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Sensitivity of MUP parameters in detecting change in early ALS

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HIGHLIGHTS

- Detailed knowledge on the earliest changes of the motor unit morphology in ALS is critical for evaluating reinnervation with electromyography, in future clinical trials with drugs promoting axonal sprouting.
- Increased motor unit duration and jitter are very sensitive and consistent markers of early motor unit adaptation in very early affected muscles in ALS.
- Mean motor unit duration is a measure easily applicable in clinical trials of compounds considered likely to be of value in promoting reinnervation.

ABSTRACT

Objectives: We aimed to identify the most appropriate MUP parameter to evaluate reinnervation in very early ALS.
Methods: We studied tibialis anterior (TA), initially of normal strength with normal MUP analysis parameters, in 15 patients with ALS of recent onset. They were studied at the initial diagnostic assessment, and then 3 and 6 months later. Spontaneous EMG activity was recorded. Conventional MUP analysis included mean amplitude, mean area, mean duration, mean number of phases, mean number of turns, % polyphasic potentials, mean jitter, % unstable pairs and % pairs with blocking. Non-parametric statistics were utilised in the analysis.
Results: Fasciculations were recorded in 72% in TA and increased jitter in 33% at study entry, but without EMG features of denervation. Mean amplitude, mean duration, mean area and the three measures of neuromuscular transmission increased significantly and linearly at each evaluation. Median duration showed the lowest variation and, together with jitter, the largest relative time effect.
Conclusions: Mean duration and mean jitter are the most effective measures of early reinnervation in a very early affected muscle, in ALS.
Significance: Mean MUP duration is a simple and easy measure that should be useful in evaluating reinnervation, for example in a future clinical trial.

1. Introduction

Electromyography is a well-standardised technique for assessing quantitative motor unit physiology, but there is little information as to the most reliable and sensitive measures for studying neurogenic disorders. This is particularly important in studies of amyotrophic lateral sclerosis (ALS). A number of measurements are available, including MUP amplitude, duration, area, polyphasicity, turns analysis, and measures of neuromuscular jitter and blocking, all of which are increased in ALS (Schwartz et al., 1976; de Carvalho and Swash 2013). None of these measures are specific for ALS, but reflect chronic partial ongoing denervation–reinnervation (Wohlfart, 1958; Erminio et al., 1959; Kimura, 2001). It is of interest to examine the relative sensitivity of these different measures in detecting the earliest changes in motor units in the disorder, and to consider whether one or more of them would be suitable for use in clinical trials of putative therapies in ALS (de Carvalho, et al., 2005a,b).

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2. Patients and methods

We studied 15 patients (9 men and 6 women) aged 45–74 years (mean 61.8 years) suspected as suffering from ALS, referred for diagnostic testing. These patients were selected on the basis of a disease duration from symptom onset of less than 12 months (mean 6.4 months). In 8 the onset was bulbar, 4 had upper limb onset and 3 lower limb onset.

In each, EMG and nerve conduction studies confirmed the diagnosis of probable (14 patients) or definite ALS, as defined by the modified El Escorial criteria (Brooks et al., 2001), applying the Awaji recommendations for neurophysiological diagnosis (de Carvalho et al., 2008). All progressed to definite ALS during subsequent follow-up. None had a family history of ALS. Patients with an associated fronto-temporal dementia were excluded because of ethical and practical concerns regarding their ability to engage with the study requirements. All patients had clinical signs of upper motor neuron involvement in lower limbs, but none had lower limb spasticity higher than Grade 2 on the modified Ashworth scale (Pandyan et al, 1999). Patients with diabetes were also excluded.

All the patients included in this study could stand unaided on the heel of at least one leg indicating clinically normal strength in tibialis anterior (TA). Further, at the time of entry to the study MUP analysis in the selected TA muscle was required to be normal according to our normative laboratory values (de Carvalho and Swash, 2013). Sequential measurements were made every 3 months for 6 months, thus 3 times in total.

2.1. Neurophysiological methods

We studied spontaneous EMG activity, MUP parameters and neuromuscular jitter.

2.1.1. Spontaneous EMG activity

In TA spontaneous activity was studied in 10 different sites, using 5 or more needle insertions, with the conventional concentric needle EMG.

Fibrillations/positive sharp waves (*fibs-sw*) were considered as present if observed as reproducible trains of fibs-sw lasting at least 1 second following needle insertion in at least two different sites in a muscle.

Fasciculation potentials (FPs) were considered as present if observed in a TA muscle completely at rest in at least one site in the muscle. We followed the definition of FPs as set out in the recommendations of the American Academy of Electrodiagnostic Medicine (2001). We accepted FPs only if their amplitude was greater than 50 μV (de Carvalho and Swash 2013). A minimum of one minute recording time was required at each of the recording sites before rejecting a site as not revealing FPs (Mills, 2011).

2.1.2. Motor unit morphology

We studied motor unit morphology in the TA muscle following routine diagnostic evaluation, as indicated in the clinical context. The TA muscle selected was always of normal strength. When both TA muscles were of normal strength the right side was preferred for study. We used a Medtronic Keypoint-Net G4 EMG machine in this investigation. The skin temperature was monitored and was always greater than 32 °C. All recordings were made with a conventional concentric needle EMG electrode (recording area 0.07 mm^2). We recorded 20 different MUPs from not less than five different sites in each muscle to quantify motor unit morphology, during a slight and regular voluntary contraction (de Carvalho et al., 2012). The "Multi-MUP analysis" program implemented on the EMG machine was used to analyze MUP data (filter setting 5 Hz–10 kHz, gain 200 mV/div and sweep speed 5 ms/div). Amplitude, duration, area, area/amplitude ratio, percentage of polyphasic potentials, mean number of phases and mean number of turns were analyzed for each MUP and mean values of these measurements were calculated for each muscle. These data were calculated automatically according to previously published standards (Stålberg et al., 1995).

2.1.3. Motor unit stability studies

We calculated the mean jitter and evaluated blocking in at least 10 different pairs of potentials in TA using a concentric needle EMG electrode (recording area 0.07 mm^2), a 1000 Hz low band pass filter, and a trigger delay line, with a sweep speed of 1 ms/div. For each potential pair, 80–100 recordings were made. The mean jitter, percent abnormal potential pairs, and percent potential blocking were analysed.

2.1.4. Ethics and statistical methods

Informed consent was obtained according to the requirements of the local Research Ethics Committee in Lisbon, Portugal, which approved the study.

Since, in general, the data did not follow a normal Gaussian distribution we used non-parametric statistics. The null hypothesis was that there was no change in the parameters studied with time. For continuous or ordinal variables we used a Wald-type statistic and ANOVA-type statistic for longitudinal data (Brunner et al., 2001). For dichotomous variables the Cochran-Q test was used (Sheskin, 2004). For multiple comparisons p values were adjusted using the Bonferroni correction. Based on these statistics a relative time effect, based on the Wald-type statistic, was considered in the whole dataset, comparing data at entry with data at 6 months. Relative time effect gives information about the impact of the change, its value at a specific time t indicates the probability that a randomly chosen observation at time t would be higher than a randomly chosen observation at any other time. Linearity was tested with the Wald-type statistic and Wilcoxon tests for paired data applied to the differences. The difference between the first and third quartile values (interquartile range, IQR) was calculated to give information about the dispersion of the data; for comparisons purposes we used IQR divide by the median (IQR/median).

3. Results

The data are summarised in Table 1. Measurements of median amplitude, area, duration, and jitter, % normal pairs and % blocking increased significantly at the 3 and 6 months evaluations compared to the baseline measurements. As required for entry to the study, all MUP parameters were normal in the TA muscles studied at the time of initial electrodiagnostic evaluation. However, 33% had abnormal jitter and 73% showed FPs at this time, although no TA muscle showed fibs-sw at the first study. The frequency of FPs did not change significantly during the study, but there were more frequent fibs-sw in the later recordings as compared with the first ($p = 0.003$).

There was a 24.6% increase in mean duration between study entry and 3 months and 13% between the 3 and 6 months studies (Fig. 1), but the IQR/median range remained consistently smaller than that of the other measures throughout the study. The mean jitter increased by 41% between study entry and the 3 month study, and by 32% between the studies at 3 and 6 months (Fig. 1). Mean MUP amplitude and area increased significantly during the study but the IQR/median ranges increased considerably as the median values increased during the study (Table 1). At 3 months jitter was abnormal in 87% of the patients and it was abnormal in all of them at 6 months; duration was abnormal in 33% at 3 months and abnormal in 73% at 6 months; amplitude

Table 1
Results of three sets of studies at study entry, at 3 months and at 6 months. Our laboratory normal values are described in de Carvalho and Swash (2013).

	Normal lab values	Entry data	3 months data	*P* value* 0–3 months	6 months data	*P* value* 3–6 months
Amplitude (μv)	Mean 661.5 ± 92.0 ULN 845.5	Mean 661.5 ± 106.6 median 687.0 IQ/median 0.22	Median 904.9 ± 391.0 median 791.0 IQ/median 0.63	0.001	Mean 1115.0 ± 644.8 median 855.0 IQ/median 0.64	0.009
Area (μv ms)	Mean 851.5 ± 158.7 ULN 1168.9	Mean 773.9 ± 187.9 median 800.0 IQ/median 0.38	Mean 1138.0 ± 594.7 median 1041.0 IQ/median 0.65	0.001	Mean 1494.0 ± 893.7 median 1248.0 IQ/median 0.75	0.01
Area/ampl	Mean 1.32 ± 0.14 ULN 1.66	Mean 1.20 ± 1.12 median 1.20 IQ/median 0.14	Mean 1.35 ± 0.24 median 1.27 IQ/median 0.26	NS	Mean 1.41 ± 0.22 median 1.46 IQ/median 0.17	NS
Duration (ms)	Mean 9.35 ± 0.93 ULN 11.2	Mean 9.09 ± 1.50 median 9.1 IQ/median 0.29	Mean 11.3 ± 2.2 median 10.7 IQ/median 0.26	<0.001	Mean 12.56 ± 2.26 median 12.9 IQ/median 0.26	0.02
% Polyphasic potentials	Observations – 5-40 ULN 35%	Mean 19.0 ± 10.56 median 20.0 IQ/median 0.75	Mean 30.0 ± 16.04 median 30.0 IQ/median 0.68	NS	Mean 30.0 ± 13.50 median 30.0 IQ/median 0.42	NS
Mean number of phases	Mean 3.47 ± 0.36 ULN 4.19	Mean 3.41 ± 0.42 median 3.4 IQ/median 0.18	Mean 4.01 ± 0.89 median 3.8 IQ/median 0.25	0.01	Mean 3.80 ± 0.58 median 3.8 IQ/median 0.16	NS
Mean number of turns	Mean 3.35 ± 0.52 ULN 4.39	Mean 3.05 ± 0.64 median 2.8 IQ/median 0.39	Mean 3.31 ± 0.79 median 3.5 IQ/median 0.30	NS	Mean 3.21 ± 0.67 median 3.3 IQ/median 0.33	NS
FPs (% patients)	Rare	73%	80%	NS	87%	NS
Fibs-sw (% patients)	0	0	27%	NS	53%	NS
Mean jitter (MCD) μs	Mean 37.0 ± 5.6 ULN 48.5	Mean 47.0 ± 14.95 median 42.0 IQ/median 0.54	Mean 64.90 ± 19.39 median 62.0 IQ/median 0.22	<0.001	Mean 84.16 ± 25.04 median 81.0 IQ/median 0.44	<0.001
% Pairs ↑ jitter	ULN 20%	Mean 20.67 ± 19.44 median 10.0 IQ/median 1.5	Mean 40.67 ± 28.40 median 30.0 IQ/median 0.50	<0.001	Mean 62.0 ± 26.78 median 60.0 IQ/median 0.67	<0.001
% Pairs blocking	ULN 10%	Mean 4.0 ± 6.32 median 0.00 IQ/median not possible	Mean 14.0 ± 12.99 median 10.0 IQ/median 1.5	<0.001	Mean 26.0 ± 15.95 median 30.0 IQ/median 0.50	<0.001

ULN – upper limit of normal as established by mean ±2.5 SD or 5% lowest/highest frequency distribution (% polyphasic potentials, % of pairs with increased jitter or blocking); IQ/median – interquartile difference, 1st–3rd/median; *p-Value corrected for multiple comparisons.

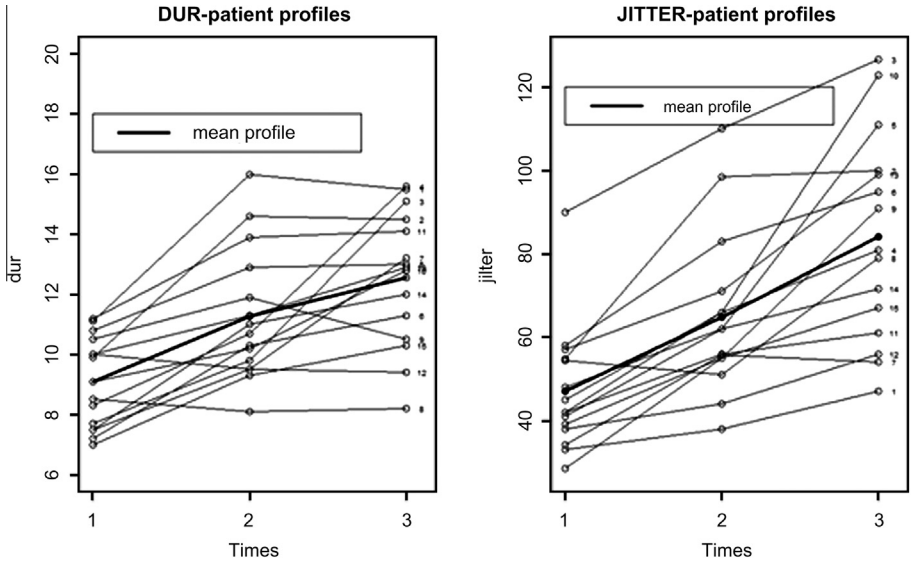

Fig. 1. Individual and mean data for the two most sensitive early measures of change during progression of chronic partial denervation. All recordings made from TA muscle with initially normal MUP parameters (see text). Note the increasingly abnormal results from time 1 (at entry) to time 2 (3 months later) and time 3 (6 months after study entry). On the *y*-axis duration (ms) and jitter (μs) are represented.

was abnormal in 40% at 3 months and in 53% at 6 months; and area was increased in 47% of the patients at 3 months and in 60% at 6 months.

We used the relative time effect statistic to evaluate change in MUP duration and jitter measurements at 6 months compared with study entry and found that the variables showing the most significant values were MUP duration and jitter (0.70) – indicating that there was a meaningful change over time. Analysis of linearity in the changes in the study data over time showed that all the parameters measured conformed to a pattern of linear progression

(Fig. 1), except for the changes in number of phases during the study, which were non-linear.

4. Discussion

In the set of TA muscles in patients with ALS with normal MUP analysis at entry, FPs were found in most muscles, an observation that confirms our previous report of the importance of FPs as one of the earliest features in the development of EMG abnormality in ALS (de Carvalho and Swash, 2013). In muscles with normal MUPs fibs-sw were not observed, as previously reported (Lambert, 1967; de Carvalho and Swash, 1998). This may represent very rapid reinnervation following denervation in the earliest phases of muscle involvement in ALS, indicating a high capacity for axonal sprouting (outgrowth) at this early stage of the disease.

Our results indicate that increased neuromuscular jitter and increased MUP duration are the most informative features associated with very early changes in a muscle in ALS. Increased jitter and blocking are characteristic of endplate dysfunction. When associated with chronic partial denervation increased jitter is a feature of terminal or nodal axonal sprouting (Brown et al., 1981; Stålberg, 1990), occurring in response to denervation of muscle fibres and the upregulation of acetylcholine receptors on their surface. When the newly reinnervated muscle fibre has immature endplate structure and function, the safety factor for neuromuscular transmission is abnormal and transmission failure occurs intermittently during continuous motor neuron firing, even at low rates (Schwartz et al., 1976; Stålberg and Falck, 1997), leading to jitter and impulse blocking. Moreover, increased jitter can also result from disturbed conductance in new axon terminal sprouts (Stålberg, 1990).

In an earlier study we found that increased jitter was a very early sign of the neurophysiological abnormality during the development of chronic partial denervation in a TA muscle in ALS (de Carvalho and Swash 2013). Increased duration of the MUP, which we found to be the most useful variable in early ALS, is a simpler measure than assessment of neuromuscular jitter, that is available to any electromyographer. MUP duration, like amplitude, depends on the number of innervated muscle fibres within the uptake area of the electrode. For a standard concentric needle electrode this is an area of about 2.5 mm radius (Nandedkar et al.,1988; Stålberg and Falck, 1997). MUP duration reflects slowed conduction in the fine sprouting axons reinnervating muscle fibres, as classically described by Wohlfart (1958). Temporal dispersion of individual single muscle fibre action potentials associated with slowed conduction in atrophic muscle fibres is less important in relation to increased duration (Nandedkar et al, 1988), but is relevant to loss of synchronicity of firing of muscle fibres within the motor unit, and therefore of increased polyphasicity (Stålberg and Karlsson, 2001). Increasing number of phases during disease progression was not observed in our study, possibly due to absence of significant muscle fibre atrophy in very early affected muscles.

Other neurophysiological measurements have been suggested as useful for assessing disease progression in ALS. Both, the motor unit number estimation, such as MUNIX (de Carvalho, et al., 2005a,b; Neuwirth et al., 2011) and the neurophysiological index (de Carvalho et al., 2005a) quantify the lower motor neuron pool but they give no information on the potential improvement in functional reinnervation. This could be derived from MUP analysis (Stålberg and Falck, 1997) and fibre density determination (Stålberg, 1990). The latter technique requires the use of reusable needle electrodes and Multi-MUP analysis, which permits a quick and readily tolerable MUP quantification is preferred (Stålberg et al., 1995).

However, to record 20 MUPs from several different muscles is impractical in routine follow-up studies. Our results suggest that MUP duration from a few target muscles might therefore be a useful measure; for example, for testing compounds considered likely to be of value in promoting reinnervation as a symptomatic therapy in ALS.

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