Clinical Neurophysiology 123 (2012) 1644–1649

Contents lists available at SciVerse ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Motor unit number index (MUNIX) versus motor unit number estimation (MUNE): A direct comparison in a longitudinal study of ALS patients

Werner A. Boekestein [a,b], Helenius J. Schelhaas [a], Michel J.A.M. van Putten [b], Dick F. Stegeman [a,c], Machiel J. Zwarts [d], Johannes P. van Dijk [a,e,*]

[a] Donders Institute for Brain, Cognition and Behavior, Department of Neurology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands
[b] Department of Neurology and Clinical Neurophysiology, Medisch Spectrum Twente, and MIRA-Institute for Biomedical Technology and Technical Medicine, University of Twente, The Netherlands
[c] Research Institute MOVE, Faculty of Human Movement Sciences, VU University, Amsterdam, The Netherlands
[d] Epilepsy Center Kempenhaeghe, Heeze, The Netherlands
[e] Klinik für Kieferorthopädie und Orthodontie, Universitätsklinikum Ulm, Germany

ARTICLE INFO

Article history:
Accepted 1 January 2012
Available online 8 February 2012

Keywords:
ALS
MUNIX
MUNE
Monitoring disease progression
HD-surface EMG

HIGHLIGHTS

- This study shows that the motor unit number index (MUNIX) and high-density motor unit number estimation (MUNE) outcomes measured on the thenar muscle are significantly correlated in ALS patients.
- After 8 months follow-up, MUNIX and high-density MUNE values in ALS patients showed significantly more decline compared to CMAP, ALS functional rating scale and MRC-scale.
- There was no significant difference in relative decline between MUNIX and high-density MUNE values, showing their equivalent potential in detecting motor neuron loss.

ABSTRACT

Objective: To evaluate how the motor unit number index (MUNIX) is related to high-density motor unit number estimation (HD-MUNE) in healthy controls and patients with amyotrophic lateral sclerosis (ALS).
Methods: Both MUNIX and HD-MUNE were performed on the thenar muscles in 18 ALS patients and 24 healthy controls. Patients were measured at baseline, within 2 weeks, and after 4 and 8 months. Clinical evaluation included Medical Research Council (MRC) scale and the ALS functional rating scale (ALSFRS).
Results: There was a significant positive correlation between MUNE and MUNIX values in ALS patients ($r = 0.49$ at baseline; $r = 0.56$ at 4 months; $r = 0.56$ at 8 months, all $p < 0.05$), but not in healthy controls. After 8 months, both MUNE and MUNIX values of the ALS patients decreased significantly more compared to MRC scale, ALS functional rating scale (ALSFRS) and compound muscle action potential (CMAP) ($p < 0.05$). There was no significant difference in relative decline of MUNIX and HD-MUNE values.
Conclusions: In ALS patients, MUNIX and HD-MUNE are significantly correlated. MUNIX has an almost equivalent potential in detecting motor neuron loss compared to HD-MUNE.
Significance: MUNIX could serve as a reliable and sensitive marker for monitoring disease progression in ALS.

1. Introduction

There is a growing interest in methods to monitor disease progression in amyotrophic lateral sclerosis (ALS). A reliable and sensitive method is relevant, for example, as an outcome measure in therapeutic trials. Besides clinical methods to monitor disease progression, such as the ALS functional rating scale (ALSFRS) and the Medical Research Council (MRC) scale, quantitative methods that are more directly related to the underlying disease process are of interest. Motor unit number estimation (MUNE) techniques (Shefner et al., 2004), and a more recently developed method providing a motor unit number index (MUNIX) (Nandedkar et al., 2004), are all based on surface electromyography (sEMG) measurements. These methods are non-invasive and, in contrast to the MRC scale and the compound muscle action potential (CMAP), are not influenced by the compensatory reinnervation process following denervation due to lower motor neuron degeneration.

* Corresponding author at: Department of Neurology – 920, Donders Institute for Brain, Cognition and Behavior, Radboud University Nijmegen Medical Center, PO Box 9101, 6500 HB Nijmegen, The Netherlands. Tel.: +31 24 3613973; fax: +31 24 3615097.
E-mail address: H.vanDijk@neuro.umcn.nl (J.P. van Dijk).

1388-2457/$36.00 © 2012 International Federation of Clinical Neurophysiology. Published by Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.clinph.2012.01.004

Most MUNE techniques are based on the ratio of the maximal CMAP divided by an average surface motor unit action potential (SMUP) (McComas et al., 1971; Bromberg, 2007). MUNE appears to be a more sensitive marker of disease progression in ALS as compared to clinical measures (Felice, 1997; Shefner et al., 2004). High-density surface MUNE (HD-MUNE) is a recently developed technique that combines high-density surface EMG with elements of two other MUNE techniques: the increment counting technique (ICT) and the adapted multiple point stimulation (aMPS) (van Dijk et al., 2008). In ICT nerve stimulation intensity is increased stepwise, starting at a sub-threshold level. Every incremental step that leads to a discrete increase in CMAP amplitude is considered as the added contribution of one single motor unit. Dividing the latest CMAP response by the number of incremental steps will provide an average MUP amplitude. However, several motor axons with similar stimulation thresholds can have a probability of firing at a certain stimulation intensity, which leads to a variable CMAP amplitude ('alternation') on repetitive stimuli (McComas et al., 1971). In MPS the nerve is stimulated at such a low intensity that only a single MUP is measured per site, at multiple sites along the nerve course. In this way, one can acquire several MUPs with a different shape and amplitude, belonging to different motor units. It can be difficult, however, to obtain a large sample of MUPs with MPS, which is beneficial for the accuracy of the motor unit estimate. This is less of a problem in the adapted form of MPS (aMPS), where the stimulus strength can be increased at a certain site as long as alternation is visible (Wang and Delwaide, 1995). The novelty of HD-MUNE is the use of high-density EMG: stimulation at each site results in simultaneous recordings from multiple densely spaced electrodes. Because of the spatial dimension added, SMUPs can be recognized in the recorded profiles and the signal can be decomposed into prints of individual MUPs. In this way, alternating MUPs can be easily recognized despite the use of incremental stimulation steps. Furthermore, small MUPs and a relative large sample size can be obtained easier. HD-MUNE in patients with ALS show a relatively good reproducibility and a steeper decline during follow up as compared to the maximal CMAP and the ALS functional rating scale (ALSFRS) (van Dijk et al., 2010). Unfortunately, the HD-MUNE technique is time-consuming and technically still relatively difficult to perform.

While the above described MUNE techniques depend on the excitation of single motor units, MUNIX has a very different and much faster approach in detecting motor unit loss. It mainly depends on maximal CMAP measurement and the recording of sEMG interference patterns at different grades of voluntary muscle contraction. In the ideal situation that all motor unit potentials are identical in size and do not overlap, the exact number of motor units can be calculated from the CMAP and the surface EMG (see Appendix A). However, as overlaps do occur and motor units are obviously not equal in size, an approximation is made using a model (Nandedkar et al., 2004, 2010). The model is described in more detail in the Appendix A. Whereas the mathematics used in MUNE results in an estimate of the actual number of motor units (providing an unbiased sample of MUPs is obtained), the index provided by the MUNIX technique has, also theoretically, an undefined relation to the actual number of motor units. Because MUNIX is a relatively easy and fast technique, it has a potential practical advantage compared to MUNE. In a recently published multicenter study (Neuwirth et al., 2011), MUNIX showed good reproducibility in healthy subjects. However, at present, it is unknown what the accuracy and thus the usefulness of the index obtained with MUNIX is.

In the current study, we compared MUNIX to HD-MUNE performed unilaterally on the thenar muscle in order to reveal the relation between MUNIX and the estimated number of motor units from HD-sEMG that we will define as our reference. In addition, we evaluate the potential of MUNIX as a measure for disease progression in eighteen ALS patients.

2. Methods

2.1. Study protocol

In ALS patients, measurements were performed on the thenar muscle of the least affected hand, or – if hands were equally affected – on the non-dominant hand. In the control group all measurements were performed on the non-dominant side. Patients were followed for a period of 8 months. Muscle strength, ALSFRS (Cedarbaum and Stambler, 1997), CMAP amplitude, HD-MUNE and MUNIX were determined at baseline, at 4 months and at 8 months. The same operator assessed the reproducibility of MUNE and MUNIX in the patient group during a separate session within 2 weeks after baseline and without knowledge of the MUNE and MUNIX results of the first visit.

2.2. Subjects

Eighteen patients, 7 women and 11 men, diagnosed with probable laboratory-supported, probable or definite ALS, according to the revised El Escorial criteria, (Brooks et al., 2000) were included in the study. All patients had clinical signs of cervical lower motor neuron involvement. Median age was 64 years (range 21–77) and median duration of symptoms was 1.7 years (range 0.8–5.5). Sixteen patients were on riluzole treatment during the entire study period. For baseline comparison, 24 healthy volunteers were measured. This group consisted of 9 women and 15 men, with a median age of 62 years (range 49–78). There was no significant age-difference between the ALS patients and the healthy controls. Apart from ALS, there was no history of neuromuscular disease in neither the patients nor the healthy controls. Carpal tunnel syndrome (CTS) was ruled out by a questionnaire and, in doubt, by a nerve conduction study according to a standard protocol. The study was approved by the medical ethical committee of the Radboud University Nijmegen Medical Center. All patients and healthy controls gave their written informed consent.

2.3. Electrophysiological measures

Measurements necessary to compute MUNIX were performed before the HD-MUNE procedure started, using the same electromyography instruments and without change of electrode positions, i.e. using the high-density electrode. As MUNIX is generally performed with a single electrode, a single electrode equivalent of 1×1 cm was calculated from the high-density electrode grid by averaging the signals from 3×3 electrodes at the position where the CMAP was maximal (van Dijk et al., 2009). The maximal CMAP was obtained by supramaximal stimulation of the median nerve at about 6 cm from the thenar eminence at the end of the HD-MUNE protocol explained below. Note that the same CMAP was used for both measures.

2.3.1. MUNIX

First, surface EMG interference patterns (SIP) were recorded during voluntary thumb abduction against resistance, provided by the operator. Two series of SIP recordings were made, each at five increasing force levels (minimal; 25%; 50%; submaximal; maximal effort). Offline, CMAP and SIP data were analyzed in a separate program written in Matlab (The Mathworks Inc., version 7.10). The signal quality was visually checked by the operator to exclude excessive noise, tremor or other artifacts. From the 2–3 s of SIP data that was recorded, 300 ms epochs were selected for further

analysis. For each of the SIP recordings the SIP area and SIP power was calculated, followed by the computation of the ICMUC for each SIP, according to Eq. (5) in Appendix A. The negative phase of the maximal CMAP was used to calculate CMAP peak amplitude, CMAP area and CMAP power. To avoid interference with volume-conducted activity of neighboring muscles that could influence the MUNIX calculation, only signals with a SIP area > 20, ICMUC < 100 and SIP area/CMAP area > 1 were used.

Using the power regression analysis according to Eqs. (6) and (7) the MUNIX outcome was calculated. A minimum of 8 out of 10 valid ICMUC-SIP area combinations were required for a reliable regression analysis. Finally, for every MUNIX outcome the MUSIX was calculated according to Eq. (8).

2.3.2. High-density MUNE

HD-MUNE was performed and analyzed as described previously (van Dijk et al., 2008, 2010). To record spatiotemporal MUPs, a flexible high-density electrode grid of 8×15 Ag–AgCl electrodes with an interelectrode distance of 4 mm (Lapatki et al., 2004) was placed over the thenar muscle. The reference electrode was placed on the first metacarpophalangeal joint of the fifth digit. The grid was attached to a 130-channel amplifier (Active-One; Biosemi, Amsterdam, The Netherlands) for amplification and digitalization (bandpass filtered at 0.16–400 Hz, sample rate 2048 Hz). The median nerve was stimulated using a computer-controlled constant-current stimulator with square wave pulses of 100 μs duration using a handheld electrode at a rate of one per second with slowly increasing stimulus strength. The stimulator electrodes were repositioned after 6–10 single MUPs were obtained at one stimulation site along the nerve, and the process was repeated. In this way, multiple points along the distal part of the nerve and around the elbow crease were used. For CMAP measurement, we used the same CMAP as obtained for the MUNIX computation.

Data were analyzed off-line using dedicated analysis software written in Matlab (The Mathworks Inc., version 7.10). Alternating MUPs were recognized and decomposed into single MUPs using spatiotemporal information. This was performed with a computer aided manual decomposition program that allowed the user to overrule automated suggested results. If MUPs from different stimulation sites were (nearly) identical, only one of these MUPs was kept for further analysis. For the MUPs obtained, the shortest latency per distal stimulation site was determined and used for alignment. In this way, phase-cancellation effects, that also occur in the CMAP recording, due to differences in axonal length and nerve conduction velocity were partly retained. MUPs from proximal stimulation sites were aligned at the onset determined per MUP in order to prevent an artificially high phase cancellation. Finally, the mean MUP was obtained by averaging the MUPs on a sample-by-sample basis, and the MUNE was calculated by dividing the mean supramaximal CMAP by the mean MUP on a sample-by-sample basis (for the complete signal) (van Dijk et al., 2008). The CMAP negative peak amplitude of a large single electrode equivalent (1×3 cm) was determined from the high-density CMAP by averaging 3×8 grid electrodes, thereby simulating a larger area electrode (van Dijk et al., 2009).

2.4. Muscle strength and functional assessment

Maximal muscle strength was measured according to the Medical Research Council (MRC) scale. The same investigator graded bilateral shoulder abduction, elbow flexion and extension, wrist flexion and extension, and thumb and fifth finger abduction (range 0–5). The MRC sumscore was calculated by summing all 14 MRC grades (maximum 70). The ALSFRS (range 0–40) was used to score activities of daily living (Cedarbaum and Stambler, 1997).

2.5. Statistical analysis

Comparison of ALS patients and healthy controls was made using the Mann–Whitney *U*-test. Reproducibility was tested using coefficient of variation (CoV), intraclass correlation coefficient (ICC), and visualized with a Bland–Altman plot. Correlation was tested using Pearson or Spearman correlation coefficient, depending on the presence of a normal distribution. To compare different measures at baseline, 4 and 8 months, Wilcoxon signed-rank test was used. All data were analyzed using SPSS (version 16) software. Results were considered significant at $p < 0.05$.

3. Results

3.1. Baseline

The mean values of the different measures at baseline are presented in Table 1, together with the subject characteristics. The presented MUNE and MUNIX were taken as the mean of test and retest values. Mean CMAP amplitude was significantly lower for ALS patients as compared to CMAP amplitude for healthy controls. Both mean MUNE and MUNIX were significantly lower in ALS patients. There was no significant decline of neither MUNE nor MUNIX with age. Mean MUSIX was not significantly higher in ALS patients. On average, 23 single MUPs (range 9–44) were used for one MUNE measurement.

MUNE and MUNIX reproducibility was assessed with a retest within 2 weeks after baseline measurement in seventeen ALS patients. MUNIX reproducibility results are provided in Fig. 1 (see van Dijk et al., 2010 for details on MUNE reproducibility). During the retest procedures, one patient was unable to relax the hand muscles sufficiently to allow valid MUNE and MUNIX measurements. The ICC for MUNE (0.86) was higher than for MUNIX (0.74). Median CoV for MUNE was 13.7%, and for MUNIX 19.4%.

In healthy controls, MUNE and MUNIX appeared uncorrelated (Fig. 2), whereas in ALS patients MUNE and MUNIX showed a significant positive correlation (at baseline, $r = 0.49$, $p < 0.05$; Fig. 3). Furthermore, there was a strong significant positive correlation between MUNIX and CMAP amplitude in both healthy controls ($r = 0.78$; $p < 0.01$) and ALS patients ($r = 0.70$; $p < 0.05$). MUNE was significantly correlated to CMAP amplitude in ALS patients ($r = 0.56$; $p < 0.05$), but not in healthy controls ($r = 0.29$; $p = 0.17$). MUSIX was not significantly correlated neither to CMAP amplitude nor to MUP size.

Table 1
Patient characteristics and mean results at baseline. Median age, median duration of symptoms, mean MRC sumscore and mean ALSFRS score are given together with the range (maximum MRC sumscore 70; maximum ALSFRS score 40); mean CMAP, MUNE, MUNIX and MUSIX values are given with standard deviation (±SD).

	ALS patients ($n = 18$)	Healthy controls ($n = 24$)
Male	11	15
Female	7	9
Median age (years)	64 (21–77)	62 (49–78)
Median duration symptoms (years)	1.7 (0.8–5.5)	
Mean CMAP amplitude (mV)	5.9 ± 1.5	7.1 ± 1.7*
Mean MUNE	158 ± 103	256 ± 85**
Mean MUNIX	91 ± 33	121 ± 31**
Mean MUSIX (μV)	81 ± 29	71 ± 13
Mean MRC sumscore	62.3 (49–69)	
Mean ALSFRS score	(28–39)	

* Signif. difference between the two groups at $p < 0.05$ level.
** Signif. difference between the two groups at $p < 0.01$ level.

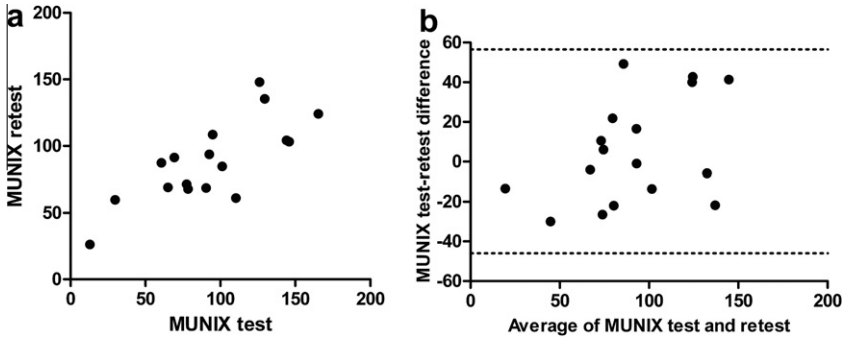

Fig. 1. Reproducibility of MUNIX in ALS patients. (a) Scatterplot of MUNIX test and retest values (on the *x* and *y*-axis, respectively), with intraclass correlation coefficient (ICC) 0.74 and median coefficient of variation (CoV) 19.4%. (b) Bland–Altman plot of MUNIX test and retest. On the *x*-axis the average values of MUNIX test and re-test are given with the 95% limits of agreement (dotted lines). On the *y*-axis the difference of MUNIX test minus MUNIX re-test is represented.

3.2. Follow-up

Over 8 months, HD-MUNE and MUNIX showed the steepest relative decline of all variables measured (both 49%); Fig. 4A, Table 1). Both measures decreased significantly more than CMAP, ALSFRS and MRC sumscore ($p < 0.05$). Although HD-MUNE showed more variability than ALSFRS scores at 4 months (CoV = 1.4), this variability was significantly reduced at 8 months (CoV = 0.6) (Table 2). A similar decrease in CoV was seen in MUNIX after 4 and 8 months (CoV, respectively 1.6 and 0.5). The relative decline over 8 months and variability of MUNIX for each individual patient is shown in Fig. 4B. The mean MUSIX of ALS patients increased respectively 24% and 52% at 4 and 8 months. During follow-up the significant correlation between HD-MUNE and MUNIX in ALS patients persisted and became slightly stronger: $r = 0.56$ at 4 months and $r = 0.56$ at 8 months (both $p < 0.05$). A non-significant correlation with MUP size was found.

4. Discussion

Monitoring disease progression in ALS with a sensitive EMG method is particularly relevant for phase II trials, where it can be used as an additional outcome measure (van Dijk et al., 2010; Shefner et al., 2011). In this perspective, it has been stated that MUNIX could be a good alternative for MUNE because of its practical advantages (Ahn et al., 2010; Neuwirth et al., 2011). In our study we performed the first formal direct comparison of MUNIX with a sensitive MUNE technique to evaluate their relation and to compare their potential of detecting disease progression in ALS. The most important finding was the significantly stronger decline of MUNIX compared to CMAP amplitude and the similar relative decline of HD-MUNE, after 8 months of follow-up. MUNIX also declined significantly more than the MRC sumscore and ALSFRS, although this comparison should be taken with care as the latter measures reflect whole body progression. Regarding the relative change in time, HD-MUNE tended to decrease slightly more than MUNIX after 4 months, but there was no significant difference between the two measures both after four and after 8 months. In healthy controls, our absolute MUNIX values were lower than MUNE values. It should be noted that MUNIX is an index of which the absolute value depends on the arbitrarily chosen SIP area of 20.

We found a significant positive correlation between MUNE and MUNIX in ALS patients. This correlation, although moderate in size, remained significant over time and slightly increased during 8 months of follow-up. However, MUNE and MUNIX did not correlate in healthy controls. This is surprisingly, as the CMAP that is used in both calculations is the same. A few points need to be addressed concerning this issue. Firstly, in the ALS patient group the wide spread in MUNIX and MUNE values (including lower values due to already degenerated muscle) could also have contributed to the significant correlation. In healthy controls, this spread was narrower, but taken into account the previously found high reproducibility of MUNIX and HD-MUNE in healthy individuals (Neuwirth et al., 2011; van Dijk et al., 2008) a significant correlation is expected. Secondly, the computation of MUNIX incorporates MUPs of the abductor pollicis brevis muscle from voluntary activation, while in HD-MUNE MUPs are obtained by stimulating the median nerve and, hence, this will include MUPs from the

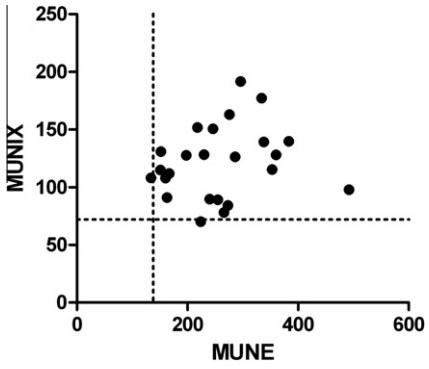

Fig. 2. Correlation of MUNE and MUNIX in healthy controls ($n = 24$). The scatterplot shows no apparent correlation between the MUNE and MUNIX values ($r = 0.19$ with $p = 0.38$). Dotted lines represent the 5% lower limit of normal.

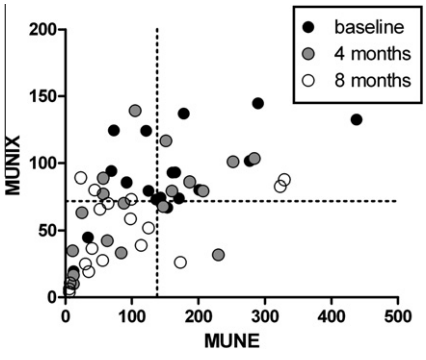

Fig. 3. Correlation of MUNE and MUNIX in ALS patients ($n = 18$). Values measured at baseline, at 4 months and at 8 months are marked differently (according to symbols in the legend). At all measuring points a significant positive correlation was present between MUNE and MUNIX values ($r = 0.49$, at baseline; $r = 0.56$, at 4 months; $r = 0.56$, at 8 months, all $p < 0.05$). Dotted lines represent the 5% lower limit of normal taken from the healthy subjects.

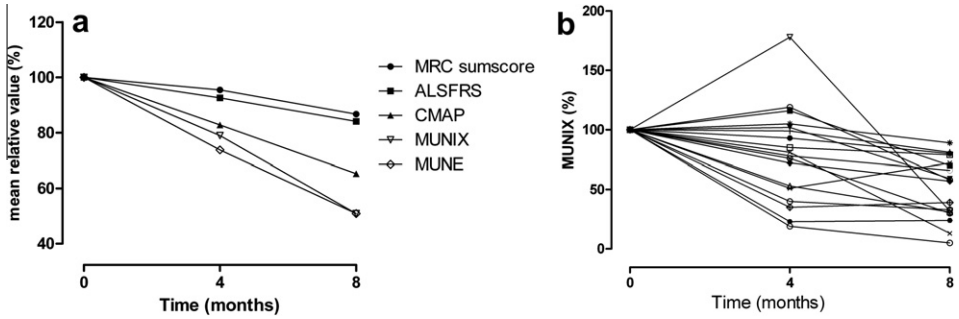

Fig. 4. (a) Relative change from baseline of different measures at 4 and 8 months follow-up in ALS patients. MUNE and MUNIX declined significantly more than the other measures at 8 months ($p < 0.05$). Abbreviations: ALS functional rating scale (ALSFRS), motor unit number estimation (MUNE), motor unit number index (MUNIX), Medical Research Council (MRC), compound muscle action potential (CMAP). (b) Relative decline of individual MUNIX values from baseline, at 4 and 8 months follow-up in ALS patients.

opponens pollicis, flexor pollicis and lumbrical I and II muscles. Thirdly, we speculate on a possible 'ceiling effect' of the MUNIX values with larger number of motor units in healthy controls. The possible mechanism could be that, for the non-denervated muscle, the first recruitment small type 1 units could rapidly lead to superimposition even at low contraction force and by this, to an 'underestimation' of the MUNIX value. Theoretically, this might influence MUNIX sensitivity at an early disease stage. Although non-significant, in ALS patients a trend of less relative decline of MUNIX compared to HD-MUNE was seen at 4 months, which might support this.

In accordance with previous reports (Ahn et al., 2010; Neuwirth et al., 2010; Nandedkar et al., 2010), we found a significant positive correlation between MUNIX and CMAP amplitude (ALS patients $r = 0.70$, $p < 0.05$; controls $r = 0.78$, $p < 0.01$). This is expected, since the CMAP is incorporated as a scaling factor in the mathematical model of MUNIX. Consequently, the influence of the CMAP on MUNIX emphasizes the need for accurate maximal CMAP measurement to reduce MUNIX variability. This means taking into account optimal active electrode position, electrode diameter and thumb position, since all of these factors can influence the CMAP amplitude (van Dijk et al., 1995; Bromberg and Spiegelberg, 1997). Moreover, it emphasizes the need to standardize electrode size when MUNIX is to be used in multi-center studies (Neuwirth et al., 2011). Note that HD-MUNE and CMAP did not correlate for the controls, which points towards another difference between the two techniques.

Our study showed that both HD-MUNE and MUNIX were reproducible in ALS patients. HD-MUNE showed a higher ICC and lower median CoV than MUNIX. Nevertheless, MUNIX median CoV was acceptable (less than 20%). Our MUNIX ICC was lower as compared to a previous report (Ahn et al., 2010). This might be explained by the different time-span between test and retest we used; whereas in our study this interval was one to 2 weeks, Ahn et al. retested at the same day. Another explanation could be the different type of muscle studied; as mentioned before, when measuring the thenar, hand and thumb position may influence the CMAP leading to higher MUNIX variability. Although the hand was positioned in a similar way during each test, no strict criteria were used for hand position.

MUSIX is considered a measure of the average amplitude of the surface-recorded motor unit potential (SMUP). Unlike most MUNE methods, the information regarding SMUP amplitude is acquired indirectly (Nandedkar et al., 2010). How these two approaches lead to matching results has not been extensively studied. One report that combined macro electromyography and MUNIX in prior polio patients did find a significant positive correlation between MUSIX and the relative macro MUP amplitude (Sandberg et al., 2011). Our results on the correlation between MUSIX and MUP size measured with HD-MUNE appeared non-significant, although there was a tendency to a moderate positive correlation. An explanation for this could be that MUP size measured with HD-MUNE contained SMUPs of all thenar muscles, while in the creation of MUSIX only the SIPs of the APB were used. MUSIX was not correlated to the CMAP amplitude in both ALS patients and healthy controls, as also found previously (Nandedkar et al., 2010).

In ALS patients, we found an average MUSIX increase of more than 50% after 8 months follow-up, indicating the presence of a strong compensatory reinnervation. MUNIX and MUSIX values together with the CMAP amplitude showed to be informative about the underlying pathophysiological process: in four patients with a relative small decline in CMAP amplitude (<30%), MUNIX dropped 41–67% and MUSIX increased 39–145%. The MRC scale of the thenar in these patients decreased slightly in two patients (from MRC 5 to MRC 4 and 4+) and remained unchanged in the other two (MRC 4). MUNE decreased in all four patients, although the decrease varied considerably (range 14–75%). Apparently, an adequate compensatory mechanism of collateral reinnervation during this stage of disease prevents fast clinical deterioration. Five other patients showed a CMAP amplitude decrease of more than

Table 2
Mean values with standard deviations (±SD) of different measures. The percental change at 4 and at 8 months are calculated with respect to the baseline mean value. *Abbreviations:* ALS functional rating scale (ALSFRS) (max = 40), Medical Research Council scale (MRC) sumscore (max = 70), compound muscle action potential (CMAP), motor unit number estimation (MUNE), motor unit number index (MUNIX), motor unit size index (MUSIX), coefficient of variation (CoV).

	ALSFRS	MRC sumscore	CMAP amplitude	MUNE	MUNIX	MUSIX
Baseline	34.8 ± 3.7	62.3 ± 6.8	5.9 ± 1.5	158 ± 103	91 ± 33	81 ± 29
4 months	32.3 ± 4.7	58.8 ± 7.3	4.9 ± 1.9	118 ± 87	69 ± 35	99 ± 70
8 months	29.5 ± 6.1	54.2 ± 11.4	4.0 ± 2.0	90 ± 97	47 ± 29	117 ± 48
4 months % change	7.3 ± 6.1	4.5 ± 4.7	17.2 ± 21.6	26.1 ± 37.3	20.9 ± 39.3	24.1 ± 66.9
CoV	0.8	1.0	1.3	1.4	1.9	2.8
8 months% change	15.9 ± 11.1	13.3 ± 11.7	34.6 ± 24.8	49.1 ± 30.2	49.1 ± 25.6	52.0 ± 65.0
CoV	0.7	0.9	0.7	0.6	0.5	1.3

50% after 8 months. MUNIX and MUNE showed an impressive decline of, respectively 68–95% and 52–93%. In these patients MUSIX also increased (57–178%), in all but one. That particular one patient already showed low MUNE and MUNIX values at baseline accompanied by a MUSIX value of 170 microvolt, indicating, even at baseline, an advanced stage of disease with barely any compensatory reinnervation capacity.

In conclusion, thenar muscle MUNIX is related to HD-MUNE in ALS patients, but not in healthy controls. In addition, the MUNIX method appears to have an equivalent potential of detecting disease progression after 8 months follow-up as compared to HD-MUNE. As MUNIX is performed rapidly and is well tolerated by patients, a multi-muscle approach seems feasible. This can be of great value in ALS, presenting itself focally, but leading to generalized motor neuron loss as the disease progresses.

Appendix A. Theoretical model for MUNIX as previously described by (Nandedkar et al., 2004, 2010):

MUNIX is based on a theoretical model where individual SMUPs are assumed having an identical shape and size, and do not superimpose. In this ideal situation, CMAP area and CMAP power are defined as:

$$\text{CMAP area} = N \times R_m \quad (1)$$

$$\text{CMAP power} = N^2 \times P_m \quad (2)$$

respectively, where N is the total number of functioning motor units, R_m the SMUP area and P_m the SMUP power. During voluntary contraction one can state that:

$$\text{SIP area} = Q \times F \times R_m \quad (3)$$

$$\text{SIP power} = Q \times F \times P_m \quad (4)$$

where a total of Q motor units are firing with a frequency of 'F Hertz. Combining Eqs. (1)–(4), we obtain:

$$N = (\text{CMAP Power} \times \text{SIP Area})/(\text{CMAP Area} \times \text{SIP Power}) \quad (5)$$

where N is also referred to as the 'ideal case' motor unit count (ICMUC), with 'ideal case' meaning the above mentioned ideal situation of the theoretical model. In reality, SMUP size is variable and superimposition of SMUPs does occur. When measuring the SIP signal, this will lead to a lower SIP area and a higher SIP power, compared to the area and power of the sum of all individual SMUPs. According to Eq. (5), ICMUC values will decrease with increasing superimposition at increasing force levels. To further address this, SIP area is used as a reflection of the force and the obtained ICMUC values are plotted against the obtained SIP area values. Their relationship is defined as:

$$\text{ICMUC} = A \times (\text{SIP Area})^{\alpha} \quad (6)$$

where A and α are constants, which numerical values are obtained from power regression analysis between the obtained ICMUC and SIP area values. The predicted ICMUC motor unit count corresponding with a SIP area of 20 mV × ms is defined as the MUNIX outcome, also briefly named 'MUNIX':

$$\text{MUNIX} = A \times (20)^{\alpha} \quad (7)$$

The SIP area of 20 is arbitrarily chosen, but is assumed to reflect a level of slight muscle contraction when no significant superimposition of SMUPs is present, hereby attempting to approach the ideal conditions of the theoretical MUNIX model. After calculation of the MUNIX value, the motor unit size index (MUSIX) value can also be computed according to:

$$\text{MUSIX} = \text{CMAP amplitude}/\text{MUNIX}. \quad (8)$$

MUSIX is considered to be a reflection of the average SMUP amplitude.

References

Ahn SW, Kim SH, Kim JE, Kim SM, Kim SH, Park KS, et al. Reproducibility of the motor unit number index (MUNIX) in normal controls and amyotrophic lateral sclerosis patients. Muscle Nerve 2010;42:808–13.

Bromberg MB, Spiegelberg T. The influence of active electrode placement on CMAP amplitude. Clin Neurophysiol 1997;105:385–9.

Bromberg MB. Updating motor unit number estimation (MUNE). Clin Neurophysiol 2007;118:1–8.

Brooks BR, Miller RG, Swash M, Munsat TL. World federation of neurology research group on motor neuron diseases El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 2000;1:293–9.

Cedarbaum JM, Stambler N. Performance of the amyotrophic lateral sclerosis functional rating scale (ALS FRS) in multicenter clinical trials. J Neurol Sci 1997;152(Suppl 1):S1–9.

Felice KJ. A longitudinal study comparing thenar motor unit number estimates to other quantitative tests in patients with amyotrophic lateral sclerosis. Muscle Nerve 1997;20:179–85.

Lapatki BG, van Dijk JP, Jonas IE, Zwarts MJ, Stegeman DF. A thin, flexible multielectrode grid for high density surface EMG. J Appl Physiol 2004;96:327–36.

McComas AJ, Fawcett PR, Campbell MJ, Sica RE. Electrophysiological estimation of the number of motor units within a human muscle. J Neurol Neurosurg Psychiatry 1971;34:121–31.

Nandedkar SD, Nandedkar DS, Barkhaus PE, Stålberg EV. Motor unit number index (MUNIX). IEEE Trans Biomed Eng 2004;51:2209–11.

Nandedkar SD, Barkhaus PE, Stalberg EV. Motor unit number index (MUNIX): principle, method, and findings in healthy subjects and in patients with motor neuron disease. Muscle Nerve 2010;42:798–807.

Neuwirth C, Nandedkar S, Stålberg E, Weber M. Motor unit number index (MUNIX): a novel neurophysiological technique to follow disease progression in amyotrophic lateral sclerosis. Muscle Nerve 2010;42:379–84.

Neuwirth C, Nandedkar S, Stålberg E, Barkhaus PE, Carvalho MD, Furtula J, et al. Motor unit number index (MUNIX): a novel neurophysiological marker for neuromuscular disorders; test-retest reliability in healthy volunteers. Clin Neurophysiol 2011;122:1867–72.

Sandberg A, Nandedkar SD, Stålberg E. Macro electromyography and motor unit number index in the tibialis anterior muscle: differences and similarities in characterizing motor unit properties in prior polio. Muscle Nerve 2011;43:335–41.

Shefner JM, Cudkowicz ME, Zhang H, Schoenfeld D, Jillapalli D. Northeast ALS consortium. The use of statistical MUNE in a multicenter clinical trial. Muscle Nerve 2004;30:463–9.

Shefner JM, Watson ML, Simionescu L, Caress JB, Burns TM, Maragakis NJ, et al. Multipoint incremental motor unit number estimation as an outcome measure in ALS. Neurology 2011;77:235–41.

Van Dijk JG, Tjon-aTsien A, van der Kamp W. CMAP variability as a function of electrode site and size. Muscle Nerve 1995;18:68–73.

Van Dijk JP, Blok JH, Lapatki BG, van Schaik IN, Zwarts MJ, Stegeman DF. Motor unit number estimation using high-density surface electromyography. Clin Neurophysiol 2008;119:33–42.

Van Dijk JP, Lowery MM, Lapatki BG, Stegeman DF. Evidence of potential averaging over the finite surface of a bioelectric surface electrode. Ann Biomed Eng 2009;37:1141–51.

Van Dijk JP, Schelhaas HJ, Van Schaik IN, Janssen HM, Stegeman DF, Zwarts MJ. Monitoring disease progression using high-density motor unit number estimation in amyotrophic lateral sclerosis. Muscle Nerve 2010;42:239–44.

Wang FC, Delwaide PJ. Number and relative size of thenar motor units estimated by an adapted multiple point stimulation method. Muscle Nerve 1995;18:969–79.