The motor unit number index (MUNIX) profile of patients with adult spinal muscular atrophy

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Highlights

- MUNIX values are significantly decreased in type III and IV SMA patients compared to healthy controls.
- The MUNIX profile of SMA patients correlates with muscle strength and disability scores.
- The MUSIX values of SMA patients are increased and suggestive of active re-innervation.

Abstract

Objective: Objective of this study is the comprehensive characterisation of motor unit (MU) loss in type III and IV Spinal Muscular Atrophy (SMA) using motor unit number index (MUNIX), and evaluation of compensatory mechanisms based on MU size indices (MUSIX).

Methods: Nineteen type III and IV SMA patients and 16 gender- and age-matched healthy controls were recruited. Neuromuscular performance was evaluated by muscle strength testing and functional scales. Compound motor action potential (CMAP), MUNIX and MUSIX were studied in the abductor pollicis brevis (APB), abductor digiti minimi (ADM), deltoid, tibialis anterior and trapezius muscles. A composite MUNIX score was also calculated.

Results: SMA patients exhibited significantly reduced MUNIX values ($p < 0.05$) in all muscles, while MUSIX was increased, suggesting active re-innervation. Significant correlations were identified between MUNIX/MUSIX and muscle strength. Similarly, composite MUNIX scores correlated with disability scores. Interestingly, in SMA patients MUNIX was much lower in the ADM than in the ABP, a pattern which is distinctly different from that observed in Amyotrophic Lateral Sclerosis.

Conclusions: MUNIX is a sensitive measure of MU loss in adult forms of SMA and correlates with disability.

Relevance: MUNIX evaluation is a promising candidate biomarker for longitudinal studies and pharmacological trials in adult SMA patients.

Keywords

SMA type III, SMA type IV, MUNIX, MUSIX, motor unit loss, biomarkers

1. Introduction

Spinal muscular atrophy (SMA) is a genetically determined lower motor neuron (LMN) disease caused by loss of function of the SMN1 gene on chromosome 5 (Finkel et al. 2015) (Lefebvre et al. 1995). The disease typically manifests with proximal and symmetrical muscle weakness and atrophy, and exhibits considerable clinical heterogeneity depending on SMN2 copy numbers (Mercuri et al. 2018). SMA type III and IV are considered slowly progressive forms of the disease, extending well into adulthood and characterized by acquisition of walking ability (Wang et al. 2007). In SMA type III, symptoms usually start after 18-month of age. Patients affected by SMA type IIIa experience symptoms before the age of 3, while SMA type IIIb patients only show symptoms after the age of 3. In type IV SMA, symptom onset is in the adulthood (Piepers et al. 2008). Both forms have a slow but relentless course with progressive muscle weakness due to motor neurons (MN) degeneration in the spinal cord and brainstem, and typically follow a proximal to distal pattern of limb weakness (Piepers et al. 2008) (Bonati et al. 2017).

Disease progression in SMA type III and IV is typically evaluated by clinical assessments; strength measurements and functional rating scales (Bonati et al. 2017). Inherently, these clinical tools suffer from considerable inter-rater variability and are suboptimal to detect subtle changes in progression. Electrophysiological measures, such as the compound motor action potential (CMAP) and motor units number estimation (MUNE), have been previously proposed as surrogate biomarkers of neurodegenerative change in clinical trials (Finkel et al. 2015). Both CMAP and MUNE are reduced in paediatric forms of SMA, and correlate with the age of the child as well as with motor function (Bromberg and Swoboda 2002) (Swoboda et al. 2005) (Lewelt et al. 2010) (Galea et al. 2001). Nevertheless, these methods have been almost exclusively applied to children with severe and rapidly progressive forms of the disease, and authoritative electrophysiological studies in adult SMA are still missing. Moreover, MUNE is technically challenging, time-consuming and invasive, and is thought to be associated with relatively low reproducibility (Swash 2017).

Motor neuron number index (MUNIX) is a more recent, non-invasive electrophysiology technique which relies on surface electromyography (EMG) and provides an estimation of the number of functional MU in a given muscle (Nandedkar et al. 2004). As opposed to other MUNE methods, MUNIX is less time-consuming and it requires only minimal nerve stimulation. Furthermore, it has a good inter-rater reliability (Neuwirth et al. 2016) (Ahn et al. 2010) and can be applied to any muscle in which a CMAP can be obtained (Nandedkar et al. 2018).

MUNIX has been applied effectively to amyotrophic lateral sclerosis (ALS) cohorts, demonstrating that it can meaningfully quantify surviving MUs both in cross-sectional (Nandedkar et al. 2010) and longitudinal study designs (Fathi et al. 2016) (Escorcio-Bezerra et al. 2017) (Neuwirth et al. 2015). Moreover, MUNIX proved indispensable in identifying MU loss in clinically asymptomatic muscles and the appraisal of surviving MUs (Fukada et al. 2016) (Neuwirth et al. 2017). MUNIX has also been proposed to be applied to other neuromuscular conditions, such as CIDP (Delmont et al. 2016), anti-MAG neuropathy (Fatehi et al. 2017) and Charcot-Marie-Tooth disease (Bas et al. 2018). The evaluation of compensatory nerve sprouting has been made possible by the detection of enlarged MUs. This can be estimated by the MU size index (MUSIX), which is calculated by dividing the CMAP amplitude by the MUNIX value (Fatehi et al. 2017).

The overall objective of this study is the characterisation of MU loss in a cohort of type III and IV SMA patients using MUNIX and the assessment of compensatory mechanisms by analysing the MUSIX profile, using healthy controls (HC) for the establishment of reference values.

2. Methods

2.1 Study population

Nineteen genetically confirmed type III (n = 14) and type IV (n = 5) SMA patients and 16 gender- and age-matched HC were recruited in a prospective cross-sectional study using standardised clinical and neurophysiological evaluations. The study protocol was approved by the Ethics Committee of the Pitié-Salpêtrière University Hospital (Paris) (NCT0288587) and all study participants provided informed consent.

All patients underwent genetic testing for mutations in the SMN1 gene. Based on current consensus criteria (Mercuri et al. 2018), patients were diagnosed as SMA type III if symptom onset occurred after 18-month of age and if they learnt to walk on time. Patients with symptom onset between 18 months and 3 years of age were classified as SMA type IIIa and patients with symptom onset between 3 and 18 years of age were diagnosed with SMA type IIIb. Patients with symptom onset after 18 years of age where stratified as type IV SMA (Wang et al. 2007).

Every patient's past medical and pharmacological history, as well as age of symptom onset, age of walking and age at diagnosis were carefully recorded.

2.2 Neuromuscular evaluation

A standardised clinical protocol was used to systematically evaluate neuromuscular performance in all muscle groups and to assess functional disability.

- Muscle strength was evaluated by manual testing (MMT) and summarised as the cumulative Medical Research Council (MRC) score of the following muscles: deltoid, biceps brachii, triceps brachii, extensor carpi, opponens pollicis and flexor digitorum profundus for upper limbs; iliopsoas, hip abductors, quadriceps femoris, hamstrings, anterior tibialis, gastrocnemius, and extensor hallucis longus for lower limbs (LL). All muscles were tested bilaterally and cumulative scores for upper (UL) and LL were used for statistical analyses (mega-score range 0–60 for UL, 0-70 for LL).

- Quantitative muscle testing by dynamometry: Dynamometric measurements were performed to quantify hand grip strength, as well as wrist, elbow, ankle, and knee extension and flexion. 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 (Seferian et al. 2015) (Li et al. 2010), that are dynamometers which measure isometric grip strength and key pinch with accuracy even in patients with considerable weakness (Allenbach et al. 2012) (Servais et al. 2013).

- **Wrist flexion and extension strength** were assessed using the MyoWrist device, which measures the maximal isometric torque with a sensitivity of 0.01 Nm (Decostre et al. 2015).

- **Ankle flexion and extension** were measured by the MyoAnkle device (Moraux et al. 2013) 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 the Biodex 3 pro-dynamometer under isometric conditions. The sensitivity of these measurements is regarded 0.7 Nm (Allenbach et al. 2012).

- SMA functional rating scale (SMAFRS) is a disease-specific instrument which appraises functional disability in 10 key domains of daily living. Each item is scored between 0 (completely dependent) and 5 (completely independent) resulting in a maximum overall score of 50 (Montes et al. 2009).

2.3 Motor unit number index (MUNIX) estimation

MUNIX is a neurophysiology technique which provides an index of functioning LMNs in a muscle. The method relies on a statistical approach based on the area and power of the supramaximally stimulated CMAP and the area and power of the surface EMG during different levels of voluntary isometric activation. Using a dedicated software, these values are integrated to compute a probable motor unit count for the estimation of functioning motor neurons (Nandedkar et al. 2004) (Nandedkar et al. 2010) (Nandedkar et al. 2018).

Recordings were acquired on a commercially available Nicolet Viking Quest® machine with disposable ground and disk electrodes (Natus® neurology), surface electrodes and a hand-held bipolar stimulator. Analysis time for sweeps was 500 msec. The high-pass filter was set to 3 Hz, and the low-pass filter to 10 kHz. MUNIX was obtained as a three-step procedure using the 2013 Natus software (version 21.1.1.200): supramaximal CMAPs (mV) were measured using the standard methods procedures; area and power of the surface EMG interference pattern (SIP) for different force levels of voluntary isometric activation were recorded (minimal to maximal, at least ten measurements) and processing of raw CMAPs and SIP data to obtain MUNIX values for each muscle (Nandedkar et al. 2004). Recordings with low SIP amplitude (< 200 µV) 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 in five muscles: abductor pollicis brevis (APB), abductor digiti minimi (ADM), deltoid, tibialis anterior (TA) and trapezius. MUNIX total score was calculated by adding the individual values of the 5 tested muscles. The motor unit size index (MUSIX) was calculated by dividing MUNIX by the CMAP amplitude (Nandedkar et al. 2010).

2.4 Statistical analysis

Statistical analyses were performed using JMP 13Pro. All the torque data were converted into percentages of normative reference strength data and predictive equations from multilinear models using age, sex, height and/or weight as covariates (Seferian et al. 2015) (Hogrel et al. 2007) (Decostre et al. 2015) (Moraux et al. 2013).

Descriptive variables such as mean, median, standard deviation, percentage and range were used to summarise quantitative measures. The Shapiro-Wilk test was applied to test for normality. Given the small sample size of study participants and that the majority of the variables showed non-normal distribution (CMAP, MUNIX and MUSIX values for ABP and ADM, MUSIX for trapezius and TA, muscle force for knee flexion and extension, elbow extension, ankle extension, MRC for the deltoid muscle and SBMAFRS values), group comparisons were performed using the 2-tailed Mann-Whitney U tests. For the same reason, non-parametric Spearman's correlation coefficient was used to assess

correlations. False Discovery Rate (FDR) was used to correct for multiple correlations. The significance level was set at $p < 0.05$.

3. Results

3.1 Clinical results

Of the 19 SMA patients (11 M: 8 F), 5 were classified as type IIIa, 9 as type IIIb and 5 as type IV. All patients reached the developmental milestone of walking between 12 and 15 months of age (mean age = 14.16 months ± 4.08, range 11-24 months). At the time of examination, 15 patients were ambulant and 4 were non-ambulant.

The mean age of the patients at the time of assessment was 43.32 years ± 14.09 while mean age of the HC (10 M:6 F, $p > 0.05$) was 39.57 years ± 13.34 ($p>0.05$). Mean age at onset of muscle weakness was 11.57 years ± 6.93 (range 1-20), and mean age at genetic diagnosis of the disease was 18.32 ± 13.20 (range 3-58). None of the patients had relevant medical comorbidities or was taking disease-modifying drugs.

3.1.1 Neuromuscular evaluation

Muscle force mega-score was 58.68 +/- 12.29 for the UL and 51.73 +/- 13.82 for the LL. Muscle weakness was predominantly proximal and affected mostly the LL. The weakness was symmetric both in the UL and LL (no significant difference was found between the left and right side for any tested muscle using a 2-tailed Mann-Whitney test, $p > 0.05$) (figure 1). Mean SMAFRS value was 39.36 ± 12.21.

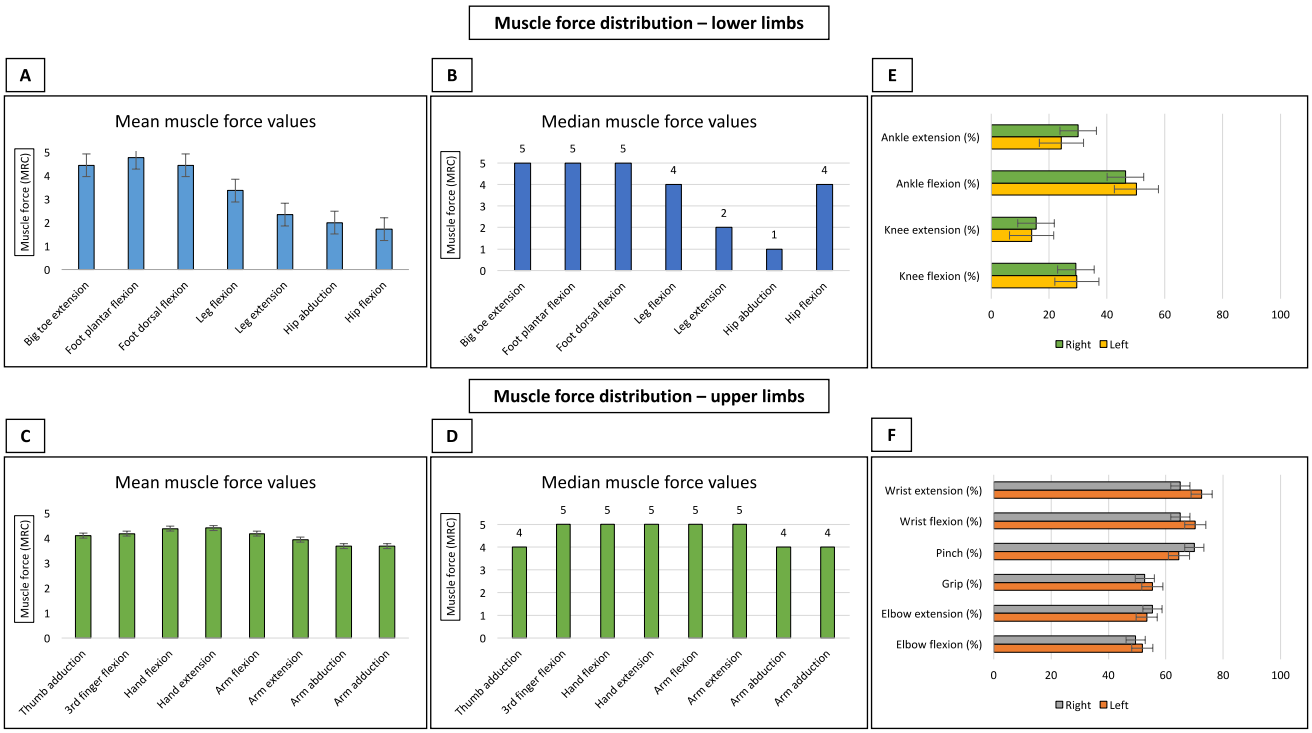

Figure 1. A and B: Patterns of muscle weakness as measured by manual muscle testing in the lower (A, B) and upper limbs (C, D) suggestive of predominantly proximal lower limb weakness. Values are expressed as mean ± standard deviation in A and C and as median values in B and D. **E and F:** The laterality of muscle weakness in the lower (C) and upper limbs (D) shows a relatively symmetric pattern. Data are presented as mean ± standard deviation.

3.2 Neurophysiology findings

Neurophysiological data are summarized in table 1. Significant CMAP reduction was only observed in muscles with considerable weakness, such as the deltoid and the TA.

Parameter	SMA	Healthy controls	p-value
CMAP APB muscle (mV)	10.01 ± 2.87	11.07 ± 2.28	0.2300
MUSIX APB muscle (μV)	79.16 ± 27.42	62.68 ± 13.80	**0.0260**
MUNIX APB muscle	144.16 ± 71.55	184 ± 52.18	**0.0320**
CMAP ADM muscle (mV)	9.24 ± 2.71	10.72 ± 2.06	0.3300
MUSIX ADM muscle (μV)	111.47 ± 44.48	66.25 ± 16.97	**0.0005**
MUNIX ADM muscle	99.37 ± 52.61	172 ± 54.48	**0.0005**
CMAP deltoid muscle (mV)	7.82 ± 5.24	13.17 ± 2.96	**0.0008**
MUSIX deltoid muscle (μV)	55.16 ± 27.42	47.06 ± 10.04	**0.0380**
MUNIX deltoid muscle	151.89 ± 114.55	289.93 ± 83.94	**0.0003**
CMAP trapezius muscle (mV)	6.36 ± 2.78	7.36 ± 1.88	0.3840
MUSIX trapezius muscle (μV)	49.47 ± 11.75	48.12 ± 12.15	0.7920
MUNIX trapezius muscle	140.11 ± 58.27	162.75 ± 59.87	0.4950
CMAP TA muscle (mV)	4.57 ± 1.73	6.10 ± 1.00	**0.0128**
MUSIX TA muscle (μV)	65.44 ± 24.19	47.75 ± 4.76	**0.0067**
MUNIX TA muscle	80.17 ± 41.06	130.37 ± 25.96	**0.0002**

Table 1. Neurophysiology findings presented as mean ± standard deviation for SMA patients and healthy controls. CMAP = compound muscle action potential (mV); MUSIX = motor unit size index (μV); MUNIX = motor unit number index.

A significant MUNIX reduction and MUSIX increase ($p < 0.05$) was observed in every tested muscle of SMA patients compared to HC regardless of their functional impairment, as shown in figure 2.

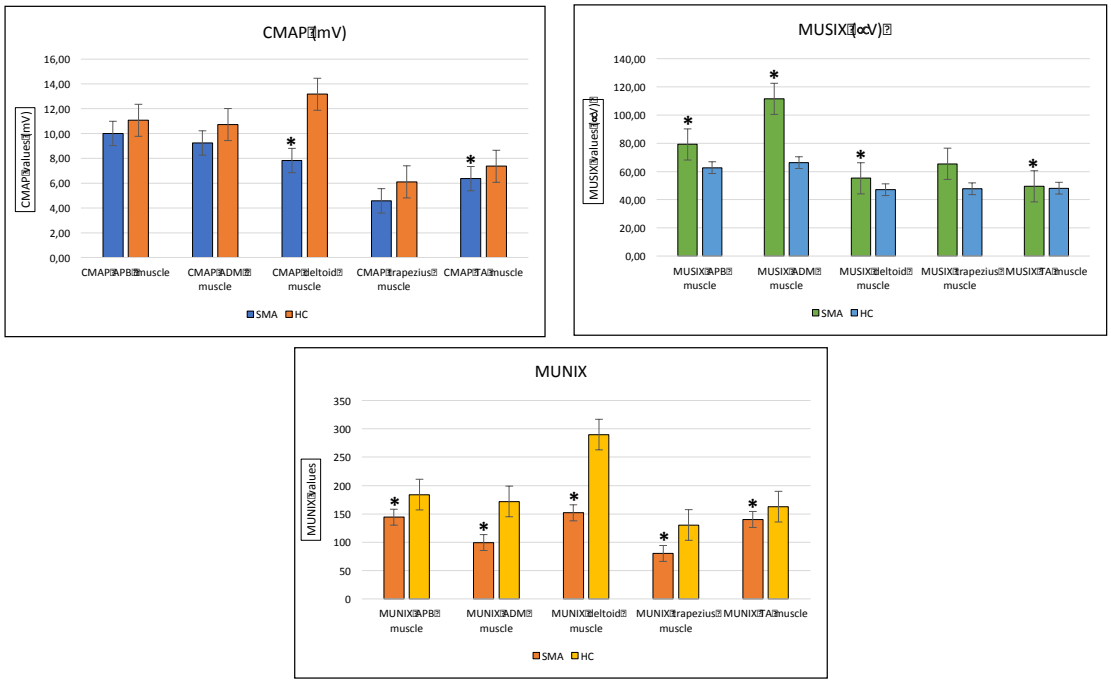

Figure 2. Graphical representation of reduced CMAP and MUNIX and increased MUSIX values in SMA patients compared to healthy controls in five muscles. * = $p < 0.05$.

In our SMA cohort, MUNIX reduction was more significant in the ADM than in the APB muscle (figure 3). When comparing the two groups, we observed that the MUNIX values were 42,2% lower in the ADM of SMA patients compared to HC, while they were only 21.6% lower in the ABP ($p = 0.0005$ and $p = 0.0032$ respectively). Keeping with this trend, we observed more significantly increased MUSIX in the ADM (47.7%) than in the APB (20.8%) ($p = 0.005$ and $p = 0.026$ respectively).

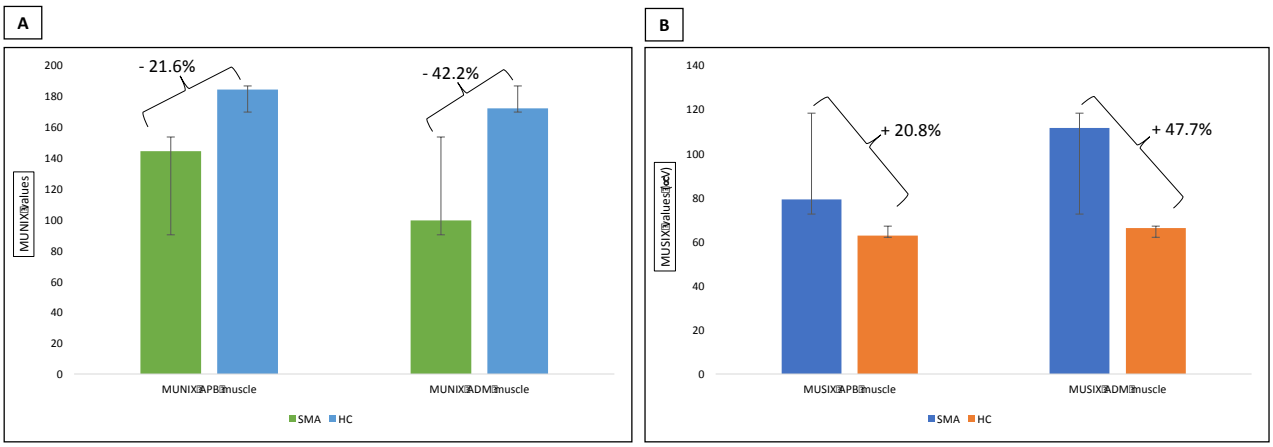

Figure 3. A. Reduction of the MUNIX in the ABP and ADM muscles: MUNIX is more significantly reduced in the ADM than in the ABP. **B.** Increased MUSIX in the ABP and ADM muscles: MUSIX was increased more significantly in the ADM muscle, suggesting active collateral axonal sprouting.

3.3 Correlations

In order to explore the relationship between neurophysiological and clinical parameters, correlations were computed for each muscle and their corresponding function.

When considering the APB, significant correlations were identified between MUNIX and grip and pinch force (rho = 0.51, p = 0.034 and rho = 0.66, p = 0.011 respectively). A significant inverse correlation was also found for the corresponding MUSIX (rho = - 0.47, p = 0.049 and rho = - 0.55, p = 0.026 respectively). Regarding the CMAP, a significant correlation was found with the pinch force (rho = 0.55, p = 0.026), but no significant association was identified between the CMAP of the APB and grip or pinch. Similar correlations were found in the ADM muscle for MUNIX and MUSIX, and no association was found between CMAP and grip or pinch force (p > 0.05) (figure 4).

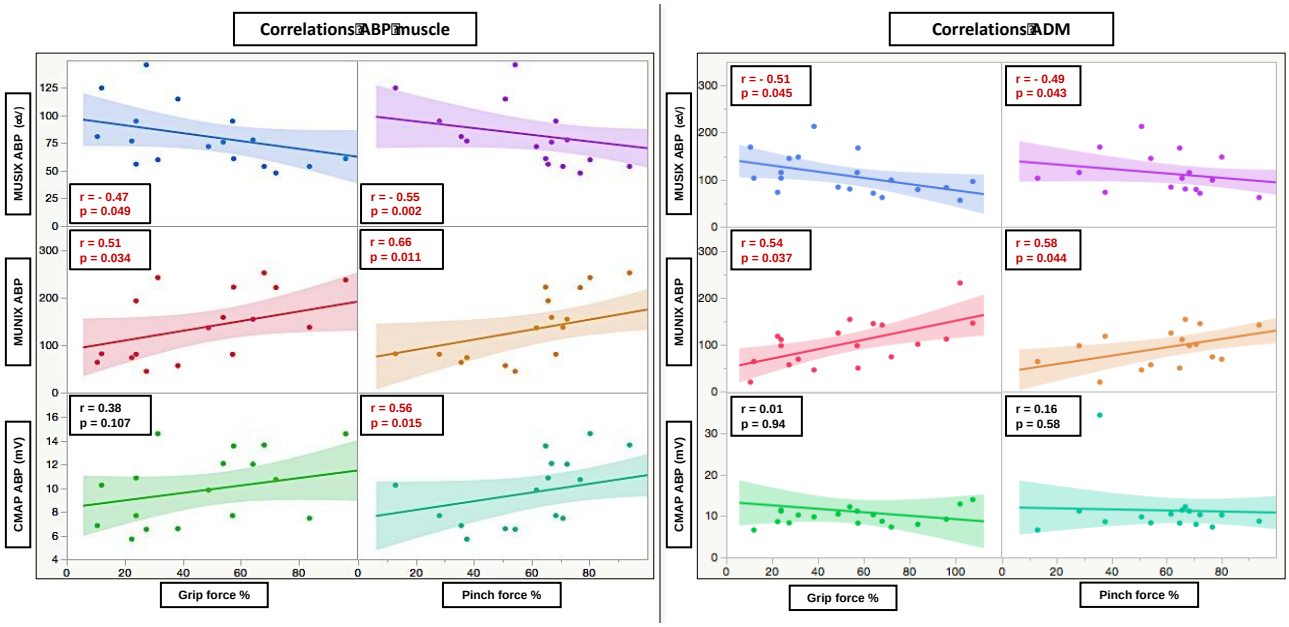

Figure 4: FDR corrected correlations described by Spearman's rho coefficient between neurophysiological parameters and muscle strength as tested by dynamometry. Significant linear correlations are observed for MUNIX and inverse correlations for MUSIX. Only a weaker trend is observed for the CMAP, suggesting that MUNIX is superior in detecting motor neurons loss.

Significant correlations were observed between MUNIX and CMAP in the deltoid muscle and the corresponding MRC score (rho = 0.79, p = 0.000 and rho = 0.78, p < 0.001 respectively), while no significant correlation was found with the MUSIX (rho = - 0.22, p = 0.34). Similar correlations were also detected between the CMAP, MUNIX and MUSIX in the TA and ankle extension strength. (figure 5).

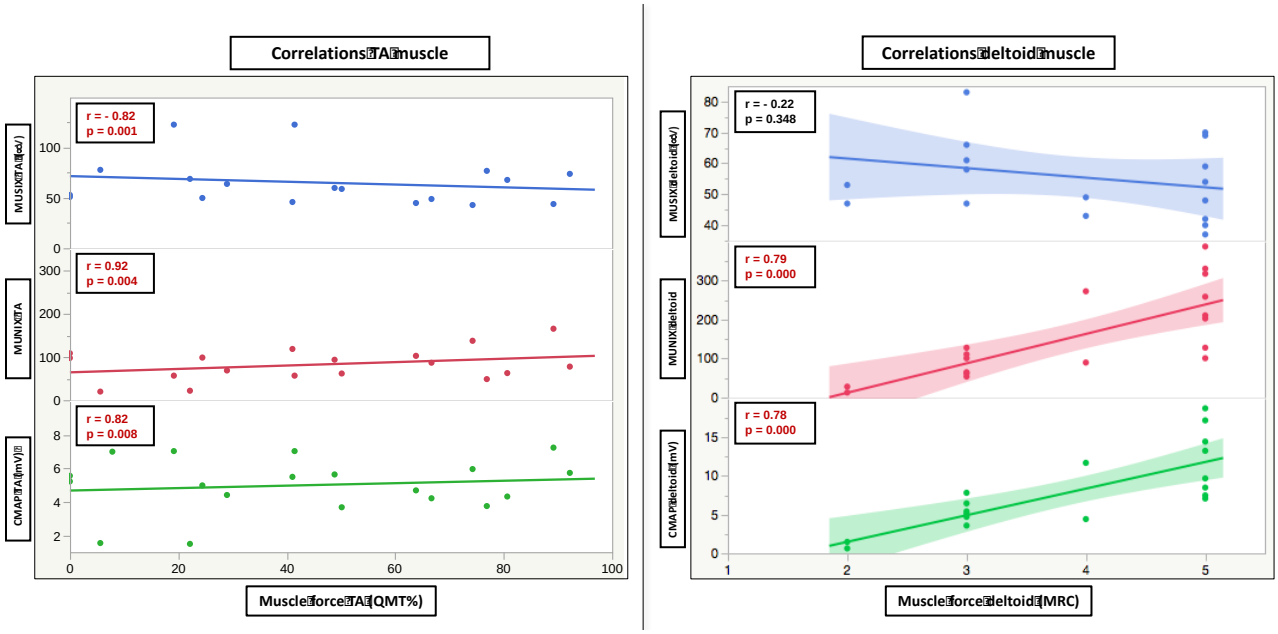

Figure 5: Correlations between the neurophysiological parameters and the corresponding muscle strength in the TA and deltoid muscles described as Spearman's rho and p value.

No significant correlation was found between the MUNIX values and the age of the patients or with disease duration. The MUNIX total score strongly correlated with the SMAFRS total score (rho = 0.79, $p < 0.001$) (figure 6).

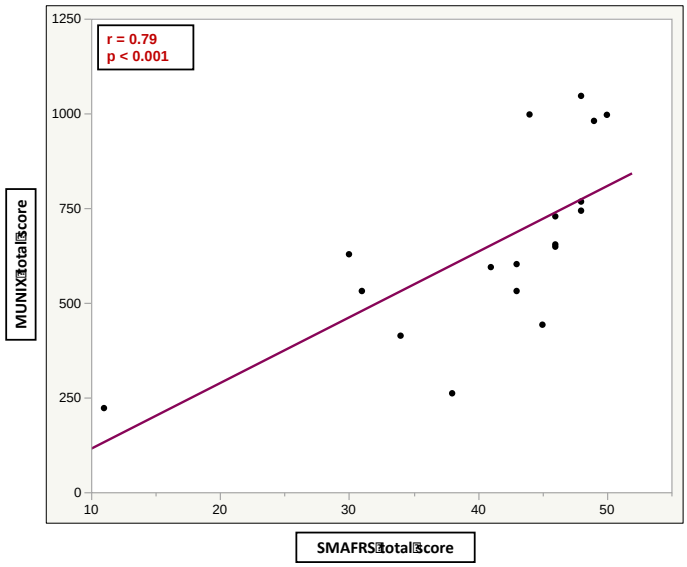

Figure 6: Graph showing the correlation between the MUNIX total score and the SBMAFRS total score described as Spearman's rho and p value (r = 0.79, $p < 0.001$).

Discussion

This study showcases the utility of MUNIX in characterising the pattern of MN loss in adult forms of SMA. We also describe significant correlation between MUNIX and muscle strength in corresponding muscles. Furthermore, we identify an association between the MUNIX total score and the SMAFRS, suggesting that the degree of denervation is closely linked to disability.

The clinical profile of the patients was assessed by a standardised neuromuscular examination including manual and quantitative muscle testing and a general functional appraisal with the SMAFRS. Our clinical findings confirm that SMA is primarily associated with proximal and symmetrical muscle weakness, more evident in the LL (Piepers et al. 2008) (Wadman et al. 2018).

In our study, MUNIX was significantly reduced in all of the tested muscles compared to HC regardless of the severity of weakness, suggesting that MUNIX can detect LMN loss in different stages of the disease, similarly to other motor neuron diseases (Fukada et al. 2016) (Escorcio-Bezerra et al. 2018).

CMAP was only significantly reduced in muscles with overt weakness (deltoid and tibialis anterior), showing near normal values in less affected muscles. These findings suggest that MUNIX may be a more sensitive as an early index of LMN degeneration than traditional neurophysiological parameters.

Interestingly, we found an association between MUNIX and muscle strength, which was more significant in the proximal muscles and in the LL, mirroring the clinical distribution of muscle weakness in SMA. We also captured a considerable increase in MU size, thought to be due to collateral sprouting from remaining axons and suggestive of re-innervation. These findings are consistent with those observed in ALS (Ahn et al. 2010) (Nandedkar et al. 2010) (Neuwirth et al. 2015), where re-innervation is more significant in patients with slower progression of the disease (Nandedkar et al. 2010). Further prospective studies are needed to specifically assess the longitudinal profile of these metrics, and preferably include several disease cohorts and phenotypes, such as slowly-progressive ALS, rapidly-progressive ALS, type III SMA, type IV SMA. A longitudinal study design and the inclusion of disease-controls would help to establish the distinguishing electrophysiological profile of these phenotypes and confirm the biomarker role of these markers. Compensatory mechanisms are relatively under-evaluated in motor neuron diseases despite their potential contribution to slowing down functional decline. Furthermore, adaptive mechanisms, such as re-innervation are seldom regarded as targets of pharmacological trials.

The close correlation between MUNIX and SMAFRS further highlights the relationship between LMN loss and disability. Finally, we underscore how, in adult SMA patients, MUNIX reduction was much greater in the ADM than in the APB muscle (figure 3). These findings clearly differentiate SMA from ALS, where the APB is usually much more affected, leading to the "split hand" phenomenon (Kim et al. 2016). Our data support the specificity of the "split hand" in relation to ALS (Kim et al. 2015) and the relevance of neurophysiological techniques for the differentiation of motor neuron disease patients (Kalita et al. 2017) and in the elucidation of pathological mechanisms underlying the different clinical presentations.

In summary, our results provide evidence that MUNIX reliably captures LMN loss and muscle involvement in SMA type III and IV. Further studies with longitudinal designs are needed to confirm the full biomarker potential of MUNIX as a diagnostic and monitoring marker, and its role in future clinical trials.

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