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REVIEW

Assessment of Motor Units in Neuromuscular Disease

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Abstract The motor unit comprises the anterior horn cell, its axon, and the muscle fibers that it innervates. Although the true number of motor units is unknown, the number of motor units appears to vary greatly between different muscles and between different individuals. Assessment of the number and function of motor units is needed in diseases of the anterior horn cell and other motor nerve disorders. Amyotrophic lateral sclerosis is the most important disease of anterior horn cells. The need for an effective biomarker for assessing disease progression and for use in clinical trials in amyotrophic lateral sclerosis has stimulated the study of methods to measure the number of motor units. Since 1970 a number of different methods, including the incremental, F-wave, multipoint, and statistical methods, have been developed but none has achieved widespread applicability. Two methods (MUNIX and the multipoint incremental method) are in current use across multiple centres and are discussed in detail in this review, together with other recently published methods. Imaging with magnetic resonance and ultrasound is increasingly being applied to this area. Motor unit number estimates have also been applied to other neuromuscular diseases such as spinal muscular atrophy, compression neuropathies, and prior poliomyelitis. The need for an objective measure for the assessment of motor units remains tantalizingly close but unfulfilled in 2016.

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Introduction

This review will discuss motor units, and focus on the electrophysiological assessment of motor units relevant to neurotherapeutics. In particular, there is a need for a measurement of the number of motor units as an endpoint for clinical trials. Amyotrophic lateral sclerosis (ALS) is the disease where a reliable method is lacking, which has stimulated the development of the assessment of motor units; however, the motor unit measures can also be applied to other disorders of motor nerves. The review will focus on methods that are being actively used in human studies and the use of these measures in clinical trials.

Anatomy of Motor Units

The lower motor neuron is the peripheral pathway for motor nerves and can also be described as the motor unit. The motor unit, as defined by Sherrington [1], includes the anterior horn cell, motor nerve, neuromuscular junction, and the muscle fibers supplied by the individual anterior horn cell (Fig. 1) [2]. Different muscles have different numbers of motor units. Not all motor units are functional and so the number of motor units measured by functional tests may be less than the total number of motor units that could be measured by anatomic methods. The number of motor units appears to decline with age, and may vary greatly among people [1, 3–5].

The motor units in muscles supplied by the median and ulnar nerves have been the most studied and there are also studies of muscles supplied by the peroneal nerve in the foot

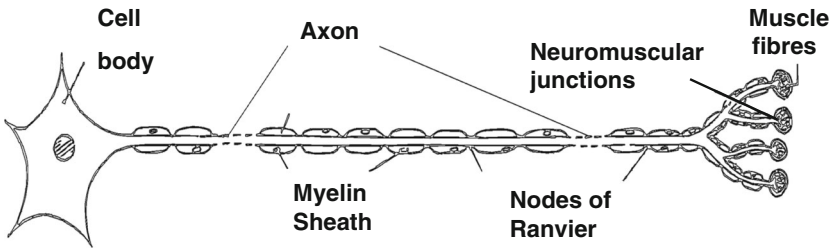

Fig. 1 The components of a motor unit from the anterior horn cell in the spinal cord through to the respective muscle fibers

or lower leg. Anatomic studies have been performed where an assumption was made that 40 % to 60 % of large-diameter myelinated fibers are alpha motor neurons [6–8], and the number of motor units was determined. Using these histologic methods the flexor digiti minimi muscle in the hand was calculated to contain approximately 130 motor units [9]. Muscles, such as the eye, requiring fine motor control have a greater ratio of numbers of motor units to numbers of muscle fibers [10].

There are different types of motor units that, in turn, define the characteristics of the muscle fibers. Type 1 muscle fibers are efficient at using oxygen, more resistant to fatigue, are used for continuous extended muscle contraction, and are the first to be recruited with voluntary muscle contraction. Type 2 fast-twitch muscle fibers use anaerobic metabolism, are used in generating short bursts of contraction, and are activated at maximal force of contraction. These type 2 fibers are preferentially assessed with methods that use maximum force analysis [11]. The conventional view is that electrical stimulation progressively activates larger-diameter motor neurons, and smaller motor neurons have the lowest threshold with voluntary contraction, although this does not necessarily apply after nerve injury [12, 13].

The integration of motor units in motor control appears highly complex [2, 14, 15]. It is likely that the motor pathway should be considered as a network from the gamma motor neuron in the cortex through to the associated muscle fibers, with multiple levels of neuronal feedback.

Need for Assessment of Motor Units

The assessment of motor units is problematic in disorders of the motor neuron. It is not possible to biopsy motor nerves to measure motor unit numbers and even in postmortem studies, where tissue is available, it is difficult to distinguish large-fiber motor and sensory axons. There is significant variability between different individuals and between different muscles. The variability in strength between and within individual muscles has meant that there is no single reliable measure for assessing muscle strength. The Medical Research Council grading is a gross estimate using a 5-point scale [16]. Modifications to standardize muscles with a composite score have been published [17], and manual strength testing has been used in recent trials [18]. Hand-held dynamometry (HHD) using grip strength to assess voluntary isometric strength has been developed for practical use and was superior to an ALS score and manual scores [19]. HHD was used in the 2 recent large international phase II–III ALS clinical trials [20, 21], as well as other recent smaller studies [22], with HHD inferior to the revised ALS functional rating scale-revised (ALSFRS-R) but better than vital capacity [23]. The methodology for HHD and manual testing has become standardized but nonlinear decline in muscle strength due to collateral reinnervation, the use of muscles such as finger flexion (which is relatively preserved in ALS), and the wide variability in normal muscle strength (whereby the strength of the manual tester can influence the result), are potential limitations of the muscle strength testing methods.

In addition, measurement of weakness is not a sufficient measure of the number of motor units because weakness due to denervation can be compensated for by collateral reinnervation if there are remaining normal motor axons supplying denervated muscle [24–26]. Collateral reinnervation after denervation is an ongoing process and accounts for the finding on muscle biopsies of fiber-type grouping with loss of the normal checkerboard pattern, and for an increased size of the motor unit action potentials on electromyography (EMG). It occurs with progressive incomplete denervation [26, 27], in nonprogressive diseases exemplified by poliomyelitis which has very large motor units, and to a lesser extent with normal aging. Collateral reinnervation can compensate for the loss of at least 50 % of the motor neuron pool [25], and probably occurs to some degree until only 5 % of the motor unit pool remains [27, 28]. From collateral reinnervation, synaptic sprouts occur such that immature reinnervating end plates form unstable connections with muscle, creating motor unit instability [29]. The effective result of collateral reinnervation is the nonlinear preservation of muscle strength despite falling motor unit number.

Electromyographic Assessment of Motor Units

Standard electrophysiology using nerve conduction studies measures a muscle compound muscle action potential (CMAP)—usually the amplitude, latency, and the F-wave response are considered. The size of CMAP of commonly tested

muscles declines with progression of ALS, and a summed score of CMAP has been used in a small clinical study [30]. However, there is a notable variation in normal values for the CMAP [31], and limited reproducibility with retesting (due to factors such as electrode placement, temperature, and noise).

EMG is a standard tool for assessing denervation and reinnervation and is crucial for diagnosing ALS. With inspection of the EMG, different motor units can be identified and there have also been a number of computerized motor unit analysis techniques to assess firing of multiple motor units, most commonly using needle EMG, and also using surface recordings. The motor unit territory can be assessed by needle EMG [32], and the decomposition and quantitation of the EMG signal was developed by Stashuk [33] as a practical method of assessing micro- and macro-EMG signals. Motor unit morphology can also be assessed using surface electrodes by using high-density grids [34].

Estimation of the Number of Motor Units

In an attempt to develop a biomarker for the loss of motor units, the field of motor unit number estimates (MUNE) was developed in the 1970s [3]. Most of the electrophysiology methods have studied the thenar and hypothenar muscles supplied by the median or ulnar nerves, and, to a lesser extent, the extensor digitorum brevis or tibialis anterior muscles supplied by the peroneal nerve, although there are many small studies of other nerve, muscle combinations. More than 7 MUNE methods have been published [35–42]. These can be divided into 3 groups: 1) methods developed >15 years ago where the methodology has been validated but the method is relatively inactive or used in few centers; 2) active methods that are used in multiple centers with validated methodology; 3) active methods that are used in few centers/research centers and the methodology requires further validation.

These will now be discussed in turn.

Established MUNE Methods

Established MUNE Methods Based on Electrical Stimulation

Previous electrical MUNE methods generally relied on the calculation of an average size of a motor unit, which could then be divided into the total CMAP to calculate a motor unit number estimate. Gradually increasing the size of the stimulus intensity to recruit additional motor units (incremental method), stimulating the nerve at different points to sample different motor axons [multiple point stimulation (MPS)], using the F-wave for analysis, or using statistical analysis of motor unit firing (statistical methods), have been used to obtain the average MU size [35–38, 40, 41]. Values of 200–300 motor units for the hand muscles, with reasonable test–retest reliability, were obtained with the incremental and MPS methods. The major limitation of the original incremental MUNE method was alternation whereby stimuli of the same strength could activate different combinations of individual motor units. Stein and Yang [43] estimated that the probability of alternation was >65 % when 10 motor units are activated by graded incremental stimulation. This led to the development of the MPS method whereby a series of low-intensity stimuli were given in different sites of a peripheral nerve. The statistical MUNE method was also developed by Daube [40] to account for the phenomenon of alternation using Poisson statistics and this method gives smaller MUNE values of approximately 100 to 150 obtained from hand muscles. More complex statistical modifications such as the weighted-average method were developed, but the statistical methods have not progressed beyond the research setting [41]. The statistical method appears limited when individual large motor units are measured at a particular stimulus level rather than a Poisson distribution of motor units. The incremental, MPS and statistical methods have all shown reasonable test–retest reproducibility and appear sensitive to disease progression [3]. Currently, the incremental and MPS methods have been combined in the multipoint incremental method described in the next subsection.

Established MUNE Methods Based on Needle or Surface EMG Methods

At a similar time to when the statistical methods were being devised, methods based on the size and pattern of the EMG signal (spike-triggered averaging) were also developed. These methods enabled more proximal muscles to be studied [44, 45]. Modifications such as decomposition spike-triggered averaging used a computerized algorithm to analyze multiple individual motor unit potentials. The aim of these methods was to calculate a mean motor unit size that can be divided into the total CMAP to determine a MUNE with reasonable reliability [3]. The need for specialized software and operator input has somewhat limited widespread applicability.

Active Validated Methods of Assessing Motor Unit Number

It remains clear that a biomarker for ALS is needed and that electrophysiological methods offer an opportunity to produce a direct measurement of motor axon loss, a key feature of disease [46]. At present, 2 methods are actively used in different centers. These are the Motor Unit Number Index (MUNIX) method, which is used largely in Europe and Asia, and the multipoint incremental method, which is favored in North America.

MUNIX

This method, developed by Nandedkar et al. [47], is noninvasive with standard nerve conduction studies (NCS) electrodes typically placed over the median or ulnar innervated hand muscles. The maximal CMAP is carefully determined. A gradual increase in force creates an EMG surface interference pattern (SIP). Five different levels of increasing voluntary effort are repeatedly tested and retested to determine the SIP. The SIP and CMAP are imported to propriety software. The power and area of the maximal CMAP and each SIP are determined. An index is determined based on an ideal case motor unit count defined as the ratio of the maximum M-wave power to its area multiplied by the ratio of the SIP area to its power. Thus, each SIP level gives an area and ideal case motor unit count. A regression curve that defines the tested muscle is produced. Rather than rely on actual force measurements, a value is computed from a small-value (20 mV/ms) to determine an index value (MUNIX) from the established exponential fitting for that muscle. There are some arbitrary criteria imposed to accept a SIP. A motor unit size index can be similarly obtained by dividing the total CMAP by the MUNIX [48, 49].

Recently longer-term studies using MUNIX have being performed, with extension of the technique to the tibialis anterior and trapezius muscles, and to earlier stages of disease [50–54]. Standardization across multiple centers has been published where 6 proximal and distal muscles were assessed over 15 months from 3 different centers [55]. The software for MUNIX is becoming more freely available on the standard Viking and Synergy EMG machines and increasing publications from different centers across the globe will increase its acceptability for use in a clinical trial. It is the method used in most centers across the globe, with 39 publications in the last 3 years and is probably the most widely accepted method for assessing motor units [56].

Multiple Point Incremental Stimulation

In North America, this MUNE method remains in active use and combines the incremental and MPS methods, where a small number of incremental motor units are studied from different sites along the nerve using standardized methodology [57]. The method was devised to overcome the problem of bias from alternation, which is the overlapping size of different motor units due to probabilistic firing at submaximal stimulus intensities. The method appears reliable and sensitive to disease progression and could be applied across multiple centers [57, 58]. Recently, a study from Poland demonstrated utility of this method from the first dorsal interosseous muscle when compared with other clinical parameters [59], and the method was applied to a study of carpal tunnel syndrome [60]. The multipoint incremental method has also been used with high-density surface EMG [61].

The advantages of the multipoint incremental stimulation method are that it does not require multiple supramaximal stimuli, which are uncomfortable, and it is relatively quick at 5 minutes per muscle. Comparisons with healthy controls and longitudinal studies have been performed and the method appears reliable in slowly progressive denervation [57]. At present, this method is actively used in a few centers but with fewer recent publications than the MUNIX method.

The challenges for both of these MUNE methods are the wide variability in normal values, some operator input is required to determine the maximal CMAP and whether to accept artifact, and the utility across different stages of ALS when there can be issues such as very low CMAP or rapid progression with unstable motor units. In addition a method that can be used in clinical trials needs to be easily applicable across multiple centers.

Electrophysiology Methods Requiring Further Validation

MUNE methods have not achieved widespread applicability and this has led to the development of other recent indirect electrophysiological methods for assessing motor units. A number of methods have been developed for application to ALS and appear promising.

Neurophysiology Index

The Neurophysiology Index, developed by Swash and de Cavalho [62, 63], relies on the formula of the CMAP size/latency × F-wave persistence, and was conceived as a practical measure utilizing typical measures obtained with standard nerve conduction studies. It has largely been studied in the ulnar nerve. There are no longer-term studies in ALS and limited recent publications.

Split Hand Index

The recent identification of the split hand offers potential as an ALS biomarker [64]. The split hand index (SHI) is calculated using the formula: SHI = APB CMAP × FDI CMAP/ADM CMAP [65], where APB is the abductor pollicis brevis, FDI is the first dorsal interosseous, and ADM is the abductor digiti minimi. There remains debate about whether the split hand phenomenon reflects peripheral or central mechanisms. Arguments for the peripheral mechanism note the different excitability properties in the respective peripheral nerves. A central explanation has support from evolutionary concepts where the thumb movements, in particular pincer grip, are specific to humans, and corticomotoneuron studies of the pincer grip that show upper motor neuron integrity is required for the precision movement [66]. The SHI is helpful clinically in that finding a value <5.2 supports ALS and it is easy to perform. However, it is not specific to ALS and there remains a

need to validate the index across multiple centers and from the whole spectrum of clinical presentations of ALS. The SHI has recently been applied to MUNIX [67].

Electrical Impedance Myography

Electrical impedance myography (EIM) developed by a single laboratory involves a set of electrodes placed over the belly of the muscle [68, 69]. A small, high-frequency electrical current is applied and the surface voltage can then be measured. The values represent the properties of the underlying tissue with changes in muscle morphology due to factors such as fibrosis, denervation, or edema.

Typically, 4 or more voltage-sensing surface electrodes are placed over the belly of the muscle. A single or multifrequency current is applied with reference to far-field electrodes. The published results show high reliability and sensitivity to disease progression. Rutkove et al. [70] showed that the rate of decline in 50 patients with ALS over 6 months was similar to that of the ALSFRS-R and HHD. This method has the advantage that more proximal muscles can be studied and muscle contraction/nerve stimulation is not required. EIM is being used in a pilot study of lumbar stem cell therapy [71]. However, the parameters for use in a multicenter clinical trial still need to be defined, and the method is used in only a few centers.

The CMAP Scan

The CMAP scan involves gradual increments in stimulus intensity with the subsequent measurement of the CMAP size [72–76]. It is attractive because it studies all of the motor units in a muscle, and has been shown to measure effectively the decline in motor unit number across a range of different rates of disease progression [74]. However, at present, applications either require off-line analysis (Bayesian MUNE) [73, 74], or have not been validated across the different stages of disease progression [77].

Other Methods for the Assessment of Motor Units

The need for objective outcome measures that can be reliably quantified and responsive to clinical deficit and disease progression has led to a search for useful quantitative imaging techniques [78].

Magnetic Resonance Imaging

Recently, magnetic resonance imaging was applied to chronic denervation in inherited neuropathy. The muscle fat fraction was serially assessed and showed correlation with functional measures [79]. Using diffusion tractography, fractional anisotropy was shown to correlate with axon pathology in ALS [80, 81]. The methods need to be standardized and reproducible across different centers and, in general, magnetic resonance imaging methods have mainly been applied to upper motor neuron dysfunction in ALS.

Ultrasound

Ultrasound has been studied in more detail and in patients with ALS. Muscle thickness and echo intensity have been studied with echo intensity correlating with survival in one study [82]. The cross-sectional area has been studied from the median and ulnar nerves showing a decline in longitudinal studies [83]. The role of ultrasound in detecting fasciculations has been highlighted [84]. The change may not correlate as well with other measures of functional decline [85], and validated multicenter studies are needed.

Peripheral Nerve Excitability

Excitability testing is useful for showing changes in disease compared with controls using the strength–duration time constant and threshold electrotonus [86]. Values for assessing motor units have not been determined and there is a requirement for specialized equipment. However, the ability to study the excitability of single motor units is potentially very promising [87].

ALS Clinical Trials

The assessment of motor units is an attractive endpoint for clinical trials of ALS because it is directly related to the pathology of disease, and MUNE methods consistently show correlation with clinical features at baseline and with disease progression. The commonly used surrogate biomarkers of disease progression in the ALS clinic include the forced vital capacity and the ALSFRS-R [88, 89], and these have been widely used in clinical trials because they show a reasonably linear decline [90]. Other simple clinical measures are also being proposed which require validation [91]. However, a measure that is targeted at the underlying pathology (loss of motor units) is needed for assessing the phenotypic heterogeneity of ALS where patients have varying degrees of involvement of upper and lower motor neurons/axons. Patients with ALS have differing rates of disease progression and while average survival is typically 2–3 years, a tail of long survivors occurs, partly due to disease pathology and also due to patient choices regarding respiratory support [88]. The severity of lower motor neuron involvement in ALS appears to be particularly relevant for survival [92], and therefore is an important measure to be evaluated in clinical trials.

The pattern of loss of motor units with disease progression is uncertain, with some groups finding a linear decline [90], and others a model of disease death favoring an exponential process of cell death [93]. A linear decline allows a more practical comparison of different stages of disease progression. Besides assessing rate of motor unit loss, MUNE has been proposed as a means of stratifying patients according to rate of progression [94].

Electrophysiological methods remain the most promising candidate as a biomarker of lower motor neuron loss, but no method has achieved widespread assessment nor consistent use in large, multicenter clinical trials. The statistical MUNE method was used in the unsuccessful multicenter creatine study, and it became clear that the method was limited as it does not account for motor unit variability as seen as advanced disease [95]. Other methods of motor unit assessment have been used in single trials of memantine [96], but methods of assessing motor units were not used in 2 recent, large international phase IIb multicenter studies [20, 21], nor other recent exploratory phase II studies [22, 97, 98]. The discussion of MUNE at recent meetings has been limited; for example, MUNIX was the only method discussed at the recent International ALS/Motor Neurone Disease Symposium in December 2015 [99].

Both the multipoint incremental motor unit number estimation and the MUNIX methods have been proposed as suitable outcome measures in ALS trials. Both are relatively easy to perform across different centers and different hand and foot muscles can be studied. The multipoint incremental method has the advantage that it is conceptually easy to understand and can be performed on standard electrophysiology machines but requires a degree of operator involvement, while MUNIX can also be performed on standard machines (that have the software installed) and has the advantage that it does not require electrical stimulation and is reasonably automated with rapid data collection. A degree of operator input is required for both methods.

The requirements for the assessment of motor units in an international multicenter clinical trial would appear to be 1) reliability of the results, 2) ease of data collection preferably by a technician or research assistant; 3) established standardized muscles and protocols; and 4) methods sensitive to disease pathology and progression.

The candidates for electrophysiological biomarkers in comparison with other measures are summarized in Table 1. The ability to show disease progression at equal to or superior rates of decline than the established ALFRS-R and manual/isometric strength has been a key feature supporting electrophysiological methods both old [100–102] and new [55].

MUNE in Other Neuromuscular Diseases and Normal Aging

Other diseases with denervation are attractive for study using MUNE and in these the influence of motor unit instability inherent to ALS is less of a problem [103]. Inherited peripheral neuropathies have been studied with significantly lower MUNE values being found in Charcot-Marie-Tooth (CMT) hereditary neuropathies type 1A, type X, and type 2 in distal ulnar nerveinnervated muscles compared with control values. There were also lower values in more proximal muscles (biceps brachii), correlating with axonal loss, but these findings were less significant than the changes in distal muscles [104, 105]. Recently MUNIX has been applied to the study of demyelinating neuropathies [106], and has an advantage over older MUNE methods that are limited by the higher stimulus intensities required in some hereditary motor neuropathies.

Normal aging has also been studied with MUNE. Healthy adults over 60 years of age demonstrate reductions of 50 % or greater in both distal (thenar, hypothenar) and more proximal muscles (biceps brachii, tibialis anterior, soleus) when compared with their younger counterparts. One study, using decomposition-enhanced STA showed that “very elderly

Table 1 Comparison of motor unit biomarkers that are used in amyotrophic lateral sclerosis (ALS)

Method	Reliability*	Ease of data collection	Standardized protocols	Sensitive to progression
ALSFRS-R [89, 90]	0.77	+++	+++	Approx. 1–2 %/month
HHD [23]	0.63	++	+	Not stated
Manual muscle [17]	0.67	++	+	1.0–1.5 %/month
MUNIX [55]	0.80	+	+	2.4–4.2 %/month
MIM [57]	9.5 %†	+	+	3 %/month
Imaging	Not established	++	No	Not established

The ALS functional rating scale-revised (ALSFRS-R) is a global measure. HHD = hand-held dynamometry; MUNIX = Motor Unit Number Index; MIM = multipoint incremental

*The coefficient of variation/correlation coefficient is not standard across the methods

†Test–retest variability in normal subjects

men" (mean age > 80 years) had even greater motor unit loss than "older men" (mean age 66 years) [107]. Motor unit loss associated with aging is a significant factor leading to age-related reductions in muscle mass, strength, and power (often referred to as "sarcopenia").

Spinal muscular atrophy is a slowly progressive disorder usually characterized by proximal greater than distal weakness, so MUNE methods developed from distal muscles have been less applicable but earlier MUNE methods have been used [108, 109]. MUNE has been applied to compression neuropathies and postpolio syndrome and has shown correlations with disease severity [110, 111].

Conclusion

In 2016, there remains a need for a lower motor neuron biomarker of disease progression. In the absence of reliable imaging, blood, or respiratory biomarkers, many centers across Europe, the Americas, Asia, and Australia remain active in the area of assessment of motor unit numbers. For use in a clinical trial, a biomarker of motor units will need to be sensitive to disease pathology, reproducible, and have utility at many clinical centers. At present, researchers studying the MUNIX and multipoint incremental methods are the most active, but it remains to be seen whether this will be translated into use in an international clinical trial.

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

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