



## Motor unit number index as an individual biomarker: Reference limits of intra-individual variability over time in healthy subjects



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### HIGHLIGHTS

- Motor unit number index (MUNIX) variation depends on the experience of the operator.
- A 20% change in MUNIX sum score is significant.
- MUNIX could be used as a biomarker in the follow-up of neuromuscular disorders.

### ABSTRACT

**Objective:** Motor unit number index (MUNIX) is proposed to monitor neuromuscular disorders. Our objective is to determine the intra-individual variability over time of the MUNIX.

**Methods:** In 11 different hospital centres, MUNIX was assessed twice, at least 3 months apart (range 90–360 days), in *tibialis anterior* (TA), *abductor pollicis brevis* (APB), *abductor digiti minimi* (ADM) and *deltoid* muscles in 118 healthy subjects. MUNIX sum score 2, 3 and 4 were respectively the sum of the MUNIX of the TA and ADM, of the TA, APB and ADM and of the TA, APB, ADM and deltoid muscles.

**Results:** The repeatability of the MUNIX was better for sum scores than for single muscle recordings. The variability of the MUNIX was independent of sex, age, interval between measurements and was lower for experienced than non-experienced operators. The 95th percentile of the coefficient of variability of the MUNIX sum score 2, 3 and 4 were respectively 22%, 18% and 15% for experienced operators.

**Conclusions:** The MUNIX technique must be performed by experienced operators on several muscles to reduce its variability and improve its reliability.

**Significance:** A variation of the MUNIX sum score  $\geq 20\%$  can be interpreted as a significant change of muscle innervation.

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## 1. Introduction

The MUNIX (Motor Unit Number IndeX) is a technique that estimates the number of motor units (MUNIX) and their size (MUSIX, muscle size index) in a given muscle (Nandedkar et al., 2004; Fatehi et al., 2018). This technique is based on the recording of the supramaximal compound muscle action potential (CMAP) and surface electromyography at different levels of voluntary muscle contractions.

The MUNIX technique has good inter and intra observer reproducibility in healthy subjects (Ahn et al., 2010; Neuwirth et al., 2011, 2016), in amyotrophic lateral sclerosis (ALS) (Furtula et al., 2013; Grimaldi et al., 2017), and in chronic inflammatory neuropathies (Delmont et al., 2016). The MUNIX declines faster than the ALS-Functional Rating Scale in ALS (Neuwirth et al., 2015) and correlates with disability scores in hereditary and inflammatory neuropathies (Delmont et al., 2016; Bas et al., 2018; Lawley et al., 2019). The MUNIX value is rapidly modified after intravenous immunoglobulins (IVIg) infusions in inflammatory neuropathies (Philibert et al., 2017; Lawley et al., 2019) and also improved after rituximab therapy in anti-MAG neuropathies (Fatehi et al., 2017).

These elements suggest that the MUNIX technique can be used to follow-up motor neuron diseases and chronic neuropathies and, in particular, to measure response to treatment. The MUNIX is now included in the secondary end-points of some clinical trials (Neuwirth et al., 2018). However, normal values of the intra-individual variability over time of the MUNIX is lacking, although being essential to know whether MUNIX variation in one given patient is a significant change in muscle innervation, not due to the variability of the measure.

We, therefore, propose to determine, in a multicentre trial, the normal limits of the intra-individual variability of the MUNIX for a group of muscles analysed in healthy individuals with an interval of at least three months between two measurements.

## 2. Methods

Healthy volunteers were recruited in 11 university departments of Clinical Neurophysiology or Neuromuscular diseases: hôpital la Timone (Marseille, France), CHU Sart Tilman (Liège, Belgium), hôpital Henri Mondor (Créteil, France), hôpital Pasteur (Nice, France), hôpital Pierre Wertheimer (Lyon, France), hôpital de Bicêtre (Kremlin-Bicêtre, France), hôpital Pierre-Paul Riquet (Toulouse, France), CHU Vaudois (Lausanne, Switzerland), hôpital Pitié-Salpêtrière (Paris, France), Hôtel-Dieu (Nantes, France), hôpital Roger Salengro (Lille, France). The study was approved by the French Ethics Committee Sud Est I (2018-94). All the subjects gave informed consent. Healthy subjects were invited to participate in this research during a doctor's appointment for general health assessment or via an advertisement in our respective faculties. A clinical examination was performed to rule out diseases that could modify the MUNIX measurement (myopathy, neuropathy, carpal tunnel syndrome, radiculopathy, or central nervous system involvement) (Neuwirth et al., 2018).

In each centre, the same investigator performed the MUNIX technique twice at least three months apart. The operators were blinded to the results of the first MUNIX assessment. Five operators were considered experienced because they had been practicing the MUNIX technique several times a month for more than 3 years. The other operators had less than 6 months of experience.

The MUNIX protocol was conducted as usual and reported in detail previously (Nandedkar et al., 2010, 2018; Fatehi et al., 2018). Briefly, at a skin temperature maintained above 32 °C, first a supramaximal CMAP was recorded to calculate the amplitude, area, and power of the negative phase of the CMAP. The recording

electrodes were moved until the maximum CMAP amplitude with a clear negative take-off was obtained. The second step was to record 10 surface interference patterns (SIPs) of electromyographic activity while the patient produces voluntary muscle contraction at five distinct force levels (about 10%, 25%, 50%, 75%, and 100% of maximal force, including 2 recordings at each force level, controlled by auditory and visual feedback). A bandpass filter of 3–3000 Hz was used as recommended for both CMAP and SIP recordings. To reduce variability caused by electrode size and type, all centres used the same self-adhesive disposable surface recording electrodes (Ref 9013S0242, Natus, Paris, France). Two types of EMG machine were used: either the Dantec® Keypoint® G4 EMG Workstation or the Nicolet® Synergy® EDX System (Natus). To be accepted the measurements had to fulfilled quality controls: SIP area >20 mV/ms, ideal case motor unit count (ICMUC) <100, SIP area/CMAP area >1, R square of the regression between ICMUC, and SIP area >0.90.

The MUNIX was assessed on the tibialis anterior (TA), abductor pollicis brevis (APB), abductor digiti minimi (ADM) and deltoid muscles of the non-dominant side. Assessment was standardized for each muscle. The subject was in half-seated position on the examination chair with stretched legs and arms on the armrest. Instructions for stimulating and recording electrode placement and muscle contraction were as follows: TA muscle: electrical stimulation of the peroneal nerve in the popliteal fossa, medial to the biceps femoris tendon; active recording electrode on the body of the TA at the upper third of a line joining the tibial tuberosity in the middle of the bimalleolar line, 2–3 cm laterally to the tibial crest; reference on the medial side of the tibial tuberosity; voluntary contraction: dorsal flexion of the foot (foot maintained at 90° of dorsal flexion of the ankle to achieve isometric contraction), knee in full extension. APB muscle: electrical stimulation of the median nerve at the wrist 6 cm proximal to the active recording electrode on the body of the APB (lateral portion of the thenar compartment) and reference on the metacarpophalangeal joint of the thumb; voluntary contraction: rising of the thumb upwards without pronation of the forearm, adduction or extension of the thumb, while the back of the hand lay flat on the armrest. ADM muscle: electrical stimulation of the ulnar at the wrist 6 cm proximal to the active recording electrode; active recording electrode on the body of the ADM and reference on the fifth digit between the second and third phalanx; voluntary contraction: abduction of the fifth digit, while the palm of the hand lay flat on the armrest. Deltoid muscle: electrical stimulation of the axillary nerve at the Erb's point with a monopolar electrode and a wide anode placed on the dorsal surface of the cervico-scapular region; active recording electrode on the middle head of the deltoid muscle, reference on the acromion; voluntary contraction: countered abduction of the arm in the frontal plane in isometric contraction, with the arm maintained at 45° from the trunk.

Collected data included age, sex, height, weight, body-mass index, interval between the 2 MUNIX assessments, MUNIX, MUSIX and CMAP values of each muscle. Regarding these latter variables (MUNIX, MUSIX and CMAP), a sum score for 2 muscles was obtained by adding the results of the TA and ADM muscles, a sum score for 3 muscles was obtained by adding the results of the TA, APB and ADM muscles, and a sum score for 4 muscles was obtained by adding the results of the TA, APB, ADM and deltoid muscles.

Quantitative data were expressed as means (standard deviation) and were compared using a Student's T-test or Mann-Whitney test whether the distribution of the values was normal or not. A two-way random, single measure intra-class correlation coefficient (ICC) was calculated to evaluate the intra-operator variability of the MUNIX technique. An ICC value >0.75 was interpreted as a good repeatability of the measures (Furtula et al., 2013). The

coefficient of variability (CV) was the absolute difference between test and retest values divided by mean value  $\times 100$ .

The normal value of the intra-individual variability over time of the MUNIX variables was calculated according to the 95th percentile of the CV and the 95% confidence interval on a Bland Altman graph. Graphs constructions, Pearson correlation coefficient ( $r$ ), linear regression, multivariable analysis by multiple linear regressions were performed using Graph Pad Prism 5 (GraphPad Software, San Diego, CA, USA) and IBM SPSS statistics, version 20 (IBM SPSS Inc, Chicago, IL, United States). A two-sided  $p$ -value  $< 0.05$  was considered as significant.

### 3. Results

118 healthy volunteers (64 females and 54 males) were enrolled from 11 participating centres (6 non-experienced and 5 experienced with the MUNIX technique). Mean age was 47yo (14), ranging from 23 to 76yo, with 32 persons above 60yo. The interval between both MUNIX assessments was 105 days (44), ranging from 90 to 360 days.

The results of the first and second MUNIX assessments are presented in the Table 1. The repeatability was good for CMAP amplitude (ICC  $> 0.75$ ) except for the TA muscle (ICC = 0.60). The repeatability was poor for MUSIX in all the tested muscles (ICC  $< 0.45$ ). Regarding MUNIX, the repeatability of MUNIX was poor for the TA, ADM, and deltoid muscles, but good (ICC  $> 0.75$ ) for the APB and the MUNIX sum scores 3 and 4 (Table 2). The variability of the MUNIX sum scores 2 and 4 was significantly lower than that of the MUNIX of the APB and deltoid muscles (Fig. 1). The variability of the MUNIX sum score 3 was significantly lower than that of MUNIX of each muscle taken separately (Fig. 1).

The variability of the MUNIX sum score 2 correlated with the variability of following variables: the MUNIX of TA muscle ( $r = 0.34$ ,  $p = 0.001$ ) and ADM muscle ( $r = 0.65$ ,  $p = 0.0001$ ), the MUSIX of ADM muscle ( $r = 0.38$ ,  $p = 0.0001$ ), the MUSIX sum score 2 ( $r = 0.45$ ,  $p = 0.0001$ ), the CMAP of TA muscle ( $r = 0.3$ ,  $p = 0.01$ ),

**Table 2**

Test-retest reliability of the MUNIX assessed with intra class coefficient correlation (ICC).

MUNIX ICC	All operators	Non-experienced operators	Experienced operators
TA	0.67	0.66	<b>0.77</b>
APB	<b>0.79</b>	0.69	<b>0.93</b>
ADM	0.66	0.53	<b>0.87</b>
Deltoid	0.69	0.60	<b>0.86</b>
Sum score 2 muscles	0.74	0.67	<b>0.87</b>
TA + ADM			
Sum score 3 muscles	<b>0.87</b>	<b>0.83</b>	<b>0.94</b>
TA + APB + ADM			
Sum score 4 muscles	<b>0.87</b>	<b>0.83</b>	<b>0.95</b>
TA + APB + ADM + deltoid			

Good repeatability is assumed when ICC  $> 0.75$  (in bold in the table). Sum scores were obtained by adding the corresponding results of the different muscles. MUNIX motor unit number index, TA tibialis anterior, APB abductor pollicis brevis, ADM abductor digiti mini.

and the CMAP sum score 2 ( $r = 0.29$ ,  $p = 0.001$ ). In multiple regression analysis, the variability of the MUNIX sum score 2 was only independently correlated with the variability of the MUNIX of ADM muscle ( $r = 0.65$ ,  $p = 0.0001$ ) (Fig. 2).

The variability of the MUNIX sum score 3 correlated with the variability of the MUNIX of APB muscle ( $r = 0.4$ ,  $p = 0.001$ ) and ADM muscle ( $r = 0.38$ ,  $p = 0.0001$ ), the variability of the CMAP sum score 3 ( $r = 0.29$ ,  $p = 0.001$ ), and the height of the subject ( $r = -0.24$ ,  $p = 0.02$ ). In multiple regression analysis, the variability of the MUNIX sum score 3 was only independently correlated with the variability of the MUNIX of ADM muscle ( $r = 0.39$ ,  $p = 0.0001$ ) (Fig. 2).

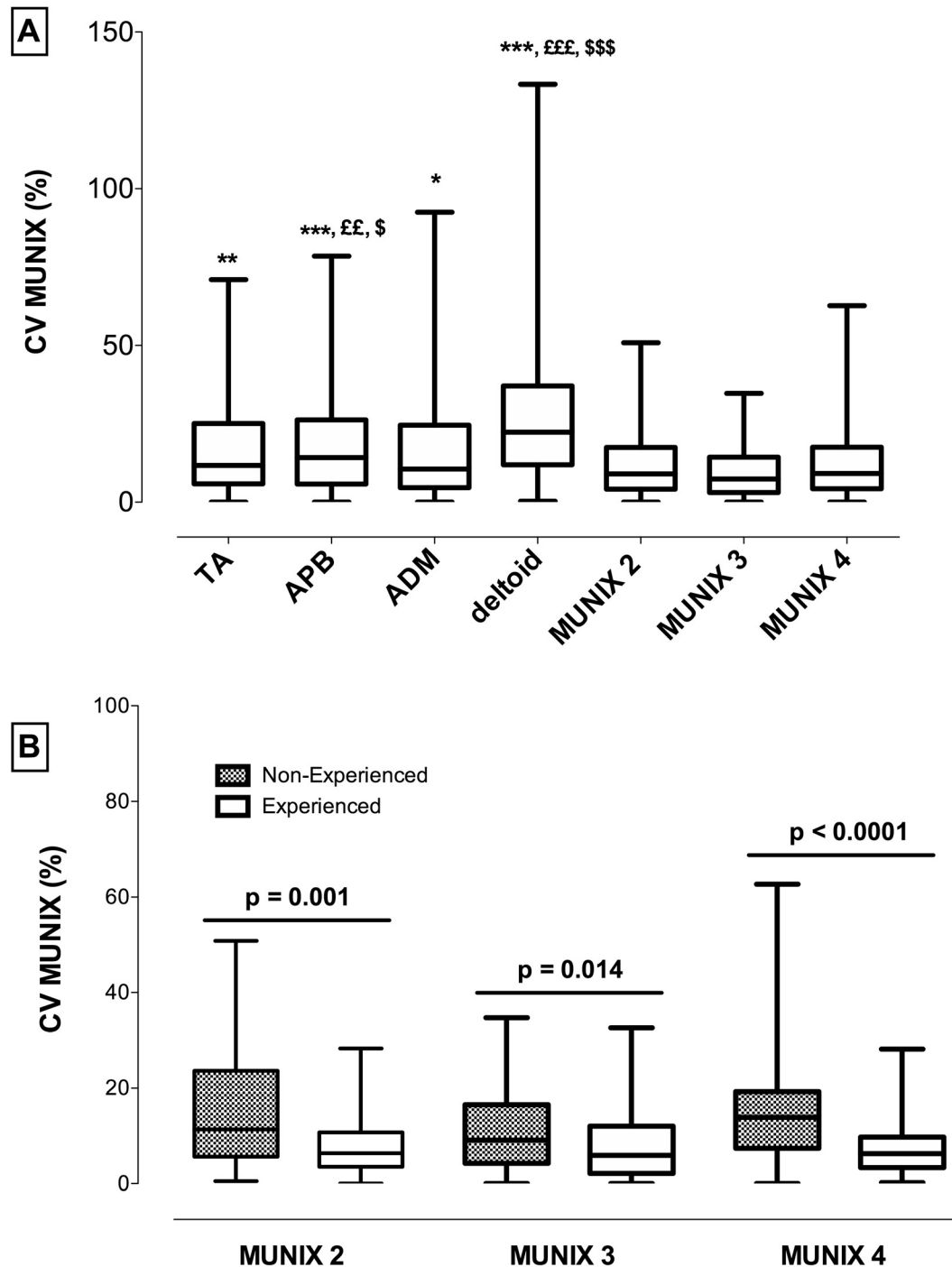
The variability of the MUNIX sum score 4 correlated with the variability of the following variables: the MUNIX of ADM muscle ( $r = 0.32$ ,  $p = 0.0001$ ) and deltoid muscle ( $r = 0.45$ ,  $p = 0.001$ ), the CMAP of the deltoid muscle ( $r = -0.33$ ,  $p = 0.0001$ ) and the CMAP sum score 4 ( $r = 0.53$ ,  $p = 0.0001$ ). In multiple analysis, the variabil-

**Table 1**  
Results of the MUNIX assessment.

	MUNIX		MUSIX		CMAP	
	Mean (SD)	5–95 percentiles	Mean (SD)	5–95 percentiles	Mean (SD)	5–95 percentiles
TA						
1st exam	118 (36)	67–182	48 (8)	38–64	5.6 (1.7)	3.3–9.4
2nd exam	121 (38)	68–193	48 (9)	38–62	5.8 (1.7)	3.3–8.7
APB						
1st exam	168 (68)	75–282	61 (16)	43–97	9.6 (2.9)	5.4–14.9
2nd exam	166 (63)	73–287	63 (17)	42–93	9.7 (2.6)	6–14.7
ADM						
1st exam	145 (41)	92–223	71 (15)	50–97	9.9 (2.2)	6.9–14.4
2nd exam	150 (43)	94–238	69 (14)	49–97	10.1 (2.2)	6.8–13.6
Deltoid						
1st exam	251 (103)	85–411	46 (11)	35–64	11.1 (4.2)	4.1–17.6
2nd exam	260 (114)	85–481	45 (11)	35–66	11 (3.9)	4.7–17.2
Sum score 2 muscles						
TA + ADM	263 (55)	185–357	119 (18)	93–155	15.5 (2.9)	11.1–20.6
1st exam	272 (62)	179–340	117 (17)	94–147	15.8 (2.9)	10.9–21.4
2nd exam						
Sum score 3 muscles						
TA + APB + ADM	432 (104)	278–616	180 (25)	149–221	25.2 (5.0)	17.5–33.1
1st exam	439 (107)	277–639	180 (26)	139–222	25.6 (4.9)	17.9–34.7
2nd exam						
Sum score 4 muscles						
TA + APB + ADM + deltoid	683 (184)	408–974	226 (30)	188–277	36.2 (8.3)	23.9–48.4
1st exam	699 (202)	388–1074	225 (31)	182–282	36.6 (8.3)	24.1–47.9
2nd exam						

Sum scores are obtained by adding the corresponding results of the different muscles.

MUNIX motor unit number index, MUSIX motor unit size index, CMAP compound muscle action potential amplitude, ADM abductor digiti mini, APB abductor pollicis brevis, TA tibialis anterior, SD standard deviation.



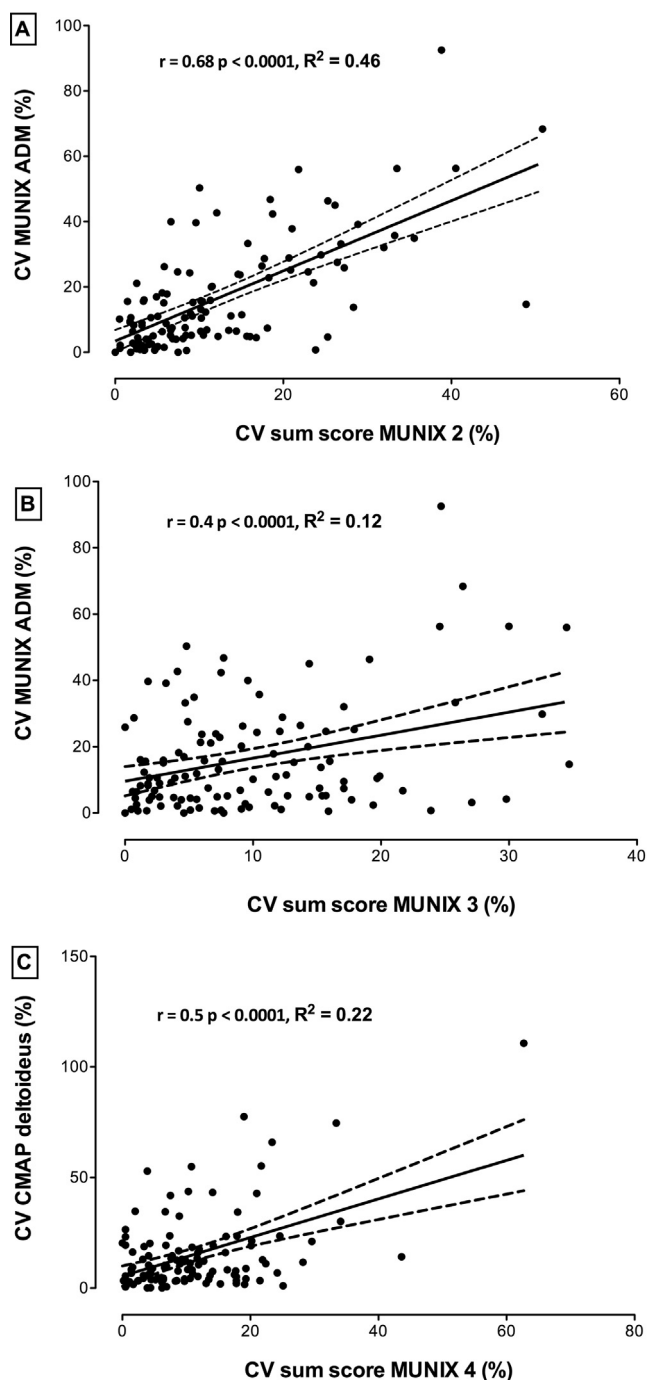
**Fig. 1.** Box plot of the coefficients of variability (CV) of MUNIX results between the two assessments. A: for the entire series of subjects. B: comparing experienced versus non-experienced operators regarding MUNIX sum scores 2, 3 and 4. TA tibialis anterior, APB abductor pollicis brevis, ADM abductor digiti minimi, CV coefficient of variability, MUNIX motor unit number index. The MUNIX sum score 2 is based on TA + ADM results, sum score 3 on TA + APB + ADM results and sum score 4 on TA + APB + ADM + deltoid results. p-values for comparisons with the MUNIX sum score 2: <sup>ss</sup> p < 0.05; <sup>sss</sup> p < 0.001. p-values for comparisons with the MUNIX sum score 3: \* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001. p-values for comparisons with the MUNIX sum score 4: <sup>££</sup> p < 0.01; <sup>£££</sup> p < 0.001.

ity of the MUNIX sum score 4 was only independently correlated with the variability of the CMAP of the deltoid muscle ( $r = 0.5$ ,  $p = 0.0001$ ) (Fig. 2).

The variability of the MUNIX sum scores 2, 3 and 4 was not related to age, gender, weight, body mass index, and test-retest interval.

Regarding the difference between experienced vs. non-experienced operators, the ICC was good ( $>0.75$ ) for all MUNIX variables in experienced operators, but only for MUNIX sum scores 3 and

4 in non-experienced operators (Table 2). In addition, the CV of the MUNIX was lower for experienced operators regarding the deltoid muscle, as well as the MUNIX sum scores 2, 3 and 4 (Table 3, Fig. 1). The reference limits of the intra-individual variability over time of the MUNIX were determined using the data from experienced operators only. According to the 95th percentile of the CV (Table 3), the upper limit for normal intra-individual variation was set at 22% for the MUNIX sum score 2, 18% for the MUNIX sum score 3, and 15% for the MUNIX sum score 4. Using the 95% limits of agreement of



**Fig. 2.** Correlation of the variability of the MUNIX sum scores. In multiple regression analysis, the variability of the MUNIX sum scores 2 and 3 was only independently correlated with the variability of the MUNIX of the abductor digiti minimi (ADM) muscle (A, B). The variability of the MUNIX sum score 4 was only independently correlated with the variability of the CMAP amplitude of the deltoid muscle (C). The MUNIX sum score 2 is based on TA + ADM results, sum score 3 on TA + APB + ADM results and sum score 4 on TA + APB + ADM + deltoideus results. TA, tibialis anterior; APB, abductor pollicis brevis; ADM, abductor digiti minimi; CMAP, compound motor action potential; MUNIX, motor unit number index; r, Pearson correlation coefficient; p, p-value; R<sup>2</sup>, R square.

the Bland-Altman plots, the upper limit for normal intra-individual variation was set at 23% for the MUNIX sum score 2, 19% for the MUNIX sum score 3, and 17% for the MUNIX sum score 4 (Fig. 3). The upper limits of variation of the CMAP sum scores 2, 3 and 4 were respectively 21%, 16% and 13%, i.e. similar to the values obtained for the MUNIX sum scores ( $p > 0.05$ ).

#### 4. Discussion

The objective of this multi-centre study was to determine the reference limits for the intra-individual variation over time ( $\geq 3$  months) of MUNIX variables in healthy subjects.

The MUNIX is one of the motor unit number estimation (MUNE) techniques. Many methods have been developed, all with particular limitations. The results provided by the MUNIX technique are usually well related to those obtained with other MUNE techniques (Benmouna et al., 2018; Higashihara et al., 2020). The MUNIX technique is valuable with regard to the good test-retest reproducibility and patient tolerability, the short duration of the evaluation, and the possibility of testing proximal muscles (de Carvalho et al., 2018).

We chose to analyse the TA, APB and ADM muscles because the sum score of their MUNIX is possibly related to the disability scores of patients suffering from inflammatory and inherited neuropathies (Delmont et al., 2016; Fatehi et al., 2017; Bas et al., 2018; Lawley et al., 2019). More distal muscles of the limbs, such as *extensor digitorum brevis* and *abductor hallucis* muscles in the feet, are usually too much involved for allowing motor responses to be recorded in pathological conditions. On the other hand, assessment of proximal muscles may be useful in clinical conditions such as motor neuron diseases (Grimaldi et al., 2017; Querin et al., 2018). In this study, we assessed MUNIX in the deltoid muscle, because, in our experience and in the literature, the recording is easier and more reliable than in other proximal muscles such as the trapezius and biceps brachii muscles (Neuwirth et al., 2016; Grimaldi et al., 2017). In addition, to improve the ability to record maximal CMAP amplitude in the deltoid muscle, the stimulation at Erb's point was performed using a monopolar technique and longer pulse duration. Finally, we chose to assess both APB and ADM muscles at the hand, since this dual evaluation may be useful to give evidence of split hand phenomenon in ALS (Zheng et al., 2019), although a simple measurement of CMAP amplitudes may be sufficient in this context. The MUNIX sum score 2, composed by ADM and TA muscle recordings, could quickly monitor the upper and lower limbs by limiting to a single muscle. However, its variability was higher than that of the MUNIX sum score composed with 3 or 4 muscles, especially in non-experienced operators. Indeed, MUNIX repeatability was good (high ICC values) only for MUNIX sum scores 3 and 4 in non-experienced operators, but even for MUNIX of individual muscles in experienced operators. However, overall, the variability of the MUNIX sum score 3 was significantly lower than that of MUNIX of each muscle taken separately.

Regarding 'temporal features', we chose an interval between two assessments of at least 3 months, because it is usually the minimal time for a treatment to be effective. However, the intra-individual variation over time of MUNIX was not affected by the interval between two measurements, which ranged between 90 and 360 days in this study.

The MUNIX values we measured at baseline were comparable to those previously published (Neuwirth et al., 2011; Cao et al., 2020). Although, MUNIX values were found to be reduced with age and sarcopenia (Cao et al., 2020), intra-individual variation overtime of MUNIX was not modified by age, sex and body mass index in our study.

Variations in the MUNIX may relate to variations in CMAP amplitude and/or MUSIX. In early stages of ALS, CMAP remains stable while MUNE is decreased and MUSIX is increased thanks to collateral reinnervation (Nandedkar et al., 2019). In the present study, performed in normal subjects, intra-individual variability over time of MUSIX was high (low ICC values), with a clearly poorer repeatability compared to that of MUNIX and CMAP sum scores. In addition, the variability of some MUNIX results corre-

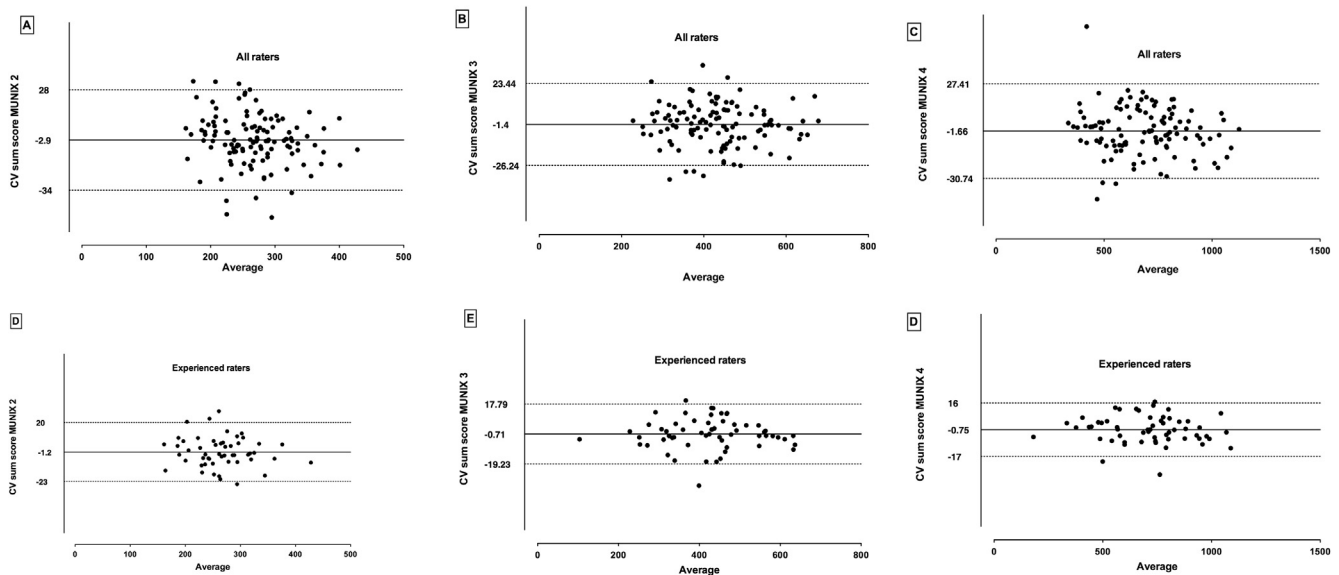


**Table 3**  
Coefficient of variability (CV) of the MUNIX protocol.

MUNIX CV	All operators		Non-experienced operators		Experienced operators		p-value
	Mean (SD)	95 centiles	Mean (SD)	95 centiles	Mean (SD)	95 centiles	
TA	17 (16)	51	11 (29)	53	5 (15)	33	NS
APB	19 (17)	55	9 (27)	65	6 (20)	41	NS
ADM	17 (17)	47	10 (29)	56	3 (7)	29	0.052
Deltoid	27 (22)	70	23 (41)	73	5 (15)	36	0.001
Sum score 2 muscles	12 (10)	33	15 (12)	38	9 (7)	22	< 0.001
TA + ADM							
Sum score 3 muscles	10 (8)	26	11 (9)	29	8 (6)	18	0.014
TA + APB + ADM							
Sum score 4 muscles	11 (10)	26	15 (11)	33	7 (5)	15	< 0.001
TA + APB + ADM + deltoid							

Coefficient of variability (CV) is the absolute difference between test and retest values divided by their mean value \* 100.

Sum scores are obtained by adding the corresponding results of the different muscles. p-value is calculated between experienced and non-experienced raters. MUNIX motor unit number index, ADM abductor digiti mini, APB abductor pollicis brevis, TA tibialis anterior, SD standard deviation, NS non-significant.



**Fig. 3.** Intra-operators MUNIX sum score Bland-Altman plots. The dotted lines represent the 95% limits of agreement, and the plain line represents the bias. A, B and C all operators; D, E and F only experienced operators. The MUNIX sum score 2 is based on TA + ADM results, sum score 3 on TA + APB + ADM results and sum score 4 on TA + APB + ADM + deltoid results. TA tibialis anterior, APB abductor pollicis brevis, ADM abductor digiti minimi, CV coefficient of variability, MUNIX motor unit number index.

lated to that of some CMAP results rather than of MUSIX. Therefore, MUNIX may depend on CMAP in healthy controls, although the repeatability of MUNIX and CMAP had independent factors. In multivariate analysis, the variability of the MUNIX sum scores 2 and 3 correlated to that of the MUNIX of the ADM muscle, indicating the predominant influence of that muscle in these sum scores. Concerning the variability of the MUNIX sum score 4, the major factor of influence was the CMAP of the deltoid muscle, a muscle that was not included in the sum scores 2 and 3. This result showed the potential influence of CMAP amplitude as a factor of variability of some MUNIX results. However, we found that the key factor of variability of the MUNIX was the level of training of the operators. The effect of training and experience was also previously reported in longitudinal studies (Neuwirth et al., 2018).

It is important to develop objective biomarkers to follow-up the evolution of neuromuscular disorders and, especially, to assess the efficacy of treatments. The results provided by nerve conduction studies (NCS) are robust and correlate with patients' disability in peripheral neuropathies (Gesquière-Dando et al., 2017). Compared to NCS variables, the MUNIX seems more sensitive to functional changes, as observed following IVIg infusions (Philibert et al.,

2017; Lawley et al., 2019). The present study demonstrates that an individual variation of a MUNIX sum score  $\geq 20\%$  over time is enough to conclude that this variation is significantly related to the disease or its treatment and not to the technique when performed by experienced operators. With this limitation, a MUNIX sum score, as calculated in this study, could be proposed as a reliable biomarker in the follow-up of motor neuron diseases and neuropathies. The sensitivity of this biomarker to give evidence for disease evolution remains to be assessed in future prospective studies investigating patients with various neuromuscular disorders.

#### Declaration of Competing Interest

Authors don't have potential conflicts of interest to be disclosed.

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