MOTOR UNIT NUMBER ESTIMATION: A TECHNOLOGY AND LITERATURE REVIEW

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ABSTRACT: *Introduction:* Numerous methods for motor unit number estimation (MUNE) have been developed. The objective of this article is to summarize and compare the major methods and the available data regarding their reproducibility, validity, application, refinement, and utility. *Methods:* Using specified search criteria, a systematic review of the literature was performed. Reproducibility, normative data, application to specific diseases and conditions, technical refinements, and practicality were compiled into a comprehensive database and analyzed. *Results:* The most commonly reported MUNE methods are the incremental, multiple-point stimulation, spike-triggered averaging, and statistical methods. All have established normative data sets and high reproducibility. MUNE provides quantitative assessments of motor neuron loss and has been applied successfully to the study of many clinical conditions, including amyotrophic lateral sclerosis and normal aging. *Conclusions:* MUNE is an important research technique in human subjects, providing important data regarding motor unit populations and motor unit loss over time.

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The ability to accurately estimate the number of functional motor neurons or motor axons in a living subject has been of great interest from the inception of modern neuromuscular physiology. The first motor unit number estimation (MUNE) method evolved from routine motor nerve conduction studies (NCS). During motor NCS, a series of small, but progressively stronger stimuli delivered to the nerve at a single site produces a progressively larger compound motor action potential (CMAP) due to activation of increasing numbers of motor units. In the original incremental stimulation method, McComas *et al.* made the assumption that each small, stepwise increase in CMAP amplitude with slight increments of stimulus intensity represented the addition of another single motor unit potential (SMUP) to the growing waveform.[1] They further reasoned that directly measuring the amplitude of each increment and averaging them together would then yield the average SMUP amplitude for that nerve and muscle. As the maximal CMAP amplitude represents the total motor unit population firing together, dividing the maximal CMAP amplitude by the average SMUP amplitude yields an estimate of the number of motor units within that nerve. This value is the MUNE, expressed in formulaic terms as: maximal CMAP amplitude / average SMUP amplitude. In addition to an estimate of motor unit number, the average SMUP size obtained with these methods also enables an assessment of the extent of collateral reinnervation in cases of denervating disease.

The ability to derive a quantitative MUNE launched an entire field of new electrophysiological investigation, and numerous MUNE methods have been developed over the last 40 years. However, virtually all MUNE methods continue to be based on the same paradigm underlying the original McComas technique in that a summated value for the total motor unit population within a nerve

Abbreviations: ALS, amyotrophic lateral sclerosis; A-MPS, adapted multiple-point stimulation; CMAP, compound motor action potential; DE-STA, decomposition enhanced spike-triggered averaging; EMG, electromyography; MPS, multiple-point stimulation; MUNE, motor unit number estimation; MUNIX, motor unit number index; NCS, nerve conduction studies; SMUP, single motor unit potential; STA, spike-triggered averaging; STAT, statistical

Key words: aging; amyotrophic lateral sclerosis; compound muscle action potential; motor unit; motor unit number estimation

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is divided by a value representing the average single motor unit to yield an estimate of motor unit numbers. The purpose of this technology review is to summarize comprehensively and compare the MUNE methods, as well as the available data regarding their reproducibility, validity, refinements, applications, and utility.

METHODS

We performed a literature search using the terms "motor unit number estimation," "MUNE," and "motor unit counting" individually and in combination with 30 related terms [incremental stimulation, multiple-point stimulation, statistical, modified, weighted, decomposition, spike-triggered averaging, motor unit, electromyography, reliability, normative, normal, thenar, amyotrophic lateral sclerosis, ALS, motor neuron disease, MND, neuropathy, Charcot Marie Tooth (CMT), carpal tunnel syndrome, entrapment neuropathy, myopathy, myotonic dystrophy, aging, polio, clinical trials, creatine, coenzyme Q, riluzole]. The search included all dates through December 2010 in the National Library of Medicine (NLM) database. Abstracts, monographs, and other reports known to the authors but not indexed in the NLM were also included, and some prominent articles that appeared after the December 2010 search terminus were included. Articles that described MUNE methods that did not appear more than once in the literature were excluded.

For analysis, human studies were stratified by the most frequently reported MUNE methods (i.e., incremental, multiple-point stimulation, spike-triggered averaging, decomposition, and statistical), whereas less frequent methods were grouped together under a "Miscellaneous" category. Articles related to other aspects of motor unit physiology without direct relevance to 1 or more MUNE methods were excluded (e.g., evoked single motor unit studies). Major issues, such as reproducibility, validation, normative data, application to specific diseases and conditions (including motor neuron disease and normal aging), technical refinements, and practicality (simplicity, time required, cost), were singled out for data extraction, compiled into a comprehensive database, and analyzed.

RESULTS

One hundred sixty-three articles were identified in the world literature using the search strategy described; 139 of the articles were relevant to human subjects and were included in this analysis.

MUNE METHODS

Incremental Stimulation. The incremental method assumes each increment in CMAP waveform size (as stimulus intensity is progressively increased during a standard motor NCS) corresponds to the addition of another single motor unit to the growing CMAP waveform and further assumes that an average SMUP size can be calculated by dividing the size of the maximal CMAP by the average size of its stepwise increases (usually numbering 8–10 or more).[1–59] Incremental stimulation was the original MUNE method and is the most widely reported to date, producing over 50 articles through 2010, more than any other method.

Normative data were generated for several muscle groups using incremental MUNE, with an overall mean MUNE of 187 for the thenar, 245 for the hypothenar, and 206 for the extensor digitorum brevis muscle groups when data from available studies were pooled (Table 1). Test–retest reproducibility in control subjects was highest for the thenar group ($r = 0.95$), with coefficients of variation ranging from 11% to 32% in other muscle groups (average of 20%; Table 1).[17,34,39,40] Reproducibility was also found to be high in patients with amyotrophic lateral sclerosis (ALS) ($r = 0.86$; hypothenar)[40] and SMA ($r = 0.83$; biceps) (Table 2).[26]

Incremental MUNE has been validated against several other measures. MUNE by surface stimulation was higher than MUNE produced by intraneural microstimulation,[45] but it was lower than anatomic counts.[1] Twitch tension values correlated better with CMAP amplitude than with MUNE itself, although dramatic declines in twitch tension were observed when MUNE was very low.[13] Incremental MUNE was a more sensitive measure of decline than other measures in ALS (forced vital capacity, strength, etc.). It had faster declines over time that correlated well with measures of patient function (e.g., Appel ALS Scale) and enabled reasonably accurate projection of survival times.[21,49] Among the diseases studied, ALS was most common,[3–5,15,17,19,21,22,35,40,49–52] followed by mono- and polyneuropathy,[7,10,27,28,30,32,53,54] muscular dystrophies,[6,14,38,55–57] aging,[13,25,48,58,59] upper motor neuron lesions,[44,47] and limb immobilization.[39]

Several technical refinements have been made to the incremental method. Soon after it was developed, investigators recognized that an individual increment in the growing CMAP waveform may not always represent activation of another single motor unit, but instead may be the result of alternating activation of 2 different motor axons with similar depolarization thresholds (a phenomenon termed "alternation").[58] Attempts to eliminate this problem spawned several other MUNE methods, including multiple-point stimulation, spike-triggered averaging, and others.[12,45,58] Other refinements focused on increased efficiency using automated and semi-automated approaches to

Table 1. Normative MUNE and reproducibility.

Muscle group	Incremental	MPS	STA	DE-STA	STAT-NW	STAT-W
Normal values						
Thenar	187 ± 58	276 ± 35	190 ± 55	249 ± 78	183 ± 22	113 ± 20
Hypothenar	245 ± 81	285 ± 103	198 ± 32	—	129 ± 22	71 ± 15
Biceps	110 ± 58	—	398 ± 150	272 ± 74	—	—
EDB	206 ± 61	290 ± 171	—	—	113 ± 24	—
Tibialis anterior	—	—	—	150 ± 43	—	—
Reproducibility						
Thenar	0.95*	0.90*	—	—	0.81*	0.79*
Hypothenar	—	—	—	—	—	0.96*
Biceps	24%†	—	0.86*	0.96*	0.86*	—
EDB	16%†	—	—	—	0.88*	—

Average normative motor unit number estimation (MUNE) and reproducibility values for the major MUNE methods, by muscle group. Averages were generated by pooling all available average values for normal subjects for each reported technique. EDB, extensor digitorum brevis; MPS, multiple-point stimulation; STA, spike-triggered averaging; DE-STA, decomposition spike-triggered averaging; STAT-NW, statistical (non-weighted); STAT-W, statistical (weighted);

**Correlation coefficient.*

†Coefficient of variation.

deliver incremental stimulation and measure waveform size.[6,12,27,41,46,47]

Incremental MUNE in its original form is based upon easily understandable assumptions and is relatively simple to perform, requiring only a basic electromyography (EMG) machine. It can be performed rapidly in 10–15 minutes. Patient discomfort is minimal and comparable to that of routine NCS. Alternation and variable motor unit population sampling may affect accuracy, but the method is reasonably reproducible in distal muscles and was the first MUNE method to chart the trajectory of motor unit loss over time in ALS.[6,12,21,41,49] It has also proven to be the MUNE method most adaptable to animal studies, where it is currently utilized widely.

Multiple Point Stimulation. The multiple-point stimulation (MPS) method[45,58,60–74] was introduced to improve upon the incremental method. By using very low levels of stimulation and selecting only the first, all-or-none SMUP at a site, a single motor unit discharge can be recorded, markedly reducing alternation. The stimulation is then performed at multiple points along the course of the nerve, enabling collection of a sample of SMUPs, which are averaged and used for calculation of a MUNE.

Articles using MPS MUNE, which included control data for the thenar muscle group, yielded a mean value of 276.[60–66,70–72] Test–retest reproducibility was good in all studies, with correlation coefficients ranging from 0.82 to 0.98 and a mean value of 0.9 (Table 1).[60,64] MPS has been used to study ALS,[62–66] SMA,[67–69] aging,[60,70] stroke,[71] post-polio syndrome,[72] and entrapment neuropathy (Table 2).[62] ALS was studied most frequently, and ALS subjects were found to have a mean thenar MUNE of 71. Normal aging was the next most commonly studied condition, and MUNE values were typically reduced by about 50% in elderly subjects in comparison to young controls. SMA, polio, polyneuropathy, and stroke were examined in a limited number of studies, and in all cases MPS was useful in demonstrating and quantifying motor unit loss or decline over time.[62,67–69,71,72]

MPS in its basic form is simple conceptually and can be performed on any EMG machine. An adequate sample of SMUPs can be collected in 15–20 minutes by an experienced operator after minimal training. The technique has undergone a number of technical refinements. Software was developed that produced a mean SMUP template

Table 2. MUNE in ALS and normal aging.

	Muscle	Average MUNE	Reproducibility
ALS			
Incremental	Mixed	49 ± 35	0.81*
MPS	Thenar	67 ± 23	0.97*
STA	Biceps	151 ± 73	32.6%†
Statistical	Mixed	40 ± 10	0.85*
DE-STA	Biceps	101 ± 126	—
Aging			
Incremental	Mixed	118 ± 70	0.95*
MPS	Thenar	139 ± 68	—
STA	Mixed	—	—
Statistical	Thenar and EDB	185 ± 45	—
DE-STA	Tibialis anterior	91 ± 22	—

Motor unit number estimation (MUNE) values (average) for patients with amyotrophic lateral sclerosis (ALS) and in patients over age 60 years, by MUNE method. Averages were generated by pooling all available average values for ALS and elderly subjects for each reported technique. EDB, extensor digitorum brevis; MPS, multiple-point stimulation; STA, spike-triggered averaging; DE-STA, decomposition spike-triggered averaging; STAT-NW, statistical (non-weighted); STAT-W, statistical (weighted).

**Correlation coefficient.*

†Coefficient of variation.

through datapoint-by-datapoint averaging of the individual waveforms, rather than by simple arithmetic averaging of fixed values for amplitude or area.[73] Datapoint averaging resulted in higher MUNEs, as the effects of phase cancellation (inherent in the maximum CMAP) were taken into account. An "adapted" MPS (A-MPS) method was introduced to increase the number of SMUPs obtainable at a single site and reduce acquisition time.[70] In this method, the first SMUP is collected (as per traditional MPS), but, in addition, the first 2 or 3 additional distinct incremental responses at the same stimulation site are also measured and used as SMUP values, making this a hybrid technique using both MPS and incremental methods. MUNEs based on A-MPS were similar to those obtained by the original method. An adapted MPS technique was recently applied to ALS with high test–retest reliability and could track MU loss (9% per month) and the effects of collateral reinnervation.[74]

The greatest disadvantage of the MPS method is its lack of applicability to larger, proximal muscles (e.g., biceps brachii, tibialis anterior), as the proximal nerves are poorly accessible for stimulation at enough sites to collect an adequate sample of SMUPs. Overall, the MPS method is a reliable and easily applied method for obtaining a MUNE in distal muscles.

Spike-Triggered Averaging. Spike-triggered averaging (STA) uses an intramuscular electrode to record an isolated single motor unit discharge (motor unit action potential) produced by voluntary contraction, while simultaneously recording the same signal with surface electrodes and averaging it over repeated discharges to extract the associated surface recorded SMUP. The intramuscular electrode is then moved to a new location, and the process is repeated to obtain a sample of SMUPs, which are then averaged for use in MUNE calculation.[75–85] STA was developed to circumvent the problem of alternation and to allow MUNE to be performed in proximal muscles. STA also provides quantitative needle EMG data.

STA MUNE has been used less commonly than the other major methods. The STA method has similar test–retest reliability as other MUNE methods ($r = 0.86$; Table 1), although there is substantial test–retest variability in normal subjects and in subjects with neurogenic conditions, such as those with ALS and CMT disease (Table 2)[80–85]; variability is less when MUNE values are low. Sources of error that contribute to variability were studied in 1 article and included the poor reproducibility of full CMAP amplitude measures when recording from proximal muscle groups due to the difficulty of delivering reliable supramaximal stimulation to the deep proximal nerves supplying them.[75] There are also a number of technical issues with the manual STA method, including sampling bias. Motor units activated at very low levels of effort are sampled preferentially and, based on the size principle of motor unit recruitment, early recruited motor units are smaller than later recruited motor units.[76–78]

The average normative value for STA in the thenar muscle group was 190, but this method also provided normative values for a proximal muscle (the biceps, 398) (Table 1). The method has been used to show motor unit loss in proximal muscles in normal aging[79,80] and in ALS[81–83] (Table 2), and it has also been used to demonstrate a correlation between loss of motor units in CMT1A and loss of strength.[84,85] MUNE values were significantly lower in CMT1A, CMTX, and CMT2 in distal muscles (hypothenar) compared with control values and also showed a trend to lower values in proximal muscles (biceps brachii), correlating with axonal loss.[84,85]

The manual STA method can be performed on any EMG machine with STA capabilities and can be applied to both proximal and distal muscles, but data gathering is more time-consuming than some other methods. Reproducibility is generally good but somewhat variable in different patient populations. A disadvantage is that it requires an intramuscular needle electrode, although needle EMG examination also provides additional diagnostic and research data not provided by other methods.

Decomposition STA. Decomposition STA MUNE (DE-STA) MUNE[86–99] is essentially an enhanced version of STA MUNE. Instead of isolating individual motor unit action potentials for the trigger source, a computerized decomposition algorithm extracts multiple motor unit potentials from a moderate intensity interference pattern and collates this information with the surface EMG waveform data to extract the surface-recorded motor unit potential corresponding to each needle-recorded motor unit action potential (MUAP). DE-STA also enables SMUP data to be gathered at higher levels of voluntary contraction, sampling a wider range of motor unit sizes. Like STA, it can also be applied to proximal and distal muscles.

Reproducibility of DE-STA MUNE was good, with correlation coefficients of 0.87 in the anterior tibialis muscle, 0.74 in the first dorsal interosseous, and 0.97 in the biceps.[92] Average normative MUNE values were 249 in the thenar muscle group, 167 in the first dorsal interosseous muscle,[88,90] 272 in the biceps,[86] 153 in the tibialis

anterior,[96,99] and 458 and 578 in the soleus of young and old subjects, respectively (Table 1).[94] The method has been tested successfully in patients with ALS[86] and CMT1X,[98] but it has not yet been employed in a sizable number of subjects with disease (Table 2).

DE-STA can usually be completed in 10–15 minutes. Although intramuscular needle recording is required, levels of patient anxiety and discomfort are similar to those experienced with STA MUNE. As in STA MUNE, the quantitative needle EMG examination provides additional data not available with other MUNE methods and is applicable to proximal muscles. DE-STA software has the potential to be retrofitted to commercial EMG systems and is freely available for research. However, as DE-STE is not currently offered as an integrated option on any commercial EMG system, its dissemination and widespread use has been limited.

The Statistical Method. The statistical (STAT) method analyzes changes in CMAP amplitude during 30 stimuli delivered at a fixed intensity at a single site. STAT MUNE assumes that the variance in CMAP size reflects the sizes of the average SMUPs that contribute to this variance through alternation, and that the motor unit population has a Poisson distribution. This train of stimuli is repeated at different levels of intensity, thereby sampling different populations of axons. A sophisticated software program then employs Poisson statistics to analyze this data and to calculate a mean SMUP size.[16,100–123] STAT MUNE was conceptualized, in part, as an attempt to more fully incorporate the larger, later recruited motor units in the sample for averaging, as these units are often less well sampled by other methods. Numerous refinements, including a weighted STAT MUNE method to improve representation of the full range of motor units, have been applied.

Articles reporting normal control data used both modified STAT MUNE methods weighted to better represent the full spectrum of motor unit sizes[102–109] and the original STAT MUNE method (see below and Table 1).[31,108,110–115] The reproducibility of STAT MUNE ranged from 0.84 using weighted analysis to a range of 0.44–0.98 with a mean correlation coefficient of 0.78 using the original method, comparable to many other MUNE techniques. Mean MUNE values for the thenar muscles were 118 (weighted) and 183 (original) compared with 71 (weighted) and 129 (original) for the hypothenar muscle group (Table 1). STAT MUNE has been used to study ALS,[31,106,110,114–118] spinobulbar muscular atrophy,[103] aging,[105,108] post-polio syndrome,[119,120] Parkinson disease,[16] peripheral neuropathy, and carpal tunnel syndrome (Table 2).[121] ALS was the most frequently studied condition, showing a mean weighted STAT MUNE of 40 in the hand muscles overall and motor unit losses of 26% (weighted)[106] and 20–50% (original)[111,112] over 6 months. Studies of normal aging showed 19% and 23% lower MUNE in subjects over age 60 when compared with younger controls, respectively.[105,108]

Many technical refinements were devised and applied to the original STAT MUNE method. The weighted STAT MUNE method[108–118] adjusted the SMUP average by applying a correction factor to insure that SMUP values derived at different stimulus levels were more proportional and accurately represented in the SMUP average. This modification improved intersubject variability and test–retest reliability, but it also produced lower MUNE values overall due to increased representation of larger units in the sample. Other studies refined and codified the methodology used by examiners for adjusting the stimulus ranges used[104,114,115,122] to improve reproducibility, whereas others piloted criteria for exclusion of smaller units from the sample, with unclear benefit.[103,107,123]

The STAT MUNE method reduces operator effort due to the high degree of automation employed in the collection of individual SMUP data, and the test can be completed in 5–15 minutes by a technician or physician. STAT MUNE can be used in distal, but not proximal, muscles. A major limitation of STAT MUNE is that it requires a software program specifically written for a proprietary EMG system. Attempts to employ STAT MUNE as a secondary outcome measure in a multicenter ALS therapeutic trial in 2007 were of limited success due to concerns that the inherent variability in size of denervating/reinnervating motor units during repeated stimulation in ALS patients introduced a confounding variable, thereby skewing the Poisson analysis.[107]

Miscellaneous MUNE Methods. Four more MUNE techniques were found in our search after excluding those methods reported only once, 3 of which were developed recently.

F-Wave MUNE. The F-waves collected routinely during standard diagnostic motor nerve conduction studies are small potentials, representing either 1 or several motor unit discharges occurring together. When a sufficient number of F-waves are collected and analyzed, identical, recurring waveforms representing SMUPs can be identified from the sample, providing a group of SMUPs for averaging and MUNE calculation.[124–127] This method is well-tolerated, requires no needle insertions and can be performed on all standard EMG systems capable of collecting F waves. An automated

method using specially designed software greatly simplifies and speeds the collection, identification, and measurement of F-waves and SMUPs from 30–45 minutes down to 15–20 minutes.[124]

The mean F-wave MUNE in the thenar muscles of normal controls is 151, and at least 1 study reported good test–retest reliability with a correlation coefficient of 0.93.[124,126] F-wave MUNE was also tested in ALS patients, yielding an average of 42, and it has been studied in stroke and Parkinson disease.[16,125–127] Unfortunately, the automated software package for F-wave MUNE was developed for an EMG system that has been out of production for many years, and it has not been adapted for use elsewhere. As the manual F-wave method is far more laborious than other MUNE methods, this technique is now used rarely and has not been developed further.

Bayesian Analysis of Statistical MUNE. The data collection for Bayesian statistical MUNE is similar to the original STAT method, but data analysis is based on Bayesian assumptions applied to the variability of motor unit firing, as well as on motor unit size. It requires complex, off-line computations available only in a limited number of centers. Bayesian MUNE was developed to provide more consistent results than STAT MUNE and its variants.[122,128–133]

From a limited number of studies, test–retest reliability of Bayesian MUNE in both control and ALS subjects was good.[122,128] MUNE averaged 80 in the hypothenar muscles of controls, and a range of 5–60 was found in ALS subjects (data pooled from median, ulnar, and fibular nerves), whereas serial studies in ALS showed decreases in MUNE over time.[132] This is a technically intensive approach to MUNE, which relies heavily upon a complex computation method, limiting its dissemination and testing to only a handful of centers worldwide.

High-Density Surface EMG MUNE. High-density surface EMG MUNE uses a grid of 120 densely spaced electrodes placed over a muscle to record SMUPs in a spatiotemporal fashion. This array reduces alternation, increases the number of identified motor units, and allows for inclusion of small motor units.[129,131–133]

The technique has shown good reproducibility in test–retest studies in controls, with a correlation coefficient of 0.88.[129,131] Mean MUNE in control subjects in the thenar eminence was 271. Reductions in MUNE values in ALS subjects over time have been demonstrated,[132] and lower MUNE values were seen in CMT1A patients and controls at older ages compared with younger controls.[131,133] However, the technique has not been applied to other muscle groups, nor have comparisons with standard MPS been completed. It requires special amplifiers and software available at only a few research centers and cannot be adapted readily for use on routine EMG systems, greatly limiting its future dissemination.

Motor Unit Number Index. Motor unit number index (MUNIX) uses a mathematical model based on the CMAP and surface EMG interference pattern to derive an index (rather than a traditional MUNE) related to the number of motor units. In contrast to all of the above methods, MUNIX does not attempt SMUP sampling or reconstruction.[133–139]

MUNIX has been tested in a number of limb muscles with good inter- and intrarater reliability, with a coefficient of variation similar to other MUNE methods (12–17% in controls and up to 25% in ALS patients).[134–136] MUNIX values vary with age and decrease over time in ALS subjects.[134,137] MUNIX is easy and rapid to perform, and requires only enough stimuli to obtain a maximum CMAP. It can be performed on any muscle in which an accurate CMAP can be obtained. It requires proprietary software for signal processing.[134,135,137–139]

CONDITIONS STUDIED

Although MUNE has been applied to many clinical conditions, ALS has been the most extensively studied, because motor unit loss is the primary factor leading to disease progression (Table 2). The incremental method,[3–5,15,17,19,21,22,35,40,49–52] MPS,[62–66] STA,[81–83] DE-STA,[86] STAT,[31,106,110,114–118] and MUNIX[134,137] have all documented reduced motor unit numbers in ALS. The thenar muscles have been studied most commonly, with mean MUNE values ranging between 40 and 70 (vs. control ranges of 113–276). High test–retest reliability has been reported for thenar MUNEs in ALS with correlation coefficients ranging from 0.81 to 0.98. MUNE has also been used to chart the rate of motor unit loss over time in ALS, showing declines of 6% per month (Incremental), 2.3% month (MPS), and 23% over 6 months (STAT). Perhaps most importantly, MUNE is more sensitive in detecting disease progression in ALS when compared with other outcome measures, including strength, pulmonary function, activities of daily living scales, and traditional electrodiagnostic measures, such as CMAP amplitude, and it has documented motor unit decline when employed as an outcome measure in clinical trials.[4,18,19,49,62,63,65,74,83]

In addition to ALS, MUNE has been used to study motor unit loss, dysfunction, and reinnervation in prior polio, SMA, CMT, acquired polyneuropathies, and entrapment neuropathies, among other diseases. In a limited number of studies, MUNE has established the relationship between motor unit loss and muscle wasting and weakness in CMT, prior polio, and SMA, and a number of studies have

demonstrated a higher sensitivity for MUNE as compared with CMAP size reductions in detecting and tracking denervating disease.[34,58,68,69,72,79]

Normal aging has also been studied with MUNE (Table 2). Healthy adults over age 60 years demonstrate reductions of 50% or greater in both distal (thenar, hypothenar) and more proximal muscles (biceps brachii, tibialis anterior, soleus)[79,80] when compared with their younger counterparts. In 1 study, DE-STA showed that very elderly men (mean age >80 years) had even greater motor unit loss in comparison to older men (mean age 66 years).[96] Motor unit loss associated with aging is a significant factor leading to age-related reductions in muscle mass, strength, and power (sometimes termed "sarcopenia").[80]

SUMMARY AND CONCLUSIONS

MUNE provides reproducible quantitative measures of the number of functional motor units in living human subjects, and most techniques also generate simultaneous measures of single motor unit size. MUNE methods are generally noninvasive and well tolerated, making them ideal for longitudinal studies. Concurrent tracking of declines in MUNE and changes in single motor unit size over time have proven useful for research into the natural history of denervating disease, as well as the compensatory effects of collateral reinnervation as motor unit numbers decline.

MUNE is not as useful as traditional EMG and NCS in clinical diagnosis and management, principally because the wide range of MUNE values in the healthy general population makes it difficult to establish valid lower limits of normal for a diagnostic MUNE study in individual patients. MUNE does provide adjunctive information regarding rates of motor unit decline and collateral reinnervation, but there is no evidence yet that MUNE data significantly improve diagnosis or clinical management. Consequently, MUNE has not been employed in routine clinical practice, and efforts have been and remain focused on MUNE as a research tool, as well as on the continual development and refinement of MUNE techniques.

The most commonly employed methods (incremental, MPS, STA, STAT) account for the vast majority of published reports, although novel approaches continue to be developed (e.g., MUNIX). The method studied most commonly in humans since 1971 has been the incremental MUNE technique; it has been cited in over 50 studies, followed by MPS and STAT MUNE, with approximately 30 each, and STA/DE-STA with 24, combined. However, over the last decade (2001–2010), the number of studies utilizing incremental MUNE [18] was surpassed by MPS [21] and STA [22], followed by STA/decomposition [17].

Normative MUNE data have been generated for many muscle groups (Table 1), but the thenar group has been tested most commonly, producing average values of 187 for incremental MUNE, 276 for MPS, 190 for STA, 249 for DE-STA, 183 for original STAT, and 113 for weighted STAT MUNE. Generally, MPS and DE-STA give the highest values, with intermediate values for STA and original STAT MUNE, and lower values for the weighted STAT method. MPS and DE-STA MUNE may yield higher values due to increased sampling of smaller motor units, whereas STA and original STAT MUNE values may be lower due to increased sampling of intermediate and large units. The weighted STAT method was designed specifically to increase large motor unit representation in the sample, resulting in smaller MUNEs. Despite these differences, however, intramethod reproducibility for each method is good, with correlation coefficients of 0.90 (MPS), 0.86 (STA), 0.87 (DE-STAT), 0.78 (original STAT), and 0.84 (weighted STAT), suggesting high consistency within each method. As MUNE provides novel information not available previously, there is no suitable "gold standard" for comparison. Anatomic counts of motor axons are not entirely accurate, as it is difficult to distinguish between large sensory axons and motor axons on histological analysis, particularly in human nerve biopsies. Although histological approaches show different counts than electrophysiological MUNE, the relative values are reasonably consistent, and MUNE correlates with clinical measures of motor unit decline.

MUNE is an excellent tool to track motor unit loss. It has been applied most extensively to the study of motor neuron diseases (Table 2). It has proven to be among the most sensitive measures of progression over time in patients with ALS, outperforming other clinical measures in head-to-head trials.[65] MUNE has also been used to demonstrate loss of motor units with normal aging and in patients with SMA, CMT, and post-polio syndrome, among others.[15,34,58,68,69,72,79,84,85]

Each MUNE method has advantages and disadvantages. Reproducibility is good and similar across methods. The original incremental and MPS methods (as well as the adapted MPS method, which combines both methods) can be mastered easily, can be performed on a basic EMG system relatively quickly (in 10–15 minutes), and are well tolerated by patients. Theoretically, the absolute accuracy of incremental MUNE may be affected by alternation, but reproducibility remains high. Concerns that the effect of small motor units may be overrepresented in MPS MUNE remain theoretical and have not affected reproducibility. Both methods are very

effective for tracking motor unit loss over time, although both are restricted to use in the distal muscle groups. The adapted MPS method may be slightly faster and easier to perform than traditional MPS MUNE, and it has been used successfully in large, multicenter clinical trials of ALS.[80]

The original STA method can also be mastered easily and can be performed on most EMG systems, but data collection and analysis are time consuming. The method also involves needle EMG examination, increasing patient discomfort. DE-STA MUNE, an extension of STA MUNE, is faster and collects extensive data but requires a software program not available on current commercial EMG systems. However, both techniques are the only MUNE methods performed easily in proximal muscle groups.

The STAT MUNE method is highly automated, reasonably comfortable for patients, and can be performed rapidly (in 10–15 minutes). Like incremental and MPS MUNE, it is restricted to distal muscles. However, it requires a software program written specifically for a proprietary EMG system, limiting its availability and increasing expense for those not already in possession of the requisite hardware. Technical concerns have also been raised regarding the influence of motor unit variability in denervating disease on STAT MUNE, and this may limit its utility in future studies of motor neuron disease.

Many other methods for estimation of motor units have been tested, including MUNIX. MUNIX differs from traditional MUNE, but it appears to be reproducible and also tracks declines in age and in ALS. It is rapid and comfortable for the patient, but it is greatly facilitated by proprietary software available on only 1 EMG system. Future studies across centers will better define the role of MUNIX and other new approaches as compared with more traditional MUNE methods.

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