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MScanFit motor unit number estimation (MScan) and muscle velocity recovery cycle recordings in amyotrophic lateral sclerosis patients

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HIGHLIGHTS

- Detailed CMAP scans and MVRCs were recorded in 26 patients with ALS.
- MScan revealed marked motor unit loss.
- MVRCs were largely unchanged, suggesting successful reinnervation of muscle fibres.

ABSTRACT

Objective: Motor Unit Number Estimation (MUNE) methods, such as the recently developed MScanFit MUNE (MScan), may be valuable in tracking motor unit loss in ALS. Muscle Velocity Recovery Cycles (MVRCs) provide information about muscle membrane properties and can reveal disease-related changes.

This study was undertaken to test the applicability of MScan to the anterior tibial muscle (TA) and to test whether the MVRCs could improve understanding of ALS pathophysiology.

Methods: Twenty-six ALS patients and 25 healthy controls were evaluated by quantitative electromyography, nerve conduction study and the two novel methods: MScan and MVRC; all in the TA and peroneal nerve.

Results: The estimated number of motor units for ALS patients (Median: 45, interquartile range: 28.5–76.5) was significantly lower than for the controls (117, 96.0–121.0) ($P = 2.19 \times 10^{-7}$). Unit size was increased only when amplitudes were expressed as percentage of CMAP. Of MVRC measurements, only relative refractory period was significantly abnormal in patients.

Conclusion: MScanFit MUNE gives a sensitive and quantitative measure of loss of TA motor units in ALS. Muscle fiber membrane properties are mostly unaffected, despite substantial denervation, presumably due to collateral reinnervation.

Significance: MScan is suitable for detecting motor unit loss in TA. MVRCs do not provide new insights in ALS.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal progressive neurodegenerative disorder affecting both upper and lower motor neurons. Although no curable treatment is available, early diagnosis is crucial for the possibility of initiating neuroprotective therapy and best possible management (Kiernan et al., 2011). Motor neuron dysfunction in ALS is comprised of axonal loss, compensatory collateral reinnervation and eventually muscle fiber loss.

Electromyography (EMG) with quantitative motor unit potential (MUP) analysis and Nerve Conduction Studies (NCS) are the main electrophysiological techniques for diagnosing ALS. Electrodiagnostic challenges include that spontaneous activity on EMG may also be found in other neurogenic disorders and in myopathies (Fuglsang-Frederiksen, 2006), and that the extent of chronic

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neurogenic MUP changes does not directly correspond to the degree of denervation (McComas et al., 1971; Gooch et al., 2014). Also, due to collateral reinnervation, the CMAP amplitude on motor NCS may not decrease notably until more than 50% of the motor units are lost (Daube, 2006; Hansen and Ballantyne, 1978). Thus, a considerable limitation of EMG and NCS is that they are unable to provide a reliable measure of motor neuron loss.

To meet this need, a number of motor unit number estimation (MUNE) methods have been developed (McComas et al., 1971; de Carvalho et al., 2018). These can only provide estimates, as no electrophysiological method allows for direct measurement of 'motor unit number', i.e. the number of functioning motor neurons or axons innervating a specific muscle (Sherrington, 1929). MScanFit (MScan), in which the number of units is estimated from a detailed stimulus-response curve or CMAP scan, is the most recent of these methods (Bostock, 2016). It meets common criticisms of traditional MUNE methods, such as: alternation, subjectivity in the evaluation process, bias in representative sampling of all motor units, time consumption and poor reproducibility. Recent studies have shown this method to be simple to perform, sensitive and with an excellent reproducibility and low coefficient of variation (CV) in ALS patients (Jacobsen et al., 2017). Up to now, MScan has only been applied to the abductor pollicis brevis muscle (Farschtschi et al., 2017; Jacobsen et al., 2017; Garg et al., 2017; Jacobsen et al., 2018). In the present study, this method is applied to the anterior tibial muscle for the first time.

In 2009, Z'Graggen and Bostock developed a technique of recording muscle velocity recovery cycles (MVRCs). Direct muscle stimulation and multifiber recording is used to assess the changes in conduction velocity (CV) of a muscle fiber action potential that follows one or more conditioning action potentials owing to early and late depolarizing afterpotentials, i.e. MVRCs (Z'Graggen and Bostock, 2009; Bergmans, 1971; Z'Graggen, Troller et al., 2011; Boërio et al., 2012). In contrast to the earlier more technically difficult and time-consuming single fiber techniques (Stalberg, 1966; Mihelin et al., 1991), this novel MVRC method is simple enough to be applied clinically and has been used to provide information about membrane abnormalities in several channelopathies and in clinical conditions (Z'Graggen, Brander et al., 2011; Tan et al., 2012; Z'Graggen et al., 2010; Tan et al., 2014; Tan et al., 2016; Tan et al., 2018; Humm et al., 2011). This method has not previously been applied to ALS patients.

In this study, we tested the applicability of MScan to the anterior tibial muscle (TA) for the first time in ALS patients. We furthermore used the MVRC technique to explore muscle fiber membrane properties in vivo in ALS patients, to test whether MVRCs could provide new insights into disease pathophysiology.

2. Material and methods

2.1. Subjects and ethical approval

Twenty-six patients with ALS or progressive muscular atrophy (PMA) (17 males and 9 females; mean age: 64.7 years, range 32–87) participated in this study. According to the Awaji-criteria (de Carvalho et al., 2008) they were distributed as: 1 definite, 7 probable, 12 possible ALS and 6 with PMA (Table 1). For simplicity, all patients in the study will be referred to as having ALS. Furthermore, 25 healthy control subjects (12 males and 13 females; mean age: 58.6 years, range 36–77) were included. Mean age and gender distribution did not differ significantly between patients and controls.

All patients were recruited from and examined at the Department of Clinical Neurophysiology, Aarhus University Hospital. Within the same session, subjects underwent clinical evaluation and the four electrophysiological examinations.

Table 1
Patient demographics.

ALS patients (n = 26)	
Age (years)	64.7 ± 2.7, range: 32–87
Sex	Males (n = 17)
	Females (n = 9)
Duration of symptoms (months)	24.4 ± 6.2, range: 2–120
Region of onset	Bulbar (n = 6)
	Upper limb (n = 8)
	Lower limb (n = 12)
ALSFRS-R	39.1 ± 1.17, range: 23–49
Diagnostic classification according to Awaji-criteria	Definite ALS (n = 1)
	Probable ALS (n = 7)
	Possible ALS (n = 12)
	Progressive muscular atrophy (n = 6)

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

Subjects were excluded if they had one of the following conditions: (1) polyneuropathy, (2) disease known to possibly induce polyneuropathy, (3) previous central or peripheral nervous system disease or (4) bleeding tendency or anticoagulation therapy.

This study was approved by the Regional Scientific Ethical Committee (1-10-72-13-17) and The Danish Data Protection Agency (1-16-02-19-17). In accordance with the Declaration of Helsinki, all patients and controls gave their written informed consent to participate in the study.

2.2. Clinical evaluation

ALS patients were evaluated clinically by a detailed neurological examination including assessment of force, deep tendon reflexes and muscle atrophy. Furthermore, they were scored using the revised ALS Functional Rating Scale (ALSFRS-R) (Cedarbaum et al., 1999) (Table 1).

2.3. Electrophysiological evaluation

The electrophysiological examinations were performed on the peroneal nerve and the anterior tibial muscle (TA), as this nerve and muscle allowed for ideal execution of all four examinations. In case of one leg being clinically more affected than the other, the less affected side was chosen for examination. Otherwise and in healthy controls, the right leg was examined. The skin was cleansed using skin prepping gel and alcohol swipes. A skin temperature over TA of 32–36 °C was retained by intermittent use of a heating lamp.

NCS and EMG were carried out using Keypoint EMG equipment (Dantec, Skovlunde, Denmark), while MScan and MVRC were recorded using a separate set up as described below.

2.3.1. Nerve conduction study (NCS)

A routine motor NCS was done on the peroneal nerve to exclude entrapment neuropathy. Supramaximal stimulation was delivered using a handheld bipolar stimulator at the ankle, just distally to capitulum fibulae and proximally in the popliteal fossa, while CMAP was recorded from m. extensor digitorum brevis, with the reference electrode at the basis of the fifth toe. A ground electrode was placed on the dorsum of the foot.

2.3.2. Electromyography (EMG)

A standard EMG with quantitative motor unit potential (MUP) analysis was done in the TA muscle using a 35-mm concentric needle electrode (Dantec). Standard filter settings (20 Hz–10 kHz), gain (100 μV/division) and sweep speed (10 ms/division) at the department were used. Quantitative MUP analysis was carried

out by sampling at least 20 different MUPs from 10 different sites in the muscle during weak voluntary contraction. Mean duration and amplitude of all potentials were evaluated. Spontaneous activity in the form of fibrillation potentials (fibs), positive sharp waves (psws) and fasciculations potentials was assessed in the 10 recording sites by observing the resting muscle for 60–90 s (Mills, 2011). An occurrence of fibs/psws in more than two sites was regarded as abnormal.

2.3.3. MScanFit MUNE (MScan)

MScan recording was controlled by the TRONDNF.QRP protocol within QtracS software (written by H. Bostock, copyright Institute of Neurology, University College London, UK). The recording set up included an isolated constant current stimulator (DS5; Digitimer Ltd), a HumBug 50 Hz noise eliminator and a D440 amplifier (Digitimer Ltd).

All recordings were undertaken by stimulating the peroneal nerve via an electrode just distally to capitulum fibulae. The active recording electrode was placed over the motor point of TA and the reference electrode over the tendon at the ankle. An optimal position for the recording electrode to obtain the maximal CMAP amplitude was localized by moving the electrode to slightly different positions over the muscle belly while stimulating the nerve. Stimuli were delivered by a standard width of 0.2 ms. In patients where it was not possible to reach supramaximal stimulation using 0.2 ms wide stimulations, the stimulus width was raised to 0.3 or 0.5 ms.

Via QtracS, the stimulus intensity was manually increased until supramaximal CMAP was reached. A pre-scan sweep of 20 supramaximal CMAPs was recorded. Following this, stimuli were delivered twice per second, with the intensity of each stimulus being 0.2% lower than the preceding one, until the CMAP was no longer recordable. Additionally, a post-scan set of 20 baseline responses were recorded. The pre- and post-scan sweeps were used to assess variability of supramaximal responses and baseline noise. Recording time for each scan was approximately 6–8 minutes.

The recordings form a detailed stimulus-response curve (Fig. 1A,D), which is S-shaped in healthy controls, while for an ALS patient it is more irregular and typically contains clear steps (gaps) as a sign of relatively large motor units due to collateral reinnervation.

2.3.4. Muscle velocity recovery cycles (MVRC) recordings

Stimulation and recording were controlled by the M3REC3.QRP protocol within QtracS software, using the same set up as described above. We successively performed: (1) MVRC recordings at rest, and (2) Frequency Ramp protocol, with intermittent repetitive stimulation at frequencies ramped from 1 to 30 Hz.

Recordings were performed in the TA as described in detail elsewhere (Tan et al., 2014; Tan et al., 2018) and summarized below.

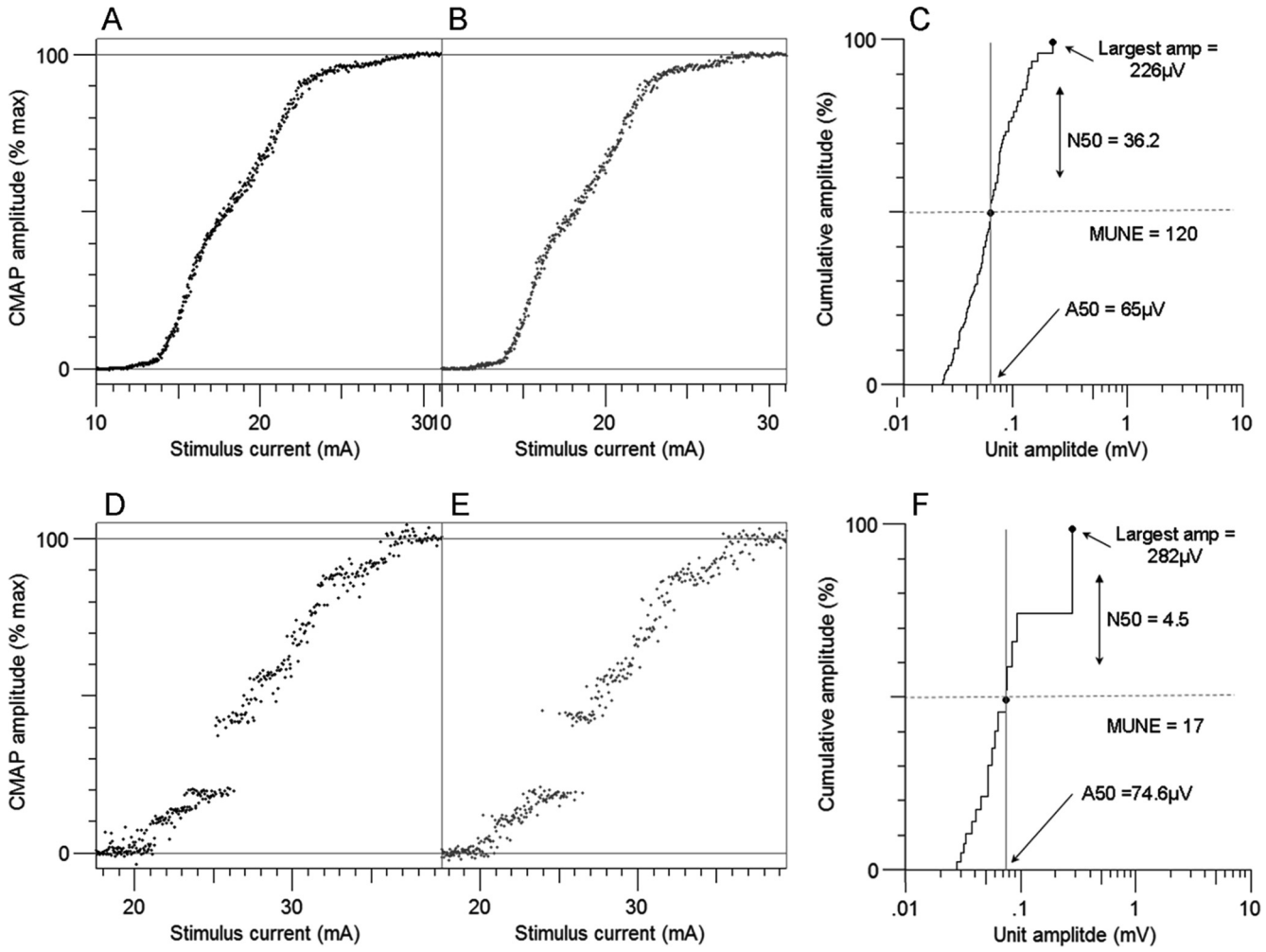

Fig. 1. Examples of CMAP scans and their MScanFit analysis from the anterior tibial muscle from a healthy control subject (A-C) and an ALS patient (D-F). A and D are the original recorded CMAP scans and B and E are simulated scans generated from the fitted models with 120 and 17 units respectively. E and F show cumulative amplitude plots of the model units ranked in order of increasing amplitude. Horizontal dashed lines are at 50%, separating the N50 larger units and the smaller ones. These lines cut the cumulative amplitude plots at the amplitude A50.

A monopolar needle electrode, which served as a cathode, was inserted perpendicularly into the distal half of the TA muscle. Stimuli (0.05-ms rectangular current pulses) were delivered through this needle. A non-polarizable surface electrode served as an anode and was placed on the skin about 1 cm distal to the cathode. Muscle recordings were obtained using a 25 mm concentric needle electrode inserted perpendicularly 20 mm proximal to the stimulating needle. The leads were taped to the skin to prevent any electrode movement. The needle electrodes were adjusted to obtain a stable negative peak response by stimulation of 3–6 mA. Once set, the stimulation current was not changed during recordings.

MVRCs were recorded following 1, 2 and 5 conditioning stimuli, all separated by 10 ms inter-stimulus intervals (ISIs). Test stimuli were delivered every 2 s. The ISI between the last conditioning stimulus and the test stimulus ranged from 1000 to 1.4 ms in 34 steps in an approximately geometric series. Recovery cycles are plotted as percentage latency change, in the form of conduction velocity slowing (%), as a function of ISI (logarithmic scale).

The Frequency Ramp protocol imitates the conventional short exercise test and characterize the effects of progressive muscle activation. A 1 s train of stimuli was delivered every 2 s. The number of stimuli in the train was successively increased by 1 from 2 to 31 stimuli, so that the mean stimulation rate was ramped up from 1 to 15.5 Hz over 1 minute. Responses were measured to the first and last stimuli in each train. Stimulus cycles with the test stimulus alone were recorded before the frequency ramp (10 cycles at 0.5 Hz) and for 30 s after the end of the ramp (15 cycles at 0.5 Hz). The details on the protocol and the interpretations were described elsewhere (Tan et al., 2014).

The recording sequence was completed within 8 minutes.

2.4. Data analysis

MScan and MVRC data were analyzed by means of the QtracP software, which was also used to generate the figures.

MScan: The MScanFit component of the QtracP software was used to obtain MScan parameters. As described in detail elsewhere (Bostock, 2016), a preliminary model is first derived from the variance and slope of scan points, and this is subsequently refined by comparing CMAP scans generated by the model with the recorded scan. CMAP scans derived from the fitted models are illustrated in Fig. 1B,E. The following MScan measurements from the fitted models are reported (see Fig. 1C,F): (1) the MUNE value, which is the estimated number of functional motor units in the muscle; (2) N50, which is the number of larger units making up 50% of the CMAP amplitude; (3) A50 (%) the smallest amplitude of the units making up the N50 larger units, expressed as a percentage of the maximum CMAP amplitude; (4) A50 (μV), the same amplitude expressed in μV, and (5) the Largest Amplitude (μV), which is the absolute size of the largest unit. N50 behaves like MUNE, but is immune to the problem of distinguishing very small units from noise. The largest amplitude (μV) and A50 (μV) provide measures sensitive to collateral reinnervation, but the largest amplitude may be expected to be more variable, as it depends on a single unit.

MVRCs: Latencies were measured from the start of the test stimulus to the negative peak of the muscle action potential. The effects of 1, 2 and 5 conditioning stimuli on the test response latency were calculated as percentage differences compared with the response to the test stimulus alone.

The excitability measures derived from MVRC recordings comprise: (1) muscle relative refractory period (MRRP), defined as the shortest interpolated ISI at which the latencies of the unconditioned and conditioned test responses were identical; (2) early supernormality (ESN), i.e. the largest percentage latency reduction of conditioned muscle action potential at ISIs < 15 ms; (3) 5ESN, the early supernormality after 5 (i.e. due to an additional 4) conditioning stimuli; (4) late supernormality (LSN), i.e. the average percentage latency reduction between ISIs of 50 and 150 ms. MRRP and ESN are very sensitive to changes in membrane potential; (5) 5XLSN, the extra late supernormality after 5 conditioning stimuli, and (6) 5XRSN, the extra residual supernormality at 950–1000 ms after 5 conditioning stimuli. MRRP and ESN are very sensitive to changes in membrane potential.

Frequency Ramp: The following measurements were recorded: (1,2) $Lat(15\ Hz)_{First}$ and $Lat(15\ Hz)_{Last}$, the latencies of the negative peak of the first and last muscle action potentials in the 15 Hz train, as percentage of baseline latency; (3,4) $Lat(30\ Hz)_{First}$ and $Lat(30\ Hz)_{Last}$, the corresponding percentage latency changes at 30 Hz, and (5) Lat(30 Hz + 30 s), the latency change 30 s after the end of the ramp. Changes in peak amplitude are not presented, since they are affected at least as much by electrode movements due to contractions of the stimulated muscle fibres as by changes in membrane potential.

Statistics: As observations were not normally distributed and variance was not equal for several of the investigated parameters, group data for patients and controls was compared using a non-parametric unequal variance t-test (Welch rank test, i.e. Welch t-test on ranked data). Results are therefore presented as medians with interquartile range. Results with $P < 0.05$ were considered significant, and 95% confidence limits calculated as 2.5 and 97.5 percentiles. The ability of a method to discriminate ALS patients from controls was evaluated with receiver operating characteristic (ROC) analyses, by determining the area under the curve (ROC-AUC). All statistical computations were performed in the QtracP software.

3. Results

All subjects completed the examinations to the full extent and tolerated them well, reporting no or minimal discomfort, mainly arising during supramaximal nerve stimulation or needle insertion.

All numerical values are provided as mean ± standard error (SE), unless otherwise stated.

3.1. NCS and EMG

None of the patients showed signs of entrapment neuropathy or demyelination of the peroneal nerve on NCS. Peak CMAP amplitudes were significantly reduced in the ALS patients compared with controls (Table 2).

On EMG, ALS patients had a significantly increased MUP duration and MUP amplitude as well as a higher occurrence of spontaneous activity (fibs/psws) and fasciculations (Table 2). When evaluating individual findings, 9 out of 26 ALS patients (34.6%) lay above the upper limit of the 95% confidence interval (95% CI) for mean MUP duration of the controls (Fig. 2A), and 10 (38.5%) lay above for MUP amplitude (Fig. 2B). In 14 patients (54%) there was abnormally increased incidence of polyphasic potentials of 12% (Buchthal and Kamieniecka, 1982), which was not the case in any of the healthy controls. Twelve of the 26 ALS patients (46.2%), but none of the controls, showed an abnormal incidence of fibs/psws.

3.2. MScan

On MScan, the ALS patients showed substantially reduced MUNE and N50 values compared to the controls (Table 2, Fig. 2C). As reflected by the parameters A50 and Largest unit (μV), motor unit size tended to be larger in ALS patients (Table 2), but this was only significant when amplitudes were expressed as a percentage of the maximum CMAP, as for A50 (%) (Table 2, Fig. 2D)

Table 2
Measures from nerve conduction studies (NCS) and electromyography (EMG), MScan MUNE, MVRC and Frequency Ramp recordings compared between controls and ALS patients. All measures also tested for correlation with ALSFRS-R among ALS patients.

	Median (1st quartile – 3rd quartile)		*P for Welch rank test*	Correlation with ALSFRS-R in ALS patients	
	Controls *n = 25*	ALS *n = 26*	Controls vs ALS	*Spearman's ρ*	*P for ρ*
NCS and EMG measures					
Peak CMAP (mV)	6.95 (6.72–7.59)	4.29 (2.62–5.72)	**5.8×10^{-7}**	0.408	**0.037**
MUP duration (ms)	13.4 (12.4–13.8)	14.7 (13.5–16.5)	**0.0025**	−0.551	**0.0036**
MUP amplitude (μV)	332 (273–419)	489 (404–681)	**5.6×10^{-5}**	−0.136	0.52
Fibs/psw	0 (0–0)	1.75 (0.63–4.88)	**3.7×10^{-8}**	−0.098	0.64
Fasciculations	0 (0–0)	4 (1–5.8)	**3.4×10^{-7}**	0.121	0.56
MScan measures					
MUNE	117 (96–121)	45 (28.5–76.5)	**2.2×10^{-7}**	0.530	**0.0053**
N50	33.8 (27.4–36.6)	12.1 (8.0–21.9)	**1.4×10^{-6}**	0.521	**0.0063**
A50 (%)	1.01 (0.95–1.10)	2.92 (1.49–4.30)	**7.5×10^{-7}**	0.494	**0.010**
A50 (μV)	73 (64–84)	85 (65–132)	0.059	0.343	0.083
Largest unit (μV)	193 (166–271)	240 (185–294)	0.19	0.370	0.060
MVRC measures					
MRRP (ms)	3.45 (3.34–3.73)	3.72 (3.41–4.09)	**0.044**	−0.303	0.13
ESN (%)	11.8 (9.4–12.8)	12.2 (9.8–13.4)	0.30	0.218	0.22
5ESN (%)	13.9 (11.9–15.0)	13.5 (11.7–15.5)	0.87	0.225	0.27
LSN (%)	3.92 (3.35–4.71)	3.91 (3.17–4.39)	0.72	0.163	0.43
5XLSN (%)	7.74 (7.49–8.78)	7.65 (6.58–9.34)	0.54	0.158	0.45
Frequency Ramp measures					
$Lat(15\ Hz)_{First}$%	94.6 (93.8–95.5)	94.1 (93.1–96.2)	0.84	−0.046	0.81
$Lat(15\ Hz)_{Last}$%	84.2 (82.3–86.0)	83.3 (81.5–87.0)	0.74	−0.136	0.51
$Lat(30\ Hz)_{First}$%	95.4 (94.1–98.2)	95.7 (93.9–98.4)	0.87	−0.002	0.94
$Lat(30\ Hz)_{Last}$%	88.1 (85.6–90.8)	90.2 (84.6–96.6)	0.32	−0.033	0.85
Lat(30 Hz + 30 s)%	102.1 (101.2–103.4)	102.9 (100.8–103.8)	0.52	−0.066	0.75

Significant P-values < 0.05 are bolded.
EMG: MUP duration = mean duration of motor unit action potentials sampled quantitatively; MUP amplitude = mean amplitude of motor unit potentials; Fibs/psw = incidence of spontaneous activity as average of number of fibrillation potentials and positive sharp waves recorded at rest; Fasciculations = number of fasciculation potentials recorded at rest.
MScan: MUNE = motor unit number estimate for the TA muscle; N50 = number of larger units making up 50% of the amplitude; A50 = amplitude of smallest of N50 units in % of CMAP and μV; Largest unit = size of largest unit in μV.
MVRC: MRRP = muscle relative refractory period; ESN = early supernormality (up to 15 ms); 5ESN = early supernormality after 5 conditioning stimuli; LSN = late supernormality (50–150 ms); 5XLSN = extra late supernormality after 5 conditioning stimuli.
Frequency Ramp: 'Lat' parameters = latency to the first or last response in train, as % of pre-ramp value, when frequency reaches 15 or 30 Hz; Lat(30 Hz + 30 s)% = percentage change in latency, 30 s after end of frequency ramp.

15 ALS patients (57.7%) lay below the lower limit of the 95% CI for the mean MUNE value of the controls (Fig. 2C), and 16 (61.5%) lay above the upper limit for A50 (%) (Fig. 2D).

There was a significant correlation between MUNE and A50 (%) (Rho = −0.986, $p = 2.2^{-17}$) and Largest unit (μV) (Rho = −0.461, p = 0.017) but not with A50 (μV) (Rho = −0.355, p = 0.072). Similarly, there was a significant correlation between N50 and A50 (%) (Rho = -0.988, $p = 1.07^{-17}$) and Largest unit (μV) (Rho = −0.532, p = 0.005) and also with A50 (μV) (Rho = −0.43, p = 0.027).

3.3. ROC analyses

The ability of the EMG and MScan methods to discriminate between ALS patients and controls by means of MUP duration and MUP amplitude for EMG and MUNE value and A50 (%) for MScan is illustrated via ROC curves in Fig. 3. From the ROC analyses we derived the area under the curve (ROC AUC) as well as the best cut-off value to maximize the accuracy (mean sensitivity and specificity) for discriminating patients from controls. Results are presented in Table 3, in which it can be seen that ROC AUC and accuracy is highest for MScan MUNE and A50 (as % of CMAP), and these measurements also provided the highest accuracy.

3.4. MVRC recordings

Results from the ALS patients of MVRCs recorded following 1 and 5 conditioning stimuli are illustrated in Fig. 4A, and the excitability measures are compared in Table 2. As can be seen in the figure, the overlap between the curves from ALS patients and controls is striking. MRRP was slightly but significantly prolonged in the ALS patients, due to 4 patients with values outside the 95% CI for healthy subjects. There was also a modest increase in the extra residual supernormality, 950 ms after 5 conditioning stimuli. Neither of these variables correlated significantly with any MScan or MUP variables, and none of the measures reflecting early and late supernormality differed significantly between patients and controls.

Furthermore, we did an analysis of the ALS patients divided into two subgroups: those with an abnormal occurrence of spontaneous activity compared to those without. This did not show a significant difference in any of the MVRC parameters.

Results from the Frequency Ramp comparing the average changes in latency and peak amplitude between patients and controls are illustrated in Fig. 4B and presented in Table 2. This protocol, with trains of stimuli of increasing rate, produced only slight changes in latency for the ALS patients, similar in size and the control group. Both the controls and the patients showed a U-shaped latency curve, with initial progressive reduction in latency reaching a plateau before gradually increasing. The degree of reduction in latency is most marked for the last in train. This is likely related to the increasing depolarizing effect of K^+ accumulating in the T-tubules during the trains of action potentials which, when mild, results in a reduction in latency, but with increasing frequency trains. But with increasing frequency trains, the depolarization subsequently reaches a degree where it starts to cause Na^+ channel inactivation, and therefore the latencies stop reducing and start to increase back towards baseline. (Tan et al., 2014)

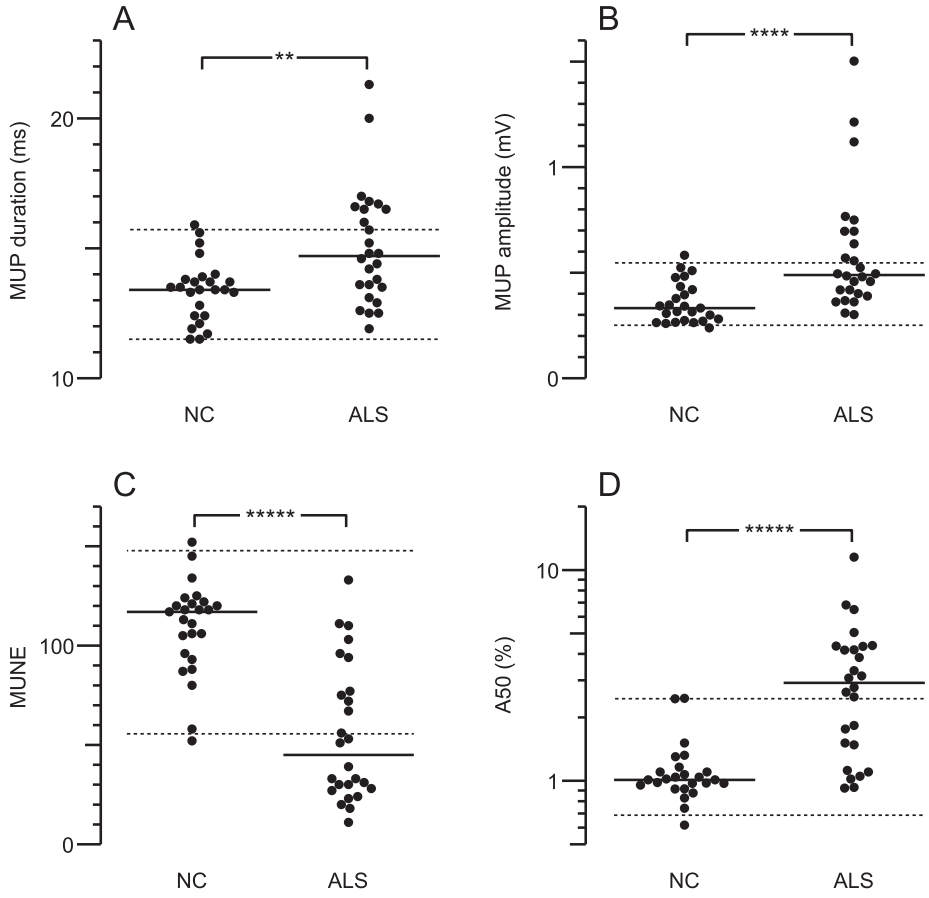

Fig. 2. Distributions of 2 EMG and 2 MScan variables between the 25 normal control subjects (NC) and 26 patients (ALS). Horizontal solid lines indicate medians, and dashed lines indicate 95% confidence limits for the normal controls. Note logarithmic *y*-axis in D for clarity. The asterisks indicate the *P* values for comparison by the Welch rank test, as listed in Table 2 (** = P < 0.01, **** = P < 0.0001, ****** = P < 0.00001). The ability of these 4 measurements to discriminate between NC and ALS are provided by ROC analysis in Table 3.

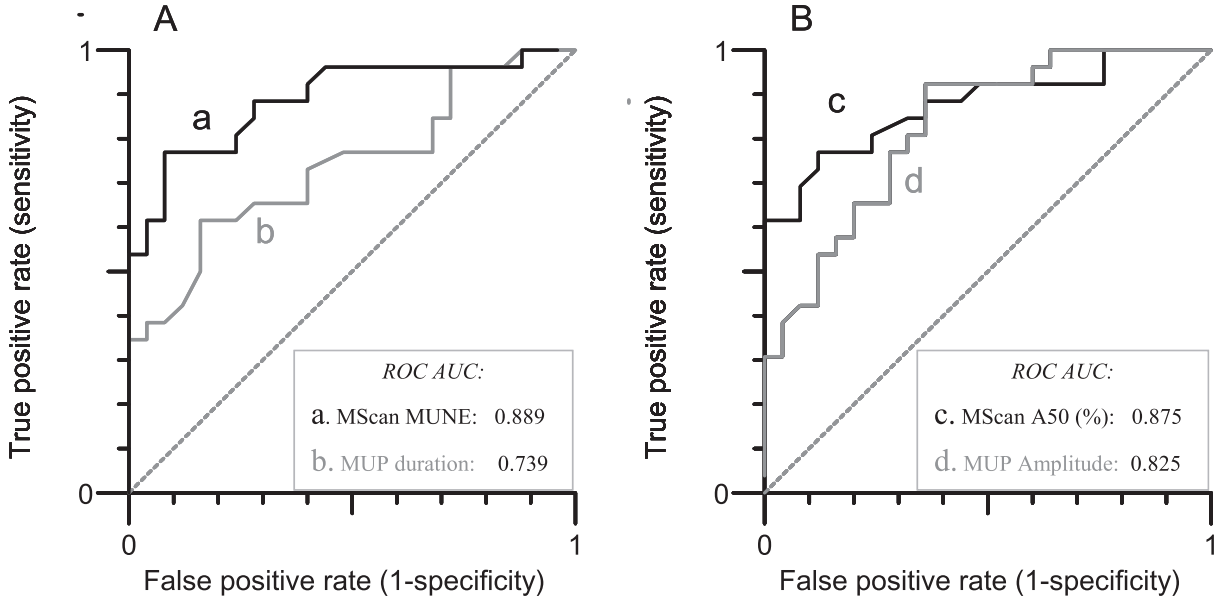

Fig. 3. ROC curves comparing area under curve (AUC) for discriminating 25 normal subjects from 26 ALS patients: A: MScan MUNE (a) vs MUP duration (b); B: A50 (%) (c) vs MUP Amplitude (d).

Table 3
Results of the ROC analysis, evaluating the accuracy of the four parameters from the EMG (MUP duration and MUP amplitude) and MScan (MScan MUNE and MScan A50) examinations in discriminating ALS patients from healthy controls.

	ROC Analysis			
	MUP duration	MUP amplitude	MScan MUNE	MScan A50 (%)
ROC AUC	0.739	0.825	0.889	0.875
Cut-off value	14.1 ms	354.0 μV	78.5 units	1.32
Sensitivity	61.5%	92.3%	76.9%	76.9%
Specificity	84.0%	64.0%	92.0%	88.0%
Accuracy	72.5%	78.4%	84.3%	82.5%

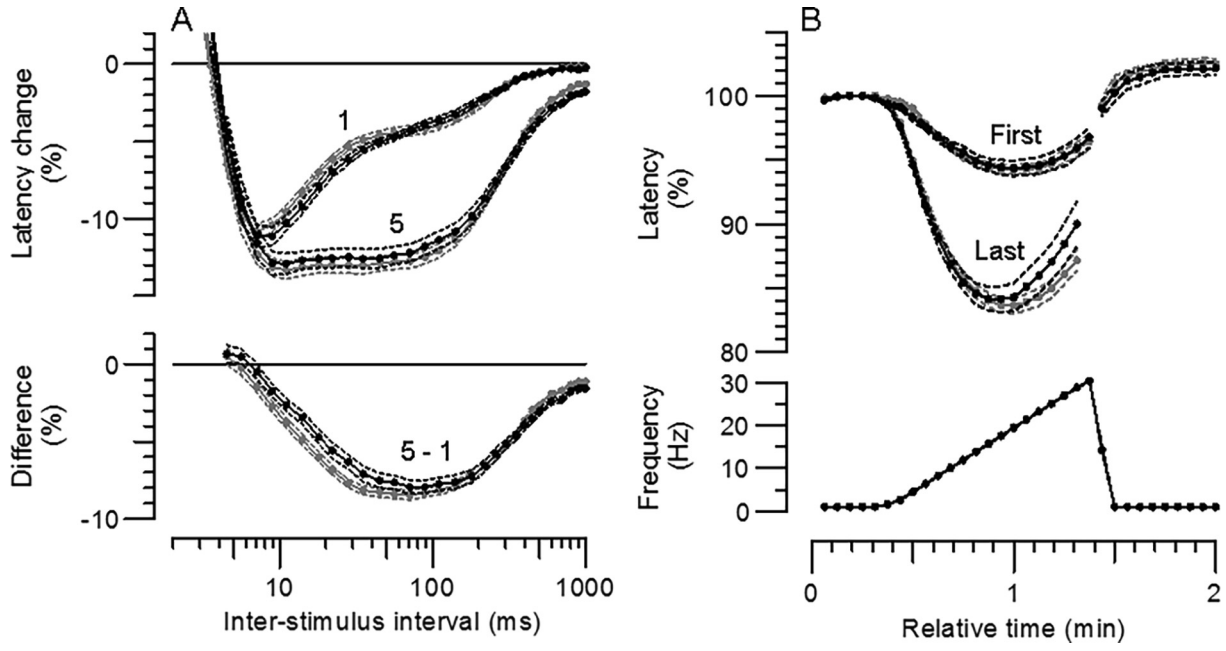

Fig. 4. (A) *Upper plot:* Muscle velocity recovery cycles (MVRCs) in ALS patients (n = 26, black) compared to normal controls (n = 25, grey) following 1 and 5 conditioning stimuli, and *Lower plot:* the differences between 1 and 5 conditioning stimuli. Percentage changes in latency are plotted as a function of interstimulus intervals (ISIs) from 2 to 1000 ms (logarithmic scale). Circles are means and dashed lines are means ± SE. (B) Muscle frequency ramp, showing changes in latency to the first and last stimulus in a train of 1 to 30 stimuli delivered in 1 s every 2 s. Means ± SE plotted as in A, with controls in grey and ALS patients in black.

4. Discussion

In the present study, we investigated the pathophysiology of ALS and the feasibility of two novel electrodiagnostic methods, MScan MUNE and MVRC. MScan was here applied in the anterior tibial muscle in ALS patients for the first time and the MVRC method has not before been used to investigate ALS.

We found that MScan MUNE is a sensitive, quantitative measure of loss of motor units in ALS patients. Despite evident ongoing neurogenic processes, interestingly, we did not find major differences in the MVRC measures between patients and controls.

4.1. MScan MUNE

From our evaluation with MScan, we found that ALS patients have a severely reduced estimated number of motor units in TA, only about half as many as the controls. This is, as expected, due to progressive loss of lower motor axons in ALS patients. Our findings thus signify that the MScan MUNE method is sensitive in detecting this abnormal motor unit loss.

Although the motor unit size tended to be larger in ALS patients, this was only significant when expressed as a percentage of maximal amplitude of CMAP (A50 (%)). Similarly, the correlation between the motor unit number and size was more prominent for the motor unit size when expressed in %. We believe the less pronounced change in unit size in TA may be because of the size and the morphology of muscle fibers, which also makes CMAP broader and often double phased.

The characteristic electromyographic signs of motor neuron disease, with long-duration and high-amplitude MUPs as well as increased occurrence of spontaneous activity and fasciculations, were all present in the ALS patients by group comparisons with healthy subjects. This clearly suggests, that compensatory collateral reinnervation is ongoing, although not all of the indicative MScan parameters were significantly increased in the patients. Nevertheless, only 9 of the ALS patients (34.6%) had a MUP duration above the upper 95% CI limit for mean MUP duration in the controls, and for MUP amplitude this was the case for 10 patients (38.5%). The challenge remains, that these methods are not able to directly quantify the degree of motor neuron loss. Looking at the MScan results, 15 ALS patients (57.7%) had a MUNE value below the 95% CI for the controls and 16 (61.5%) had an A50 (%) amplitude above the upper limit. Thus, more patients had abnormal MScan values than abnormal MUP duration or amplitude.

The revised ALS Functional Rating Scale (ALSFRS-R) is a global measure of function in ALS and the most commonly used primary outcome measure in clinical ALS trials. For an objective biomarker to provide a credible outcome measure for potential ALS treatments it should correlate well with ALSFRS-R. Table 2 shows that

this is the case for MUP duration and numbers of units (MUNE and N50), which all have $P < 0.01$, although these tests were limited to one extremity, which may not be affected with bulbar or upper limb onset disease. It is notable, however, that measures of absolute unit size (MUP amplitude, A50 (μV) and Largest Unit (μV)) were not significantly correlated with ALSFRS-R. This may be because the size of the TA muscle dictates that the collateral reinnervation takes place over sufficiently long distances that it increases MUP duration more than amplitude. Besides, MUP amplitude is highly dependent on the positioning of the EMG needle in relation to the motor units. Another possible explanation may be the time course in ALS, as the disease does not last long enough before patients die for reinnervation to become complete, as for example is the case in poliomyositis.

It should be noted, that there is no 'gold standard' method to directly measure the motor unit number, and hence no method for direct comparison to determine sensitivity and specificity in more detail. The ROC analysis, however, suggests a great ability to discriminate ALS patients from controls with regards to MUNE value (AUC = 0.889) with an accuracy of 84%. As for EMG, the ROC analysis indicates an accuracy of MUP duration of just 72.5% (AUC = 0.739) in distinguishing ALS patients from controls, and MUP duration is otherwise regarded the most important EMG parameter in identifying chronic neurogenic change. The MScan parameters thus seem sensitive in discriminating ALS patients from healthy controls compared to MUP duration and amplitude of the EMG.

In a previous study by Jacobsen et al. (2017), MScan was shown sensitive in detecting abnormal loss of motor units in the abductor pollicis brevis (APB) muscle in ALS patients. It was also shown to have an excellent reproducibility and low coefficient of variation, superior to that of two older MUNE methods (Multiple point stimulation MUNE and MUNIX). MScan has moreover been shown sensitive in following the progression of motor unit loss in these ALS patients. Jacobsen et al. (2018). In the present study we applied MScan to TA muscles in ALS patients for the first time, and its success in discriminating patients from controls expands the clinical utility of MScan as a tool for quantifying the degree of motor unit loss.

As compared to other MUNE methods, MScan has the advantage that analysis takes the entire motor unit population into account, and not simply a sample from this. Other advantages include, that it is quick and easy to perform, with recording and analysis lasting 5–10 minutes each, and that it is almost fully automated, which reduces the risk of operator related bias.

A compelling feature of MUNE is that it is not affected by collateral reinnervation, in contrast to EMG, NCS and clinical measures.

It should be emphasized, that the conventional methods, EMG and NCS, are essential for observing spontaneous activity and for excluding differential diagnoses. Thus, MUNE is not suggested to replace conventional diagnostic methods, but rather as a supplementary tool, which could aid in diagnosing and following pathophysiological disease progression in ALS patients.

4.2. MVRC recordings

The MVRC recordings seemed highly interesting to apply in patients with motor neuron disease, as it could possibly reveal changes in membrane properties of muscles fibers affected by substantial denervation and expand the knowledge of disease pathophysiology affecting the muscle. The present study is the first to apply this MVRC recording technique in patients with ALS.

Our most remarkable finding is actually the striking similarity between MVRCs recorded from ALS patients and controls. There was only a barely significant prolongation of muscle relative refractory period (MRRP) in the patient group. As an isolated finding, with the other MVRC parameters not being significantly different from controls, we do not put much emphasis on this finding.

We also found that there was no significant difference in the MVRC parameters between ALS patients with an abnormal occurrence of spontaneous activity and those without. As fibs/psws should be related to membrane instability, this lack in significant changes in MVRCs in ALS patients with spontaneous activity in TA is rather surprising.

Measures from the Frequency Ramp protocol were also indifferent between patients and controls, but as this protocol imitates the procedure and effects of a short exercise test, we did not really expect to make any significant findings in the ALS patients.

The changes in muscle membrane properties in these neurogenic muscles thus seem minor, despite substantial axonal loss and denervation. An explanation may simply be that the compensatory process of collateral reinnervation is effective enough to keep the muscle fibers rather healthy, at least initially. There is however a slight risk that we may have tended to examine healthy spots in the muscle during MVRC recordings, due to a patchy distribution of pathologically altered areas in the muscle. Thus, when adjusting the recording needle to obtain a stable response for measurements, we might unknowingly have favored a healthier spot with a more optimal response.

Note, the potential of the MVRC method primarily lies in studying pathophysiology by means of muscle ion channel dysfunction in vivo, and, as advocated by Tan et al. (2018), not in being a diagnostic utility.

4.3. Limitations

There are some limitations of the present study. First of all, we examined just one muscle in the lower limb in all patients, regardless of type of onset and regions showing clinical affection. Consequently, the TA muscle may have been completely unaffected in patients with primarily bulbar or upper limb onset disease. Secondly, we always examined the patient's less affected limb, in which atrophy and loss of strength was less pronounced. If we had examined a clinically affected muscle in all patients, we may potentially have found more striking differences Thirdly, almost half of the patients reached a diagnostic certainty of just 'possible ALS' according to Awaji criteria, indicating not very progressed state of disease. These factors may have blunted our results, particularly of the MVRC recordings, and may also help explain the rather low sensitivities of the MScan parameters and MUP duration in the ROC analysis. On the other hand, evaluation of patients not very affected by disease and of the least affected side may also reflect the everyday clinical situation and challenge well.

A technical challenge was that we had to raise the stimulus width from the standard 0.2 ms to 0.3 or 0.5 ms to reach supramaximal stimulation in the MScan. We do however not expect this to have changed the outcoming parameters, as stimulus intensity was decreased exponentially in steps of 0.2% during the recording.

In this study we compared ALS patients only to healthy controls, not to patient controls, and the problem of differentiating ALS from ALS mimicking disorders has therefore not been addressed.

4.4. Conclusion

We here show that MScan MUNE is sensitive in detecting and quantifying abnormal loss of motor units in the anterior tibial muscle ALS patients. Despite this ongoing neurogenic process with substantial denervation, muscle fiber membrane properties, reflected by the MVRC parameters, seem largely unchanged, presumably owing to compensatory collateral reinnervation.

More research is needed to further explore the potential of MScan as a clinical tool. On the other hand, MVRC recordings are

not suggested for diagnostic purposes in ALS, but rather to provide pathophysiological information in vivo.

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Declaration of Competing Interest

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