Video Article

CMAP Scan MUNE (MScan) - A Novel Motor Unit Number Estimation (MUNE) Method

Anna B. Jacobsen[1], Hugh Bostock[2], Hatice Tankisi[1]

[1]Department of Clinical Neurophysiology, Aarhus University Hospital

[2]Institute of Neurology, Queen Square House

Correspondence to: Hatice Tankisi at hatitank@rm.dk

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Abstract

Like other methods for motor unit number estimation (MUNE), compound muscle action potential (CMAP) scan MUNE (MScan) is a non-invasive electrophysiologic method to estimate the number of functioning motor units in a muscle. MUNE is an important tool for the assessment of neuropathies and neuronopathies. Unlike most MUNE methods in use, MScan assesses all the motor units in a muscle, by fitting a model to a detailed stimulus-response curve, or CMAP scan. It thereby avoids the bias inherent in all MUNE methods based on extrapolating from a small sample of units. Like 'Bayesian MUNE,' MScan analysis works by fitting a model, made up of motor units with different amplitudes, thresholds, and threshold variabilities, but the fitting method is quite different, and completed within five minutes, rather than several hours. The MScan off-line analysis works in two stages: first, a preliminary model is generated based on the slope and variance of the points in the scan, and second, this model is then refined by adjusting all the parameters to improve the fit between the original scan and scans generated by the model.

This new method has been tested for reproducibility and recording time on 22 amyotrophic lateral sclerosis (ALS) patients and 20 healthy controls, with each test repeated twice by two blinded physicians. MScan showed excellent intra- and inter-rater reproducibility with ICC values of >0.98 and a coefficient of variation averaging 12.3 ± 1.6%. There was no difference in the intra-rater reproducibility between the two observers. Average recording time was 6.27 ± 0.27 min.

This protocol describes how to record a CMAP scan and how to use the MScan software to derive an estimate of the number and sizes of the functioning motor units. MScan is a fast, convenient, and reproducible method, which may be helpful in diagnoses and monitoring disease progression in neuromuscular disorders.

Video Link

The video component of this article can be found at https://www.jove.com/video/56805/

Introduction

Motor system movement is dependent on the motor unit, which refers to an individual motor nerve fiber together with the muscle fibers it activates, and motor unit number is the number of anterior horn cells or axons innervating a single muscle[1]. During the denervation and reinnervation processes, healthy axons take over the role of axons that are lost by collateral sprouting. Therefore, compound muscle action potential (CMAP) amplitude does not give the necessary information about the degree of motor unit loss. CMAP amplitude may only start to fall when more than 50% of motor units are lost. Similarly, the magnitude of abnormal spontaneous activity or motor unit potential (MUP) changes does not correlate with the degree of denervation.

Overall, there is no electrophysiological technique that allows for simple, direct measurements of motor unit number. Instead, an estimate of motor unit number (MUNE) is used to assess lower motor neuron loss[2]. Several MUNE methods have been developed since the implementation of the first method, incremental stimulation MUNE, which was introduced in 1971 by McComas[3]. Most methods have been based on measuring several surface-recorded motor unit potentials (sMUP) and dividing the maximal CMAP by the average sMUP amplitude. Such methods include incremental stimulation[4], multiple point stimulation (MPS)[5], and spike-triggered averaging[6]. Other MUNE methods have used statistical techniques based on the probabilistic nature of the firing of a motor unit in response to a stimulus[7,8,9,10]. This variability means that firing different combinations of motor units leads to variability in the size of the CMAP responses. Motor unit number index (Munix) is a more recently introduced method, which uses the surface interference patterns recorded during voluntary contractions to estimate the average size of sMUP[11,12].

These MUNE methods all suffer from one or more limitations, such as the presence of subjectivity, dependence on the absolute CMAP amplitude, bias in the selection of units, the long time needed to sample enough units, or the long time required to analyze the results. A new MUNE method has recently been developed, 'CMAP scan MUNE` (MScan), to overcome these limitations[13]. This method avoids the problems inherent in unit selection by taking account of the contribution of all units to the CMAP, as measured in a detailed stimulus-response curve, or

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CMAP scan[14,15]. It also avoids the extended analysis time of a similar, model-fitting method[9,10], by using new algorithms[16]. In a recent study, the reproducibility of MScan in estimating the number of motor units was better than two more traditional methods, MPS MUNE and Munix[13]. Additionally, MScan could show motor unit loss in earlier stages of Amyotrophic Lateral Sclerosis (ALS) than MPS MUNE and Munix. MScan was faster than MPS MUNE and as fast as Munix[13].

This paper describes the methodology of MScan in detail. It also summarizes the previously reported intra- and inter-rater reproducibility of MScan in patients with ALS and healthy control subjects[13], which may enable the reader to judge whether the method would be appropriate for a planned study.

Protocol

All subjects must give their written consent prior to examination, and the recording protocol must be approved by the appropriate local ethical review board(s). All methods described here were approved by the Regional Scientific Ethical Committee and the Danish Data Protection Agency.

Note: The recordings are made with the "TRONDNF" recording protocol, which is a part of the software (see the Table of Materials). Other equipment used is a bipolar stimulator, a 50 Hz noise eliminator, an amplifier, and an analogue-to-digital (A/D) board (also recommended is an audio amplifier for feedback of electromyogram (EMG) activity, and a muting box to cut off the sound during electrical stimulation). Motor unit number estimation by the MScan method involves three stages: 1) preparation of the subject (as for nerve excitability studies), 2) recording the CMAP scan, and 3) analyzing the results with MScan software. The recording procedure described below is specific to the software and instruments we use (see the **Table of Materials**); these will need to be adapted for other software and hardware.

1. Preparation of the Subject

1. Screen the subjects to ensure that they do not have any history of nervous system disorders (particularly neuropathy and carpal tunnel syndrome), other than the disease group that will be investigated.
2. Instruct the subject in detail about the examinations and request written consent.
    1. Inform the subject that when the recording starts, the power will gradually be increased to a maximum followed by a stepwise decrease, and the examinations will take about 5 - 6 min.
    2. Explain that the subject will experience a tickling feeling in the hand and fingers.
    3. Inform the subject that the power can be switched off immediately at any moment during the recording, if the subject feels too much discomfort.
3. Clean the subject's hand and forearm with skin prep gel and alcohol.
4. Place the active recording electrode over the abductor pollicis brevis muscle and the reference electrode on the metacarpo-phalangeal joint of the thumb (**Figure 1**).
5. Place a ground electrode on the dorsum of the hand.
6. Connect these electrodes to the pre-amplifier (**Figure 1**).
7. Tape the fingers together to eliminate noise and artefacts due to voluntary movement (**Figure 1**).
8. Maintain the skin temperature between 32 °C and 36 °C with a warming lamp.

2. Recording the CMAP Scan

Note: All software actions described below are specific to the software and instruments we use (see the **Table of Materials**); these will need to be adapted for other software and hardware.

1. Start the semi-automated computerized system.
2. Select 'MScan-R' recording protocol from the 'Select recording protocol' form.
3. Accept default settings for the pre-amplifier and the stimulator.
4. On the 'Select recording parameters' form, enter in the 'Output file' box a 2- or 3-letter operator prefix, then click on the "O.K." button.
5. When the program displays the Raw EMG input, select either 'MScan parameters' (scan step, interstimulus interval, and stimulus width) manually, or accept the default parameters: stimulus width of 0.2ms, scan step of 0.2%, and interstimulus interval of 0.5 s. Click on "OK" or press the "Escape" key to continue.
   Note: The default parameters were accepted in this study. Filter settings were 3 Hz -3 kHz.
6. Place a repositionable bipolar stimulating electrode on the median nerve at the wrist to find the site of lowest threshold. Click "OK" to start stimulation.
7. The next display shows the stimulus (initially 15% max output) and the EMG response; adjust the electrode position to find site of lowest threshold (*i.e.* largest response). Adjust the position of the recording electrodes if necessary to ensure that the shape of the CMAP is diphasic with a single peak, if possible. If needed, adjust stimulus strength with the 'Insert' and 'Delete' keys. Then, click "OK" to continue.
8. 'Modified' response appears, with 'window', in which response is measured, indicated by horizontal magenta line. Ensure that the short green line before the window (indicates the baseline) is a flat line between stimulus artifact and response. If necessary, set the window start by depressing right mouse button, drag the cursor to the right, and then release the button for window end.
   Note: CMAP peak height is measured from baseline to the upward peak within window, and is indicated by vertical blue line.
   Click "OK" to continue.
9. Replace the repositionable electrode with a non-polarizable adhesive stimulating cathode electrode and place an anode 2 cm proximally along the median nerve; bottom trace now shows high gain EMG. Encourage the subject to find the most relaxed position for their hand to minimize spontaneous activity. Click "OK" to continue.

10. Increase the stimulus intensity manually by pressing the "Insert" key until the stimulus current is above the level for the maximal amplitude of the CMAP; first, increase by steps of 3% in stimulus intensity, and then for fine adjustments use steps of 1%.
11. Check the window and peak measurement before clicking on the "OK" button to start CMAP scan.
12. Note that after the responses to 20 supramaximal stimuli (pre-scans) are recorded, the stimulus intensity is automatically decreased in small steps, from supramaximal stimulation until there is no longer a discernible motor response, then a further set of 20 CMAPs (post-scans) is recorded.
    Note: The data are plotted as a 'CMAP scan', or a detailed stimulus response curve with the amplitude of the motor response on the y-axis and stimulus intensity on the x-axis. In healthy subjects, this creates an S-shaped curve, whereas in patients with reduced numbers of motor units, as in ALS, the curve develops a stepped appearance (**Figure 2**).
13. Notice that in addition to the feedback achieved from the muting-box, it is possible to check in the middle panel on the left-hand side if the subject is relaxed. Look at the CMAP (modified) on the left top panel, and on the left bottom panel, the decrease in stimulus intensity (threshold). Follow the CMAP Scan on the right-hand side.
14. Finish the recording by clicking on the "OK" button in the lower right corner unless a repeat scan is required and save the data.
    Note: When completing the 'Legends and scaling' form it is necessary to replace all the question marks.

3. MScan Analyses

Note: All software actions described below are specific to the software and instruments we use (see the **Table of Materials**); these will need to be adapted for other software and hardware.

1. **Fitting a model to the CMAP scan**
    1. To analyse the recording offline, start the analysis program and click on "O.K." to select the the last recording for analysis.
    2. Click on the "select 'Fit MScan QZD file" from the "MScanFit" menu.
       Note: One can instead select "Fit MScan on MEM file". It is alo possible to select "Fit MScan on dat (mA mV) file" in case the CMAP Scan was recorded with equipment different from that used here. See below how to create a DAT file.
    3. Observe that the program first generates a preliminary model, and then goes on to optimize the fit. No user intervention is required until the multi-coloured progress bar in the 'Optimization' box is complete and the "Stop" button is grayed out.
    4. The preliminary model provides a first guess of a model, derived from the slope and variance over successive parts of the scan. Note that the original scan (in black) and a scan generated from the model (in magenta) may be plotted side by side. Follow the model and improvements in the model on the text display and alternative displays.
       Note: Alternative displays may be selected at any time according to the options in the 'Data to plot', 'Plot amplitudes as', and 'Plot type' boxes.
    5. Perform optimization to improve the fit to the recorded CMAP by making serial adjustments to minimize the difference between the simulated and recorded CMAP scans[16].
       Note: This runs several optimizations in succession, starting with the preliminary model, as previously described in detail[15]. If the optimization increases the number of units, then the next attempt at fitting the scan starts by generating a model with more units, whereas if the first optimization reduced the number of units, the second attempt starts with a model with fewer units. If all works well, the optimization procedure zeroes in on the best number of units to fit the scan.
    6. Notice that when running the optimization procedure, the optimization panel also shows a multi-colored progress bar.
    7. Use different plotting options at the "plot type" box, *i.e.*, contour plots, error v N units, model units, cumulative amplitude to follow the optimization process.
       Note: The changes in the model during the optimization procedure are continually updated according to the latest model and the improvements in the model may be followed by different plotting options and on the text display.
        1. Contour maps
            1. Utilize contour maps to assess the accuracy of the model by blurring the points and generating an error score based on the difference in the x-y distributions[15]. Select "Diff" and "Contour map" displays to see the differences between the recorded and model CMAP scans as a contour map. It is these differences that the optimization process tries to minimize.
               Note: 'Contour maps' enables the probability density of obtaining a given response with a given stimulus. The error score depends on the difference between the original and modeled contour map, and this can be visualized by selecting the 'Diff' option in the top panel and the 'Contour plot' in the Plot type panel. The red lines indicate that there was a greater density of points in the original scan, and the green lines indicate more points in the modeled scan. The difference plot at the top indicates the stimulus intensities at which the greatest errors are found.
        2. Error v N units
            1. Follow the optimization procedure by selecting the log-log 'Error v N units' plot. Each stage of the optimization procedure is plotted in a different color, corresponding to the bars in the 'Optimization' panel.
        3. Model units
            1. Use this to see how the mean CMAP amplitude is made up by the recruitment of the different axons in the model; the top part shows the peak amplitudes and threshold distributions of the individual motor units.
        4. Cumulative amplitude
            1. Note that the units are plotted in order of increasing size, rather than threshold. The black curve plots the cumulative unit number, while the red curve shows cumulative amplitude.

2. When the optimization process is complete, view the results of the analysis on the 'MScanFit text display'. The available MScan parameters, *i.e.*, number of units, median amplitude, largest unit size are shown on the text display.

3. Click on the "OK" button in the 'Save fit to MEM file' box to save the model in the MEM file for further analyses.

4. CMAP Scan MUNE Using .DAT Files

Note: An alternative and free version of the software enables analysis of CMAP Scans recorded by other equipment.

1. Generate a.DAT file containing the CMAP scan to be analyzed.
   Note: This must be a text file in a standard 2-column format, with the stimulus intensity in mA in one column and CMAP amplitude in mV in another column.
2. Generate a suitable.DAT file by copying two columns from a spreadsheet file into a text editing software, and then designating the extension as.DAT rather than.TXT.
   1. Perform all the other steps of the MScan analysis as previously described, after starting the stand-alone program and selecting the.DAT file.
3. Find the freeware program, manual, and specimen data on the University College London FTP site (Host: 144.82.46.62, User name: QtracW, Password: Hg32wK5e).

Representative Results

The following results were obtained in a recent study, in which the MScan method was compared with two established techniques: multiple point stimulation MUNE (MPS) and motor unit number index (Munix)[13]. The results show that with the technique described in this protocol, consistent results with excellent reproducibility can be achieved. The method can differentiate ALS patients from healthy controls in an earlier stage of the disease than CMAP and it is quick and easy to use[13]. This suggests that the method may be suitable for clinical use.

Patients versus healthy controls: A total of 168 MScan recordings were performed in 22 patients and 20 healthy subjects, with two recordings by two different observers for each subject. The median number of motor units was significantly lower in patients (32.4, range:1 - 123.5) than healthy controls (111.1, range: 71.75 - 166.8), $p = 1.2 \times 10^{-6}$.

Reproducibility: The coefficient of variation (CV) for MScan MUNE values (mean ± SE) for intra-rater variability was 8.6 ± 1.6%, and for inter-rater variability was 9.7 ± 1.3%. The intra-rater variability was similar between the two observers. When patients and healthy controls were analyzed separately, the CV value for patients was 14.7 ± 2.7%, while for healthy controls it was 9.6 ± 1.3%, and for patients and controls combined it was 12.3 ± 1.6%.

Intraclass correlation coefficients (ICC): ICC values showed excellent intra-rater agreement for both observers. There was no difference in intra-rater ICC values between Observer 1 (0.983, confidence interval: 0.968 - 0.991) and Observer 2 (0.985, confidence interval: 0.972 - 0.992), and the ICC values also showed excellent inter-rater agreement (0.993, confidence interval: 0.987 - 0.996).

Sensitivity and specificity of MScan and CMAP: Figure 3 illustrates in a ROC curve how MScan and CMAP amplitude can distinguish 22 ALS patients from 20 healthy subjects. The best cut-off MScan MUNE value for distinguishing between healthy controls and patients was 75.5. This yielded a sensitivity of 92.5% and specificity of 80.7%. MScan MUNE was compared with the corresponding CMAP amplitude and evaluated by the area under the curve (AUC) as a measure of how well they were able to distinguish between patients and healthy subjects. MScan had an AUC (0.903), which was significantly higher than the CMAP amplitude (0.845).

Recording times: The mean recording time (mean ± SE) for all recordings for patients was 6.08 ± 0.28 min, while for healthy controls it was 6.48 ± 0.29 min, and for patients and controls combined, it was 6.27 ± 0.20 min.

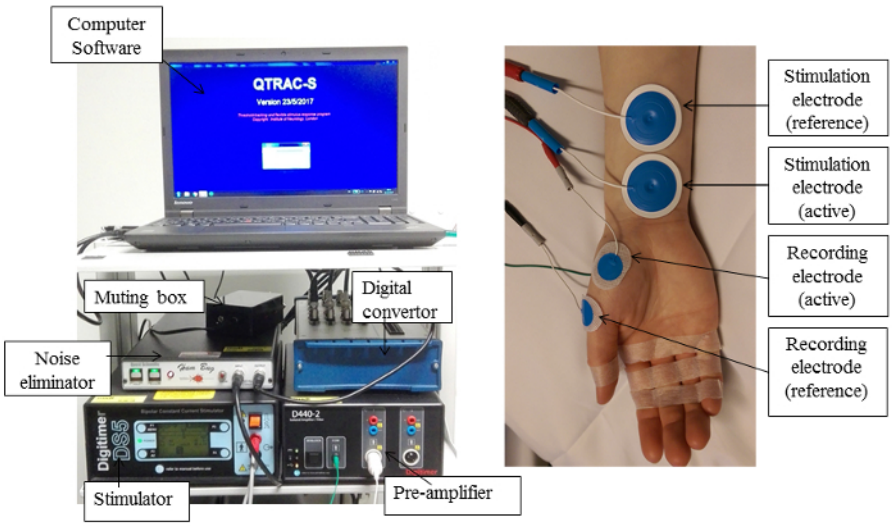

Figure 1: A picture of the CMAP scan MUNE (MScan) set-up. Please click here to view a larger version of this figure.

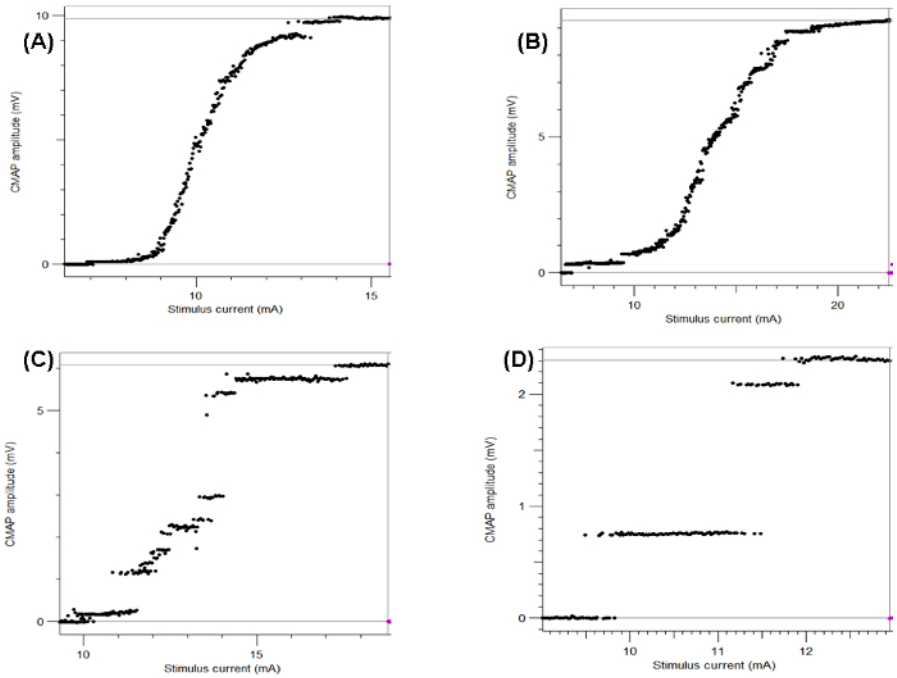

Figure 2: Examples of MScan recordings: (**A**) a healthy subject, (**B**) an ALS patient with normal MScan, (**C**) an ALS patient with moderate loss of motor units, and (**D**) an ALS patient with severe loss of motor units Please click here to view a larger version of this figure.

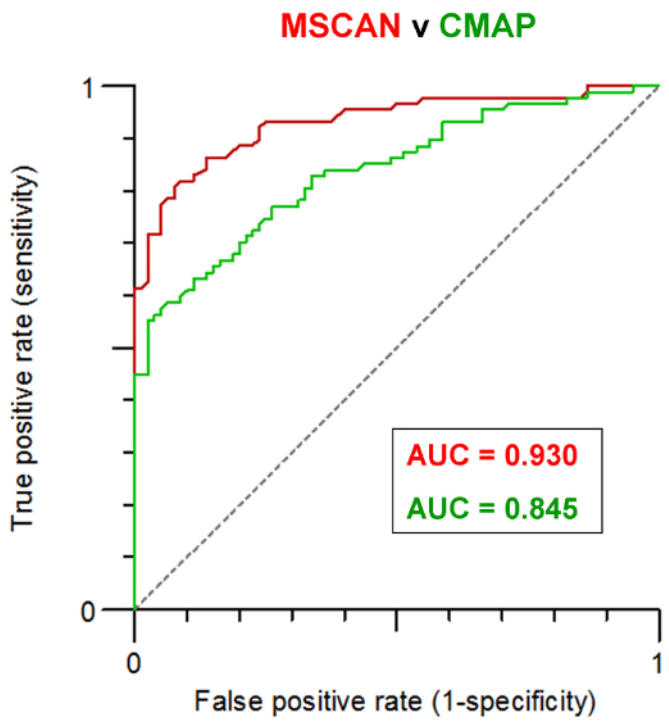

Figure 3: ROC curve comparing area under the curve (AUC) of MScan and CMAP amplitude. Two different methods were used for the analyses. Both methods made use of the fact that the same subjects were used for each test**.** Pa I refers to the method of DeLong[17] *et al*. and Pb refers to the method of Hanley and MacNeil[18]. Please click here to view a larger version of this figure.

Discussion

Critical steps within the protocol: MScan is a highly automated procedure, but as with all EMG methods, care should be taken to obtain consistent results. In the preparation stage, it is important to achieve relaxation, since spontaneous activity or movement artefacts during the CMAP scan introduce spurious variance in the CMAP and confound the generation of the preliminary model.

Modifications and troubleshooting: We found that taping of the fingers, and use of auditory EMG feedback to help find the best limb position, is helpful to avoid some variance.

Limitations of the technique: Because of the finite number of points in the CMAP scan and the stochastic nature of the responses and the modelling, one cannot expect to find exactly the same number of units after each analysis of the same recording. Even with ideal recordings, an absolute error of about 7% in the MUNE values is unavoidable[16]. This degree of uncertainty in the results is inevitable with the quick fitting procedure used. Another limitation of MScan is that it is not suitable in proximal muscles such as quadriceps femoris and deltoideus. So far, only the abductor pollicis brevis muscle has been used[13,19], but in other distal muscles, both in the upper and lower extremities including the anterior tibial muscle, we believe MScan can be applied successfully. Several supramaximal stimuli may be unpleasant, but we did not experience any subject who could not complete the examination.

A further limitation is that the recording method as described here uses specialized software and associated nerve excitability testing equipment, at present available in less than 100 departments worldwide. It is, however, possible to apply a freeware program to CMAP scans generated with other equipment. If linear CMAP scans are used, we would recommend that the step size not exceed the 0.2% used in our recordings. Motor unit thresholds have a coefficient of variation of about 2%, and 0.2% steps are required to provide adequate information about each unit.

Significance with respect to existing methods: MScan is a method that takes into account most of the limitations that have been found in other MUNE methods. We found excellent intra- and inter-rater reproducibility, and intra-rater reproducibility did not differ between experienced and non-experienced observers. In our recent study, quoted above[13], the overall CV in different recordings from the same subjects was 12.3% for MScan, which compared favorably with CVs of 24.7% for MPS MUNE and 21.5% for Munix. This reproducibility could be important in assessing potential treatments for neurodegenerative diseases such as ALS, since the number of patients required to detect an improvement is expected to increase with the square of the variability of the measurements. In distinguishing the ALS patients from healthy controls, using receiver-operator characteristic (ROC) curves, MScan discriminated slightly better, *i.e.* had a slightly higher area under the curve (AUC = 0.930) than MPS MUNE (0.899) and both discriminated significantly better than Munix (0.831) and CMAP amplitude (0.831). Additionally, MScan is a quick method to perform the recordings (~6 min). The analysis is also fast and takes less than 5 min.

Future applications: In conclusion, MScan is a method that may have the potential to be implemented in the clinic for diagnosis and follow-up of neuromuscular disorders such as ALS. Further studies with other patient groups, and larger groups, are warranted. Studies on the application of MScan in different muscles should also be conducted.

Disclosures

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.

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