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Long-Term Dynamics of CSF and Serum Neurofilament Light Chain in Adult Patients With 5q Spinal Muscular Atrophy Treated With Nusinersen

Isabell Cordts,[1,*] Cornelia Fuetterer,[2,*] Annika Wachinger,[1] Ricarda von Heynitz,[1] Tobias Kessler,[3,4] Maren Freigang,[5] Anna Lisa Quinten,[6] Bogdan Bjelica,[7] Svenja Brakemeier,[8] Elke Hobbiebrunken,[9] Tim Hagenacker,[8] Susanne Petri,[7] Jan Christoph Koch,[10] Andreas Hahn,[6] Paul Lingor,[1] Marcus Deschauer,[1] Rene Günther,[5] Markus Weiler,[3] Bernhard Haller,[2] and Emily Feneberg[1]

Correspondence
Dr. Feneberg
emily.feneberg@tum.de

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Abstract

Background and Objectives

The availability of disease-modifying therapies for 5q-associated spinal muscular atrophy (SMA) has heightened the need to identify suitable biomarkers. This study investigates neurofilament light chain (NfL) concentrations during long-term nusinersen treatment in adult SMA.

Methods

In a retrospective study of prospectively collected data, NfL concentrations in the CSF (cNfL) and serum (sNfL) were measured in patients with SMA from 8 German centers and in neurologic controls using a single-molecule array (Simoa) assay. NfL concentrations and clinical characteristics, including the clinical scores Hammersmith Functional Motor Scale Expanded (HFMSE), Revised Upper Limb Module (RULM), and Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R), were analyzed for defined treatment intervals (T1–T4 [loading phase until 4 months], T5–T8 [until 23 months], T9–T12 [until 37 months], and T13–T19 [until 60 months]). Linear mixed models with a random intercept were used to assess the changes in NfL levels during treatment, considering time and covariates as fixed effects.

Results

One hundred thirteen adult patients with SMA (median age 35, 46% female), with a treatment duration of maximum 60 months, and 52 controls were included. At baseline, NfL concentrations were significantly higher in SMA {cNfL median, 585 (interquartile range [IQR] 428–787) pg/mL; sNfL, 11 (IQR 8–14) pg/mL} than in controls (cNfL, 420 [IQR 323–662] pg/mL; sNfL, 8 [IQR 6–12] pg/mL) (cNfL, $p = 0.021$; sNfL, $p = 0.030$). Median differences for all clinical scores were the highest for T5–T8 compared with the loading phase (Δ HFMSE, 0.6 [IQR 0.1–1.4], $p = 0.017$; Δ RULM, 0.9 [IQR 0.4–1.3], $p < 0.001$; Δ ALSFRS-R, 0.7 [IQR 0.4–1.0], $p < 0.001$), but not for subsequent intervals. Longitudinal analysis revealed a significant decrease of NfL concentrations during each treatment interval compared with the loading phase ($p < 0.05$, respectively) except for sNfL in T13–T19. Even among patients with no measurable clinical improvement (Δ HFMSE ≤ 0), more than 50% showed declining cNfL and sNfL levels up to T13–T19.

Discussion

NfL decreased during nusinersen treatment, suggesting its potential as a pharmacodynamic response marker in adult SMA. However, in patients without detectable clinical improvement, our study cannot determine whether they represent a more sensitive outcome measure or are not clinically meaningful.

*These authors contributed equally to this work as co-first authors.

[1]Department of Neurology, Klinikum rechts der Isar, TUM School of Medicine and Health, Technical University of Munich, Germany; [2]Institute of AI and Informatics in Medicine, TUM School of Medicine and Health, Technical University of Munich, Germany; [3]Department of Neurology, Heidelberg University Hospital, Germany; [4]Clinical Cooperation Unit Neuro-oncology, German Cancer Consortium (DKTK), German Cancer Research Centre (DKFZ), Heidelberg, Germany; [5]Department of Neurology, University Hospital Carl Gustav Carus Dresden, Germany; [6]Department of Child Neurology, University Hospital Giessen, Germany; [7]Department of Neurology, Hannover Medical School, Germany; [8]Department of Neurology, and Center for Translational Neuro- and Behavioral Sciences (C-TNBS), University Medicine Essen, Germany; [9]Clinic for Paediatric and Adolescent Medicine, University Medicine Göttingen, Germany; and [10]Department of Neurology, University Medicine Göttingen, Germany.

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Glossary

ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised; **AUC** = area under the curve; **BMI** = body mass index; **cNfL** = cerebrospinal fluid; **HFMSE** = Hammersmith Functional Motor Scale Expanded; **IQR** = interquartile range; **NfL** = neurofilament light chain; **RULM** = Revised Upper Limb Module; **sNfL** = serum NfL; **TUM** = Technical University of Munich.

Introduction

5q Spinal muscular atrophy (SMA) is a neurodegenerative motor neuron disease caused by biallelic variants in the *SMN1* gene, resulting in insufficient survival of the motor neuron (SMN) protein. The clinical phenotype of SMA is wide, ranging from severely disabled infants who do not reach motor milestones to less-affected individuals who remain ambulatory until an older age. Nusinersen, an antisense oligonucleotide that modulates the splicing of *SMN2* and leads to increased SMN protein production, was the first among now several disease-modifying drugs approved for SMA. Preapproval clinical trials have demonstrated remarkable improvements in motor function and survival in pediatric patients,[1,2] prompting approval for patients with SMA of all subtypes and ages. The therapeutic benefit of the drug in older patients with a longer disease duration has been addressed by multiple studies after approval[3-6]; however, the used clinical outcome measures encounter limitations in certain subpopulations.[3]

Neurofilaments are useful in the diagnosis and prognosis of neurodegenerative disorders including amyotrophic lateral sclerosis (ALS).[7-10] It is important that neurofilaments have shown promise for pediatric SMA in several biomarker categories[11]: levels were elevated in SMA infants compared with controls[1,12,13] (diagnostic biomarker), correlated with motor function[12] (prognostic biomarker), rapidly declined following treatment with nusinersen[13,14] (response biomarker), and were associated with motor milestone in presymptomatic individuals[15] (risk biomarker). However, the utility of neurofilaments as suitable biomarkers in adolescent and adult SMA populations has not yet been established. To date, neurofilaments have been analyzed cross-sectionally between adult patients with SMA and controls[16-18] or longitudinally during short nusinersen treatment observations,[16,17,19] yielding mostly no significant changes. Hence, this study aimed to assess the long-term neurofilament light chain (NfL) CSF and serum dynamics in a large, multicenter cohort of adult patients with SMA being treated with nusinersen.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

This study was approved by the local ethics committee of the Technical University of Munich (TUM) (approval no. 733/21 S-KK), as well as the local ethics committees of the centers involved. All patients provided written informed consent.

Sample Collection and Study Participants

Patient samples were collected from 8 German neurologic centers: departments of neurology in Munich (n = 17), Dresden (n = 23), Essen (n = 6), Giessen (n = 26), Göttingen (n = 14), Hannover (n = 16), Heidelberg (n = 24), and the Clinic for Pediatric and Adolescent Medicine in Göttingen (n = 1). Most patients were enrolled in the German Network for Motor Neuron Diseases (MND-NET) and/or the SMArtCARE registry.[20] Clinical data and biomaterials were prospectively collected at the respective sites. For our study, we conducted a retrospective analysis of these prospectively collected data and biofluids. Inclusion criteria for the final analysis were an age of ≥18 years, genetically confirmed 5q SMA, and treatment with nusinersen at the time of sample acquisition. All patients received nusinersen according to the prescription information, with 4 loading doses of 12 mg on days 0 (T1, baseline), 14 (T2), 28 (T3), and 63 (T4), followed by a maintenance dose of 12 mg once every 4 months. Sample collection was regularly performed at each center on the respective treatment day. Individuals without available CSF or serum samples during the loading phase (T1–T4) were excluded from the final analyses.

Demographic characteristics, including age, sex, weight, height, body mass index (BMI), and clinical parameters, for all patients from the respective sites were centrally compiled for our project. Patients were classified according to their SMA type (1–3) and functional status (walkers, sitters, and non-sitters) at baseline.[21] Disease duration was calculated based on the age at disease onset and the initiation of nusinersen treatment. Disease-relevant scores, including the Hammersmith Functional Motor Scale Expanded (HFMSE), Revised Upper Limb Module (RULM), and Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised (ALSFRS-R), were routinely collected during nusinersen administration. The HFMSE (33 items) and RULM (22 items) assess motor and arm function, with higher scores indicating better function. The ALSFRS-R evaluates functional status across 12 items, including respiratory and bulbar function, with higher values reflecting a less severe disease stage.

All control samples were collected from a single center (TUM). Biofluids from patients with various neurologic conditions undergoing diagnostic procedures are routinely stored in the local biobank. Corresponding pairs of CSF and serum samples from individuals without neurodegenerative or CNS inflammatory diseases, of similar age and sex to adult patients with SMA, were identified from the local biobank's

data collection platform. The control group with single measurements was patients with primary headache and idiopathic facial paresis. In addition, longitudinal controls of patients with idiopathic intracranial hypertension had consecutive CSF and serum samples at irregular intervals, often due to clinical deterioration. Demographic data were available for all our control participants.

Sample Analysis

Samples were shipped on dry ice and centrally collected and stored at −80°C at TUM. On receipt of all samples, NfL concentrations in the CSF (cNfL) and serum (sNfL) were measured using a single molecular array (Simoa) immunoassay with a fully automated HD-X analyzer (Quanterix, Lexington, MA). Measurements were performed using the NfL advantage kit from Quanterix according to the manufacturer's instructions (Lot: 503729). While multicentric samples were randomized, CSF and serum samples from the same patient, whether from single or longitudinal time points, were measured together on a single plate to minimize intra-individual variability. For quality control, CSF and serum pools were measured in duplicate per plate, in addition to duplicates of the control samples included in the NfL kits. The mean coefficients of variations for the kit controls and pools were 7.8 (intra-assay) and 14.0 (interassay), respectively.

Statistical Analysis

Continuous variables are described as median and interquartile range (IQR), with the first and third quartiles in square brackets (median [IQR Q1–Q3]). Differences in baseline characteristics of SMA and control groups were tested using *t* tests for quantitative variables and χ^2 tests for qualitative variables. As NfL levels were not normally distributed, logarithmic values were used for specific analyses. Mann-Whitney *U* tests were performed to compare NfL levels between patients with SMA and controls. When SMA subgroups (types, functional status) were compared with the control group, resulting *p* values were adjusted for multiple testing using the Holm procedure (p*). The optimal cutoff level for dichotomising values was chosen based on the highest Youden index, and the area under the curve (AUC) was calculated. The association of baseline NfL levels with baseline characteristics was examined with a Spearman rank correlation coefficient (ρ) and the corresponding 95% CI provided in square brackets [CI lower bound to upper bound]. To investigate the correlation between cNfL and sNfL levels, between-subject correlations were calculated,[22] including all available data over time for each participant, weighted by the number of combined measures available for each individual. The corresponding correlation coefficient (r) and CI are provided. To assess changes in NfL levels and clinical scores, we first selected 3 time points: T5, representing the first measurement after the loading phase of nusinersen when a therapeutic concentration is expected to be achieved; and T9 and T13, chosen to maintain consistent 4-month intervals. Individual treatment time points (T5, T9, and T13) were compared with baseline (T1) using a paired Wilcoxon signed-rank test for both the NfL levels and clinical scores. Since no linear relationship between treatment time and NfL levels could be assumed, we defined treatment intervals (T5–T8, T9–T12, and T13–T19), corresponding to earlier time points, but with an extended final interval to accommodate the reduced number of available data points. Intervals were compared with the loading phase (T1–T4) using paired *t* tests. To investigate HFMSE and NfL changes during nusinersen therapy in relation to patients' baseline HFMSE scores, we analyzed the changes in HFMSE scores and NfL levels during each treatment interval relative to the initial assessment, defined as the average HFMSE or corresponding NfL level for T1–T4. We then investigated the development of NfL levels over time by fitting linear mixed-effects models with NfL levels as the dependent variable and either time as a continuous fixed covariate or treatment intervals as fixed covariates, allowing us to analyze the nonlinear relationship between time and NfL levels more flexibly. In all the models, a random intercept for each participant was considered. For each mixed model, the estimated regression coefficients (b) and corresponding p values are provided for the different covariates. In the final analysis, we assessed the change in NfL levels and HFMSE scores from each treatment interval compared with the loading phase. All statistical analyses were performed using R version 4.2.2.[23]

Data Availability

Data are available from the corresponding on reasonable request.

Results

Demographic and Characteristics of the Study Groups

A total of 126 adult patients with SMA were assessed for eligibility. After excluding individuals without any samples during the loading phase (n = 13), 113 patients remained for the final analysis, including 6 individuals with SMA type 1, 39 with type 2, and 68 with type 3. The control group comprised 52 patients without neurodegenerative or inflammatory diseases of the CNS (primary headache, n = 32; idiopathic facial paresis, n = 20). There were no significant differences in age or sex between patients with SMA and controls (Table 1). The characteristics of all patients with SMA and the control group are summarized in Table 1 and the SMA subgroup characteristics in eTable 1.

In some cases, the timing of nusinersen treatment deviated from the intended schedule (e.g., a patient was unable to attend their scheduled visit). Consequently, actual treatment time points differed from intended ones, as shown in eTables 2 and 3. The median duration of nusinersen treatment was 14.3 months, corresponding to T7. The longest observation period for patients with available cNfL and sNfL data corresponded to samples measured after 59.9 months of treatment (T19).

Table 1 Study Group Characteristics of Patients With SMA and Controls

Demographics	SMA (n = 113)	Controls (n = 52)	*p* Value
Age (y), median (IQR)	35 (27–44)	35 (26–44)	0.163
Sex, female, n (%)	52 (46.0)	24 (46.2)	0.987
Weight (kg), median (IQR)[a]	60.0 (44.5–76.0)	79.0 (65.8–90.0)	<0.001
Height (m), median (IQR)[a]	1.7 (1.5–1.7)	1.7 (1.7–1.8)	<0.001
BMI (kg/m^2), median (IQR)[a]	21.5 (18.3–26.7)	25.7 (22.5–28.4)	0.006
Disease characteristics			
Age at onset (y), median (IQR)	2.0 (1.0–8.0)		
Disease duration, (y), median (IQR)	25.5 (17.5–33.3)		
SMN2 copy number, n (%)[b]			
1	1 (0.9)		
2	8 (7.3)		
3	61 (60.0)		
4+	39 (35.9)		
SMA type, n (%)			
Type 1	6 (5.3)		
Type 2	39 (34.5)		
Type 3	68 (60.2)		
Functional status, n (%)			
Nonsitters	30 (26.5)		
Sitters	49 (43.4)		
Walkers	34 (30.1)		
Scoliosis and medical devices, n (%)			
Scoliosis	86 (76.1)		
Previous scoliosis surgery	37 (32.7)		
Wheelchair-use	31 (27.4)		
Feeding tube	29 (25.7)		
Duration of nusinersen treatment (y), median (IQR)	14.3 (2.1–29.8)		
Clinical scores at baseline[c]			
HFMSE score (of 66), median (IQR)	11.0 (2.5–37.0)		
RULM score (of 37), median (IQR)	19.0 (13.8–34.3)		
ALSFRS-R score (of 48), median (IQR)	32.0 (28.0–38.8)		
Sample collection			
At baseline, n for CSF/serum (paired)	88/78 (68)	52/52 (52)	
During nusinersen treatment, n for CSF/serum (paired)			
T1–T4	113/87 (87)		
T5–T8	97/77 (77)		
T9–T12	91/73 (70)		
T13–T19	44/49 (40)		

Continued

Table 1 Study Group Characteristics of Patients With SMA and Controls *(continued)*

Demographics	SMA (n = 113)	Controls (n = 52)	*p* Value
Baseline NfL levels			
cNfL (pg/mL), median (IQR)	585.0 (428.0–787.0)	419.5 (322.8–662.3)	
sNfL (pg/mL), median (IQR)	11.1 (8.0–13.8)	8.0 (5.9–12.0)	

Abbreviations: ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised; BMI = body mass index; cNfL = neurofilament light chain in the cerebrospinal fluid; HFMSE = Hammersmith Functional Motor Scale Expanded; IQR = interquartile range; RULM = Revised Upper Limb Module; SMA = spinal muscular atrophy; sNfL = neurofilament light chain in serum.
[a] Height and weight (and BMI) were available for 111 patients with SMA and 50 controls.
[b] Data for *SMN2* copy number were available for 109 patients with SMA. For 2 individuals in the group with an *SMN2* copy number of 3, the copy number was reported to be 3–4 (due to an inherent limitation of the method used for copy number determination).
[c] Data for clinical scores were available for 62 (RULM and HFMSE) and 66 (ALSFRS-R) patients with SMA.

As treatment with nusinersen was not started at the same time in all individuals, the treatment duration varied across patients, and the total number of samples for each treatment time point decreased over time (eFigure 1). Paired serum and CSF samples were available for most cases (Table 1).

NfL Levels at Baseline

At baseline, cNfL levels were higher in patients with SMA (585.0 [IQR 428.0–787.0] pg/mL) compared with those in the control group (419.5 [IQR 322.8–662.3] pg/mL, p = 0.021) (Figure 1A). Similarly, sNfL levels were higher in patients with SMA (11.1 [IQR 8.0–13.8] pg/mL) compared with those in the controls (8.0 [IQR 5.9–12.0] pg/mL, p = 0.030) (Figure 1A). A cutoff concentration of 428 pg/mL for cNfL and 7.98 pg/mL for sNfL discriminated SMA from controls with a sensitivity of 74% and a specificity of 52% (AUC, 0.63 [CI 0.54–0.72]) for cNfL, and a sensitivity of 76% and specificity of 51% (AUC, 0.61 [CI 0.51–0.71]) for sNfL.

Testing the SMA types against the control group with adjustment for multiple testing, significantly higher cNfL levels were only observed in patients with type 3 (649.5 [IQR 106.0–8,460.0] pg/mL, p^* = 0.021, Figure 1A, eTable 4). When comparing the functional status, only sNfL levels (12.1 [IQR 9.2–15.0] pg/mL) in sitters were significantly elevated when compared with controls (p^* = 0.018, Figure 1B, eTable 4). NfL levels did not significantly differ between SMA patients with high (≥35 points) and low (<35 points) HFMSE score. Further subgroup comparisons are presented in eTable 5.

Characteristics of NfL Levels and Correlations With Clinical Variables at Baseline

At baseline, NfL levels in paired CSF and serum samples showed a positive correlation (SMA, ρ = 0.26 [CI 0.02–0.47], p = 0.032; controls, ρ = 0.60 [CI 0.39–0.75], p < 0.001, Figure 1C). NfL levels correlated with age in both patients with SMA (cNfL, ρ = 0.23 [CI 0.02–0.42], p = 0.030; sNfL, ρ = 0.30 [CI 0.09–0.49], p = 0.007) (Table 2) and controls (cNfL, ρ = 0.73 [CI 0.58–0.84], p < 0.001; sNfL, ρ = 0.46 [CI 0.21–0.65], p < 0.001).

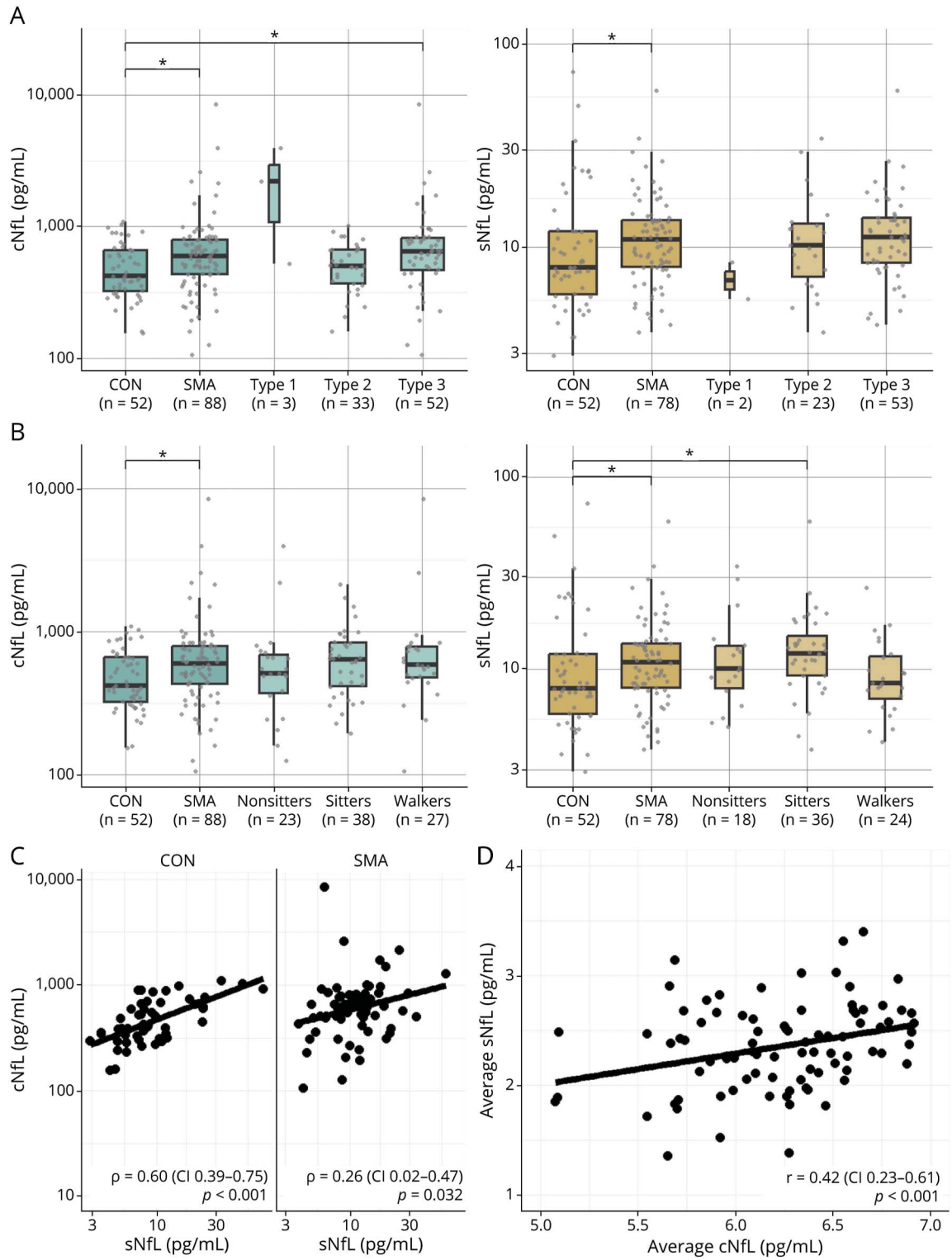
Furthermore, cNfL, but not sNfL, was significantly positively correlated with BMI in patients with SMA (ρ = 0.29 [CI 0.09–0.47], p = 0.006). There was a strong positive correlation between sNfL, but not cNfL levels, and disease duration (ρ = 0.49 [CI 0.30–0.64], p < 0.001); however, disease duration was also strongly correlated with age (ρ = 0.83 [CI 0.76–0.88], p < 0.001). When focusing on a subset of patients with type 2 and 3 SMA (n = 32) with a similar age range (31.5 [IQR 25.0–37.3] years for type 2; 31.0 [IQR 25.0–38.0] years for type 3), a positive significant correlation persisted between disease duration and sNfL (ρ = 0.47 [CI 0.20–0.68], p = 0.002) but not cNfL levels. In a subset of age-matched walkers and nonsitters (n = 26), the correlation between disease duration and sNfL persisted (ρ = 0.60 [CI 0.33–0.78], p < 0.001). No significant correlations were observed between baseline NfL concentrations and baseline clinical scores (Table 2).

Characteristics of NfL, Clinical Scores, and Their Correlations Over Time

First, we analyzed the characteristics of NfL levels in patients with SMA over time. On calculating the between-subject correlation between cNfL and sNfL levels for all included treatment time points, a significant positive association was found (r = 0.42 [CI 0.23–0.61], p < 0.001; Figure 1C).

In a next step, clinical scores and NfL values were analyzed over the course of the treatment period. The clinical scores at T1 were compared with a single time point of treatment (T5, T9, and T13). Clinical scores were significantly higher at T5 (median difference Δ HFMSE, 2.0 [CI 1.0–3.0], p = 0.001; Δ RULM, 1.0 [CI 0.0–1.5], p = 0.009; Δ ALSFRS-R, 1.0 [CI 1.0–2.0], p = 0.001) and at T9, but only for RULM and ALSFRS-R (Δ RULM, 1.5 [CI 0.5–2.5], p = 0.002; Δ ALSFRS-R, 1.0 [CI 0.0–0.5], p = 0.041) (eTable 6). When comparing NfL concentrations of these time points with T1, cNfL levels were significantly decreased at T9 (Δ −91.0 [CI −182.5 to −15.5], p = 0.017), but not at T5 and T13 (Figure 2A, eTable 7). Levels of sNfL were significantly lower at T5 (Δ −1.1 [CI −2.2 to −0.1], p = 0.033), but not at T9 and T13 (Figure 2A, eTable 7).

Figure 1 Baseline NfL Levels in Patients With SMA and Controls



cNfL and sNfL concentrations at baseline in all patients with SMA, as well as categorized by SMA types (A) and functional status (B) on a logarithmic scale. Paired *t* test of subgroups compared with controls, adjusted for multiple testing with the Holm procedure. Spearman correlation (ρ, CI and *p* value) of cNfL and sNfL levels at baseline for CON (n = 52) and SMA (n = 68) (C). Between-subject correlation of logarithmized cNfL and sNfL levels for all included treatment time points (D). CON = controls; cNfL = neurofilament light chain in the cerebrospinal fluid; SMA = spinal muscular atrophy; sNfL = neurofilament light chain in the serum.

To gain a more detailed understanding of how the clinical scores and cNfL and sNfL levels changed throughout the treatment period, we defined 4 intervals, starting with the loading phase (T1–T4) and 3 advancing treatment intervals (T5–T8, T9–T12, and T13–T19). The medians of the average clinical scores and NfL concentrations during each

Table 2 Spearman Correlation of Baseline Logarithmized cNfL and sNfL Levels With Clinical Characteristics of Patients With SMA

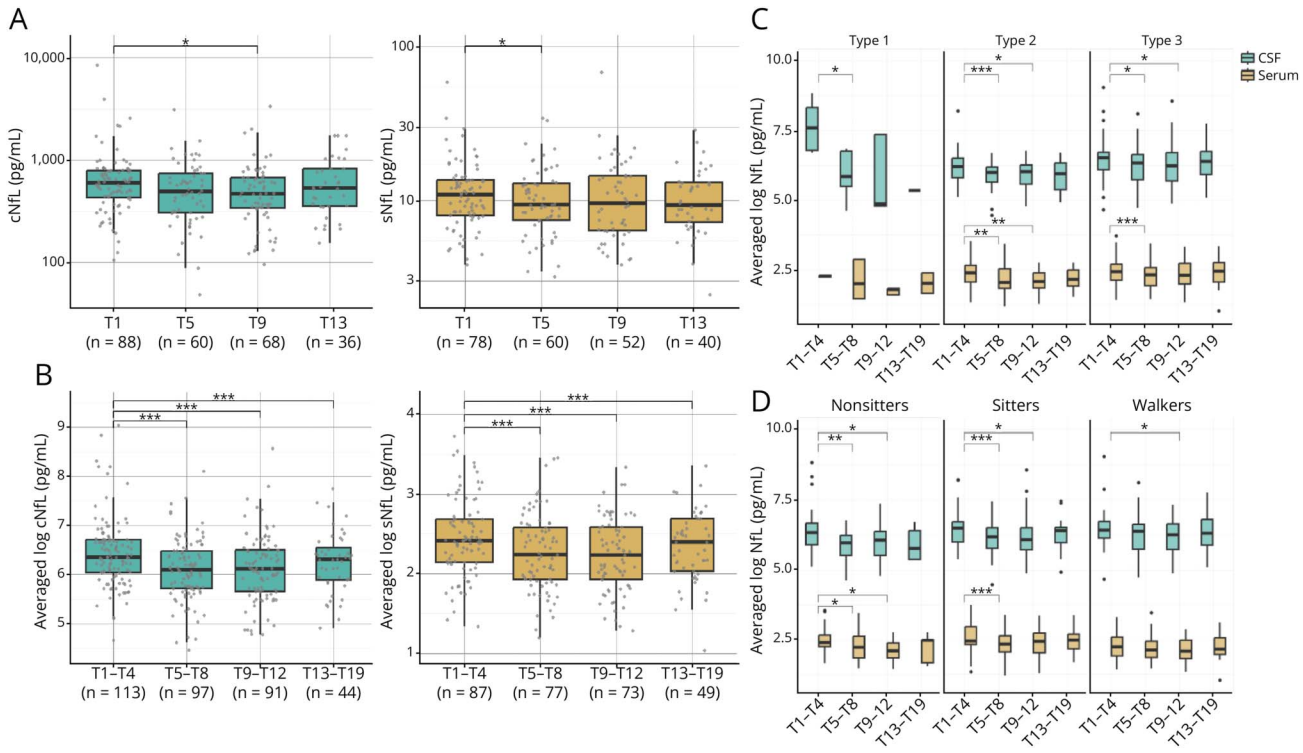
Clinical category	Sample size, n for CSF/serum	Correlation with cNfL ρ (CI)	p Value	Correlation with sNfL ρ (CI)	p Value
Age	88/78	0.23 (0.02 to 0.42)	0.030	0.30 (0.09 to 0.49)	0.007[a]
BMI	88/78	0.29 (0.09 to 0.47)	0.006	0.16 (−0.06 to 0.37)	0.161
Disease duration	82/78	0.14 (−0.08 to 0.34)	0.22	0.49 (0.30 to 0.64)	0.001[a]
HFMSE	71/74	0.12 (−0.11 to 0.35)	0.310	−0.15 (−0.36 to 0.09)	0.215
RULM	73/74	0.03 (−0.20 to 0.26)	0.774	−0.13 (−0.34 to 0.11)	0.285
ALSFRS-R	56/63	0.05 (−0.21 to 0.31)	0.692	−0.19 (−0.42 to 0.06)	0.130

Abbreviations: ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised; BMI = body mass index; ρ = rho; cNfL = neurofilament light chain in cerebrospinal fluid; HFMSE = Hammersmith Functional Motor Scale Expanded; RULM = Revised Upper Limb Module; sNfL = neurofilament light chain in serum.
[a] Age and disease duration were strongly correlated.

treatment interval were compared with those of the loading phase. Clinical scores were higher for T5–T8 (Δ HFMSE, 0.6 [CI 0.1–1.4], $p = 0.017$; Δ RULM, 0.9 [CI 0.4–1.3], $p < 0.001$; Δ ALSFRS-R, 0.7 [CI 0.4–1.0], $p < 0.001$) but did not reach statistical significance for the remaining treatment intervals when compared with those of the loading phase (Table 3).

When analyzing all patients with SMA, both cNfL and sNfL concentrations were significantly lower at all treatment intervals compared with the loading phase ($p < 0.001$) (Figure 2B, eTable 8). Comparing the decrease of NfL levels at each time interval with the loading phase by subgroups, significant decreases were found for T5–T8 in SMA type 1

Figure 2 NfL Levels in SMA Patients at Different Time Points and Intervals of Nusinersen Treatment



(A) Comparison of NfL levels during nusinersen treatment in patients with SMA at individual treatment time points (T5, T9, and T13) compared with baseline (T1) on a logarithmic scale, differences tested using a paired Wilcoxon signed rank test. (B) Comparison of averaged logarithmized NfL levels for each treatment interval (T5–T8, T8–T12, and T13–T19) compared with the loading phase (T1–T4) using paired *t* tests. Averaged logarithmized NfL levels for each treatment interval, for the different SMA types (C) and functional status (D) using paired *t* tests. cNfL = neurofilament light chain in cerebrospinal fluid; SMA = spinal muscular atrophy; sNfL = neurofilament light chain in serum.

Table 3 Averaged Clinical Scores at Baseline and Change for Nusinersen Treatment Intervals

Treatment interval	HFMSE, median (IQR)/median difference (CI)	*p* Value	RULM, median (IQR)/median difference (CI)	*p* Value	ALSFRS-R, median (IQR)/median difference (CI)	*p* Value
T1–T4	11.5 (3.8 to 40.3)		19.0 (13.4 to 34.6)		32.8 (28.0 to 39.0)	
T5–T8 vs T1–T4	0.6 (0.1 to 1.4)	0.017	0.9 (0.4 to 1.3)	<0.001	0.7 (0.4 to 1.0)	<0.001
T9–T12 vs T1–T4	0.4 (−0.7 to 1.5)	0.523	0.5 (−0.0 to 1.1)	0.070	0.4 (−0.1 to 1.0)	0.115
T13–T19 vs T1–T4	−0.1 (−1.3 to 1.4)	0.843	0.7 (−1.5 to 1.2)	0.057	0.1 (−0.8 to 0.9)	0.774

Abbreviations: ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale–Revised; HFMSE = Hammersmith Functional Motor Scale Expanded; IQR = interquartile range; RULM = Revised Upper Limb Module.
p Values are obtained by a paired Wilcoxon signed-rank test.

(only cNfL, $p = 0.021$), type 2 (cNfL, $p < 0.001$; sNfL, $p = 0.003$), type 3 (cNfL, $p = 0.041$; sNfL, $p = 0.001$), nonsitters (cNfL, $p = 0.005$; sNfL, $p = 0.019$), and sitters (cNfL, $p < 0.001$; sNfL, $p < 0.001$). In T9–T12, NfL levels decreased in SMA type 2 (cNFL, $p = 0.021$; sNfL, $p = 0.010$), type 3 (only cNfL, $p = 0.015$), nonsitters (cNfL, $p = 0.045$; sNfL, $p = 0.018$), sitters (only cNfL, $p = 0.032$), and walkers (only cNfL, $p = 0.024$). In T13–T19, NfL levels were not significantly different from the loading phase in any of the subgroups (Figure 2, C and D, eTables 9 and 10).

To investigate whether the HFMSE values during therapy changed depending on the patients' initial HFMSE scores, we analyzed the changes in HFMSE for each treatment interval relative to the initial HFMSE scores during the loading phase. At all treatment intervals, patients with low initial HFMSE values (below the median of 11.5) showed minor changes in the HFMSE. In patients with initially moderate HFMSE values, most demonstrated an increase in the score during treatment. In patients with high initial values (>60), the average difference was restored to nearly zero (eFigure 2). By contrast, except for a small range at T13–T19, consistent average decreases in cNfL and sNfL were observed under nusinersen treatment, independent of the initial HFMSE values (eFigure 2).

We then investigated the time trend as a continuous fixed effect in a random intercept model for each categorized treatment interval, which showed a significant decrease in cNfL concentration during the loading phase ($b = -0.11$, $p = 0.007$) (eTable 11). In a model considering categorized treatment intervals, and the variables with previously shown influence on NfL levels (age and BMI) as fixed effects, both cNfL and sNfL levels were lower at each time interval when compared with those at T1–T4 ($p < 0.001$, $p < 0.001$, and $p = 0.007$, respectively), with the exception of sNfL at T13–T19 (Figure 3, A and B, eTable 12). We then used a longitudinal control group consisting of 7 patients with idiopathic intracranial hypertension to compare NfL dynamics over time in controls. In this cohort, cNfL and sNfL levels were measured over a period of 76 months, and constant values were observed (eFigure 3).

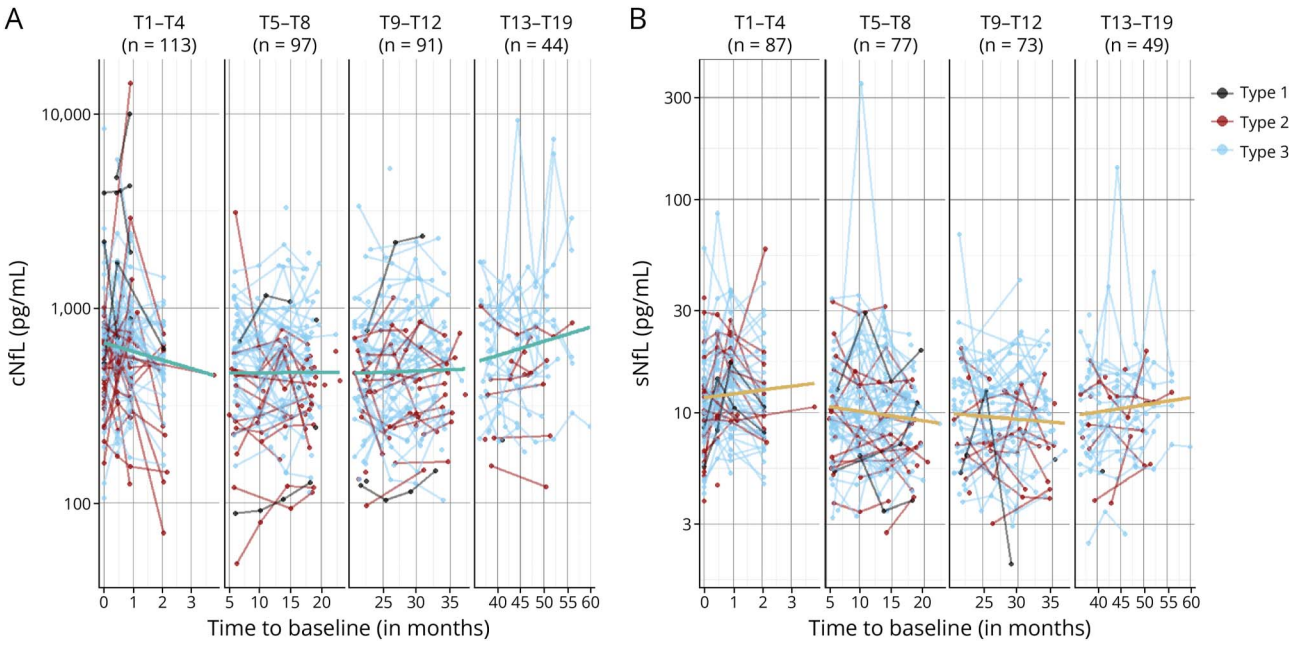
When using a mixed model and interpreting the coefficient estimates (b) observed in treated patients with SMA in relation to average NfL levels at T1–T4, we observed a geometric mean of cNfL levels that was ∼29% lower at T5–T8 ($b = -0.33$, $p < 0.001$), ∼27% at T9–T12 ($b = -0.31$, $p < 0.001$), and ∼20% at T13–T19 ($b = -0.23$, $p = 0.007$). The geometric mean of sNfL levels at T5–T8 was ∼18% lower compared with that at T1–T4 ($b = -0.19$, $p < 0.001$), ∼17% at T9–T12 ($b = -0.18$, $p < 0.001$), and ∼9% at T13–T19 ($b = -0.08$, $p = 0.116$; eTable 12). Age was associated with higher cNfL and sNfL levels ($b = 0.02$ and $p < 0.001$). This equates to an approximate increase of 2% per year from the original NfL scale. BMI had a positive but nonsignificant effect of less than 1%. The results of applying the mixed model to SMA types 2 and 3 separately are given in eTable 13 and 14, respectively. When using the covariate disease duration instead of age in our model, the same effect size and similar CIs were observed for disease duration and age ($b = 0.02$, $p < 0.001$; eTable 15).

We then assessed the association between NfL dynamics and changes in HFMSE. In SMA patients with a measurable clinical improvement (Δ HFMSE >0), a decrease in NfL levels was observed in at least 40% of the patients (cNfL, 56% in T5–T8, 57% in T9–T12, and 47% in T13–T19; sNfL, 68% in T5–T8, 68% in T9–T12, and 42% in T13–T19; Figure 4). Furthermore, in patients with SMA without a measurable clinical improvement (Δ HFMSE ≤0), a decrease in NfL levels was observed in at least 50% of the patients (cNfL, 69% in T5–T8, 65% in T9–T12, and 52% in T13–T19; sNfL, 73% in T5–T8, 67% in T9–T12, and 60% in T13–T19; Figure 4).

Discussion

As currently used motor scales are inappropriate for capturing subtle changes in locomotor ability, identifying treatment response biomarkers is important. By using ultrasensitive Simoa, which is known for its superior sensitivity compared with other methodologies,[4,10,18,19,24] we were able to detect considerably higher cNfL and sNfL levels in adult patients with SMA than in controls. However, there was significant

Figure 3 Longitudinal Trends of NfL Levels Across Nusinersen Treatment Intervals for SMA Types 1-3



Changes in cNfL (A) and sNfL (B) levels over time based on categorized treatment intervals for SMA types 1–3. Color lines represent the linear trend of NfL levels for CSF (blue) and serum (yellow) over time in each interval using a random intercept linear mixed-effects model. cNfL = neurofilament light chain in cerebrospinal fluid; SMA = spinal muscular atrophy; sNfL = neurofilament light chain in serum.

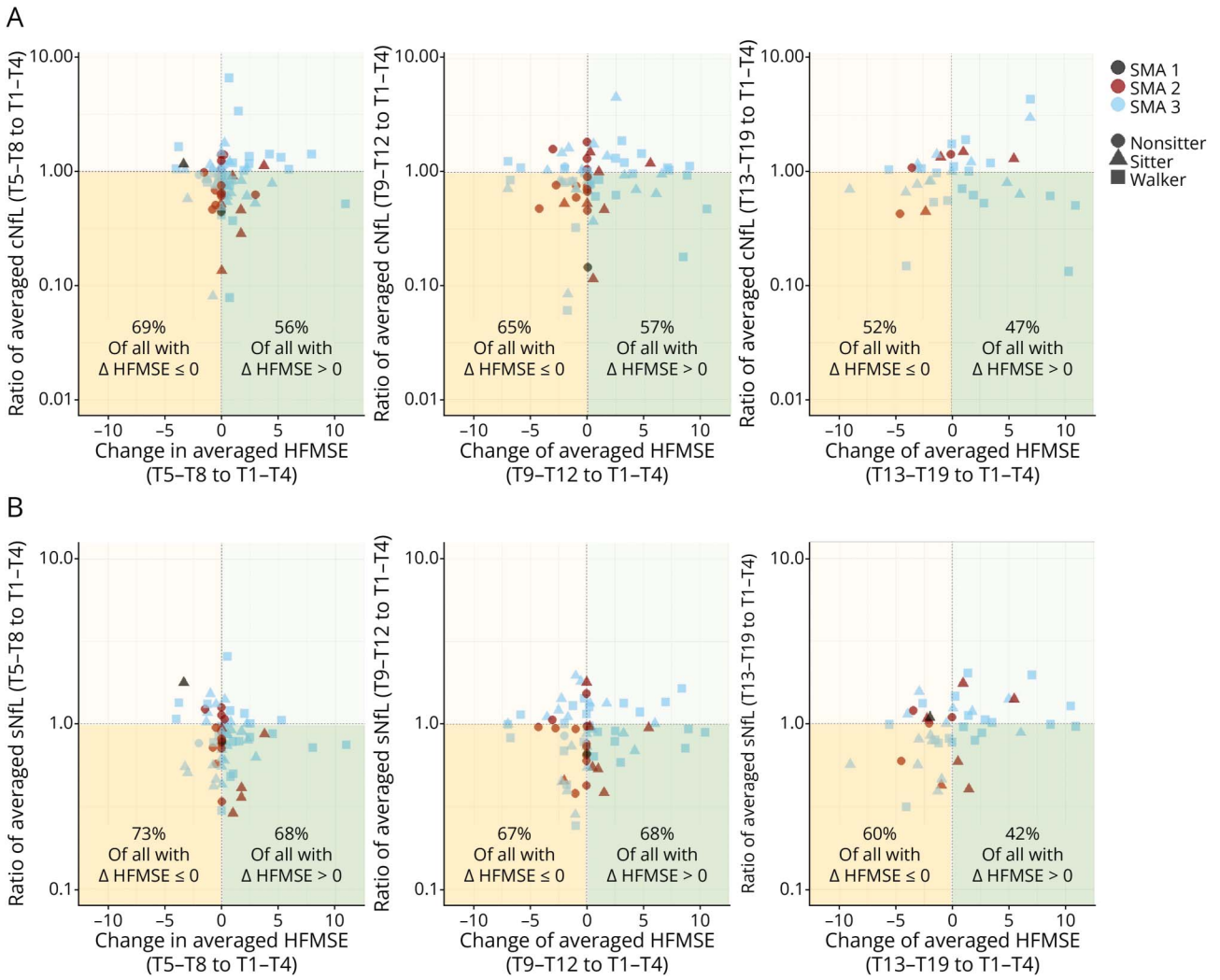
overlap in the distributions of the 2 groups, and the specificity to discriminate patients from controls was low. The correlation between CSF and sNfL levels suggests that sNfL is a surrogate marker for cNfL in adult patients with SMA. Furthermore, cNfL and sNfL levels were strongly correlated with age in both patients with SMA and controls, which is in line with the well-documented effect of age on neurofilament concentrations.[25]

When comparing SMA subgroups with controls and adjustment for multiple testing, significantly higher NfL levels were observed only in the CSF of SMA type 3 and in the serum of sitters. We believe this effect is primarily due to the larger sample sizes in these groups. Although previous studies associated higher neurofilament levels with worse motor performance,[16,17,26] our study found no correlation between NfL levels with baseline clinical scores. We then assessed the effect of disease duration and found a positive correlation between sNfL levels and disease duration, consistent with recent reports.[26] However, owing to the multicollinearity of age and disease duration, our data cannot determine whether disease duration affects neurofilaments independently of age. When analyzing a subset of individuals with a similar age range, only sNfL notably correlated with disease duration; however, the sample size was limited. Future studies with larger age-matched cohorts and normalizing neurofilament values with parameters such as creatinine concentration, an estimate of preserved muscle mass, may provide further insights.[24]

When assessing neurofilament dynamics during therapy, it is essential to thoroughly evaluate analytical approaches. Like many previous studies, we initially conducted cross-sectional analyses between individual treatment time points and baseline, revealing sporadically lower NfL levels. After dividing the treatment phase into intervals, we observed significantly lower cNfL and sNfL levels across all intervals in the combined analysis of all patients with SMA. In the longitudinal analysis, we identified a pronounced decrease in cNfL, but not in sNfL, during the loading phase, which could reflect the initial central effect of nusinersen. A recent study also indicated a rapid decline in NfL during the loading phase.[27] However, this trend was not observed for pNfH, possibly due to its longer half-life, resulting in a delayed representation of neurofilament dynamics. Another study in SMA infants enrolled in the ENDEAR trial observed a drastic reduction in pNfH concentrations during nusinersen treatment, especially during the loading phase[14]; however, levels also steadily declined in the sham control group. In these untreated SMA infants experiencing progressive motor decline, the decrease in neurofilaments has been attributed to a fulminant motor neuron loss, signifying a transition from an active to a subacute disease phase. Our analysis of a longitudinal control group found no changes in NfL levels, reducing the likelihood that the initial decline was due to repeated lumbar punctures.

To analyze changes in NfL levels and estimate their longitudinal trajectories over the treatment period, we used a linear mixed model, similar to a recent study in ALS.[10] We

Figure 4 Changes in NfL Levels in Relation to HFMSE Scores in SMA Patients Treated With Nusinersen



Visualization of the changes in cNfL (A) and sNfL (B) levels along with the changes in HFMSE scores for each individual patient and each treatment interval compared with the loading phase. For patients without a measurable clinical improvement (Δ HFMSE ≤0, yellow) and patients with a clinical improvement (Δ HFMSE >0, green), the percentages of individuals with decreasing NfL levels are displayed. cNfL = neurofilament light chain in cerebrospinal fluid; HFMSE = Hammersmith Functional Motor Scale Expanded; SMA = spinal muscular atrophy; sNfL = neurofilament light chain in serum.

considered the time of treatment as a fixed effect and categorized the treatment period into intervals to account for the nonlinear relationship between time and NfL. Significantly lower NfL levels were observed in both CSF and serum for all treatment periods, except T13–T19 for sNfL, compared with the loading phase. The largest decrease was observed at T5–T8 (treatment months 5–23), accompanied by significant improvements in HFMSE, RULM, and ALSFRS-R. In a recent large observational study of adult patients with SMA treated with nusinersen, an overall increase in motor scores at 14, 26, and 38 months was observed.[6] When analyzing the same time points as in the previous study, we observed a marked improvement in HFSME until 14 and 26 months but not thereafter (data only shown for 6, 22, and 38 months). Notably, the authors demonstrated that clinical improvement in HFMSE scores was lower in more severely affected patients, with significant differences for SMA type 2 and nonambulatory patients observed only at 14 months but not at later time points. We subsequently analyzed HFMSE dynamics relative to baseline values and observed that, on average, clinical improvement was only measurable in patients with initially moderate HFMSE values but not in those with very low or very high baseline values. This aligns with previous assertions that the utility of HFMSE and RULM may be constrained by floor and ceiling effects in individuals with severe and mild impairments, respectively.[28,29] Comparing our study cohort with the baseline characteristics of the previously mentioned 14-month analysis,[6] we observed a higher proportion of severely affected individuals, including more nonambulant patients (70% vs 58%) and those with prior scoliosis surgery (33% vs 19%), along with lower baseline HFMSE scores (median 11 vs mean 25). Thus, the lack of detectable clinical changes at later treatment times in our study may reflect the insufficient sensitivity of scores in these

patients. By contrast, we demonstrated consistent decreases in cNfL and sNfL levels during nusinersen treatment, independent of initial HFMSE values, for up to 60 and 37 months, respectively. The lack of reduction in sNfL after 37 months could be due to fewer individuals at later time points or a diminishing effect of nusinersen. If more frequent or higher doses of nusinersen could improve efficacy is currently under investigation.[30] Notably, the use of NfL as a biomarker in adult patients with SMA is limited to group-level analyses that detect and control confounding factors, while its application to individual patients in clinical settings remains uncertain and requires reliable cutoff values and a better understanding of its utility in monitoring disease progression.

As other SMN-targeted medications for SMA have emerged, biomarkers could prove valuable for patient stratification before therapy. Currently, there is no consensus on the optimal timing for treating presymptomatic individuals with 4 *SMN2* copies.[31] In the context of surveillance strategies targeting asymptomatic individuals, rising biomarkers such as NfL levels could indicate early biochemical changes signalling the transition to the symptomatic stage of SMA. Furthermore, neurofilaments, most likely in combination with other robust biomarkers, may assist in monitoring disease progression during treatment and selecting different therapeutic approaches. This is particularly relevant because combination therapies (e.g., onasemnogene abeparvovec followed by nusinersen or risdiplam) or treatment switches (e.g., from nusinersen to risdiplam, or vice versa) are now possible.[32-34]

Our study has several limitations. First is the lack of a sham control group for SMA. Our longitudinal control group was solely used to see whether repeated lumbar punctures affect NfL concentrations. In recent years, most patients with SMA have received disease-modifying therapies, significantly limiting the opportunity to conduct studies in untreated cohorts. Unlike in ALS, and other patient groups, where extensive longitudinal studies have established NfL as a valuable biomarker, our understanding of NfL dynamics in SMA is less comprehensive.[10,35,36] However, the general stability of individual NfL levels over time suggests that any significant deviation from an individual's baseline value—whether an increase or a decrease—may indicate an abnormal biological process.

Another limitation is the clinical scores used to assess motor function. It remains uncertain whether these scores truly capture relevant changes from the patient's perspective. Recent evidence shows that the minimal detectable change for the HFMSE varies significantly with age and functional status in SMA, indicating that interpreting these values, including the smallest score changes deemed meaningful by patients, should be approached cautiously when assessing treatment responses in large, heterogeneous cohorts.[37] Therefore, patient-orientated outcome measures have been suggested as valuable supplements for assessing treatment efficacy, particularly in severely affected adults, while also offering the opportunity to monitor further symptoms such as ventilation and swallowing.[38] Unfortunately, patient-orientated outcome measures are not routinely used to evaluate patients with SMA and were not available in our multicenter study.

While patients with SMA exhibit higher baseline NfL levels than controls, one might assume that a decline to "normal" levels is beneficial. However, we could not demonstrate a direct correlation between NfL changes and clinical improvements in at least 50% of cases. Thus, our study cannot determine whether NfL changes represent a more sensitive outcome measure or if they do not correspond to clinically meaningful changes for patients. Therefore, the long-term clinical benefits for individuals whose motor function changes are inadequately captured by standard scores but show decreasing NfL values still need to be demonstrated in future studies.

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Author Contributions

I. Cordts: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. C. Fuetterer: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. A. Wachinger: major role in the acquisition of data; analysis or interpretation of data. R. von Heynitz: major role in the acquisition of data; analysis or interpretation of data. T. Kessler: major role in the acquisition of data; analysis or interpretation of data. M. Freigang: major role in the acquisition of data; analysis or interpretation of data. A.L. Quinten: major role in the acquisition of data; analysis or interpretation of data. B. Bjelica: major role in the acquisition of data; analysis or interpretation of data. S. Brakemeier: major role in the acquisition of data; analysis or interpretation of data. E. Hobbiebrunken: major role in the acquisition of data; analysis or interpretation of data. T. Hagenacker: major role in the acquisition of data; analysis or interpretation of data. S. Petri: major role in the acquisition of data; analysis or interpretation of data. J.C. Koch: major role in the acquisition of data; analysis or interpretation of data. A. Hahn: major role in the acquisition of data; analysis or interpretation of data. P. Lingor: major role in the acquisition of data; analysis or interpretation of data. M. Deschauer: major role in the acquisition of data; analysis or interpretation of data. R. Günther: major role in the acquisition of data; analysis or interpretation of data. M. Weiler: major role in the acquisition of data; analysis or interpretation of data. B. Haller: major role in the acquisition of data; study concept or design; analysis or interpretation of data. E. Feneberg: drafting/revision of the manuscript for content, including medical

writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data.

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