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Alix, J.J.P., Neuwirth, C., Gelder, L. et al. (20 more authors) (2019) Assessment of the reliability of the motor unit size index (MUSIX) in single subject "round-robin" and multi-centre settings. Clinical Neurophysiology, 130 (5). pp. 666-674. ISSN 1388-2457

https://doi.org/10.1016/j.clinph.2019.01.020

Assessment of the reliability of the motor unit size index (MUSIX) in single subject "round-robin" and multi-centre settings

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Abstract

Objective

The motor unit size index (MUSIX) is incorporated into the motor unit number index (MUNIX). Our objective was to assess the intra-/inter-rater reliability of MUSIX in healthy volunteers across single subject “round robin” and multi-centre settings.

Methods

Data were obtained from i). a round-robin assessment in which 12 raters (6 with prior experience and 6 without) assessed six muscles (abductor pollicis brevis, abductor digiti minimi, biceps brachii, tibialis anterior, extensor digitorum brevis and abductor hallucis) and ii). a multi-centre study with 6 centres studying the same muscles in 66 healthy volunteers. Intra/inter-rater data were provided by 5 centres, 1 centre provided only intra-rater data.

Intra/inter-rater variability was assessed using the coefficient of variation (COV), Bland-Altman plots, bias and 95% limits of agreement.

Results

In the round-robin assessment intra-rater COVs for MUSIX ranged from 7.8% to 28.4%. Inter-rater variability was between 7.8% and 16.2%. Prior experience did not impact on MUSIX values. In the multi-centre study MUSIX was more consistent than the MUNIX. Abductor hallucis was the least reliable muscle.

Conclusions

The MUSIX is a reliable neurophysiological biomarker of reinnervation.

Significance

MUSIX could provide insights into the pathophysiology of a range of neuromuscular disorders, providing a quantitative biomarker of reinnervation.

Highlights

Motor unit size index (MUSIX) is a rapid, non-invasive means of assessing reinnervation.

Using healthy volunteers reliable data were provided by both experienced and non-experienced raters

Results across single subject and multi-centre settings demonstrate high reliability/reproducibility.

Keywords

Motor unit number index; motor unit size index; motor unit; motor neuron; amyotrophic lateral sclerosis; electrophysiology

Introduction

Motor unit number techniques have been significantly developed since their inception over 30 years ago (McComas et al. , 1971). The motor unit number index (MUNIX), first reported in 2004 (Nandedkar et al. , 2004), has grown in popularity in recent years due to its ease and speed of application across different muscles (Nandedkar et al. , 2010). As a result, it has been employed across a range of neuromuscular conditions, from amyotrophic lateral sclerosis (ALS) (Neuwirth et al. , 2015, Escorcio-Bezerra et al. , 2016) to demyelinating peripheral neuropathies (Delmont et al. , 2016, Philibert et al. , 2017). In addition to providing an index of the number of functional motor units, the MUNIX computation also includes a size index, termed the motor unit size index (MUSIX) which is derived from dividing the compound muscle action potential (CMAP) amplitude by the MUNIX value (Nandedkar et al. , 2004, Nandedkar et al. , 2010). The MUSIX has the possibility of detailing the reinnervation process (e.g. reflecting motor unit size) in neuromuscular disorders which may reveal important information on the natural course of such conditions and the response to new treatments.

Although reports on the MUNIX technique have documented a change in the MUSIX in patients with ALS (Nandedkar et al. , 2010, Escorcio-Bezerra et al. , 2016), the MUSIX has received little attention. The first step in evaluating its ability to provide novel insights into pathophysiological changes in human subjects is to assess its reliability. We have previously assessed MUNIX in this regard, reporting its reliability in healthy subjects across two settings: a round-robin style assessment of a single healthy subject by multiple raters and a multi-centre study of 66 healthy volunteers (Neuwirth et al. , 2011, Neuwirth et al. , 2016). In order to ascertain the reliability of the MUSIX, we have analysed data from those studies in order to gauge its utility as a parameter in clinical trials of neuromuscular diseases.

Methods

The MUNIX/MUSIX procedure has been described in detail previously (Nandedkar et al. , 2004, Nandedkar et al. , 2010). A CMAP is recorded and optimised for maximal amplitude using standard neurophysiological techniques and the test subject then asked to contract the given muscle with differing force levels. The computation from these data has been described previously (Nandedkar et al. , 2004).

The studies concerning the round-robin MUNIX assessment (Neuwirth et al. , 2016) and the multi-centre evaluation of healthy volunteer subjects have been reported previously (Neuwirth et al. , 2011). The multi-centre study was approved by the relevant Human Studies Committees in each centre and written consent was obtained for both studies. Briefly, for the round-robin assessment a two-day training course was undertaken by neurophysiologists from across Europe at an ENCALS (European Network for the Cure of ALS) meeting in Dublin 2015. 12 participants were included in the study, 6 of whom had already completed a prior qualification process for a longitudinal study and were termed "experienced raters". 6 participants with no prior experience of MUNIX/MUSIX, but participated in the training, were also included and termed "non-experienced raters". All 12 raters completed two MUNIX/MUSIX assessments of a single healthy subject (M.W) on consecutive days. Assessments were performed using a Keypoint Dantec Focus EMG system. All muscles tested were on the right side: abductor pollicis brevis (APB), abductor digiti minimi (ADM), biceps brachii (BB), tibialis anterior (TA), extensor digitorum brevis (EDB) and abductor hallucis (AH).

In the multi-centre study healthy volunteers were recruited from six centres – Aarhus, Denmark; Lisbon, Portugal; Milwaukee, Wisconsin, USA; Nijmegen, Netherlands; St. Gallen, Switzerland and Uppsala, Sweden (Neuwirth et al. , 2011). The demographic details and inclusion/exclusion criteria have been reported previously; briefly potential participants with any medical condition that could impact on MUNIX measurements were excluded (Neuwirth et al. , 2011). The study was approved by the relevant Human Studies Committee in each centre and informed consent obtained. Each centre used the same electrodes and measurements were made on Keypoint Classic- and Synergy machines. Recordings were undertaken on right APB, ADM, BB, TA and AH muscles. The EDB muscle was also measured in 32 healthy volunteers across four centres. As documented previously, two investigators performed measurements twice in alternation in four centres (Neuwirth et al. , 2011). In one centre only intra-rater variability was assessed (i.e. the same investigator performed measurements twice). In another centre, a first examiner undertook measurements twice and a second examiner performed the recordings once.

Statistics

The coefficient of variation (COV; standard deviation/mean) was calculated for each muscle. Variability was calculated by: 100*(difference of test-retest)/(mean of test-retest). Welch's t-test was used to compare MUSIX values for the experienced and non-experienced rater groups in the round-robin assessment. A hypothetical MUSIX reference value was generated for the round-robin assessment in the same way reported by Neuwirth et al., except on this occasion substituting MUSIX for MUNIX (Neuwirth et al. , 2016). Briefly, for each muscle the 6 largest CMAPs taken from the mean of test-retest measurements were determined for each muscle. Of these 6, the 3 test-retest measurements with the smallest variability were used to calculate the "reference" CMAP amplitudes and the corresponding MUSIX values. The difference between the observed measurements and the hypothetical reference was determined and reported as accuracy, expressed as a percentage of the reference value.

For the multi-centre data, inter-rater variability in each study centre was assessed through Bland–Altman plots on log-transformed data. Displayed are the line of equality (i.e. where the data would lie if both examiners had exact agreement), the mean difference between examiners (bias), and 95% limits of agreement (LOA) for each comparison. The LOA represent the interval of two standard deviations of the measurement differences either side of the mean difference, confidence intervals surrounding these LOA were calculated using the MOVER (method of variance estimates recovery) method (Zou, 2013). Analyses were performed in Statistical Analysis Software, version 9.3.

Results

In the single healthy subject round-robin assessment, test-retest data are summarised in figure 1 and documented in detail in tables 1 and 2. Intra-rater COVs for MUSIX ranged from 7.8% in APB to 28.4% in EDB (**table 1**). Inter-rater COV ranges were from 7.8% (BB) to 16.2% (AH) (**table 2**). Welch's t-test found no evidence of a difference in the values obtained between experienced and non-experienced examiners for any of the muscles examined ($0.19 < p < 0.79$, **table 3).** Analysis of the relative mean and standard deviation of the MUSIX measurements of the experienced and non-experienced groups revealed a high degree of accuracy for all muscles, with the possible exception of ADM and EDB (**figure 2**).

Figure 1 here

	All Raters		Experienced Raters		Non-experienced Raters	
	COV	**Variability**	**COV**	**Variability**	**COV**	**Variability**
Muscle						
APB	8.8	12.4	9.7	13.8	7.8	11.1
ADM	9.7	13.7	10.2	14.5	9.1	12.9
BB	12.5	17.7	16.2	23.0	9.4	13.3
TA	10.1	14.3	8.9	12.6	11.2	15.9
EDB	24.0	33.9	19.6	27.7	28.4	40.1
AH	14.8	20.9	12.0	16.9	17.6	24.9

Table 1. Intra-rater COV (%) and variability (%) for MUSIX measurements in individual muscles in the single healthy subject study.

	Mean of measurements COV	Measurement 1 COV	Measurement 2 COV
Muscle			
APB	11.1	14.6	12.5
ADM	11.2	15.8	12.4
BB	7.8	14.4	13.0
TA	9.3	12.7	12.4
EDB	14.4	21.9	20.7
AH	16.2	14.3	24.8

Table 2. Inter-rater variability for MUSIX expressed as COV (in %) in individual muscles for the first and second measurement and mean of both values (single healthy subject round-robin study).

	MUSIX All Raters	MUSIX Experienced Raters	MUSIX Non-experienced Raters	Welch's t tests (Experience. Vs. Non-experienced.)
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**p-value**
Muscle				
APB	56.12 (7.8)	54.32 (7.16)	57.93 (7.66)	0.61
ADM	67.25 (9.46)	70.28 (9.75)	64.21 (8.5)	0.79
BB	44.16 (5.93)	43.40 (6.64)	44.86 (5.41)	0.32
TA	49.01 (6.02)	48.49 (5.83)	49.53 (6.41)	0.53
EDB	72.04 (17.16)	71.71 (16.60)	72.36 (18.43)	0.45
AH	61.83 (13.57)	66.64 (13.87)	57.02 (11.92)	0.19

Table 3. Mean and standard deviation (SD) of MUSIX values across the groups. All muscles grouped together for Welch's t test.

Figure 2 here

In the multi-centre study of healthy volunteers, MUSIX inter-rater variability for each muscle was examined for all centres, except one where there was only a single examiner (**table 4; complete dataset shown in supplemental table 1)**. AH was the least reliably measured muscle in centres 2 – 5, and the second least for centre 1. Box plots of the LOA to assess the variability of MUSIX, MUNIX and CMAP over all centres and muscles demonstrated that, on average, the MUNIX measurements had a greater inter-rater variability (figure 3; see also supplemental table 1). Example inter-rater Bland–Altman plots for APB MUSIX are shown in figure 4 (plots for all other muscles can be found in supplemental figure 1), and reveal few points beyond the LOA.

Centre	Muscle	Mean Difference (bias)	Standard Deviation Difference	Lower LOA	Upper LOA
1	APB	-0.03	0.13	0.21	-0.28
	ADM	-0.01	0.1	0.19	-0.2
	BB	0	0.04	0.08	-0.08
	TA	-0.03	0.09	0.15	-0.21
	AH	0.05	0.015	0.33	-0.24
	EDB	-	-	-	-
2	APB	-0.04	0.09	0.14	-0.21
	ADM	-0.02	0.11	0.2	-0.24
	BB	0.01	0.07	0.14	-0.12
	TA	0.02	0.09	0.18	-0.15
	AH	-0.03	0.14	0.24	-0.3
	EDB	-0.09	0.14	0.18-	-0.36
3	APB	-0.06	0.1	0.14	-0.25
	ADM	-0.04	0.09	0.14	-0.22
	BB	0	0.09	0.17	-0.17
	TA	-0.03	0.07	0.1	-0.16
	AH	-0.1	0.18	0.26	-0.46
	EDB	-0.1	0.18	0.26	-0.46
4	APB	0.03	0.1	0.22	-0.16
	ADM	0.01	0.09	0.18	-0.17
	BB	-0.02	0.04	0.07	-0.1
	TA	0	0.05	0.11	-0.11
	AH	-0.01	0.17	0.32	-0.34
	EDB	0.02	0.11	0.22	-0.19
5	APB	0	0.08	0.16	-0.15
	ADM	0	0.08	0.16	-0.17
	BB	-0.02	0.04	0.06	-0.1
	TA	0	0.06	0.12	-0.13
	AH	0.05	0.15	0.35	-0.25
	EDB	0.05	0.17	0.39	-0.13

Table 4. MUSIX inter-rater variability across all muscles for the different centres.

Intra-rater MUSIX variability was assessed in a similar fashion for all examiners, except examiner 8 for whom only data from one visit was available (table 5, full data set supplemental table 2). Example MUSIX intra-rater Bland-Altman plots for the APB muscle for 4 examiners are shown in figure 5 (remaining plots for APB and all other muscles available in supplemental figure 2). AH was again the muscle with the most variation between measurements, although EDB was fairly similar. In addition, APB and ADM also showed more variable measurements that BB or TA. As with inter-rater variation,

MUNIX was more variable than either MUSIX or CMAP (figure 6). Lastly, MUSIX variability had only a very limited relationship to CMAP, with the CMAP measurement contributing between 0.3% and 3.3% to MUSIX variability (table 6).

Examiner	Muscle	Mean Difference (bias)	Standard Deviation Difference	Lower LOA	Upper LOA
1	APB	-0.03	0.12	-0.26	0.21
	ADM	0	0.11	-0.21	0.22
	BB	0	0.03	-0.07	0.07
	TA	0	0.06	-0.12	0.12
	AH	0	0.12	-0.24	0.23
	EDB	-0.01	0.07	-0.14	0.12
2	APB	0.05	0.13	-0.21	0.3
	ADM	0.01	0.12	-0.21	0.24
	BB	0	0.06	-0.13	0.12
	TA	0	0.11	-0.21	0.22
	AH	0.04	0.12	-0.2	0.28
	EDB	-	-	-	-
3	APB	-0.03	0.12	-0.26	0.210
	ADM	0.03	0.12	-0.21	0.27
	BB	-0.02	0.05	-0.12	0.08
	TA	-0.04	0.08	-0.2	0.11
	AH	-0.03	0.14	-0.3	0.24
	EDB	-0.03	0.11	-0.24	0.17
4	APB	0.01	0.08	-0.16	0.17
	ADM	0.05	0.12	-0.18	0.29
	BB	0.03	0.09	-0.15	0.2
	TA	-0.02	0.09	-0.2	0.15
	AH	-0.05	0.12	-0.28	0.18
	EDB	0.02	0.17	-0.32	0.36
5	APB	-0.07	0.07	-0.21	0.08
	ADM	-0.01	0.11	-0.23	0.2
	BB	0.01	0.07	-0.12	0.14
	TA	-0.01	0.06	-0.12	0.1
	AH	0.09	0.14	-0.19	0.36
	EDB	0.02	0.17	-0.32	0.36
6	APB	-0.02	0.06	-0.14	0.1
	ADM	-0.02	0.08	-0.18	0.14
	BB	0.01	0.06	-0.12	0.13
	TA	0.04	0.09	-0.14	0.21

	AH	0.15	0.21	-0.27	0.58
	EDB	0.01	0.06	-0.12	0.13
7	APB	0.03	0.06	-0.9	0.16
	ADM	0.04	0.09	-0.15	0.22
	BB	0.01	0.04	-0.07	0.08
	TA	0.01	0.04	-0.07	0.09
	AH	0.04	0.13	-0.21	0.28
	EDB	0	0.07	-0.15	0.14
9	APB	0	0.08	-0.16	0.15
	ADM	0.01	0.05	-0.08	0.11
	BB	0	0.05	-0.1	0.09
	TA	-0.02	0.07	-0.17	0.12
	AH	0.01	0.1	-0.18	0.21
	EDB	0.06	0.09	-0.12	0.25
10	APB	0.01	0.09	-0.16	0.18
	ADM	-0.01	0.08	-0.17	0.15
	BB	0	0.04	-0.08	0.08
	TA	0.03	0.06	-0.08	0.15
	AH	0.03	0.13	-0.22	0.27
	EDB	-0.02	0.15	-0.32	0.28
11	APB	0.02	0.07	-0.13	0.17
	ADM	0.01	0.07	-0.13	0.15
	BB	-0.04	0.05	-0.15	0.07
	TA	0.01	0.05	-0.09	0.1
	AH	0.02	0.08	-0.14	0.18
	EDB	0.02	0.08	-0.14	0.18

Table 5. MUSIX intra-rater variability across all muscles for the different examiners in the multi-centre study on healthy volunteers.

Figure 4 here

		All centres
MUSIX~CMAP	**ABP**	0.26
	ADM	0.95
	BB	3.25
	TA	1.80
	AH	1.44
	EDB	7.11

Table 6. Linear regression analysis (multiple R-Squared). Relative contribution (%) of CMAP variability for total MUSIX variability. The data are pooled from all participating centres. For the EDB muscle this is only centres A, B, D and E

Discussion

There is increasing interest in the MUNIX/MUSIX technique as a rapid, non-invasive means to monitor progression in neuromuscular disease. In two previous reports our team detailed the reliability of the MUNIX values (Neuwirth et al. , 2011, Neuwirth et al. , 2016), but little attention was given to the consistency of the MUSIX. Since the rate of symptom progression in neuromuscular diseases is determined by both the amount of axon loss and effective reinnervation (Gordon et al. , 2004, Van Asseldonk et al. , 2006), MUSIX could provide valuable information regarding the reinnervation process. Such data could provide important insights into how certain conditions progress and how they respond to new treatments. This paper combines data from two previous studies to investigate whether the MUSIX can be a reliable measurement of motor unit size for future clinical studies.

The dominant finding of the round-robin study is that the coefficient of variability for MUSIX is below 20% for all muscles, except EDB. This finding is similar to the initial report of MUNIX variability, in which the small muscles of the foot (AH and EDB) were also the worst performing (Neuwirth et al. , 2016). The reasons for this are multiple. As the MUNIX values obtained for EDB are often smaller than other muscles (which was especially true in the single healthy volunteer study) any variability in the absolute values of EDB measurements will result in a larger relative variability and COV. Furthermore, the peroneal nerve branch to EDB is susceptible to injury and so may changes may not reflect the disease process of interest. Regarding AH, multiple small foot muscles contribute to the recorded measurements of the AH CMAP (Nandedkar et al. , 2007) and as some individuals find activation of this muscle difficult, the recordings may be susceptible to volume conducted artefact. It is therefore possible that the muscles contributing to the CMAP are different to those activated during voluntary contraction. We would suggest that such technical pitfalls outweigh the benefit of including AH in study protocols and advocate omission from future studies.

Interestingly, in the multi-centre study MUSIX was more consistent, both in intra- and inter-rater assessments, than the MUNIX. Our analyses suggest that one explanation of this observation is that MUSIX is independent of CMAP variability, which is in keeping with prior reports and the opposite of MUNIX, for which variability is highly dependent on the CMAP (Nandedkar et al. , 2010). We have previously documented that the relative contribution of the CMAP to MUNIX variation may be as high as 90% which emphasises the importance of CMAP optimisation to the MUNIX measurement (Neuwirth et al. , 2011). The underlying cause of MUSIX variation is less clear but may be found in the distribution of surface interference patterns (SIP) across weak and strong contractions.

Overall, comparing the performance MUNIX and MUSIX in the other muscles sampled across both study settings reveals a similar level of performance (Neuwirth et al. , 2011, Neuwirth et al. , 2016). Encouragingly, there was no significant difference in the values obtained for MUSIX between those with prior experience in the technique and those unfamiliar with it (but with training in standard nerve conduction studies/EMG). This would suggest that that following training, obtaining high quality recordings can be rapidly achieved without prior experience. We would suggest that MUSIX values are an even more reliable than MUNIX values.

However, in keeping with the original multi-centre MUNIX paper, our new analysis demonstrates that there is a degree of variability between different centres and different muscles (Neuwirth et al. , 2011). As noted in the report of Neuwirth et al., training is likely to be important in ensuring consistency of the MUNIX/MUSIX technique across individuals/centres and a training experience appeared to negate any effects of unfamiliarity with the technique in our round-robin study (Neuwirth et al. , 2016, Nandedkar et al. , 2018). This training course based approach was not implemented for the multi-centre study which may explain some of the variation. Recent multi-centre studies (e.g. SOPHIA and

the ongoing Biogen trials) have required a training course attendance and completion of test-retest assessments on healthy volunteers prior to commencing recordings on patients, which appears to reduce variability (Neuwirth et al. , 2018). Several members of our team have recently published guidelines on performing the MUNIX/MUSIX technique which should provide a further step towards standardisation of the technique (Nandedkar et al. , 2018).

Although only limited data are available for MUSIX in conditions such as ALS, studies have shown the expected inverse relationship between MUNIX and MUSIX in such patients (Nandedkar et al. , 2010, Escorcio-Bezerra et al. , 2016), with one study documenting a 50% increase in MUSIX over 8 months (Boekestein et al. , 2012). As emphasised in previous papers focusing on the MUNIX, the MUSIX is not an anatomical measure of motor unit size but an index underpinned by the MUNIX and CMAP measurements and their own relationship to the number of motor axons (Nandedkar et al. , 2010, Neuwirth et al. , 2011). The relationship to actual motor unit size is therefore unknown. The MUSIX is calculated by dividing the CMAP amplitude by the MUNIX and, as noted many years ago, different methods to derive motor unit size may result in quite different values (Doherty et al. , 1993), thus making comparisons, even between neurophysiological techniques, difficult. However, a significant positive relationship has been reported between MUSIX and motor unit potential amplitude obtained through macro-EMG, in keeping with the assertion that MUSIX relates to the size of the motor unit (Sandberg et al. , 2011). Nonetheless, preliminary studies have documented the expected increase in MUSIX in conditions such as ALS and so the technique may provide valuable insights into which muscles undergo compensatory re-innervation and which do not. Such information may also be of value in other conditions, for example, in spinal muscle atrophy and neuropathies, providing researchers with a further measurement relevant to the pathophysiology of neurogenic diseases. The rapidity and ease of application of MUNIX/MUSIX will hopefully facilitate uptake and enhance our understanding the interplay between denervation and compensatory re-innervation over time in progressive conditions such as ALS.

Conclusion

We conclude that the MUSIX measurement can provide a reliable surrogate marker of re-innervation. The index warrants consideration in future longitudinal studies in diseases with either axon or motor neuron loss and compensatory reinnervation, such as ALS.

Acknowledgements

The authors wish to thank Kathleen Baster, Statistical Services Unit, University of Sheffield for statistical advice. J Alix and L Gelder were supported by the National Institute for Health Research (NIHR) Sheffield Biomedical Research Centre (Translational Neuroscience). The work was also supported by the Swiss ALS Foundation. Dr. Barkhaus acknowledges funding from the Dolores and Carroll Fund from the Milwaukee Medical Foundation.

Conflict of interest

The authors declare no conflicting interests.

List of abbreviations

ADM – abductor digiti minimi

AH - Abductor hallucis

ALS – amyotrophic lateral sclerosis

APB – abductor pollicis brevis

CMAP – compound muscle action potential

COV – coefficient of variation

EDB – extensor digitorum brevis

BB – Biceps brachii

EMG – electromyography

LOA- limits of agreement

MOVER - method of variance recovery for ratios

MUNIX – motor unit number index

MUSIX – motor unit size index

SD – standard deviation

SIP – surface interference pattern

TA – tibialis anterior

Supplemental figures/tables

Supplemental table 1 here.

Complete dataset for table 4.

Supplemental table 2 here. Complete dataset for table 5.

Figure 1. Test-retest results for MUSIX in individual muscles in the single subject round-robin study. The dotted line represents a hypothetical reference value (see methods).

Figure 2. Relative mean and standard deviation of MUSIX measurements in individual muscles of the experienced group (filled circles) and non-experienced group (empty circles) compared to the hypothetical reference values, expressed as accuracy (%).

Figure 3. Box plots demonstrating the distribution of inter-rater limits of agreement for CMAP/MUNIX/MUSIX across the different centres. The horizontal line represents the median, the larger open circles the mean.

Figure 4. Example inter-rater MUSIX Bland-Altman plots across participating centres for the APB muscle.

Figure 5. Example intra-rater MUSIX Bland-Altman plots for 4 examiners and the APB muscle.

Figure 6. Box plots demonstrating the distribution of intra-rater limits of agreement for CMAP/MUNIX/MUSIX pooled across the examiners. The horizontal line represents the median, the larger open circles the mean.

Supplemental figure 1 here

Supplemental figure 1. MUSIX inter-rater Bland-Altman plots the remaining muscles studied. A, ADM; B, BB; C, TA; D, AH, EDB.

Supplemental figure 2 here

Supplemental figure 2. MUSIX intra-rater Bland-Altman plots for the examiners who provided such data to the multi-centre study.

A, remain APB plots. B, ADM plots; C, BB plots; D, TA plots; E, AH plots; F, EDB plots.

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