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Electrodiagnostics: MUNE and MUNIX as methods of estimating the number of motor units – biomarkers in lower motor neurone disease

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ABSTRACT

Routine quantitative electromyography is used for the assessment of the presence of lower motor neurone involvement and its consequences, including primary denervation and compensatory reinnervation of muscle fibres. However, it is not useful for the assessment of the motor unit number reserve. The need for a valid biomarker to evaluate lower motor neurone disease progression in such diseases as amyotrophic lateral sclerosis, and for use in clinical trials, has led to a number of studies of the methods that allow assessment of the number of motor units. In this review, motor unit number estimation (MUNE) methods with incremental stimulation and the recently developed motor unit number index (MUNIX) method, along with their technical and clinical aspects, are presented as methods which reflect motor unit loss in neurogenic processes. These electrodiagnostic tests may allow a valuable assessment of disease progression and the efficacy of new therapeutic methods in clinical trials in diseases with lower motor neurone degeneration.

Key words: motor unit, motor unit number estimation, MUNE, motor unit number index, MUNIX, amyotrophic lateral sclerosis

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Introduction

Routine quantitative needle electromyography (EMG) is used for the assessment of the presence of lower motor neurone involvement and its consequences, such as primary denervation and compensatory reinnervation of muscle fibres. However, it is not useful for the evaluation of the number of motor units. In neurogenic processes such as amyotrophic lateral sclerosis (ALS), abnormal needle EMG recording reflects the effects of two overlapping processes that occur in the muscles: acute denervation and reinnervation. In the initial stage of the disease, the loss of anterior horn cells results in acute motor fibre denervation. Afterwards, in the stage of secondary muscle fibre innervation, this denervation is compensated by sprouting axonal collaterals from surviving motor units into denervated muscle fibres. Routinely, the concentric needle electrode used in EMG records the combined action potentials generated by several fibres within a motor unit territory of 5–15 mm. This is known as the motor unit activity potential, or MUAP). MUAP parameters are increased in the stage of secondary reinnervation. This occurs due to an enlarged motor unit area and dispersion, which results from differences in the duration of potential components caused by abnormal neuromuscular transmission in immature axonal collaterals. Finally, in the stage of decompensation, MUAP parameters decrease due to continuous loss of motor units and a decrease in their area. Neurophysiological changes in motor units in ALS undergo continuous evolution along with a dynamic reorganisation.

Due to these overlapping processes, MUAP parameters do not appear to correlate with clinical muscle dysfunction. Pseudo-normal MUAP parameters may be observed even in the terminal stage of the disease, because MUAP parameters do not reflect the motor unit number but rather the effect of the denervation-reinnervation processes.

It is worth pointing out that conventional EMG abnormalities reveal denervation and reinnervation changes caused by lower motor neurone degeneration, but do not reflect the actual motor unit number.

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Motor unit number estimation

The importance of the motor unit as a crucial element in the production of force and movement was first recognised by Sherrington [1]. Subsequently, numerous studies have dealt with the structure and function of motor units. Studies on the methods estimating the number of motor units in individual muscles are still needed because there is a need for a valid biomarker to assess disease progression and to estimate potential treatment effects.

In 1971, McComas et al. described an attractively simple method for counting motor units. Recording from the extensor digitorum brevis, the authors maximally stimulated the deep peroneal nerve at the ankle and obtained a maximal compound motor action potential (CMAP) [2].

Then, starting from subthreshold stimulation levels, they gradually increased stimulus intensity until a quantal response was seen, representing the first motor unit activated. With further stimulus intensity increases, quantal increases in the response were recorded. Up to 11 discrete increments were recorded, with each increment assumed to represent the addition of one motor unit. The amplitude of the resultant response was divided by the number of increments to yield an estimate of the amplitude of a single unit; this value was divided by the maximum CMAP to give the estimate of the number of motor units. The incremental motor unit number estimation (MUNE) technique was soon applied to upper extremity muscles supplied by median, ulnar and radial nerves [3–5].

MUNE techniques have been used to quantify the proportion of surviving lower motor neurones in ALS. The results of multiple studies have confirmed that MUNE, when applied longitudinally, may reflect the rate of disease progression [6–9].

Assuming that the maximal CMAP is the sum of all single motor unit potentials, the universal rule for MUNE with incremental stimulation is that MUNE may be calculated as a ratio: the average size of a surface-detected single motor unit action potential (SMUP) should be divided by the maximum CMAP. SMUP is acquired by averaging several potentials of an increased amplitude with stimulation of an increasing intensity using the 'all or none' method.

Many techniques for estimating the average amplitude of single motor units have been suggested, but most have been limited by sampling bias and/or a lack of reproducibility. Different techniques for MUNE have been practiced, among them the spike-triggering averaging method using a voluntary muscle contraction to activate the motor unit, and the multiple point stimulation method with stimulation at multiple sites along the nerve.

One of the MUNE methods, with incremental stimulation in Shefner's modification, starts by obtaining CMAP in the most distal point with a maximal amplitude using supramaximal stimuli. During the second step of the test, the stimulating electrode is positioned in the following three locations: at the wrist crease; 4 cm proximal to the wrist crease; and in the cubital fossa [6, 10]. A standard three-site motor conduction programme is used with traces set to superimpose.

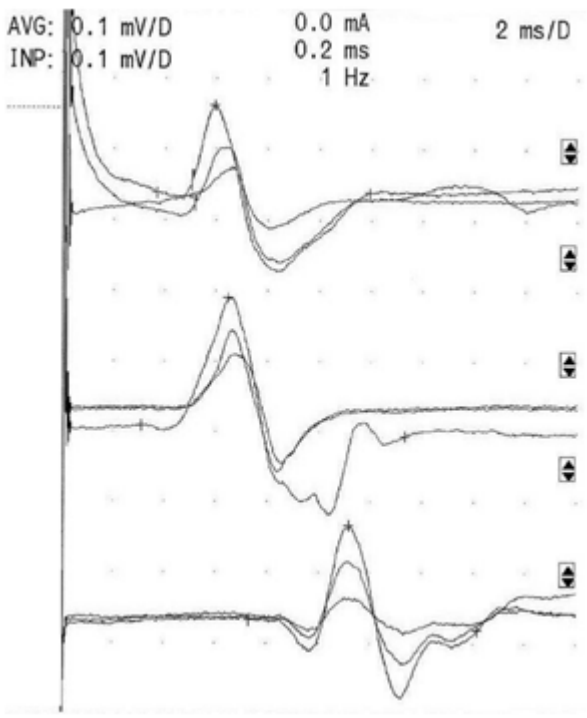

Figure 1. The second step in MUNE calculation: Three incremental responses in three sites of stimulations of the ulnar nerve (motor potentials)

Then, subthreshold stimuli are applied at the rate of 1/s, with stimulus intensity slightly increased until an 'all or none' response is recorded. For both initial and incremental responses, the minimum negative peak amplitude considered to be acceptable for recording is 25 μV. When the initial response has been obtained, two more incremental responses (of more than 25 μV) are recorded. Stimulus location is then moved to the second and third sites, and the same procedure of incremental stimulation is repeated [8] (Fig. 1).

The amplitude of three maximal responses from the three sites is totalled and divided by 9 to obtain the mean amplitude of an average surface-detected SMUP. The maximum CMAP amplitude is then divided by the SMUP amplitude to calculate the number of motor units [6, 10].

From the practical point of view, MUNE has some advantages. For example, it is not invasive and it is not unpleasant for the patient because only CMAP is obtained with a supramaximal impulse, and subsequent responses for a single motor unit are obtained with a very low current. For MUNE, cooperation with the patient is not required, so it can be used even in small children [11]. However, MUNE also has the disadvantage that it is useful only for the distal muscles. Due to response variability, extensive experience is needed to ensure that the proper curve is selected and the result is valid. Opinions have been voiced that the traditional

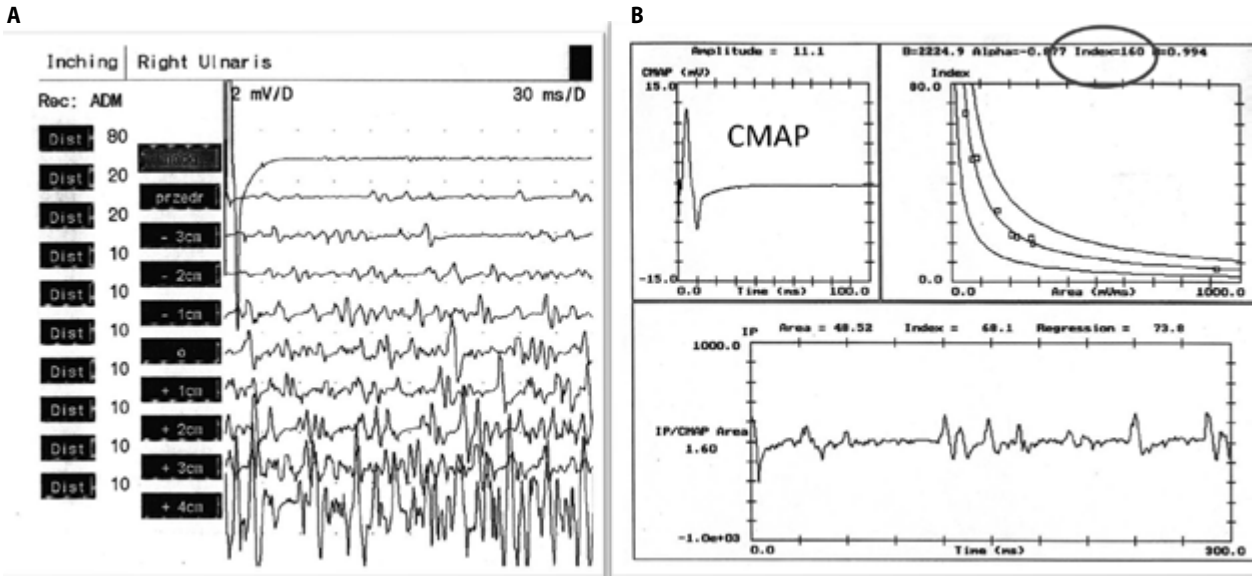

Figure 2 **A.** The surface electromyography interference pattern (SIP) of abductor digiti minimi is recorded when the patient maintains a constant isometric force of contraction at different force levels. This is used in conjunction with the CMAP to compute the so-called 'ideal case motor unit count (ICMUC)'. **B.** A plot of ICMUC versus SIP area is made, and the relationship of data points is modelled as a power regression equation and the MUNIX is calculated (from the own archives of EMG Laboratory) [14]

stimulation-based MUNE techniques are technically challenging, time consuming, and potentially limited by motor unit instability [12–13].

For these reasons, a surface-based electromyography MUNE technique that does not require nerve stimulation, something that has been termed the motor unit number index (MUNIX), has been developed in order to overcome some of the limitations of the MUNE method [13].

Motor unit number index

The MUNIX method is one of the recently developed electrophysiological approaches which may provide information about the degree of motor unit loss[14]. The MUNIX technique is a means of expressing the number of active motor units in a muscle as an index, rather than providing a direct measure of their total number [15].

MUNIX is a non-invasive method that can be applied to any muscle in which CMAP can be evoked by supramaximal nerve stimulation. The result of the examination is directly related to the number of functioning motor neurones in a given muscle. MUNIX uses a mathematical model based on CMAP and the surface interference pattern (SIP) following their import into an analysis software created by Nandedkar et al. [14, 16].

In the first step of MUNIX, a supramaximal CMAP is obtained by a standard tendon belly application of the surface electrodes to optimise the amplitude of the motor potential. Because obtaining maximal CMAP is crucial for the MUNIX result, it is recommended to reposition the recording electrode up to 5 times in order to record an optimised CMAP [17–18].

The next step is EMG signal recording during voluntary effort. The patient activates the examined muscle by resisting the examiner in an isometric contraction. It is recommended to avoid isotonic contraction and limb movement. The EMG signals should have spikes with an amplitude of 200 μV or more. The force is increased from minimal to maximal in 5–9 steps. At each force level, the patient maintains a steady contraction while SIP is recorded. The area and power of the CMAP and SIP are used to calculate the ideal case motor unit count (ICMUC). The MUNIX results are calculated using an automated method. The result is presented as a plot and a numeric value reflecting the number and size of motor units recruited at various force levels [18] (Fig. 2A, B).

Just as in MUNE with the incremental stimulation method, a strong correlation between CMAP amplitude and MUNIX in healthy controls has been found (Fig. 3). However, recurrent stimuli could result in habituation and a decreased potential amplitude, and thus intervals between the stimuli are required. A lower, underestimated CMAP amplitude has a dramatic effect on the reduction of MUNIX. When a potential is recorded with a stimulus artifact, the power cannot be measured accurately. Due to the need to perform a gradual effort, a good cooperation with the patient is crucial.

Our results suggest that MUNIX is a method that could be useful not only for the estimation of disease progression but also as a complementary method for the initial assessment of muscle [19]. From a practical point of view, the global MUNIX seems to be more useful for general patient assessment.

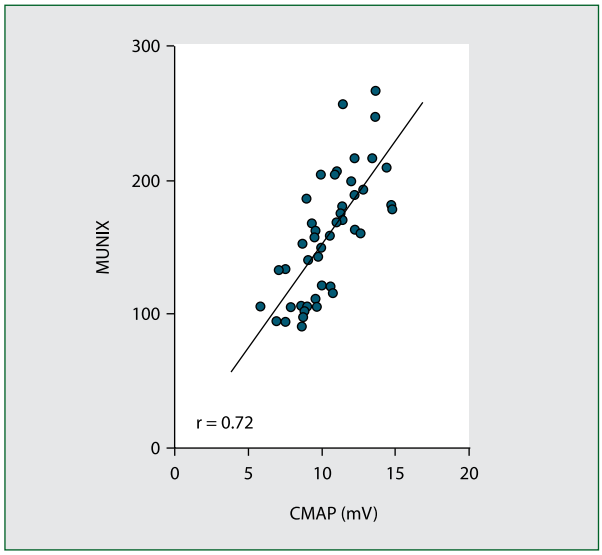

Figure 3. A strong correlation was found between CMAP amplitude and MUNIX results in healthy controls [14]

Use of MUNIX in amyotropic lateral sclerosis and other neurogenic processes

Amyotrophic lateral sclerosis is an adult-onset, progressive lethal neurodegenerative disorder characterised by a selective dysfunction and loss of upper and lower motor neurones. It leads to quadriplegia and respiratory insufficiency within a few years from the onset of initial symptoms. ALS shows a characteristic variability of onset and rate of disease progression which, together with a clinical heterogeneity, makes quantification of the symptoms problematic. It is therefore important to develop strategies that would allow objective assessment of the disease progression and the prediction of outcomes. The diagnosis of ALS is based on a clinical evaluation together with conventional EMG [20–21].

Amyotrophic lateral sclerosis

The reliability and sensitivity of MUNIX as a tool to monitor the progression of motor unit loss during the course of the disease in ALS patients has been reported in many studies. Some of them focused only on a single muscle. In the study by Boekestein, the MUNIX and HD-MUNE (high density MUNE) of the thenar muscle was evaluated. Patients with ALS were assessed at baseline, within two weeks, and after four and eight months. There was a significant positive correlation between MUNE and MUNIX values in ALS patients. After eight months, both MUNE and MUNIX values in the ALS patients had decreased significantly more compared to the Medical Research Council (MRC) scale, ALS functional rating scale (ALSFRS), and CMAP ($p < 0.05$) [22].

In the study by Fathi, MUNIX was recorded in the abductor pollicis brevis and tibialis anterior muscles bilaterally in ALS patients, with two measurements, one at the first visit and the second at a follow-up visit. The consistency of reproducibility of MUNIX in 30 ALS patients during the course of the disorder was analysed. A significant correlation between the first and the second MUNIX measurement in all tested muscles was found. A statistically significant good reproducibility of MUNIX in all four measured muscles was obtained at the follow-up visit [23].

Even more valuable are studies with several MUNIX examinations assessing disease progress and with evaluation of the distal as well as proximal muscles.

In a large clinical trial (27 centres participating in the Biogen study, 792 individual test-retest measurements), MUNIX was measured in a set of six muscles: the abductor pollicis brevis, abductor digiti minimi, first dorsal interosseous, biceps brachii, tibialis anterior, and extensor digitorum brevis. The aim was to analyse the reliability of MUNIX measurements and possible pitfalls in implementing the method in clinical trials. Mean coefficient of variation (COV) of all raters at the first measurements was 12.9% ± 13.5% (median 8.7%). The need for repeated tests ranged from 0 to 43 (mean 10.7 ± 9.1, median 8). The biceps brachii muscles showed the highest repetition rates. Evaluation of the biceps brachii failed in approximately two thirds of cases due to contamination of the CMAP by co-stimulation of other nearby nerves, such as the musculocutaneous nerve, with volume conducted signals from wrist and finger flexors or even the triceps muscle. MUNIX variability correlated considerably with the variability of CMAP. The authors concluded that MUNIX showed generally good reliability, but was rater-dependent and that ongoing support for the raters was needed [24].

Neuwirth et al. reported the rate of MUNIX decline in ALS during a series of examinations at three-month intervals after the diagnosis. Three centres measured MUNIX in 49 ALS patients every three months in six muscles (abductor pollicis brevis, abductor digiti minimi, biceps brachii, tibialis anterior, extensor digitorum brevis, and abductor hallucis) on the less affected side. The decline in MUNIX in initially non-wasted, clinically strong muscles (manual muscle testing, MMT grade 5) was analysed before and after the onset of weakness. The average monthly relative MUNIX loss was 5.0% before, and 5.6% after, the onset of weakness. Although the preclinical loss of motor units is a well-known feature of ALS and can be detected by needle EMG very early in the course of the disease even in a clinically normal muscle, MUNIX seems to be a valuable measurable marker of preclinical abnormalities. In that study, the rate of MUNIX change was significantly higher compared to the ALS functional rating scale ALSFRS and CMAP change over 12 months prior to the onset of muscle weakness. This makes MUNIX a good

biomarker candidate for disease progression, and possibly pharmacodynamic response [25].

In one of our studies, we analysed MUNIX in ALS patients (15 patients) and we found a significant correlation between the global MUNIX score and the clinical dysfunction as measured by the ALSFRS-R scale ($P < 0.05$). The global MUNIX score showed a higher monthly decline (4.3%) compared to ALFRS-R (0.7%) and the MRC global score (0.5%). This study also confirmed that the MUNIX method is a sensitive, reliable, and accurate tool reflecting both motor dysfunction and disease progression in ALS. We have found this approach to be more reliable and technically easier in distal muscles with less atrophy and a better strength.

The results of our study suggest that MUNIX is a method that may be useful not only for estimation of disease progression but also as a complementary method for initial muscle assessment. We also made some practical observations. The MUNIX method may be affected by an inappropriate CMAP recording caused by technical or anatomical problems. It is crucial to find the optimal location of the recording electrode, and to repeat CMAP recording to ensure that the motor response with a maximal amplitude is obtained. A lower, underestimated CMAP amplitude has a dramatic effect on the reduction of MUNIX. When a potential is recorded with a stimulus artifact, the power cannot be measured accurately and the same problem affects MUNIX too [19].

To date, the usefulness of this method for assessing the dynamics of disease-related changes has been studied mainly in non-treated subjects. One of the most recent studies focused on choosing an optimal monitoring tool after intraspinal transplantation of adipose tissue-derived regenerative cells in three patients with amyotrophic lateral sclerosis. The treatment did not prove effective in terms of reversing or delaying disease progression, but a number of observations were made. Of all methods used, MUNIX proved to be the first and the most sensitive tool for identifying fine changes at the muscle level. It was markedly more sensitive than ALSFRS R and MRC. In that study, dynamometry was the closest measurement to MUNIX both in the upper and the lower limb [26].

Spinal muscular atrophy

In one of the most recent studies, hand muscle innervation pattern was studied by the MUNIX method in 38 adult patients with genetically confirmed 5q spinal muscular atrophy (SMA). Data was compared to that of healthy controls and ALS patients and correlated with typical disease-relevant scores and other clinical and demographic characteristics. By calculation of the MUNIX ratios, the authors identified a specific hand muscle wasting pattern for SMA which is different to the split hand in ALS. MUNIX parameters strongly correlated with established disease course parameters, independently of disease stages [27].

Polyneuropathy

The MUNIX technique was also assessed unilaterally in the abductor digiti minimi, the abductor pollicis brevis, and the tibialis anterior muscles in 14 patients with chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) on two different occasions by two blinded examiners, and the MUNIX score was calculated by adding the results for the abductor digiti minimi, the abductor pollicis brevis, and the tibialis anterior muscles. The intraclass correlation coefficient (ICC) was high for inter- and intravariability for all three examined muscles, and the combined MUNIX scores from the first and the second evaluations were strongly correlated to each other. The MUNIX score was significantly correlated with MRC testing and also with the overall neuropathy limitation scale and the Rasch-built overall disability scale. The authors concluded that the MUNIX technique estimates the axonal loss and the number of functional motor units and that the MUNIX score may be a good instrument to evaluate CIDP patients during their follow-up [28].

In one study, a short-term effect of intravenous immunoglobulins (IVIg) in multifocal motor neuropathy (MMN) was evaluated using MUNIX. MUNIX was assessed longitudinally in seven MMN patients and 17 healthy controls in the abductor pollicis brevis and abductor digiti minimi muscles. All MMN patients were evaluated on the first day of IVIg infusion, five MMN patients were evaluated 22 days after IVIg infusion, and three MMN patients were evaluated one month after two IVIg infusions. The authors concluded that MUNIX seems to be a reliable and sensitive tool for monitoring the short-term efficacy of IVIg in MMN [29].

MUNE or MUNIX

Interestingly, in the study by Higashihara, evaluation by MUNE with multipoint stimulation and MUNIX was performed in 15 healthy subjects at three different time-points by the same examiner. ICC and COV values for MUNIX and MUNE were excellent across the three tests (0.80 and 0.77, respectively), although COV values were significantly lower for MUNIX than for MUNE ($P < 0.01$). In addition, the test-retest reproducibility was better for MUNIX, a finding largely attributable to poor reproducibility of the single motor unit action potential area. The authors concluded that MUNIX demonstrated better intra-rater reproducibility and may be a more reliable neurophysiological biomarker than MUNE [30].

In conclusion, MUNIX seems to be valuable tool for monitoring the progression of diseases with neurogenic processes. While multiple studies have confirmed the usefulness of MUNIX for monitoring ALS progression, the application of MUNIX in other diseases with lower motor neurone degeneration needs further assessment.

MUNIX is recommended because it shows good repeatability, is less time-consuming, can be used for both distal and proximal muscles, and requires less electrical stimulation. However, currently there are only few pharmacological studies using MUNIX which would test the utility of this approach. MUNE with incremental stimulation can be recommended for the distal muscles, and for those occasions when there is no possibility of patient cooperation. The crucial issue regarding the application of both MUNIX and MUNE is the electromyographist's experience and his or her attention to some very important technical aspects.

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