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Why averaging multiple MUNIX measures in the longitudinal assessment of patients with ALS?

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HIGHLIGHTS

- Mean-MUNIX relative change values are less dispersed than that of single-MUNIX in patients with ALS.
- Mean-MUNIX is more sensitive to detect significant relative change over time in ALS patients.
- Mean-MUNIX is a more reliable approach that can potentially reduce sample size and study costs.

ABSTRACT

Objective: To assess the impact of averaging multiple MUNIX trials on the follow-up of patients with amyotrophic lateral sclerosis (ALS).
Methods: We determined the percent relative change (%RC) of MUNIX, in healthy subjects and patients with ALS, by subtracting the MUNIX value in the second visit from the first. Both the mean of a set of three MUNIX (mean-MUNIX) and the first MUNIX sample (single-MUNIX) were evaluated. Then, we studied the sensitivity to detect relative changes over time and the statistical dispersion of the %RC from these two parameters.
Results: We found that the mean-MUNIX %RC has lower mean coefficient of variation than the single-MUNIX %RC in all muscles. The mean-MUNIX also resulted in more ALS patients with significant %RC, i.e., outside reference limits.
Conclusion: The mean-MUNIX resulted in less dispersed values of %RC in patients with ALS and thus, increased the precision of the technique. The mean-MUNIX resulted also in an increase in the sensitivity to track changes over time in these patients.
Significance: The mean-MUNIX should be considered in any ALS follow-up study as a more reliable approach and as a way of potentially reducing the sample size needed for the study.

1. Introduction

Amyotrophic lateral sclerosis (ALS), a terminal degenerative disease of the upper and lower motor neurons (LMN), lacks reliable lower motor neuron disease progression biomarker. Clinical assessment, functional scales, and routine electrophysiological parameters are insensitive in detecting subtle lower motor neuron degeneration as muscle strength and compound muscle action potential (CMAP) can remain stable despite progressive subclinical LMN loss and reinnervation (Neuwirth et al., 2017).

The Motor Unit Number Index (MUNIX) is a promising electrophysiological biomarker of motor unit loss in ALS, with performance comparable to the motor unit number estimation (MUNE) techniques (Furtula et al., 2013; Boekestein et al., 2012). To serve as reliable LMN degeneration biomarker, extensive MUNIX analytical validation is required (Benatar et al., 2016) and precision is a crucial attribute. Precision can be defined as the degree of agreement between consecutive measures, and testing the reproducibility is a way of assessing it (Westgard et al., 2010; Joint Committee for Guides in Metrology, 2017). Besides the reproducibility, the dispersion of the distribution (measured by the coefficient of variation

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(CV)) is also closely related to the precision. Thus, with a more precise technique repeated measures are more reproducible and less dispersed (Joint Committee for Guides in Metrology, 2017).

MUNIX studies assessing reproducibility in patients with ALS revealed good MUNIX intraclass correlation coefficient (ICC), however, the intra-individual variability was suboptimal (around 15–20%) in multiple muscles (Ahn et al., 2010; Nandedkar et al., 2011; Escorcio-Bezerra et al., 2016). Our group demonstrated that averaging a set of consecutive MUNIX measures (mean-MUNIX) significantly reduced the intra-individual variability in healthy controls, indicating that the mean-MUNIX is a more reproducible parameter (Escorcio-Bezerra et al., 2017).

Prior studies demonstrated that MUNIX values declines over time in ALS patients (Neuwirth et al., 2015; Nandedkar et al., 2011). This decline can be quantified as percent relative change (%RC) within a time period. We hypothesize that, considering the mean-MUNIX is a more reproducible parameter (Escorcio-Bezerra et al., 2017), the distribution of the %RC values from an ALS cohort will be less dispersed with mean-MUNIX than with single-MUNIX.

In the current study we determined and compared the dispersion of the %RC values, measured by the coefficient of variation (CV), obtained with mean-MUNIX and single-MUNIX from a prospective cohort of ALS patients. We also aimed to evaluate which of the two parameters is more sensitive to detect significant relative changes over time in the motor unit number from these patients.

2. Methods

2.1. Subjects

Prospective patients diagnosed with laboratory-supported probable, clinically possible, probable or definite ALS according to the World Federation of Neurology Revised El Escorial criteria (Brooks et al., 2000) and also patients diagnosed with progressive muscular atrophy (PMA) were prospectively recruited from the Neuromuscular Diseases Center, Universidade Federal de Sao Paulo (UNIFESP, Brazil). Patients with PMA were considered to have a form of ALS with pure lower motor neuron (LMN) findings in at least two segmental body regions (bulbar, cervical, thoracic or lumbar) in the absence of another neurological condition to account for the findings (Kim et al., 2009). Other neurological disorders such as focal neuropathies or polyneuropathies were excluded with complementary exams when necessary, including electromyography. Muscles with compound muscle action potential (CMAP) values below 1 mV or with MRC muscle strength graded below III were excluded from the study.

Age and sex-matched healthy subjects with no history of polyneuropathy, neuromuscular diseases, diabetes, or hypothyroidism were recruited (control group) and were used to determine normal neurophysiological parameters. Part of this control cohort has been previously published (Escorcio-Bezerra et al., 2017).

The UNIFESP Research Ethics Board approved this study. All participants provided written informed consent.

2.2. Study protocol

MUNIX procedure was performed as described in two previously published studies by our group (Escorcio-Bezerra et al., 2016, 2017). We used the same mathematical model originally described by Nandedkar and colleagues (Nandedkar et al., 2004, 2010), with seven progressive surface interference patterns (SIP) for the power regression curve. Also, in every MUNIX sample, the CMAP with the highest amplitude (baseline to negative peak) was obtained.

MUNIX was performed in the first and second study visits, which were three months apart for the control group and between three to six months for the ALS group. In each visit we performed the MUNIX of the tibialis anterior (TA), abductor pollicis brevis (APB) and abductor digiti minimi (ADM) muscles, following this order. We repeated the same sequence until we collected three trials of MUNIX in each muscle (i.e., a total of nine MUNIX trials per study visit). After each measurement, the marks from the electrode placement were erased. During the second visit the same protocol was repeated and the examiner did not have access to the MUNIX or the CMAP values from the first visit. We also calculated the MUNIX sum score, which is the sum of the MUNIX values from the three muscles.

For each muscle we calculated the average of three MUNIX trials, i.e., the mean-MUNIX. The values of the mean-MUNIX were contrasted to those of the single-MUNIX (the value of the first MUNIX trial).

2.3. Measures and statistical analysis

For each muscle from controls and ALS subjects, the %RC of the mean-MUNIX and single-MUNIX, between the first and second visits was determined using the following formula:

$$[(\text{second visit} - \text{first visit})/\text{first visit}] \times (100)$$

Table 1
Single and mean MUNIX values from control and ALS groups in the first and second visits. Based on control data, the reference limits of the single and mean-MUNIX relative change (technical variability) was determined using the mean %RC ± 2 times the SD (*). To demonstrate how the %RC values are spread out using the single or mean-MUNIX, we calculated the coefficient of variability (CV) of the %RC. Overall, mean-MUNIX was associated with less dispersed %RC values and narrower reference limits. Data are mean ± SD. Mean-MUNIX = the average of three MUNIX measurements in a given muscle; single-MUNIX = one single MUNIX measurement (the first value collected); TA = tibialis anterior; APB = abductor pollicis brevis; ADM = abductor digiti minimi; SD = standard deviation; %RC = percent relative change.

	Single-MUNIX					Mean-MUNIX				
	First visit	Second visit	Mean %RC	CV of the %RC	%RC reference limits*	First visit	Second visit	Mean %RC	CV of the %RC	%RC reference limits*
Control group (N = 21)										
TA	172 ± 38	167 ± 41	−2.7 ± 14	5.2	−31 to 25%	174 ± 41	167 ± 40	−3.7 ± 10	2.6	−23 to 16%
APB	197 ± 65	192 ± 46	−0.9 ± 19	19.5	−36 to 38%	197 ± 56	196 ± 54	−0.6 ± 5.5	8.9	−11 to 10%
ADM	199 ± 44	184 ± 49	−7 ± 18	2.6	−43 to 29%	197 ± 46	185 ± 46	−5.9 ± 8.4	1.4	−23 to 11%
Sum score	568 ± 131	543 ± 112	−3.6 ± 12	3.2	−27 to 19%	568 ± 126	547 ± 122	−3.6 ± 5.8	1.6	−14 to 7%
ALS group (n = 21)										
TA	113 ± 42	91 ± 49	−22 ± 28	1.3		117 ± 40	88 ± 44	−28 ± 22	0.8	
APB	101 ± 54	71 ± 45	−34 ± 27	0.8		105 ± 52	73 ± 45	−35 ± 25	0.7	
ADM	125 ± 48	97 ± 40	−20 ± 27	1.4		127 ± 40	97 ± 45	−26 ± 22	0.8	
Sum score	345 ± 105	266 ± 105	−24 ± 17	0.7		351 ± 94	262 ± 105	−27 ± 16	0.6	