Following Disease Progression in Motor Neuron Disorders with 3 MUNE methods

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Ethical statement: We confirm that we have read the Journals's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Conflict of interest: HB receives royalties from UCL for sales of his Qtrac software used in this study. The other authors have no potential conflicts of interest. All authors have approved the final article.

Abstract

Introduction: The objective of this study was to evaluate a recently developed motor unit number estimation (MUNE) method, MScanFit MUNE (MScan), as a measure of disease progression in amyotrophic lateral sclerosis (ALS) compared to compound muscle action potential (CMAP) amplitude and two traditional MUNE methods.

Methods: ALS patients were evaluated clinically by ALS Functional Rating Scale-Revised (ALSFRS-R). MScan, multiple point stimulation MUNE (MPS) and motor unit number index (MUNIX) were performed in the abductor pollicis brevis (APB) muscle at baseline (27 patients), 4 months (23 patients) and 8 months (16 patients).

Results: Of the 5 measures, MScan registered the largest decline (8.7% per month) compared with MPS (3.4%), MUNIX (4.8%), CMAP amplitude (2.0%) and ALSFRS-R (1.9%). Only MScan and ALSFRS-R registered significant decrements over 4 and 8 months.

Discussion: MScan may be a sensitive objective tool for quantifying motor unit loss in ALS.

Keywords: Motor unit number estimation, Amyotrophic lateral sclerosis, follow-up study, MScan, motor unit loss, MUNIX, Multiple point stimulation MUNE, ALS functional rating scale

1. Introduction

Despite intensive research and clinical trials, amyotrophic lateral sclerosis (ALS) remains a progressive disease with a fatal outcome. One of the key challenges in finding a treatment to the disease is a lack of sufficiently sensitive measures to evaluate potential drugs or treatments in clinical trials. A useful and reproducible follow-up method may facilitate assessment of treatments aimed at reducing or preventing motor neurone degeneration as seen in ALS.

Several motor unit number estimation (MUNE) methods have been developed as an attempt to follow the progression of ALS, and have been shown to be a more sensitive tool of disease progression than clinical measures [1, 2]. These methods have limitations, including subjectivity, time required, or complexity of performance or analysis.

For these reasons, a new MUNE method was recently developed, MScanFit MUNE (MScan) [3]. This method records a detailed stimulus-response curve or 'compound muscle action potential (CMAP) scan' [4] and then fits a model that comprises the thresholds, threshold variability and amplitudes of all the motor units contributing to the CMAP. The MScanFit program takes full account of the probabilistic nature of motor unit firing, and also incorporates an allowance for the neuromuscular instability that may occur in ALS. The method is simple, quick and easy to use and has shown promising results on simulated data [3] andin a few clinical studies. [5-8]

In our first clinical study, we compared the reproducibility in patients with ALS and in healthy subjects of MScan with two other well-established MUNE methods, multiple point stimulation (MPS) and motor unit number index (MUNIX) [5]. The results showed that MScan was significantly more reproducible than MPS or MUNIX. Moreover, MScan had the ability to differentiate ALS patients from healthy controls at an earlier stage of the disease than MPS or MUNIX. In another recent study, we found that MScan was more sensitive for revealing abnormalities in ALS than

quantitative motor unit potential analysis parameters. [8] These results suggested the potential utility of MScan in the clinic and in research studies, where the ability to detect motor unit loss may be helpful in ALS and other neuromuscular disorders. The potential of MScan for longitudinal studies remains unanswered. Therefore, the aim of this study was to evaluate the potential of MScan as a measure of disease progression in ALS and progressive muscular atrophy (PMA) compared to MPS and MUNIX, the ALS Functional Rating Scale-Revised (ALSFRS-R) and CMAP amplitude.

2. Methods

2.1 Subjects

Twenty-seven patients with ALS or PMA were included in this study between December 2015 and February 2017. For purposes of simplicity, all patients will be referred to in the study as having ALS. All patients enrolled in this study had undergone an exclusionary workup for ALS mimics prior to entry, for confirmation of diagnosis, including blood and cerebrospinal fluid tests, magnetic resonance imaging and electromyographic studies. Twenty-three patients completed 4 month follow-up examinations and 16 completed 8 month follow-up examinations. Fig 1 shows a schematic flow chart of the patient inclusion and follow-up. Drop out from the study was either due to death, severe physical weakness or mental health reasons. Patients were categorized according to the revised El Escorial criteria [9]. At baseline, five of the patients were categorized as definite, five as probable, twelve as probable laboratory-supported, and one as possible ALS. Four had only lower motor neuron signs and were categorized as PMA. Patients were recruited from the departments of Neurology and Neurophysiology at Aarhus University Hospital. All participants signed informed consent. The project was approved by the Regional Scientific Ethical Committee and the Danish Data Protection Agency. Exclusion criteria were carpal tunnel syndrome, polyneuropathy or conditions that could cause polyneuropathy.

2.2. Clinical examinations

All patients underwent a detailed neurological examination during which and deep tendon reflexes were tested. The ALSFRS-R was used to score the patients' functional ability [10].

2.3 Electrophysiological examinations

In all patients, a routine motor and sensory nerve conduction study and three MUNE methods (MPS, MUNIX and MScan) at baseline, 4 months, and 8 months. Recordings were made on the thenar muscles on the least affected side. The subject's hand was cleansed with skin prepping gel and alcohol. A warming lamp kept the skin temperature between 32 and 36°C. The active electrode was placed over the abductor pollicis brevis muscle (APB) and the reference electrode was placed on the interphalangeal joint of the thumb. A ground electrode was placed on the dorsum of the hand. All electrophysiological examinations except for MScan were done using a Keypoint EMG machine, version 2.11 (Dantec, Skovlunde, Denmark)**.** Most of the measurements were done by the same examiner (ABJ) who remained blinded to the previous recordings. Final calculations of MUNE values were made after all the data had been collected and were done by the same examiner who had performed the recordings. The three MUNE methods were performed in accordance with earlier protocols [5] in the following order: 1) MPS, 2) MUNIX and 3) MScan. The examiner had equal experience with MScan, MUNIX and MPS.

2.4. Multiple point stimulation MUNE

Supramaximal percutaneous stimulation was delivered to the wrist by a handheld bipolar stimulator to record the median nerve CMAP. The CMAP amplitude was measured from baseline to negative peak. A surface-recorded motor unit potential (SMUP) with a minimum amplitude of ≥ 25 μv was measured as an all-or-none response by gradually increasing the stimulus intensity. Next, the stimulator was moved to a new site along the median nerve and the procedure repeated until 10

SMUPs had been recorded with different amplitudes, sizes and shapes. [11-13] In patients with less than 10 SMUPs, we recorded as many as possible. The MUNE value was calculated by dividing the CMAP amplitude by the average amplitude of the recorded SMUPs.

2.5. Motor Unit Number Index

A supramaximal CMAP was measured again with the same recording electrodes kept at the same position. Next, the patient was asked to perform an abduction of the thumb while the examiner provided manual resistance. Ten contractions were performed with increasing isometric force and ten electromyographic surface interference patterns (SIP) were recorded with a minimum of 20 mV/ms. There was a break of 30 seconds between each recording to avoid fatigue. The data from the recordings were processed in an excel file to obtain MUNIX values. MUNIX uses a mathematical calculation based on the CMAP and SIP to estimate the motor unit number [14].

2.6. MScanFit MUNE

The new MUNE method, MScan, was recently developed as part of the software QTRAC [3]. The TRONDNF recording protocol was used for recordings. After recording a maximal CMAP by stimulating the median nerve at the wrist, the stimulus was gradually reduced from supramaximal level to subthreshold in 0.2% steps to generate a detailed and inverted stimulus-response curve or CMAP scan [4]. The stimulus-response curve describes the amplitude of the motor response as a function of stimulus current, due to recruitment of more and more motor units with increasing stimulus intensity. In MScan, a model is fitted to the recorded stimulus-response curve (scan) to obtain an estimate of motor unit number and the distribution of motor unit sizes and thresholds. The model in the QTRAC program consists of N motor units each defined by three parameters: size, threshold and relative spread of threshold, defined as the coefficient of variation of the threshold, expressed as a percentage (100 x (standard deviation of threshold)/threshold). The number of motor

units was estimated offline automatically, using the MScanFit component of the QtracP analysis program.

2.4 Statistics

MUNE values ranged from 1 to 251 and were positively skewed. Since we were interested in % changes rather than absolute changes, the MUNE values were normalised by log-transformation before making paired comparisons. Log transformation was not considered appropriate for the ALSFRS-R and CMAP values, which were negatively skewed. Following Shefner et al. [15] the six patients with average MUNE values less than 10 at baseline were excluded from the assessments of decline in MUNE values, since these values were excessively sensitive to small changes, and for MPS MUNE and MUNIX inappropriately registered an increase from baseline to 4 months. Changes from baseline to 4 and 8 months were assessed by paired *t*-tests . Within-subject changes from baseline to 4 months, and from 4 months to 8 months, were also combined to estimate a mean percentage change per month, on the assumption that the decline in motor unit numbers with time is exponential. [16] Correlation analyses between the MUNE methods and ALSFRS-R were done with Spearman's rank correlation. Results with p-values<0.05 were considered significant. All analyses were done with the QtracP software.

3. Results

Patient demographics are summarized in Table 1. Among the patients, 18 (67%) were newly diagnosed while 9 (33%) were included in the study relatively late after the time of diagnosis.

Fig. 2 shows the absolute values for MScan, MPS, MUNIX, ALSFRS-R and CMAP between baseline, 4 and 8 months for each patient. As depicted in the figure, the baseline values are very spread indicating that the patients were at different stages in the disease at the baseline recordings.

Moreover, it is also noticeable that the change of values varies from patient to patient, that is some of the patients are fast progressers with a steep decline and some are slow progressers with a less steep decline or even a small incline

Table 2 shows the geometric mean values of MPS, MScan, MUNIX, and the mean values of CMAP amplitude and ALSFRS-R at baseline and after 4 and 8 months. Fig. 3 illustrates the declines in MUNE and other measures over 4 and 8 months.

When evaluating each of the methods' ability to detect a change during follow-up (Table 2), we found that only ALSFRS-R and MScan registered significant changes, and MScan registered the steepest average decline of 8.7% per month. MPS, MUNIX and CMAP amplitude did not change significantly at any time point.

The greater decline of MUNE values than of CMAPs seen in Table 2 implies that SMUP amplitudes increased slightly. Thus the mean SMUP amplitude estimated by MScan increased by $47 \pm 17\mu V$ over 4 months and by $68 \pm 27\mu V$ over 8 months ($P = 0.011$, $n = 19$, and $P = 0.026$, $n = 14$, respectively). For MPS and MUNIX the corresponding SMUP amplitude increases were not significant: MPS $0 \pm 18\mu V$ and $13 \pm 47\mu V$ ($P = 0.92$, $n = 19$, and $P = 0.77$, $n = 14$); MUNIX $15 \pm 11\mu V$ and $39 \pm 27\mu V$ ($P = 0.21$, $n = 18$, and $P = 0.17$, $n = 13$).

Correlations between the 5 measured variables are listed in Table 3. Table 3(a) shows strong correlations between the 3 MUNE measurements, especially between MScan and MPS[4]. MScan and MPS also correlated better than MUNIX with ALSFRS-R. Table 3(b) and (c) show that correlations between the MUNE changes over 4 and 8 months were weaker, but still statistically significant. On the other hand, changes in ALSFRS-R were not significantly related to any of the MUNE changes.

3. Discussion

This study showed the follow-up potential of the new promising MUNE method, MScan, compared with two other MUNE methods, MPS and MUNIX and with CMAP and the clinical measure ALSFRS-R. Only MScan and ALSFRS-R registered a significant progression of ALS. MScan measured the steepest decline both between baseline and 4 and 8 months respectively. The results provide evidence for the potential of MScan as a tool in detecting motor unit loss over time in ALS.

MUNE methods have long been of interest in research. They have been shown to be more sensitive in measuring disease progression in ALS than clinical measures and ALSFRS-R [1, 2, 17, 18] and may predict survival of ALS patients better than measures of strength or function[19]. Previous studies with ALS patients found that incremental stimulation MUNE showed a significantly larger decline than CMAP, ALRFRS-R and upper motor neuron measurements [15, 20]. A larger decline in MUNE values than CMAP can be explained by collateral reinnervation, and we presume that that is the explanation for the significant increase in mean SMUP amplitude that we found by MScan. In a study with 17 ALS patients, high density-MUNE and MUNIX decreased significantly more over a period of 8 months compared with manual muscle testing, ALSFRS-R and CMAP. [21] In a similar study, however, the decline of MUNIX was similar to ALSFRS-R, and CMAP. [22] In another study, high density-MUNE was sensitive to motor neuron loss early in the disease course when compared to other clinical measures. [23]

MUNE remains a research tool rather than a routine tool used in the clinic. Some of the reasons for this are the various limitations such as subjectivity or the difficulty or length of time for performing or analyzing the recordings. A recent study, however, showed that the new MUNE method, MScan, takes into account most of the limitations that have been found in other MUNE methods. It had excellent reproducibility both with regard to inter- and intra-rater measurements, and was simple

and quick to perform and analyse. Also, MScan had the ability to differentiate ALS patients from healthy controls in an earlier stage of the disease than MPS or MUNIX [5].

In contrast to the above mentioned studies, we did not find any significant decline in CMAP amplitude or in the two traditional MUNE methods, MPS and MUNIX over 8 months. An explanation for this could be that some patients were included relatively late after the onset of the symptoms. That means that at baseline, some patients had progressed quite far in their disease process and had severe atrophy of hand muscles. Most patients with slow progression underwent all of the follow-up examinations, whereas patients with fast progression were more likely to drop out. However, MScan was able to detect significant disease progression at both 4 and 8 months of follow-up. This suggests that MScan is a more sensitive tool than the two traditional MUNE methods for measuring motor unit loss over time in patients with ALS.

The small number of patients is a limitation in this study but is comparable with other similar studies, and the number of drop-outs is in line with that expected in this fatal disorder. The small number of patients did not allow us to do analyses for sub-groups of patients with different disease onset (bulbar vs limb) or category (PMA vs ALS). The progression rate is different in these sub-groups which may be expected to influence the sensitivity of MUNE methods. A limitation of MScan is that the recording method as described here uses the specialized Qtrac software and nerve excitability testing equipment. However, a freeware program is available which can be applied to CMAP scans generated with any EMG machine.[3]

The mean percentage decline in MUNE per month found for MScan in this study (8.7%) is comparable with the highest rates of decline previously reported for other MUNE methods, e.g. 8.94% for MPS [15] and 8.1% for MUNIX and high-density surface MUNE [21] reported as 49.1% decline over 8 months. Other MUNE studies have reported more modest rates of decline, e.g. 3.2-

3.7% and 5-5.6% for MUNIX [18, 24] or 2.35% for MPS [25], which are closer to those we found for MPS (3.4%) and MUNIX (4.8%). All studies have reported a slower rate of decline for ALSFRS-R, similar to our value of 1.9% per month, e.g. 2.3%, [18] 3.5% [24] and 1.07% [25]

We found strong correlations between the 3 MUNE measurements, especially between MScan and MPS, as found in our previous study [4]. However, a significant decline was found only for MScan. One explanation for this discrepancy may be that the other methods were more affected by recording from other muscles in patients with severe hand muscle atrophy. Even though the % changes in MPS and MUNIX over 8 months were not statistically significant, those changes correlated well with the changes in MScan (Table 3c) but not with ALSFRS-R. This may reflect the fact that the MUNE measurements were all restricted to the thenar muscle, whereas ALSFRS-R is a global measure, not based on a single muscle. ALSFRS-R may be influenced by its self-reported nature. [26] This can be influenced by many factors, such as day to day variations in a patient's symptoms, the patient's memory, and the manner in which the questions are asked, which can be impacted inadvertently by the clinician. Some patients lose the ability to speak and are dependent on nonverbal methods to communicate and answer questions, which can also lead to errors. Another factor that may explain the lack of correlation between ALSFRS-R and MUNE values may be the tendency for ulnar-mediated thenar muscle fibres to be recruited in atrophic muscles, and this could also account for some of the increases in CMAP amplitude and MUNE with time (Fig. 2). To avoid this possibility, it would seem desirable in future studies to perform recordings in an additional muscle such as tibialis anterior [27] or in multiple muscles [18] rather than only in APB.

In conclusion, this study found that MScan was the most sensitive of the three MUNE methods for detecting disease progression in ALS. MScan is a reproducible and sensitive method which is quick and easy to perform, and is as a more objective measure than ALSFRS-R. MScan therefore appears to be a useful follow-up method for assessing the effects of therapeutic agents for ALS in future drug trials..

ABBREVIATIONS

ALS: Amyotrophic lateral sclerosis

MUNE: Motor unit number estimation

CMAP: Compound muscle action potential

MPS: Multiple point stimulation

MUNIX: Motor unit number index

ALSFRS-R: revised ALS functional rating scale

PMA: Progressive muscular atrophy

APB: Abductor pollicis brevis muscle

SMUP: Surface-recorded motor unit potential

SIP: Surface interference patterns

MRC: Medical Research Council

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Table 1. Baseline patient demographics

Patients (n=27)	
Age (years)	64.9 ± 9.7
Sex	Females (n=9), Males (n=18)
Duration of symptoms (months)	31.4 ± 32.8
ALSFRS-R	35.8 ± 10.7
Region of onset	Bulbar (n=5) Upper limb (n=13) Lower limb (n=9)

Values expressed as mean ± standard deviation, n=number of patients.

Table 2. Changes in MUNE and CMAP values over 4 and 8 months.

	MSCAN MUNE	MPS MUNE	MUNIX	ALSFRS-R	CMAP (mV)
Baseline ($n = 27$)	24.40 ×÷ 1.33	23.82 ×÷ 1.33	46.54 ×÷ 1.38 ($n = 26$)	36.22 ± 2.0	8.61 ± 1.00
4 months ($n = 23$)	20.71 ×÷ 1.33	26.86 ×÷ 1.33	67.19 ×÷ 1.29 ($n = 21$)	33.7 ± 2.08	8.38 ± 1.06
4 months (% of baseline) ($n = 19$)	76.5 ×÷ 1.11	97.3 ×÷ 1.13	100.8 ×÷ 1.12 ($n = 18$)	90.5 ± 2.2	91.3 ± 6.5
P for paired t-test (baseline v 4mo) ($n = 19$)	**0.017**	0.81	0.90 ($n = 18$)	**0.00032**	0.31
8 months ($n = 16$)	13.83 ×÷ 1.53	20.8 ×÷ 1.47	48.78 ×÷ 1.56 ($n = 14$)	33.0 ± 2.24	8.46 ± 1.65
8 months (% of baseline) ($n = 14$)	48.1 ×÷ 1.37	80.2 ×÷ 1.24	63.3 ×÷ 1.46 ($n = 13$)	87.4 ± 3.8	91.6 ± 13.4
P for paired t-test (baseline v 8mo) ($n = 14$)	**0.037**	0.33	0.25 ($n = 13$)	**0.0038**	0.48
Mean change per month (%)	-8.7 ± 3.0	-3.4 ± 2.6	-4.8 ± 3.6	-1.9 ± 0.5	-2.0 ± 1.8

Baseline, 4-month and 8-month values for all patients tested, and 4-month and 8-month values as percentages of baseline for patients tested at both times. MUNE values expressed as geometric means, with standard errors as a factor, while ALSFRS and CMAP values expressed as arithmetic mean ± SE. Similarly, MUNE paired t-tests were performed on logged values. P values in bold indicate significant changes. The mean change per month was estimated from 19 changes from 0-4 months and 14 changes from 4-8 months. Numbers of MUNIX values are reduced since MUNIX examinations could not be performed in all patients due to inability to make voluntary contraction.

Table 3. Correlations between MUNE methods, ALSFRS and CMAP amplitudes.

		MScan	MPS	MUNIX	CMAP	ALSFRS-R
(a) Correlations over all values (*n* = 66)	MScan		**0.918**	**0.868**	**0.850**	**0.597**
	MPS	**0.918**		**0.850**	**0.873**	**0.568**
	MUNIX	**0.868**	**0.850**		**0.919**	**0.451**
(b) Correlations between % changes from baseline to 4 months (*n* = 19)	MScan		**0.686**	**0.530**	**0.616**	0.050
	MPS	**0.686**		**0.463**	**0.744**	0.063
	MUNIX	**0.530**	**0.463**		**0.507**	-0.091
(c) Correlations between % changes from baseline to 8 months (*n* = 14)	MScan		**0.830**	**0.732**	0.477	0.407
	MPS	**0.830**		**0.795**	**0.634**	0.335
	MUNIX	**0.732**	**0.795**		**0.901**	0.059

For MUNE correlations with CMAP amplitude, the amplitude obtained during the particular

MUNE recording is used. Statistically significant correlations (*P*<0.05) are shown in bold type.

Figure legends:

Figure 1. Flowchart of patient inclusion and follow-up

* One did not fulfill the diagnosis of amyotrophic lateral sclerosis (ALS) or progressive muscular atrophy (PMA) and 2 had atrophy of the abductor pollicis brevis muscle that was too severe to permit performance of MUNE.

** Severe physical weakness or mental health reasons.

*** Death (3 patients) or severe physical weakness or mental health reasons (4 patients).

Figure 2. Absolute values for MScan MUNE, MPS MUNE, MUNIX, ALSFRS-R and CMAP amplitude for each of the patients at baseline and after 4 and 8 months. Lines connect values for the same patient. Missing points are due to drop-out.

Figure 3 Percentage changes in MUNE values (geometric means) and mean ALSFRS-R and CMAP amplitude at 4 and 8 months.