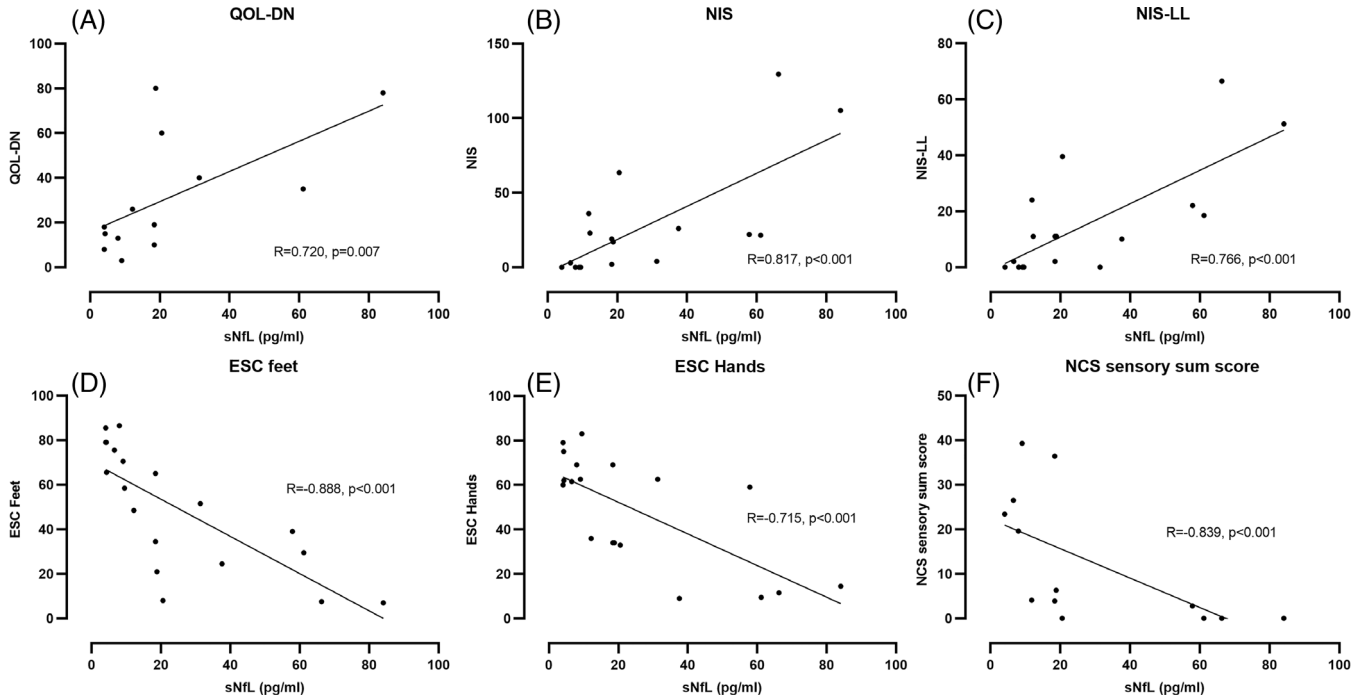

FIGURE 3 Strongest correlations between sNfL levels and disease severity scores at baseline. There is a strong correlation between sNFL levels and (A) QOL-DN ($R = 0.720$, $P = .007$), (B) NIS ($R = 0.817$, $P < .001$), (C) NIS-LL ($R = 0.766$, $P < .001$), (D) ESC in feet ($R = -0.888$, $P < .001$), (E) ESC in hands ($R = -0.715$, $P < .001$) and (F) NCS sensory sum score ($R = -0.839$, $P < .001$)

with a sensitivity of 85.7% and a specificity of 100% (Youden index of 0.86).

3.4 | NfL levels correlates with peripheral neuropathy severity (Figure 3)

We found a significant correlation between sNfL levels and most clinical and electrophysiological disease severity scores, either at baseline or *T*1 (Appendix, Table A2). The strongest correlations were found with the PND score at *T*1 ($R = 0.778$, $P < .001$), QOL-DN at baseline and *T*1 ($R = 0.720$, $P = .007$ and $R = 0.723$, $P < .001$ respectively), NIS score at baseline and *T*1 ($R = 0.817$, $P < .001$ and $R = 0.775$, $P < .001$ respectively), NIS-UL subscore at *T*1 ($R = 0.732$, $P < .001$ respectively), NIS-LL subscore at baseline and *T*1 ($R = 0.766$, $P < .001$ and $R = 0.777$, $P < .001$ respectively), ESC in feet at baseline and *T*1 ($R = -0.888$, $P < .001$ and $R = -0.707$, $P < .001$), ESC in hands at baseline ($R = -0.715$, $P < .001$), NCS motor sum score at *T*1 ($R = -0.886$, $P < .001$ respectively) and NCS sensory sum score at baseline and T1 ($R = -0.839$, $P < .001$ and $R = -0.785$, $P < .001$ respectively).

With multiple linear regression analysis, we estimated the predictive value for disease severity of several parameters at baseline and T1 (age, sex, BMI, ongoing treatment, presence of diabetes, TTR mutation, birthplace, and sNfL levels). sNfL level was the only variable to be a predictor of every disease severity score, although not always reaching significance (appendix, Table A3). Of note, age was not a strong predictor of disease severity, except for the NIS-LL score at baseline ($\beta = 0.665$, $P = .01$) and the NCS sensory sum score at *T*1 ($\beta = -0.634$, $P = .007$), neither was the presence of diabetes, who was a significant predictor only for the PND score ($\beta = 1.994$ $P = .003$) and R-ODS score ($\beta = -11.231$, $P = .02$) at baseline.

3.5 | NfL tends to increase over time in hATTR patients (Figure 4)

sNfL levels was stable during the 1 year follow-up in the whole cohort, with a median (IQR) sNfL of 15.3 (6.9, 36.0) pg/ml at baseline and 15.1 (8.3, 36.4) pg/ml at *T*1 ($P = .154$). However, sNfL tended to increase over 1-year time in symptomatic patients, with a median (IQR) sNfL of 19.7 (12.1, 58.7) pg/ml at baseline and 28.0 (12.1, 50.9) pg/ml at *T*1 ($P = .426$) and in asymptomatic patients, with a median (IQR) sNfL of 5.4 (4.2, 8.3) pg/ml at baseline 7.5 (4.9, 11.2) pg/ml at *T*1 ($P = .094$).

In symptomatic patients, sNfL levels tended to increase in the six untreated patients, with a median (IQR) sNfL of 16.4 (8,2, 43.5) pg/ml at baseline and 23.1 (11.4, 50.9) pg/ml at *T*1, the four patisiran-treated patients, with a median (IQR) sNfL of 42.6 (18.5, 79.6) pg/ml at baseline and 47.1 (25.6, 103.9) pg/ml at *T*1 and the two tafamidis-treated patients (sNfL values from baseline to *T*1 of 11.8 and 18.4 to 20.8 and 17.1 pg/mL respectively). In the two symptomatic patients that were started on patisiran during the follow-up, there was a decrease tendency in sNfL levels (sNfL values from baseline to T1 of 57.9 and 31.3 to 37.3 and 8.2 respectively). We did not identify any patient who worsened significantly during the follow-up. Both

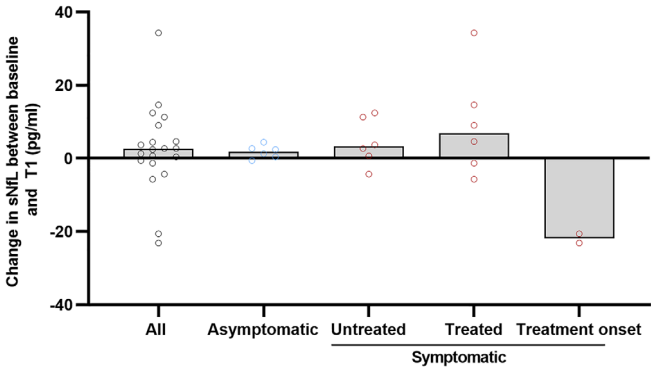

FIGURE 4 Variation of sNfL concentration between baseline and *T1*. All six untreated patients underwent liver transplantation. Of the six patisiran-treated patients, two also underwent liver transplantation. Treatment onset group corresponds to both patients who initiated patisiran during the follow-up. The top/bottom of the gray box represents the median. Median sNfL variation is +2.6 pg/mL in all hATTR patients, +1.9 pg/mL in asymptomatic hATTR patients, +3.2 pg/mL in symptomatic untreated hATTR patients, +6.8 pg/mL in symptomatic treated patients and −21.9 pg/mL in symptomatic hATTR patients who switched to patisiran

patients who started patisiran during the follow-up were not treated because of disease worsening but because diagnosis had just been confirmed at baseline.

4 | DISCUSSION

To our knowledge, this is the first prospective study to have assessed sNfL levels, in a one-year follow-up, in patients with hATTR amyloidosis who have undergone a comprehensive and thorough clinical (with measurement of several functional scores) and electrophysiological evaluation.

In this study, we have shown that sNfL levels are significantly higher, about 3.6-fold, in symptomatic hATTR patients compared to asymptomatic ones. Because amyloid levels tend to increase physiologically beyond the age of 60,[30] with possible axonal loss related to amyloid deposits, we specifically looked for a difference between our under 60-year-old symptomatic and asymptomatic patients. We confirmed that sNfL levels were also significantly higher in symptomatic hATTR patients (3.4-fold) in this population. There was also a significant difference in the sNfL levels of symptomatic hATTR patients, compared with a healthy control cohort, with a median Z score of 2.52. Those data are consistent with the results of previous studies on the subject, which found sNfL levels 4–10 times higher in symptomatic hATTR patients compared to asymptomatic ones or controls.[14-17] However, we found no significant difference between asymptomatic hATTR patients and healthy controls (Z score − 0.29). Therefore, in our study, there seems to have a correlation between elevation of sNfL level and symptoms onset.

In the previous studies, mean sNfL levels in symptomatic patients were much higher (between 66.4 and 69.4 pg/mL) than in our study (between 32.3 and 35.0 pg/mL).[14,16,17] There are a couple of reasons for this difference. First, we included younger hATTR symptomatic patients, with a mean age of 47.3-years, compared to a mean age between 59 and 65.8-years in the other studies.[14,16,17] Second, we probably included patients with a milder neuropathy. For example, the median PND score was I in our study, with 36% of patients with a PND > I, against a median PND score of II, with 74% of patients with a PND > I in Ticau's study.[17]

In Maia's study, it was suggested that a sNfL level of 10.6 pg/mL (mean age of hATTR patients 43.5 years) could discriminate asymptomatic with symptomatic hATTR patients, either PND I or PND > I, with a sensitivity of 96.2% and 92.3% respectively, and a specificity of 93.8% for both.[15] In another study, the threshold was set at 37 pg/mL (mean age 60.5 years for hATTR patients and 58.6 years for healthy controls), to discriminate healthy controls from hATTR patients with polyneuropathy, with a sensitivity of 84.9% and specificity of 96.4%.[17] In our cohort, a threshold at 11.7 pg/mL could discriminate symptomatic with asymptomatic patients with a sensitivity of 85.7% and a specificity of 100%. This cut-off depends on patient age, especially in patients over 60 years, which is why further studies are needed to determine an age-dependent cut-off. The best option may be not to use a unique sNfL cut-off, but to base individual cut-off on percentiles and z-scores from a reference healthy control population, depending on age and BMI.[18] Using the Z scores of our data, compared to this reference healthy control population, we could define a Z score threshold at 1.45, which could discriminate symptomatic with asymptomatic patients with a sensitivity of 85.7% and a specificity of 100%.

In addition to the correlation with symptoms onset, sNfL levels increase in parallel with clinical severity. In our study, there was a significant correlation between sNfL levels and most disease severity scores, with the strongest correlations being with NIS score, ESC in feet and NCS sensory sum scores. One of the strongest correlations was found with the NIS-LL subscore, a score that has been shown to be one of the most sensitive in the follow-up of patients with hATTR polyneuropathy.[31] This correlation between sNfL levels and some disease severity scores (PND, NIS, CMTES-R, and mNIS+7) was already suggested in previous studies.[14-16]

sNfL level tends to increase over time, particularly in symptomatic hATTR patients. In our study, the increase was not significant during the one-year follow-up, as we were unable to identify any patient who significantly worsened, nor asymptomatic patients who became symptomatic. This would have allowed us to determine whether this worsening was associated or preceded with a significant increase in sNfL levels, which would have confirmed the utility of sNfL as a marker of disease onset. The prediction of clinical worsening by an elevation of sNfL level could indeed favor a more rapid therapeutic modification. Data from Berends et al. (unpublished) demonstrated a rise of sNfL levels in an asymptomatic hATTR patient, a few years before the neuropathy was detected with NCS.[32]

Finally, we also showed that the introduction of gene silencing therapy with patisiran in two of our patients was associated with a

reduction in sNfL levels (mean reduction −21.9 pg/mL). This had already been demonstrated by Ticau et al., with a reduction of sNfL levels lasting at least 18 months.[17] In the patisiran global open-label extension study (unpublished data), reduction of sNfL levels under patisiran was sustained during the 4.5 and 5 years of follow-up.[33] This illustrates that patisiran treatment effectively stops the neuronal destruction process and supports the hypothesis of a certain degree of reversibility of the neuronal damage responsible for the amyloid neuropathy symptoms. This reversibility was already suggested in the APOLLO trial, where a substantial proportion of patient improved certain clinical severity scores, such as the mNIS+7, after patisiran introduction.[8]

Our study has several limitations. First, our sample of patients with hATTR amyloidosis is small and heterogeneous (asymptomatic, symptomatic treated, and untreated patients) making correlations and comparative analyses difficult to interpret. Second, we included a patient considered symptomatic (patient number 2, Table A1) because she had undergone liver transplantation several years earlier. Nevertheless, this patient is now asymptomatic, with a NIS score of 0, normal electrophysiological examinations, and very low sNfL level (4.1 and 6.8 pg/mL at baseline and *T*1 respectively). It is possible that this patient was paucisymptomatic at the time of the liver transplantation, and that the transplantation led to a reversibility of the few existing symptoms, explaining the current asymptomatic state. In any case, this underlines the difficulty of classifying those patients who have very few nonspecific sensory symptoms. In addition, complete electrodiagnostic data was not available for all hATTR patients (14/20). Finally, an already mentioned limitation is the relative short duration of follow-up, which did not allow detection of significant differences in sNfL levels or of clinically worsening patients. This underlines that monitoring sNfL over such a period of time, provides few relevant information on clinical worsening. It might be useful to do a longer-term follow-up of sNfL levels in hATTR patients, with annual dosing over 5 years for example.

In conclusion, sNfL is a promising biomarker of peripheral neuropathy in hATTR amyloidosis. In this prospective study, we showed that it can reliably distinguish between asymptomatic and symptomatic patients and is well correlated with peripheral neuropathy severity, as measured with functional and electrophysiological scores. It may be a reliable biomarker to detect disease onset. However, it is difficult to determine a definite sNfL cut-off over which neuropathy would appear, and the use of deviation measure (like Z score) from age- and BMI-adjusted cohort should be considered. We could not confirm whether sNfL elevation precedes the onset of neuropathy, but recent data in the literature suggest it. Larger scale studies with longer longitudinal follow-up are needed to confirm our data.

CONFLICT OF INTEREST

Marie Théaudin received travel grants, speaker honoraria, fees for advisory boards from Alnylam and Sobi; research grant from Pfizer. All the other authors report no disclosures.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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APPENDIX A

Disease severity scores: PND stratifies patient disability into six stages: PND 0 no impairment; PND I sensory disturbances but preserved walking capacity; PND II impaired walking capacity but ability to walk without a stick or crutches; PND IIIa walking only with the help of one stick or crutch and IIIb with the help of two sticks or crutches and PND IV confined to a wheelchair or bedridden. We converted this score in ordinal variable from 0 to 4 (0 = 0, I = 1, II = 2, IIIa = 3, IIIb = 4, IV = 5). FAP stage stratifies patients disability into four stages: FAP 0 no impairment; FAP I mild symptoms limited to lower limbs, ambulatory; FAP II moderate symptoms, needs support for ambulation; FAP III severe symptoms, bedridden or wheelchair bound. NIS is a composite score of clinical impairments (weakness, reflex loss and sensory loss), range 0–180, with higher score indicating more impairment. We distinguished the upper limb NIS score (NIS-UL) from the lower limb NIS score (NIS-LL). CADT is a questionnaire which evaluates the main symptoms of autonomic dysfunction, range 0–16 in females and 0–20 in males, with lower scores indicating more autonomic symptoms. SFN-SIQ is a questionnaire which evaluates somatic and autonomic symptoms related to small fiber neuropathy, range 0–15 for the screening and 0–45 for the follow-up, with higher score indicating more symptoms. R-ODS is a disability score, range 0–48, with lower scores indicating more disability. Norfolk QOL-DN Is a questionnaire of quality of life, range −4–136, with higher scores indicating worst quality of life.

For the measurement of ESC with Sudoscan, the lower limit of the norm was set at 60 μS for hands and 70 μS for feet.

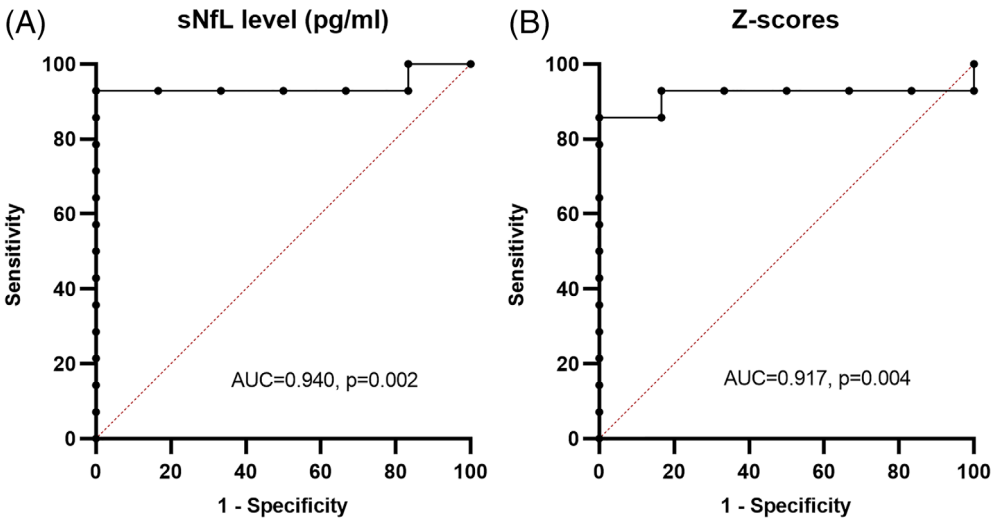

FIGURE A1 ROC curves of sNfL levels (A) and Z-scores (B) for hATTR patients, illustrating the sensitivity and the specificity in differentiating asymptomatic and symptomatic patients at baseline. AUC = area under the curve

TABLE A1 Clinical and demographic characteristics and sNfL levels of each 20 hATTR patients.

	Sex	Age (years)	Sym.	LT (Y/N) and delay (years)	Treatment	PND	FAP	QOL-DN	RODS	NIS	NIS-LL	NIS-UL	SFN-SIQ	CADT	Handgrip (R/L)	ESC (F/H)	S. sum score	M. sum score	sNFL (pg/ml)
1	M	52	Y	Y (16.7)	Patisiran	I	I	80	42	17	11	6	11	13	120/100	21/34	6.3	9	18.9
					Patisiran	II	I	64	42	19	14	5	11	14	113/98	9.5/43	2.6	12.5	33.5
2	F	42	Y	Y (17.9)	None	I	I	8	48	0	0	0	3	15	76/70	85.5/79	23.4	15.4	4.1
					None	0	0	7	47	0	0	0	1	16	80/79	84/81	12.1	15.9	6.8
3	F	60	Y	Y (9.9)	Patisiran	IV	III	78	23	105.25	51.25	54	7	9	40/30	7/14.5	0	3.5	84.1
					Patisiran	IV	III	67	25	111	63.5	47.5	9	11	30/35	18.5/21	0	4.4	118.4
4	F	46	Y	Y (11.4)	None	0	0	NA	46	0	0	0	0	16	70/80	58.5/83	NA	NA	9.5
					None	0	0	2	48	0	0	0	0	16	70/75	73/81	NA	NA	13.2
5	F	43	Y	Y (11.1)	None	I	I	26	43	23	11	12	11	13	100/100	48.5/36	NA	11.1	12.2
					None	I	I	15	43	14	4	10	9	13	100/95	58/42	22.2	12.4	12.9
6	F	39	N	N	None	0	0	3	48	0	0	0	4	14	58/60	70.5/62.5	39.3	18.2	9.0
					None	0	0	3	47	1	0	1	7	9	70/68	68/65	56.9	18.3	11.7
7	F	56	Y	Y (17.8)	None	II	I	60	25	61.5	39.5	22	5	11	50/50	8/33	0	4.6	20.5
					None	II	I	59	26	47.5	37.5	10	8	9	40/45	11.5/18.5	0	4.5	32.9
8	M	71	Y	N	None	II	I	NA	43	22	22	0	7	13	62/74	39/59	2.8	9.2	57.9
					Patisiran	II	I	77	25	22	14	8	6	15	70/62	72/58.5	4.8	9.1	37.3
9	M	41	Y	N	Tafamidis	IIIb	II	NA	25	36	24	12	9	16	70/80	NA	4.1	5.2	11.8
					Tafamidis	II	I	67	16	25.75	19.75	6	8	16	65/90	6/25.5	0	5.5	20.8
10	F	69	Y	N	Patisiran	IIIb	II	NA	29	129.5	66.5	63	6	10	NA	7.5/11.5	0	0	66.3
					Patisiran	IIIb	II	68	26	112.25	54.25	58	9	9	32/30	24.5/26	0	0.6	60.6
11	F	36	N	N	None	I	I	15	45	0	0	0	1	16	NA	65.5/62	NA	NA	4.3
					None	0	0	13	45	0	0	0	4	12	74/60	73.5/67.5	38.8	16.6	6.7
12	M	32	N	N	None	0	0	NA	NA	0	0	0	0	NA	NA	79/75	NA	NA	4.2
					None	0	0	2	48	0	0	0	0	20	120/140	NA	27.3	21.6	3.6
13	F	48	N	N	None	I	I	13	48	0	0	0	7	12	100/72	86.5/69	19.6	15.8	8.0
					None	I	I	8	47	0	0	0	4	14	98/64	82/69	26.7	16.3	8.4
14	M	39	Y	N	Patisiran	I	I	10	40	19	11	8	6	9	70/70	34.5/34	3.9	6.2	18.4
					Patisiran	I	I	29	40	21	18	3	7	10	80/80	13.5/45	0	6.8	23.0
15	F	54	N	N	None	I	I	NA	48	3	2	1	2	16	65/50	75.5/61.5	26.5	13.4	6.6
					None	I	I	14	47	2	2	0	6	11	66/64	72/69	23.4	14.4	11.0
16	F	27	N	N	None	I	I	18	45	0	0	0	3	16	NA	79/60	NA	NA	4.1
					None	I	I	33	45	8	2	6	9	10	74/60	78.5/56.5	38.8	16.6	5.4
17	M	39	Y	Y (0.4)	None	I	I	35	43	21.5	18.5	3	12	17	100/90	29.5/9.5	0	1.3	61.2

TABLE A1 (Continued)

	Sex	Age (years)	Sym.	LT (Y/N) and delay (years)	Treatment	PND	FAP	QOL-DN	RODS	NIS	NIS-LL	NIS-UL	SFN-SIQ	CADT	Handgrip (R/L)	ESC (F/H)	S. sum score	M. sum score	sNFL (pg/ml)
					None	I	I	27	46	16	10	6	8	14	110/110	33.5/33	0	2.6	56.9
18	F	38	Y	Y (−1.1[a])	None	I	I	NA	47	26	10	16	5	16	NA	24.5/9	NA	NA	37.6
					None	II	I	111	24	18	8	10	10	8	48/38	10/8	NA	NA	48.9
19	F	31	Y	N	None	I	I	40	46	4	0	4	7	16	100/95	51.5/62.5	NA	22.4	31.3
					Patisiran	I	I	20	45	2	2	0	7	14	100/82	35/69.5	NA	22.1	8.2
20	F	35	Y	N	Tafamidis	I	I	19	48	2	2	0	8	15	NA	65/69	36.4	14	18.4
					Tafamidis	I	I	14	48	0	0	0	4	14	98/95	46.5/71.5	27.7	14.1	17.1

Note: First line of each patient is the baseline, and second line is the follow-up at *T*1.

Abbreviations: Age, Age at baseline; delay, delay between transplantation or treatment and baseline; F, feet; F, female; H, hand; L, left; LT, Liver transplantation; M, male; N, no; NA, not available; R, right; Sens, sensory; Sym, symptomatic; Y, yes.

[a]Patient 18 had a liver transplant 1.12 years after baseline.

TABLE A2 Correlation between sNfL level and clinical/electrophysiological score at both baseline and *T*1. Significant *P*-values are in bold

	R (Spearman)	*P*-value
PND	0.569	**.009**
	0.778	**<.001**
FAP	0.485	**.030**
	0.667	**.001**
R-ODS	−0.568	**.011**
	−0.621	**.004**
CADT	−0.328	.170
	−0.310	.183
SFN-SIQ	0.585	**.007**
	0.571	**.009**
QOL-DN	0.720	**.007**
	0.723	**<.001**
NIS	0.817	**<.001**
	0.775	**<.001**
NIS-UL	0.684	**<.001**
	0.732	**<.001**
NIS-LL	0.766	**<.001**
	0.777	**<.001**
Handgrip right	−0.109	.709
	−0.440	.052
Handgrip left	−0.152	.601
	−0.256	.276
ESC feet	−0.888	**<.001**
	−0.707	**<.001**
ESC hands	−0.715	**<.001**
	−0.682	**.001**
NCS motor sum score	−0.643	**.012**
	−0.886	**<.001**
NCS sensory sum score	−0.839	**<.001**
	−0.785	**<.001**

Note: First line of each patient is the baseline, and second line is the follow-up at *T*1.

TABLE A3 Multiple regression analysis result; prediction of disease severity score from sNfL levels. Significant *P*-values are in bold

	B-value	*P*-value
PND	0.0306	**.002**
	0.0282	**<.001**
R-ODS	−0.133	.07
	−0.144	.08
CADT	0.010	.64
	−0.044	.06
SFN-SIQ	0.0781	**.04**
	0.0570	**.03**
NIS	0.695	**.01**
	0.841	**<.001**
NIS-UL	0.382	**.002**
	0.379	**<.001**
NIS-LL	0.404	**.006**
	0.465	**<0.001**
ESC feet	−0.782	**.002**
	−0.621	**.01**
ESC hands	−0.863	**<.001**
	−0.671	**<.001**
NCS motor sum score	−0.133	.051
	−0.157	**.004**
NCS sensory sum score	−0.276	**.049**
	−0.342	**.002**

Note: First line of each patient is the baseline, and second line is the follow-up at T1.