



Development of new outcome measures for adult SMA type III and IV: a multimodal longitudinal study

Giorgia Querin, Timothée Timothée Lenglet, Rabab Debs, Tanya Stojkovic, Anthony Behin, François Salachas, Nadine Le Forestier, Maria del Mar Amador, Gaëlle Bruneteau, Pascal Laforêt, et al.

► To cite this version:

Giorgia Querin, Timothée Timothée Lenglet, Rabab Debs, Tanya Stojkovic, Anthony Behin, et al.. Development of new outcome measures for adult SMA type III and IV: a multimodal longitudinal study. *Journal of Neurology*, 2021, 268 (5), pp.1792-1802. 10.1007/s00415-020-10332-5 . hal-03230903

HAL Id: hal-03230903

<https://hal.science/hal-03230903>

Submitted on 17 Jan 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Development of new outcome measures for adult SMA type III and IV: a multimodal longitudinal study

Giorgia Querin^{1,2,3} · Timothée Lenglet^{4,5} · Rabab Debs⁴ · Tanya Stojkovic¹ · Anthony Behin¹ · François Salachas⁵ · Nadine Le Forestier^{5,6} · Maria Del Mar Amador⁵ · Gaëlle Bruneteau⁵ · Pascal Laforêt^{7,8} · Sophie Blancho⁹ · Véronique Marchand-Pauvert² · Peter Bede^{2,5,10} · Jean-Yves Hogrel¹¹ · Pierre-François Pradat^{2,5,12,13}

Received: 6 September 2020 / Revised: 19 November 2020 / Accepted: 19 November 2020 / Published online: 2 January 2021
 © Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract

Objective The aim of this study was the comprehensive characterisation of longitudinal clinical, electrophysiological and neuroimaging measures in type III and IV adult spinal muscular atrophy (SMA) with a view to propose objective monitoring markers for future clinical trials.

Methods Fourteen type III or IV SMA patients underwent standardised assessments including muscle strength testing, functional evaluation (SMAFRS and MFM), MUNIX (abductor pollicis brevis, APB; abductor digiti minimi, ADM; deltoid; tibialis anterior, TA; trapezius) and quantitative cervical spinal cord MRI to appraise segmental grey and white matter atrophy. Patients underwent a follow-up assessment with the same protocol 24 months later. Longitudinal comparisons were conducted using the Wilcoxon-test for matched data. Responsiveness was estimated using standardized response means (SRM) and a composite score was generated based on the three most significant variables.

Results Significant functional decline was observed based on SMAFRS ($p=0.019$), pinch and knee flexion strength ($p=0.030$ and 0.027), MUNIX and MUSIX value in the ADM ($p=0.0006$ and 0.043) and in TA muscle ($p=0.025$). No significant differences were observed based on cervical MRI measures. A significant reduction was detected in the composite score ($p=0.0005$, $SRM=-1.52$), which was the most responsive variable and required a smaller number of patients than single variables in the estimation of sample size for clinical trials.

Conclusions Quantitative strength testing, SMAFRS and MUNIX readily capture disease progression in adult SMA patients. Composite multimodal scores increase predictive value and may reduce sample size requirements in clinical trials.

Keywords Adult SMA · Outcome measures · Longitudinal progression · MUNIX · Clinical trial

Abbreviations

6MWT	Six-minute walk test
APB	Abductor pollicis brevis
ADM	Abductor digiti minimi
ALS	Amyotrophic lateral sclerosis
CMAP	Compound muscle action potential
CSA	Cross-sectional area
EMG	Electromyography
FDR	False discovery rate
FOV	Field of view

GM	Grey matter
LL	Lower limbs
MFM	Motor function measure
MN	Motor neuron
MND	Motor neuron disease
MMT	Manual muscle test
MRC	Medical Research Council
MRI	Magnetic resonance imaging
MU	Motor unit
MUNIX	Motor unit number index
MUSIX	Motor unit size index
SC	Spinal cord
SIP	Surface interference pattern
SMA	Spinal muscular atrophy
SMAFRS	SMA functional rating scale
SMN	Survival motor neuron
SCT	Spinal cord toolbox

Querin Giorgia and Lenglet Timothée have equally collaborated to the study.

✉ Pierre-François Pradat
pierre-francois.pradat@aphp.fr

Extended author information available on the last page of the article

SRM	Standardized response mean
TA	Tibialis anterior
TE	Echo time
TR	Repetition time
UL	Upper limbs
WM	White matter

Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive lower motor neuron disease (MND) due to biallelic loss of function of the survival-motor-neuron 1 (SMN1) gene on chromosome 5q13 [1]. Four main phenotypes are typically distinguished based on age of symptom onset and reaching of motor milestones [2, 3]. SMA type III and IV are slowly progressive forms of the disease extending well into adulthood [4, 5]: in SMA type III, symptoms start after 18-months of age, while in type IV symptom onset is after the age of 18 years [6]. Both forms typically present with a proximal to distal pattern of limb weakness [7] and with a relatively slow progression rate that tends to a plateau over time [3, 6–8]. Patients normally learn to walk unassisted, but they can lose the ability to ambulate over time [6, 7, 9].

The recent emergence of efficient disease-modifying therapies [10, 11] has highlighted an unmet need for sensitive outcome measures and biomarkers in adult SMA. Currently, disease progression in type III and IV SMA is mostly evaluated by strength measurements and functional rating scales [5, 12, 13]. Nevertheless, it is widely recognized that these tools may be suboptimal to detect subtle progressive changes in a slowly progressing condition and that quantitative approaches are required [14, 15]. Among these, specifically developed scales including performed tasks and dynamometric strength evaluation have been showed to increase sensitivity also in patients with severe neuromuscular involvement [16]. Moreover, the motor unit number index (MUNIX), a non-invasive electrophysiology technique [17], has been demonstrated to effectively detect MN loss in adult SMA and to correlate with functional disability [18]. At the same time, quantitative cervical spinal cord (SC) MRI has been shown to detect isolated grey matter degeneration in adult SMA, and to be correlated with clinical measures [19]. These findings provide the rationale to appraise their efficacy as monitoring outcome measures.

The objective of this study was to evaluate the comparative sensitivity profiles of putative outcome measures in adult SMA over a 24-month follow-up period and to assess their individual and combined predictive value.

Methods

Study population

Twenty-five genetically confirmed patients with type III and IV SMA were recruited in a prospective longitudinal study [18, 19]. Fourteen out of the 25 participants (9 type III and 5 type IV SMA) were followed-up in a 24-month longitudinal arm including standardized clinical, neurophysiological and neuroimaging evaluations.

All patients underwent genetic testing for mutations in the *SMN1* gene and for *SMN2* copy number. Based on current consensus criteria [20], patients were diagnosed as SMA type III if symptom onset occurred after 18 months of age and if they learnt to walk on time. Patients with symptom onset after 18 years of age were stratified as type IV SMA [4]. Past medical history, medications and age of symptom onset were recorded. Patients with relevant comorbidities, with any contraindication to MRI, with comorbid spinal pathology, and patients with spinal arthrodesis, which could interfere with high-quality MRI images acquisition and processing, were excluded from the study. Patients were tested at baseline and after 24-month follow-up period.

Standard protocol approvals, registrations and patient consents

The protocol was approved by the Ethics Committee of the Pitié-Salpêtrière University Hospital (Paris, France, NCT0288587) and all study participants provided informed consent.

Neuromuscular evaluation

The standardized neuromuscular evaluation comprised the following:

Muscle strength evaluation by manual testing (MMT) of individual muscles and cumulative Medical Research Council (MRC) scores: deltoid, biceps brachii, triceps brachii, extensor carpi, abductor pollicis brevis (APB) and flexor digitorum profundus for upper limbs (UL); iliopsoas, hip abductors, quadriceps femoris, hamstrings, tibialis anterior (TA), gastrocnemius and extensor hallucis longus for lower limbs (LL). All muscles were tested bilaterally and cumulative scores for UL and LL were used for statistical analyses (sum score range 0–60 for UL, 0–70 for LL).

SMA functional rating scale (SMAFRS) is a disease-specific instrument which appraises functional disability

in ten key domains of daily living. Each item is scored between 0 (completely dependent) and five (completely independent) resulting in a maximum overall score of 50 [12].

The 6-min walk test (6MWT) measures the distance a person can walk within 6 min and provides a composite proxy of cardiopulmonary and neuromuscular abilities. It is widely used as functional index in a number of neurological conditions including SMA [21, 22].

The motor function measurement scale (MFM) is a purpose-designed scale for evaluating functional impairment in neuromuscular conditions [23]. It is composed of 32 items grouped in 3 functional domains: standing and transfers (D1 sub-score), axial and proximal motor function (D2 sub-score) and distal motor function (D3 sub-score). Cumulative scores are typically expressed as percentage of the maximum possible score; the lower the total score, the more severe the functional impairment. The MFM is applicable to both ambulant and non-ambulant patients and is recognised as one of the most reliable tools for assessing motor function in SMA [24].

Quantitative muscle testing by dynamometry: dynamometric measurements were performed to quantify hand grip and pinch strength, as well as wrist, elbow, ankle, and knee extension and flexion strength. All tests were performed under standardised conditions assessing muscle strength bilaterally. The patients were given uniform instructions to produce maximal voluntary isometric contractions. For each muscle group, the maximum of two reproducible trials was recorded as the maximum voluntary isometric contraction.

Distal UL strength was assessed using the MyoGrip and MyoPinch devices [25], i.e. dynamometers which measure isometric grip strength and key pinch with accuracy even in patients with considerable weakness [16, 26, 27].

Wrist flexion and extension strength were assessed using the MyoWrist device, which measures the maximal isometric torque with a sensitivity of 0.01 Nm [28].

Ankle flexion and extension were measured by the MyoAnkle device [29] which is validated to measure the isometric ankle extension and flexion torque with a sensitivity of 0.01 kg. The distance between the head of the fifth metatarsal bone and the lateral malleolus was used to compute the torque.

Knee and elbow extension and flexion torques were assessed using a Biodex 3 Pro dynamometer in isometric conditions. The sensitivity of these measurements is regarded 0.7 Nm [25].

The strength was expressed in percentage of predicted normal values. All muscles were tested bilaterally. The mean value on both sides was retained for the statistical analysis.

Motor unit number index (MUNIX) estimation

Recordings were acquired on the same commercially available Nicolet Viking Quest[®] machine at baseline and on follow-up by the same experienced neurologist (TL).

Analysis time for sweeps was 500 ms. The high-pass filter was set at 3 Hz, and the low-pass filter at 10 kHz. MUNIX was obtained as a three-step procedure using the 2013 Natus software (version 21.1.1.200):

- 1- Supramaximal CMAPs (mV) were measured using the standard method;
- 2- area and power of the surface EMG interference pattern (SIP) for different strength levels of voluntary isometric activation were recorded (minimal to maximal, at least ten measurements);
- 3- raw CMAPs and SIP data were processed to obtain MUNIX values for each muscle [17].

Recordings with low SIP amplitude ($< 200 \mu\text{V}$) were rejected to avoid interference with volume-conducted activity of neighbouring muscles that could influence MUNIX calculation. All the subjects were tested on the right side of the body. CMAP and MUNIX were recorded at the two evaluations in the same five muscles: APB, abductor digiti minimi (ADM), deltoid, TA and trapezius. The motor unit size index (MUSIX) was calculated by dividing MUNIX by the CMAP amplitude.

Cervical spinal cord MRI acquisition protocol

SMA patients underwent 3 T cervical SC MR imaging (MAGNETOM Prisma, Siemens Healthcare, Erlangen, Germany, 64-channel head and neck coil). Structural imaging between C2 and C7 spinal levels included a sagittal 3D T2-weighted fast spin-echo SPACE sequence and an axial 2D T2*-weighted multi-echo gradient echo (MEDIC) sequence to provide high GM-WM contrast [30]. The main imaging parameters for the 3D-T2-weighted sequences included voxel size = $0.8 \times 0.8 \times 0.8 \text{ mm}^3$, FOV = $256 \times 256 \text{ mm}^2$, 72 sagittal slices, TR/TE = 1500/131 ms, acceleration factor $R = 2$. T2*-weighted imaging parameters were voxel size = $0.5 \times 0.5 \times 5 \text{ mm}^3$, FOV = $180 \times 180 \text{ mm}^2$, TR/TE = 470/17 ms, flip angle = 30° and seven slabs. T2*-weighted images were acquired at the middle of each cervical vertebral level and perpendicular to the SC axis.

Spinal cord MRI data processing

Data processing was performed using the Spinal Cord Toolbox v3.1 (SCT) [31]. Raw data were first reviewed for acquisition quality and incidental findings, based on which images from two subjects were excluded due

to motion artefacts and inadequate image quality. Total cross-sectional area (CSA) was extracted at each vertebral level from the T2-weighted images using *PropSeg* [30] and expressed in mm². GM and WM were automatically segmented on the T2*-weighted images using the *DeepSeg* tool of the SCT [32]. White (WM) and grey matter (GM) CSA were calculated in mm².

Statistical analysis

Statistical analyses were performed using JMP®, Version 13 Pro (SAS Institute Inc., Cary, NC).

Descriptive variables such as mean, median, standard deviation, percentage and range were used to summarize quantitative measures. The Shapiro–Wilk test was applied to test for normality. Comparisons between baseline and follow-up evaluation were conducted using the Wilcoxon signed rank-sum test for matched data. Responsiveness of each measure to change over time was estimated as standardized response means (SRM) value. SRM were calculated for all outcome measures by taking the average of the paired difference over the 2 years divided by the standard deviation of these differences. A high SRM (> 0.8) was considered indicative of high level of responsiveness to changes in value [33].

The false discovery rate (FDR) correction was used to account for multiple comparisons. Statistical significance level was set at $p < 0.05$.

A composite score was computed by adding the scores of the three most significant variables. Each variable was normalized to the predicted 100%. The mean value of the three percentages was used to compute the composite score.

Power analyses were conducted to estimate the sample size required in a hypothetical clinical trial to detect significant statistical differences in progression over the course of 2 years. Sample sizes needed to evaluate a variety of expected drug effects were calculated for reference, including halting disease progression (i.e., assuming mean progression estimates from the current sample among untreated and no progression among treated patients). An α level of 0.05, 80% power, and a treated-to-untreated ratio of 1:1 were assumed.

Data deposition

Anonymized data will be shared by request from any qualified investigator for the sole purpose of replicating procedures and results presented in the article and as long as data transfer is in agreement with European Union legislation on the general data protection regulation.

Results

Neuromuscular evaluation

The mean age of the patients at baseline was 43.5 years \pm 12.1. Nine patients were classified as having SMA type III and five patients were categorized as SMA type IV based on age of symptom onset. Ten patients were males and four were females. Both at baseline and follow-up visit, ten patients were ambulant and four were non-ambulant: all the non-ambulant patients had SMA type III. Eleven patients had 4 *SMN2* copies, and the remaining three patients had three of them. Muscle strength testing revealed predominantly proximal muscle weakness primarily in the lower limbs. No significant correlations were observed between muscle strength and *SMN2* copy numbers.

On neuromuscular evaluation, significant functional decline was observed on SMAFRS ($p = 0.019$, SRM = 0.61*), pinch ($p = 0.030$, SRM = −0.77) and knee flexion strength ($p = 0.027$, SRM = −0.43). Within these variables, the pinch strength had the better SRM, followed by the SMAFRS score.

No other significant changes were observed in any of the variables recorded longitudinally.

The same variables exhibited longitudinal change when considering SMA type III and by SMA type IV patients separately (p-values for SMA type III: SMAFRS score = 0.016, pinch strength = 0.004, knee flexion strength = 0.05. p-values for SMA type IV patients: SMAFRS score = 0.016, pinch strength = 0.047, knee flexion strength = 0.05).

Motor unit number estimation (MUNIX)

No significant differences were observed between the baseline and the 24-month visit for the CMAP in any of the examined muscles (all $p > 0.05$).

A significant reduction was observed between the two timepoints in the MUNIX value for the ADM ($p = 0.0006$, −31.2%, SRM = −1.18) and the TA muscle ($p = 0.025$, −13.1%, SRM = −0.54). Moreover, a significant increase was detected in the MUNIX value for the ADM ($p = 0.0043$, +41.8%, SRM = 0.84).

No further longitudinal changes were captured by the other variables. MUNIX reduction in the ADM was the most significant and responsive variable based on its SRM value.

Table 1 summarizes our longitudinal data as mean, standard deviation, p-value of the comparison between the two means (at V1 and V2) and responsiveness to change as described by the SRM value. The longitudinal profile of significant variables is visually represented in Fig. 1.

Table 1 Longitudinal parameters and their responsiveness to change

Parameter	SMA V1	SMA V2	p value	SRM
Upper Limbs strength sum score	58.7 ± 12.7	57.8 ± 11.9	0.678	0.00
Lower limbs strength sum score	53.6 ± 12.4	52.7 ± 10.8	0.481	0.00
SMAFRS score	40.4 ± 10.3	38.3 ± 10.9	0.019	−0.61
6MWT (m)	341.3 ± 247.6	333.3 ± 264.9	0.318	−0.15
MFM1%	51.3 ± 30.4	47.0 ± 32.5	0.095	−0.11
MFM2%	94.0 ± 8.7	92.0 ± 11.4	0.165	−0.32
MFM3%	98.9 ± 2.8	97.1 ± 4.6	0.201	−0.32
MFM tot%	77.7 ± 15.3	74.8 ± 17.3	0.169	−0.27
Grip (%)	56.5 ± 34.8	54.7 ± 35.4	0.233	−0.42
Pinch (%)	70.1 ± 33.0	62.1 ± 32.5	0.030	−0.77
Wrist flexion (%)	70.2 ± 46.2	71.5 ± 47.9	0.412	−0.06
Wrist extension (%)	68.3 ± 31.8	66.1 ± 39.2	0.299	−0.17
Ankle flexion (%)	52.3 ± 31.6	53.4 ± 27.8	0.525	−0.29
Ankle extension (%)	78.3 ± 39.6	66.8 ± 41.4	0.628	−0.53
Knee flexion (%)	33.9 ± 31.8	31.3 ± 34.2	0.027	−0.43
Knee extension (%)	16.0 ± 35.8	14.7 ± 32.4	0.373	−0.36
Elbow flexion (%)	46.2 ± 33.0	47.1 ± 34.2	0.285	−0.06
Elbow extension (%)	53.8 ± 58.7	49.0 ± 57.6	0.376	−0.15
CMAP APB muscle (mV)	10.2 ± 2.9	10.4 ± 2.9	0.740	−0.20
MUNIX APB muscle (a.v.)	148.4 ± 72.0	143.6 ± 66.3	0.660	−0.19
MUSIX APB muscle (μV)	79.5 ± 28.6	82.1 ± 43.7	0.440	0.07
CMAP ADM muscle (mV)	9.9 ± 2.2	9.3 ± 2.2	0.150	−0.34
MUNIX ADM muscle (a.v.)	111.2 ± 45.7	76.4 ± 54.1	0.001	−1.18
MUSIX ADM muscle (μV)	100.0 ± 32.9	172.2 ± 97.1	0.004	0.84
CMAP deltoid muscle (mV)	8.5 ± 5.3	7.5 ± 4.6	0.120	−0.48
MUNIX trapezius muscle (a.v.)	155.7 ± 55.8	130.4 ± 63.9	0.100	−0.50
MUSIX trapezius muscle (μV)	50.6 ± 13.0	49.6 ± 12.1	0.370	−0.08
CMAP TA muscle (mV)	4.9 ± 1.7	4.6 ± 1.9	0.180	−0.24
MUNIX TA muscle (a.v.)	84.4 ± 40.5	73.3 ± 37.5	0.025	−0.54
MUSIX TA muscle (μV)	66.4 ± 26.0	67.3 ± 15.7	0.560	0.04

Neuromuscular and neurophysiological parameters described as mean value and standard deviation at baseline (V1) and after 24 months' observation time (V2). The *p* value describes the comparison between the two time-points by a Wilcoxon signed rank-sum test. SRM is a measure of effect size reported for change over 2 years. All results are FDR corrected for multiple comparisons

6MWT 6-minute-walk test, *MFM* motor function measure, *SMAFRS* spinal muscular atrophy functional rating scale, *CMAP* compound motor action potential, *MUNIX* motor unit number index, *MUSIX* motor unit size index, *APB* abductor pollicis brevis, *ADM* abductor digiti minimi, *TA* tibialis anterior, *SRM* standardized response means, *FDR* false discovery rate, *a.v.* absolute value

The same variables exhibit significant change when considering patients affected by SMA type III and by SMA type IV separately (*p* values for SMA type III: MUNIX in the ADM muscle = 0.007, MUNIX in the TA muscle = 0.035, MUSIX for the ADM muscle = 0.006. *p* values for SMA type IV patients: MUNIX in the ADM muscle = 0.0002, MUNIX in the TA muscle = 0.025, MUSIX for the ADM muscle = 0.0029).

Spinal cord MRI analyses

No significant differences were noted in global GM and WM CSA between the baseline and the 24-month scans as summarized in Table 2.

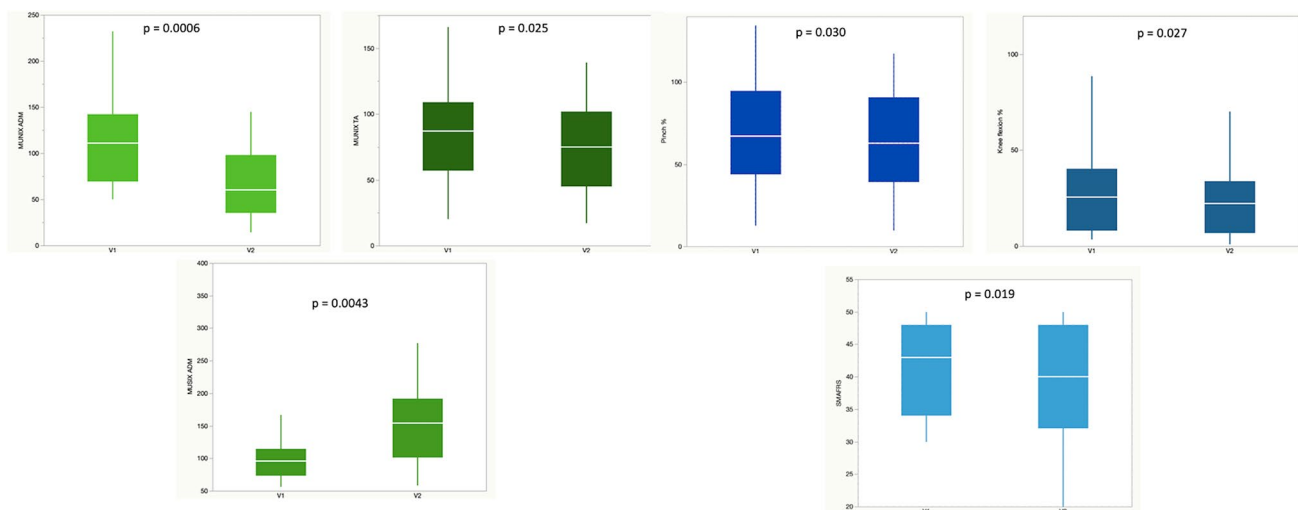


Fig. 1 Visual representation of significant parameters over time, represented as mean and standard deviation. MUNIX=motor unit number index, MUSIX=motor unit size index, ADM=abductor digit minimi. TA=tibialis anterior

Table 2 Cervical spinal cord atrophy in adult SMA patients

Parameter	SMA V1	SMA V2	<i>p</i> value
C2 vertebral level global CSA (mm ²)	75.7 ± 5.5	73.8 ± 5.4	0.18
C2–C3 vertebral level global CSA (mm ²)	72.7 ± 6.7	72.0 ± 5.5	0.45
C3–C4 vertebral level global CSA (mm ²)	74.2 ± 7.7	72.1 ± 6.6	0.35
C4–C5 vertebral level global CSA (mm ²)	72.2 ± 8.5	67.6 ± 9.3	0.25
C6–C7 vertebral level global CSA (mm ²)	59.6 ± 5.3	55.2 ± 5.6	0.10
C2 vertebral level GM CSA (mm ²)	11.8 ± 2.5	12.4 ± 0.9	0.78
C2–C3 vertebral level GM CSA (mm ²)	12.9 ± 1.4	13.1 ± 1.0	0.62
C3–C4 vertebral level GM CSA (mm ²)	13.7 ± 2.7	14.5 ± 3.0	0.82
C4–C5 vertebral level GM CSA (mm ²)	13.5 ± 2.7	13.8 ± 3.1	0.59
C5–C6 vertebral level GM CSA (mm ²)	13.0 ± 2.7	13.2 ± 3.9	0.54
C6–C7 vertebral level GM CSA (mm ²)	9.6 ± 2.1	10.1 ± 2.8	0.71
C2 vertebral level WM CSA (mm ²)	58.1 ± 9.0	52.6 ± 3.7	0.07
C2–C3 vertebral WM CSA (mm ²)	56.2 ± 10.7	51.3 ± 7.1	0.06
C3–C4 vertebral WM CSA (mm ²)	56.7 ± 13.1	54.6 ± 9.3	0.27
C4–C5 vertebral level WM CSA (mm ²)	55.1 ± 15.5	52.5 ± 14.5	0.31
C5–C6 vertebral level WM CSA (mm ²)	51.4 ± 8.8	53.7 ± 6.8	0.38
C6–C7 vertebral level WM CSA (mm ²)	44.7 ± 7.0	43.4 ± 8.5	0.84

Cervical spinal cord atrophy parameters between C2 and C7 defined as global, GM and WM CSA (mm²). Results are expressed as mean value and standard deviation and *p* values are FDR corrected for multiple comparisons

CSA cross-sectional area, GM grey matter, WM white matter, FDR false discovery rate

Definition of a proposed composite score

A composite score was generated by adding the scores of the three most responsive parameters of the longitudinal analyses (MUNIX value in the ADM, pinch strength and SMAFRS score) for the whole group of patients. These

parameters encompass three different aspects of SMA; muscle strength, functional disability in routine daily activities and electrophysiological quantification of motor neuron loss. A significant reduction of the composite score was observed over time ($p = 0.0005$) and a good SRM value was computed (SRM = −1.52) (Fig. 2).

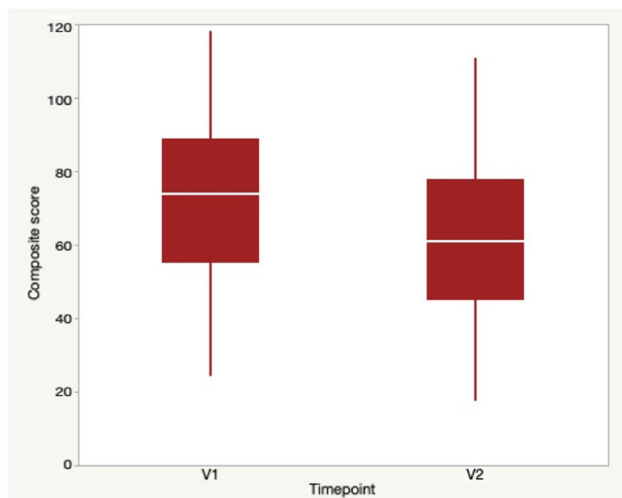


Fig. 2 Modification over time of the composite score expressed as mean value and standard deviation

Estimated sample size for trials

Estimated clinical trial sample size is highly dependent on the expected treatment effect; the smaller the effect size, the larger the sample required. For example, with the inclusion of SMA type III and IV patients of all disease severities, a total of 54 patients (27 in the treatment and 27 in the placebo arm) would be required to detect a 50% reduction in disease progression (i.e., half the deterioration seen in the current sample) over 2 years compared to 16 patients (8 in each group) needed to detect halting in progression.

Sample size was estimated for each parameter which captured longitudinal change, as evidenced by higher SRM values.

As shown in Table 3, 172 subjects would be required to demonstrate a ‘halting effect’ when considering the knee flexion strength, while only 25 participants would be

required when considering the MUNIX in the ADM, according to the higher SRM value. Crucially, the composite score, having the highest SRM between the parameters, would require a smaller patient sample to demonstrate disease halting or improvement of the motor phenotype.

Discussion

In this study, we report a 24-month longitudinal protocol testing the tracking sensitivity of different outcome measures of disease progression in a cohort of adult type III and IV SMA patients. As putative effective therapies for adult SMA are being developed [11, 12, 34], sensitive longitudinal outcome measures are urgently required. This is the first longitudinal study of adult SMA patients providing comparative analyses of different modalities to use a sufficiently long follow-up interval to detect progressive changes in slowly progressive SMA phenotypes. Accordingly, our findings allow the ranking of the monitoring potential of putative outcome measures in adult SMA and potentially their role in detecting response to therapy.

The most sensitive measure to monitor disease progression in this study is the MUNIX value in the ADM and TA muscles, the knee flexion and the pinch strength and the SMAFRS score. Among the considered outcome measures, cervical SC MRI was the least sensitive one. Despite evidence that SC MRI detects GM atrophy in the same cohort of adult SMA patients [19], no significant longitudinal change was observed over time, suggesting that MNs loss may be progressive early in the course of the disease and that SC atrophy may remain relatively stable in adult patients with a chronic and long-lasting motor impairment. The limited performance of SC MRI as a biomarker of disease progression could also be explained by the small dimension of the GM at the cervical level, which requires high-resolution image acquisitions. Improvements in MRI sequences and

Table 3 Sample size estimates for a placebo-controlled trial of 2 years

Parameter	50% Reduction of progression	75% Reduction of progression	Halting of progression	20% improvement	50% Improvement
Composite score	54	25	16	12	9
MUNIX ADM	93	43	25	18	13
Pinch%	211	95	55	36	17
SMAFRS score	334	150	85	60	39
MUNIX TA	418	187	106	75	49
Knee flexion %	682	306	172	121	78

Sample size estimates are based on projected mean change in the parameters score for variable treatment effects over 2 years. The numbers represent the total sample size based on equally sized treatment and control groups

MUNIX motor unit number index, *ADM* abductor digiti minimi, *TA* tibialis anterior, *SMAFRS* spinal muscular atrophy functional rating scale

acquisition techniques may improve tracking sensitivity and provide novel insights about the evolution of anterior horn atrophy in SMA, while further MRI evaluation of the patients after a long observation time (5–10 years) could better describe SC modifications in such a slowly progressive disease.

Functional scales and quantitative muscle strength testing are widely used to evaluate disability in SMA and other MNDs [12, 13]. They are considered highly reproducible in multicentric protocols [35, 36]. In our study, no significant decline was observed in manually evaluated muscle strength over time, but quantitative muscle testing captured reduction in knee flexion and pinch strength between the two time points. Interestingly, a significant decline was also observed in SMAFRS, which reflects the impact of muscle weakness on daily activities [12].

The most significant longitudinal measure in our cohort was the MUNIX value of the ADM and the TA muscles. Our results strongly support the statement that MUNIX is a sensitive outcome of MNs loss over time even in patients with considerable disease duration. MUNIX is a relatively recently developed method to quantify MUs loss but has already been evaluated in a range of neuromuscular conditions [37–39]. It is a widely validated and reproducible tool which has already been used in multicentric trials in ALS and international training programs have been set up to ensure standardized assessments [40–43]. In SMA, MUNIX has already been shown to be more responsive to MUs depletion than the CMAP, even in muscles with minimal clinical involvement [18]. Moreover, MUNIX captures SMA-specific patterns of MN loss which is characterized by a predominant involvement of the ulnar aspect of the hand [44, 45]. MUNIX value in the ADM muscle was the most responsive parameter in our study ($SRM = -1.18$) and it was paralleled by a significant increase in the MUSIX, suggesting that, even in long lasting disease phenotypes, collateral reinnervation is still present as a compensatory mechanism. Our neurophysiology data also mirror our quantitative muscle strength findings; pinch strength exhibited the most prominent decline over time, implying that the evaluation of distal and less severely involved muscles could provide superior sensitivity from an outcome measure point of view. A practical limitation of MUNIX is that it is difficult to obtain and to reproduce in very weak muscles, thus limiting its application in severely disabled patients [40]. The apparent discrepancy described in our patients between a significant loss of the pinch strength and a relative stability of the MUNIX value in the ABP could be in part explained by the divergent linearity of the two measures and by the high variability of the MUNIX value compared to the limited size of the study group.

Finally, our data demonstrate that the definition of a composite score encompassing different modalities of evaluation

reduces the number of subjects required to enroll in a hypothetical clinical trial. Our proposed score was composed of the SMAFRS, pinch strength and MUNIX of the ADM. Natural history study initiatives in other MNDs have shown that instead of single measures, panels of multiple indices are best suited to track progressive changes and detect response to therapy [46]. The composite score proposed by this study not only incorporates both functional and electrophysiological aspects of the disease, but also its sensitively captures progressive changes over time, making it a promising outcome measure in clinical trials. We recognize that the composite score needs to be interpreted with care, as the subscores are likely to progress at different speed and may have divergent linearity. Therefore, further validation in independent samples is required to demonstrate its longitudinal sensitivity. Moreover, the clinical relevance of progressive changes in the score remains to be established. Nevertheless, its high responsiveness to longitudinal changes underlines the importance of integrating multiple dimensions of disease dynamics that cannot be captured by a single measure.

This study is not without limitations. The study cohort was relatively small due to the low incidence of type III and IV SMA and also due to the fact that the majority of SMA patients are now under disease-modifying therapy. Second, the population was clinically heterogeneous, including patients with diverse functional status and disease duration. A larger cohort size and inclusion of type II adult SMA patients would be required to validate the responsiveness of the proposed outcome measures. A meta-analysis of other natural history studies (NatHis-SMA, NCT02391831) [27], or pooling data from studies including young adults may provide additional insights regarding the disease trajectory of SMA across various clinical stages.

Moreover, patients were evaluated after 24-months' observation time. This was imposed by the very slow disease progression of adult type III and VI patients, which implies that a long observation period is needed to test and validate even the most reliable outcome measure. We nevertheless recognize that the majority of ongoing and up-coming clinical trials testing disease-modifying drugs usually has a shorter duration (12 months in average) and that our study should be repeated considering a shorter observation time to make the results more easily comparable with those issued from therapeutic trials.

Finally, even if we were able to demonstrate that several quantitative parameters are effective in tracking disease progression in adult SMA patients, the direct implication of this modifications in clinical practice and their relation to patients' perceived disability is not linear and often not clear. Further studies are, therefore, needed to better characterize relations between the evolution of quantitative parameters and the real clinical evolution of the patients, possibly combining them with real-life inspired evaluation tools.

In conclusion, this study demonstrates the ability of MUNIX and quantitative muscle testing to detect progressive motor impairment in adult SMA. Our findings underscore the value of MUNIX as a sensitive monitoring index in adult SMA and the utility of combining complementary indices into a composite score [47]. The critical appraisal of the sensitivity profiles of putative outcome measures and biomarkers in SMA may contribute to the design of future clinical trials and ultimately to a better identification of efficient disease-modifying treatments.

Acknowledgements We are grateful for the generosity of all SMA patients who have kindly participated in this study. This study was supported by the *Association Française contre les Myopathies* (AFM-Téléthon). We also thank the *Institut pour la Recherche sur la Moelle Épineuse et l'Encephale* (IRME) for their support of this study. Peter Bede is supported by the Health Research Board (HRB EIA-2017-019), the Andrew Lydon scholarship, the Irish Institute of Clinical Neuroscience (IICN), the Irish Motor Neuron Disease Association (IMNDA) and the Iris O'Brien Foundation. The rest of the authors have no specific sponsors to declare.

Funding Supported by the Association Française contre les Myopathies (AFM-Téléthon, NCT0288587).

Compliance with ethical standards

Conflicts of interest This study was sponsored by the Association française contre les myopathies (AFM-Téléthon). Dr. Giorgia Querin, Dr. Timothée Lenglet, Dr. Rabab Debs, Dr. Tanya Stojkovic, Dr. Anthony Behin, Dr. François Salachas, Dr. Nadine Le Forestier, Dr. Maria del Mar Amador, Dr. Gaëlle Bruneteau, Dr. Sophie Blancho, Dr. Véronique Marchand-Pauvert: report no disclosures. Prof Pascal Laforêt receives funding for research and as an advisor or speaker from Sanofi Genzyme and Spark Therapeutics. Dr. Peter Bede is supported by the Health Research Board (HRB EIA-2017-019), the Andrew Lydon scholarship, the Irish Institute of Clinical Neuroscience (IICN), the Irish Motor Neuron Disease Association (IMNDA) and the Iris O'Brien Foundation. Dr. Jean-Yves Hogrel is co-inventor of the Myogrip and MyoPinch devices and did consultancy for Biogen and Sarepta. Dr Pierre- François Pradat receives funding as an advisor and speaker from Biogen.

References

- Lefebvre S, Bürglen L, Reboullet S et al (1995) Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* 80:155–165
- Munsat TL, Davies KE (1992) International SMA consortium meeting (26–28 June 1992, Bonn, Germany). *Neuromuscul Disord* 1992(2):423–428
- Wadman RI, Wijngaarde CA, Stam M et al (2018) Muscle strength and motor function throughout life in a cross-sectional cohort of 180 patients with spinal muscular atrophy types 1c–4. *Eur J Neurol* 25:512–518
- Wang CH, Finkel RS, Bertini ES et al (2007) Consensus statement for standard of care in spinal muscular atrophy. *J Child Neurol* 22:1027–1049
- Mercuri E, Lucibello S, Pera MC et al (2019) Long-term progression in type II spinal muscular atrophy: a retrospective observational study. *Neurology* 93:e1241–e1247
- Piepers S, van den Berg LH, Brugman F et al (2008) A natural history study of late onset spinal muscular atrophy types 3b and 4. *J Neurol* 255:1400–1404
- Deymeer F, Serdaroglu P, Parman Y, Poda M (2008) Natural history of SMA IIb: muscle strength decreases in a predictable sequence and magnitude. *Neurology* 71:644–649
- Bonati U, Holiga Š, Hellbach N et al (2017) Longitudinal characterization of biomarkers for spinal muscular atrophy. *Ann Clin Transl Neurol* 4:292–304
- Montes J, McDermott MP, Mirek E et al (2018) Ambulatory function in spinal muscular atrophy: age-related patterns of progression. *PLoS ONE* 13:e0199657
- Mercuri E, Darras BT, Chiriboga CA et al (2018) Nusinersen versus Sham control in later-onset spinal muscular atrophy. *N Engl J Med* 378:625–635
- Mendell JR, Al-Zaidy S, Shell R et al (2017) Single-dose gene-replacement therapy for spinal muscular atrophy. *N Engl J Med* 377:1713–1722
- Montes J, Gordon AM, Pandya S, De Vivo DC, Kaufmann P (2009) Clinical outcome measures in spinal muscular atrophy. *J Child Neurol* 24:968–978
- Finkel R, Bertini E, Muntoni F, Mercuri E, ENMC SMA Workshop Study Group (2014) 209th ENMC International Workshop: outcome measures and clinical trial readiness in spinal muscular atrophy 7–9 November 2014, Heemskerk, The Netherlands. In: *Neuromuscular disorder*, vol 25. Elsevier, The Netherlands, pp 593–602
- Kariyawasam DST, D'Silva A, Lin C, Ryan MM, Farrar MA (2019) Biomarkers and the development of a personalized medicine approach in spinal muscular atrophy. *Front Neurol* 10:898
- Montes J, Glanzman AM, Mazzone ES et al (2015) Spinal muscular atrophy functional composite score: a functional measure in spinal muscular atrophy. *Muscle Nerve* 52:942–947
- Seferian AM, Moraux A, Canal A et al (2015) Upper limb evaluation and one-year follow up of non-ambulant patients with spinal muscular atrophy: an observational multicenter trial. *PLoS ONE* 10:e0121799
- Nandedkar SD, Nandedkar DS, Barkhaus PE, Stalberg EV (2004) Motor unit number index (MUNIX). *IEEE Trans Biomed Eng* 51:2209–2211
- Querin G, Lenglet T, Debs R et al (2018) The motor unit number index (MUNIX) profile of patients with adult spinal muscular atrophy. *Clin Neurophysiol Off J Int Fed Clin Neurophysiol* 129:2333–2340
- Querin G, El Mendili M-M, Lenglet T et al (2018) The spinal and cerebral profile of adult spinal-muscular atrophy: a multimodal imaging study. *NeuroImage Clin* 21:101618
- Mercuri E, Finkel RS, Muntoni F et al (2018) Diagnosis and management of spinal muscular atrophy: part 1: recommendations for diagnosis, rehabilitation, orthopedic and nutritional care. *Neuromuscul Disord* 28:103–115
- Dunaway Young S, Montes J, Kramer SS et al (2016) Six-minute walk test is reliable and valid in spinal muscular atrophy. *Muscle Nerve* 54:836–842
- Montes J, Dunaway Young S, Mazzone ES et al (2019) Nusinersen improves walking distance and reduces fatigue in later-onset SMA. *Muscle Nerve* 60:409–414
- Vuillerot C, Payan C, Iwaz J, Ecochard R, Bérard C (2013) MFM spinal muscular atrophy study group. responsiveness of the motor function measure in patients with spinal muscular atrophy. *Arch Phys Med Rehabil* 94:1555–1561
- Bertini E, Dessaud E, Mercuri E et al (2017) Safety and efficacy of olesoxime in patients with type 2 or non-ambulatory type 3 spinal

- muscular atrophy: a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Neurol* 16:513–522
25. Allenbach Y, Benveniste O, Decostre V et al (2012) Quadriceps strength is a sensitive marker of disease progression in sporadic inclusion body myositis. *Neuromuscul Disord* 22:980–986
 26. Servais L, Deconinck N, Moraux A et al (2013) Innovative methods to assess upper limb strength and function in non-ambulant Duchenne patients. *Neuromuscul Disord* 23:139–148
 27. Chabanon A, Seferian AM, Daron A et al (2018) Prospective and longitudinal natural history study of patients with type 2 and 3 spinal muscular atrophy: baseline data NatHis-SMA study. *PLoS ONE* 13:e0201004
 28. Decostre V, Canal A, Ollivier G et al (2015) Wrist flexion and extension torques measured by highly sensitive dynamometer in healthy subjects from 5 to 80 years. *BMC Musculoskelet Disord* 16:4
 29. Moraux A, Canal A, Ollivier G et al (2013) Ankle dorsi- and plantar-flexion torques measured by dynamometry in healthy subjects from 5 to 80 years. *BMC Musculoskelet Disord* 14:104
 30. Martin AR, De Leener B, Cohen-Adad J et al (2017) A novel MRI biomarker of spinal cord white matter injury: T2*-weighted white matter to gray matter signal intensity ratio. *AJNR Am J Neuroradiol* 38:1266–1273
 31. De Leener B, Lévy S, Dupont SM et al (2017) SCT: Spinal Cord Toolbox, an open-source software for processing spinal cord MRI data. *NeuroImage* 145:24–43
 32. Gros C, De Leener B, Badji A et al (2019) Automatic segmentation of the spinal cord and intramedullary multiple sclerosis lesions with convolutional neural networks. *NeuroImage* 184:901–915
 33. Husted JA, Cook RJ, Farewell VT, Gladman DD (2000) Methods for assessing responsiveness: a critical review and recommendations. *J Clin Epidemiol* 53:459–468
 34. Oldenburg D, Guberina N, Stolte B et al (2019) Radiation exposure of image-guided intrathecal administration of nusinersen to adult patients with spinal muscular atrophy. *Neuroradiology* Epub 61:565–574
 35. Hogrel J-Y, Payan CA, Ollivier G et al (2007) Development of a French isometric strength normative database for adults using quantitative muscle testing. *Arch Phys Med Rehabil* 88:1289–1297
 36. Hogrel JY, Ollivier G, Desnuelle C (2006) Manual and quantitative muscle testing in neuromuscular disorders. How to assess the consistency of strength measurements in clinical trials? *Rev Neurol* 162:427–436
 37. Fathi D, Mohammadi B, Dengler R, Bösel S, Petri S, Kollwe K (2016) Lower motor neuron involvement in ALS assessed by motor unit number index (MUNIX): long-term changes and reproducibility. *Clin Neurophysiol Off J Int Fed Clin Neurophysiol* 127:1984–1988
 38. Bas J, Delmont E, Fatehi F et al (2018) Motor unit number index correlates with disability in Charcot–Marie–Tooth disease. *Clin Neurophysiol* 129:1390–1396
 39. Fatehi F, Grapperon A-M, Fathi D, Delmont E, Attarian S (2018) The utility of motor unit number index: a systematic review. *Neurophysiol Clin Clin Neurophysiol* 48:251–259
 40. Neuwirth C, Nandedkar S, Stålberg E et al (2011) Motor Unit Number Index (MUNIX): reference values of five different muscles in healthy subjects from a multi-centre study. *Clin Neurophysiol Off J Int Fed Clin Neurophysiol* 122:1895–1898
 41. Neuwirth C, Braun N, Claeys KG et al (2018) Implementing Motor Unit Number Index (MUNIX) in a large clinical trial: real world experience from 27 centres. *Clin Neurophysiol Off J Int Fed Clin Neurophysiol* 129:1756–1762
 42. Neuwirth C, Burkhardt C, Alix J et al (2016) Quality control of motor unit number index (MUNIX) measurements in 6 muscles in a single-subject “Round-Robin” setup. *PLoS ONE* 11:e0153948
 43. Lingor P, Weber M, Camu W et al (2019) ROCK-ALS: protocol for a randomized, placebo-controlled, double-blind phase IIa trial of safety, tolerability and efficacy of the Rho kinase (ROCK) inhibitor Fasudil in amyotrophic lateral sclerosis. *Front Neurol* 10:293
 44. Swash M (2019) Reading the palm with MUNIX: a “reversed split hand” in spinal muscular atrophy. *Clin Neurophysiol Off J Int Fed Clin Neurophysiol* 130:305–306
 45. Günther R, Neuwirth C, Koch JC et al (2019) Motor Unit Number Index (MUNIX) of hand muscles is a disease biomarker for adult spinal muscular atrophy. *Clin Neurophysiol Off J Int Fed Clin Neurophysiol* 130:315–319
 46. Chipika RH, Finegan E, Li Hi Shing S, Hardiman O, Bede P (2019) Tracking a fast-moving disease: longitudinal markers, monitoring, and clinical trial endpoints in ALS. *Front Neurol* 10:229
 47. Ard MC, Raghavan N, Edland SD (2015) Optimal composite scores for longitudinal clinical trials under the linear mixed effects model. *Pharm Stat* 14:418–426

Affiliations

Giorgia Querin^{1,2,3} · Timothée Lenglet^{4,5} · Rabab Debs⁴ · Tanya Stojkovic¹ · Anthony Behin¹ · François Salachas⁵ · Nadine Le Forestier^{5,6} · Maria Del Mar Amador⁵ · Gaëlle Bruneteau⁵ · Pascal Laforêt^{7,8} · Sophie Blancho⁹ · Véronique Marchand-Pauvert² · Peter Bede^{2,5,10} · Jean-Yves Hogrel¹¹ · Pierre-François Pradat^{2,5,12,13}

¹ Centre de Référence Maladies Neuromusculaires Paris-Est, APHP, Hôpital Pitié-Salpêtrière, Service de Neuromyologie, Paris, France

² Laboratoire D’Imagerie Biomédicale, Sorbonne Université, CNRS, INSERM, Paris, France

³ Institut de Myologie, I-Motion Adultes Plateforme, Paris, France

⁴ Département de Neurophysiologie, APHP, Hôpital Pitié-Salpêtrière, Paris, France

⁵ APHP, Centre Référent SLA, Hôpital Pitié-Salpêtrière, Paris, France

⁶ Département de Recherche en Éthique, EA 1610: Etudes Des Sciences Et Techniques, Université Paris Sud/Paris Saclay, Paris, France

⁷ Neurology Department, Nord/Est/Ile de France Neuromuscular Center, Raymond-Poincaré Hospital, Garches, France

⁸ INSERM U1179, END-ICAP, Versailles Saint-Quentin-en-Yvelines University, Montigny-le-Bretonneux, France

⁹ Institut Pour La Recherche Sur La Moelle Epinière Et L’Encéphale (IRME), Paris, France

- ¹⁰ Computational Neuroimaging Group, Academic Unit of Neurology, Trinity College, Dublin, Ireland
- ¹¹ Institute of Myology, Neuromuscular Investigation Center, Paris, France
- ¹² Northern Ireland Centre for Stratified Medicine, Biomedical Sciences Research Institute Ulster University, Altnagelvin Hospital, Derry/Londonderry C-TRIC, UK
- ¹³ Département de Neurologie, 47 Boulevard de l'Hôpital, 75634 Paris cedex 13, France