Changes in motor unit numbers in patients with ALS: a longitudinal study using the adapted multiple point stimulation method*

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METHOD: The adapted multiple point stimulation (AMPS) method for calculating motor unit numbers (MUNE) was applied in 12 patients with amyotrophic lateral sclerosis (ALS) before riluzole therapy (T₀) and again after 4, 8 and 12 months of treatment.

RESULTS: Paired Student’s t-test indicated a significant decrease of thenar MUNE and compound muscle action potential (CMAP) size at each 4-monthly interval, while average surface motor unit potential (SMUP) size did not change significantly over time. The rate of motor unit (MU) loss at month 4 was more than 20% in six patients (group 1) and less than 20% in six other patients (group 2). Comparison of groups 1 and 2 by Mann-Whitney U-testing indicated that percent changes in thenar MUNE and CMAP size compared to baseline were significantly different at months 4, 8 and 12, while no difference between the two groups was found for average SMUP size variations. In the group with a slow rate of MU loss, CMAP size remained stable, while in the group with a rapid rate of MU loss, there was a dramatic reduction in size of the CMAP. A positive correlation was found between percent change in thenar MUNE at T₄ and at T₈ (P < 0.001).

CONCLUSION: AMPS is a useful technique to document MUNE, SMUP size and CMAP size changes over time in patients with ALS. (ALS 2002; 3: 31–38)

Keywords: motor unit – MUNE – ALS

Introduction

Motor unit number estimate (MUNE) quantization seems to be the most reliable procedure for measuring the loss of motor neurons in clinical trials.¹–⁴ In the perspective of new clinical trials of promising drugs intended for patients with amyotrophic lateral sclerosis (ALS), longitudinal studies are essential. MUNE techniques might therefore provide a useful approach.

Since 1971,⁵ various procedures have been proposed for calculating MUNE. All these techniques rely on the same basic principle. The MUNE is determined through division of the supramaximal compound muscle action potential (CMAP) size by the average surface motor unit potential (SMUP) size. Average SMUP size estimates can be made by measuring all-or-nothing responses at threshold stimulation,⁵–⁷ by recording single motor unit (MU) F-waves,⁸ from spike-triggered averaging⁹ or from measurements of CMAP variance.¹⁰ In the present study, the adapted multiple point stimulation (AMPS) method was chosen.⁷,¹¹ AMPS is a simple, non-invasive, painless, rapidly executed procedure, which does not require any specific recording system or software. This method is thus easily applicable in routine clinical use. In addition, AMPS seems to be devoid of methodological bias.

The current study was designed, over a period of one year, to analyse thenar MUNE and the size of the remaining MU and CMAP variations by using the AMPS method in patients with ALS.

Patients and methods

Participants

Data were collected from 20 consecutive patients with ALS after having obtained their informed consent. These patients underwent motor and sensory nerve conduction
measurements in upper and lower limbs bilaterally, and
concentric needle recordings from at least three limbs,
including the thenar muscles where MUNE were obtained.
All patients fulfilled the clinical and electrophysiological
criteria for definite ALS. Moreover, patients were screened
to exclude any additional pathology affecting peripheral
erves, muscles or neuromuscular junctions. We excluded
carpal tunnel entrapment neuropathy on the basis of clini-
cal examination and electrophysiological tests (motor con-
duction velocity with terminal latency index and palmar
stimulation of sensory fibres). None received drug therapy
initially, in particular riluzole; but all patients received
riluzole after the first electrophysiological and clinical eval-
uation.$T_0$.

AMPS was performed on the right side or, if amyotro-
phy was present at baseline, on the side with the lesser
thenar atrophy. AMPS was applied at baseline $T_0$ and after
4, 8 and 12 months of a riluzole therapy ($T_4$, $T_8$, $T_{12}$). MUNE,
average SMUP size and CMAP size were measured in
all patients at $T_0$ and each 4-month interval. With a view
to normalizing the results, percent changes compared to
baseline were calculated for the three variables using the
following formula:

$$\left(\frac{T_0 \text{ value-control visit value}}{T_0 \text{ value}}\right) \times 100.$$ 

Results in patients were also compared with those from 70
healthy control subjects of both sexes and ages ranging
from 19 to 93 years. As the three variables studied depend
on age, each patient’s result was assessed by comparing it
with the normal limits in terms of age.$^7,13$

Thirteen patients with ALS completed this study over
the one-year period. Four patients died (two died 9 and 11
months after the onset of symptoms); three patients were
lost to follow-up for unknown reasons; one additional
patient was eliminated because of missing data. The results
for the remaining 12 patients are analyzed in the present
paper.

**AMPS technique**

**Electrode placement**

AMPS was originally designed for median-innervated
thenar muscles. The stigmatic and reference recording elec-
trodes consisted of silver foil 4 cm long by 0.8 cm wide.
The stigmatic electrode was positioned transversely over
the thenar eminence, as close as possible to the muscle
endplates. The reference electrode was attached over the
proximal phalanx of the thumb. The ground electrode
(silver foil) was fixed over the dorsum of the wrist. In this
longitudinal study, a meticulous replication of electrode
placement was sought by measuring in each patient the
precise distance between the stimulating electrode and the
tip of the thumb. The stimulating electrodes (Medelec
Model LBS 53051; interelectrode distance: 2.5 cm) were
moved over the median nerve from the wrist to the elbow
with the cathode distal.

**Stimulation and recording systems**

All studies were performed on a Nicolet Viking IV EMG
machine (Nicolet Instrument Corp.). SMUP and com-
pound motor responses were recorded with a gain of
50–100 µV/division and were evoked by constant-current
square stimuli of 0.05 ms duration, at a rate of 1 Hz, with
a weak intensity gradually increased from a subthreshold
value by incremental steps of 0.1–0.5 mA. CMAP were
evoked by stimuli of 0.2 ms duration at a rate of 1 Hz,
stimulating electrodes placed 7 cm proximal to the stig-
matic electrode (supramaximal intensity was defined as
150% of the stimulus intensity giving a maximal
response). Surface recordings were filtered with a
20 Hz–5 kHz bandpass. The hand temperature was main-
tained above 30°C.

**Description of the AMPS method**

AMPS was a two-step procedure. The first step consisted of
estimating the MU size by collecting and averaging 10
well-identified SMUP after stimulation at distinct points
along the course of the median nerve between the wrist
and the elbow. At each stimulation site, only two or three
SMUP were successively evoked by incremental stimula-
tion. The second step consisted of eliciting CMAP by
supramaximal stimulation of the median nerve at the wrist
7 cm from the stigmatic electrode. By dividing the CMAP
size by the average SMUP size, a MUNE was obtained.

Each SMUP was randomly incorporated in the estima-
tion. However, to avoid alternation and ensure that any
increment of the motor response corresponded to the ac-
tivation of one single MU, some specific criteria had to be
met. SMUP had to be evoked:

- with distinct thresholds
- in an all-or-nothing fashion
- with no fractioning of the compound motor responses
to successive identical stimuli
- in an orderly and reproducible manner.

Except for the first SMUP evoked at each selected stimu-
lation point, the precise morphology of successive SMUP
was not visualized, the overall potential representing, as it
did, two or three units. It was theoretically possible to
record the same SMUP twice from two different stimula-
tion points in any one trial because their morphology
might not be recognizable. To ensure that selected SMUP
were different, the morphology of an individual SMUP
could be reconstructed by subtracting from each com-
pound motor response the preceding one obtained from
the same stimulus site with a lower intensity. This recon-
struction took place automatically when point-by-point
differences were measured between successive digitized
traces.

SMUP were eliminated from the estimation in the
following circumstances: similar morphology at two dis-
tinct stimulation points, SMUP which did not fulfil selec-
tion criteria, and predominantly positive SMUP, as they
were supposed to be elicited from distant muscles, such as
lumbrical muscles.
Results

The clinical data and results of longitudinal testing for the 12 patients with ALS are shown in Table 1. In addition, analyzes were also performed on two subsets of six patients: patients 1–6 with a rate of MU loss at $T_4$ of more than 20% (group 1), and patients 7–12 with a rate of MU loss at $T_4$ of less than 20% (group 2) (Tables 1 and 2, Figure 1).

Age, sex and disease duration

Mean ages of the 12 patients with ALS were: 54.8 $\pm$ 9.9 years (range: 36–69); 52.8 $\pm$ 10.4 years in group 1; and 56.8 $\pm$ 10.0 in group 2. There was no significant difference between the mean ages of groups 1 and 2. There were four men and two women in each group. The overall mean disease duration (from the onset of symptoms) at baseline $T_0$ was 21.2 $\pm$ 9.0 months. In group 1, the mean disease duration was 20.5 $\pm$ 9.7 months and in group 2, 21.8 $\pm$ 9.2 months. There was no significant difference between the two groups.

Thenar muscle involvement

At baseline, clinical thenar amyotrophy was present in five of 12 patients with ALS: two in group 1 (33%) and three in group 2 (50%). After one year of riluzole therapy, thenar amyotrophy was observed in 10 of 12 ALS patients: six in group 1 (100%) and four in group 2 (66%).

Needle electromyography (EMG) of the thenar muscles using a standard concentric needle indicated chronic motor denervation (fibrillation potentials in fewer than four of 10 needle muscular insertions, and increased size of MU potential) in eight patients with ALS: three in group 1 (50%) and five in group 2 (84%). Subacute motor denervation (fibrillation potentials in more than four of 10 needle muscular insertions and increased size of MU potential) was observed in three patients from group 1 (50%) and none from group 2 (0%). In patient 7 (group 2), there was no EMG evidence of thenar muscle involvement.

Thenar MUNE

At baseline $T_0$, thenar MUNE was decreased in all patients with ALS (Table 1). Mean thenar MUNE in the 12 patients were 38.5 $\pm$ 25.5 at $T_4$, 29.8 $\pm$ 23.8 at $T_8$, 23.8 $\pm$ 21.1 at $T_{12}$ and 15.3 $\pm$ 18.4 at $T_{12}$ (Table 1). Paired Student’s $t$-test indicated a significant decrease of thenar MUNE at each 4-monthly interval (Table 2). Among the four patients who died before month 12 (patients withdrawn from the longitudinal analysis), two presented a very high rate of MU loss (>50% at $T_4$). At $T_{12}$, the percent change compared to baseline reached on average 60.8% (Table 1). Comparison of group 1 (rate of MU loss more than 20% at $T_4$) and group 2 (rate of MU loss less than 20% at $T_4$) by Mann-Whitney U-testing indicated that percent changes compared to baseline were significantly different at $T_4$, $T_8$ and $T_{12}$ (Table 2).

Average SMUP size

Average SMUP size remained within normal limits in patient 7 at $T_0$, patients 6 and 8 at $T_8$, and patient 8 at $T_{12}$; while all other average SMUP size values were above the upper limit of normal (Table 1). Mean average SMUP sizes in the 12 patients with ALS were 357 $\pm$ 162 $\mu$V.ms at $T_4$, 391 $\pm$ 204 $\mu$V.ms at $T_8$, and 336 $\pm$ 217 $\mu$V.ms at $T_{12}$ (Table 1). Comparison of different means obtained at $T_0$, $T_4$, $T_8$ and $T_{12}$ by using paired Student’s $t$-test did not indicate any significant difference (Table 2).

CMAP size

Thenar CMAP size remained within normal limits in seven patients with ALS at $T_0$ (four from group 1 and three from group 2), five patients at $T_4$ (two from group 1 and three from group 2), three patients (from group 2) at $T_8$ and two patients (from group 2) at $T_{12}$; and was decreased in other patients (Table 1). Mean thenar CMAP sizes in the 12 patients were 11.6 $\pm$ 7.1 mV.ms at $T_0$, 9.4 $\pm$ 6.5 mV.ms at $T_4$, 6.7 $\pm$ 5.8 mV.ms at $T_8$, and 4.4 $\pm$ 5.0 at $T_{12}$ (Table 1). Paired Student’s $t$-test indicated a significant decrease of thenar CMAP size at each 4-monthly interval (Table 2).

Relationship between variables

Correlation coefficients between percent changes, compared to baseline $T_0$, in the three variables derived by using AMPS are shown in Table 3. Changes in thenar MUNE were correlated with changes in CMAP size but were not correlated with changes in average SMUP size. The latter was correlated with changes in CMAP size only when variations at month 4 in both variables were compared.

Percent changes in thenar MUNE or CMAP size calculated during distinct AMPS evaluations over time were correlated; while no correlation was found concerning changes in average SMUP size.

Figure 1 shows the positive correlation between the rate of MU loss at $T_4$ and the rate of MU loss at $T_{12}$. The six patients with a rate of MU loss greater than 20% at $T_4$ (group 1) had a rate of MU loss of more than 50% at $T_{12}$. Five of the six patients with a rate of MU loss less than 20% at $T_4$ (group 2) had a rate of MU loss less than 50% at $T_{12}$.
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Patients 1–12: 8M, 4F

Mean: 54.8 21.2
SD: 9.9 9.0

Patients 1–6: 4M, 2F

Mean: 52.8 20.5
SD: 10.4 9.7

Patients 7–12: 4M, 2F

Mean: 56.8 21.8
SD: 10.0 9.2

Table 1
Summary of clinical and electrophysiological observations on 12 patients with amyotrophic lateral sclerosis

TMI = thenar muscle involvement; MUINE = motor unit number estimate; SMUP = surface motor unit potential; CMAP = compound muscle action potential; V = variation compared to baseline (T0); T0, T4, T8 = evaluation by using the adapted multiple point stimulation method after 4, 8 and 12 months of riluzole therapy; DD = disease duration; CMD = chronic motor denervation; SAMD = subacute motor denervation.
Discussion

First of all, the reliability and practicality of AMPS will be discussed.

Assumptions related to the MU recruitment by incremental stimulation

SMUP selection criteria were established to avoid alteration. We could therefore postulate that any increment of the motor response was due to activation of an additional MU.

The sample of selected MU had to be representative of the whole population. Theoretically, techniques using incremental stimulation or near threshold SMUP activation might all be biased toward large MU. However, the arguments developed by McComas and colleagues, Galea and colleagues and Doherty and Brown are evidence against this possible bias. Moreover, considering the size distribution of SMUP evoked by techniques using incremental stimulation, a wide distribution with more small units than large ones was observed. This distribution was also nearly identical to those obtained by techniques in which MU were not recruited at threshold intensity as in the F-response method, or procedures using a MU recruitment based on voluntary contraction or techniques estimating the MU size by measuring the MU twitch.
Validity

AMPS results were reproducible in control subjects as well as in patients with motor denervation. In 10 normal subjects, when thenar MUNE and average SMUP size estimation by the AMPS method were repeated by the same examiner on two successive days, the coefficients of variation between results from the two trials were 10.4% and 9.5% respectively. In patients with ALS, when AMPS was performed twice in the same session with new electrode placements for the second trial, the coefficient of variation for both MUNE and average SMUP size was 4% between the two trials.

Ease of application

AMPS was a fast procedure, which could be completed in about 15 minutes. Moreover, AMPS did not require any specific recording system or software. Thus, AMPS was easily applicable for clinical purposes. With the AMPS technique, it was possible to obtain a MUNE in all 70 volunteers studied, without technical failures. Moreover, in our experience with patients presenting with severe axonal loss, particularly in the 12 patients with ALS in the current study, MUNE were easier to obtain than in healthy volunteers. Indeed, as previously reported, there was less overlap of MU thresholds when denervation was severe, and alternation was therefore less noticeable. On the other hand, in partially denervated muscles the accuracy is also higher, since the sample of units is a greater fraction of the whole population. When the MU number was very low, it was sometimes possible to evoke more than three SMUP without alternation from the same point using incremental stimulation. Therefore, two or three different stimulation points along the course of the median nerve were enough to derive a MUNE. If there were less than 10 MU remaining in some patients, the best way to derive a MUNE was sometimes to find one stimulation point from which it was possible to activate the few remaining MU without alternation. In these particular cases, AMPS was very similar to the initial estimation technique described by McComas and colleagues.

Finally, AMPS was non-invasive and painless, as only surface electrodes and low stimulation intensities were used.

Thenar MUNE

At baseline T₀, after a mean disease duration of 21.2 ± 9.0 months, all 12 patients with ALS had decreased thenar MUNE compared with the normal limits in terms of age previously defined in a group of 70 healthy control subjects. In patient 7, there was neither thenar amyotrophy nor electromyographic evidence of motor denervation in thenar muscles (Table 1). Thus, in patients with ALS, decreased MUNE was probably one of the most sensitive signs of motor denervation. Paired Student’s t-test pointed out a significant decrease of thenar MUNE every 4 months (Table 2). After one year, the MUNE decline reached on average 60.8% compared to baseline (Table 1). A more severe evolution has been found by several authors, who established that on average the MU population halved in each 6-month period, with a drop of around 75% in the first year after diagnosis. This might be due to the fact that only patients who survived one year after baseline were included in the study. Two patients, with an acute form of ALS, presented very high rate of MU loss (>50% at T₁), and died before T₁₂, and were therefore withdrawn from analysis.

Two subsets of six patients were defined on the basis of the rate of MU loss at T₄, more (group 1) or less (group 2) than 20% compared to baseline. From a statistical point of view, these two groups did not differ in terms of age, disease duration from the onset of symptoms and sex. Comparison by Mann-Whitney U-testing of percent changes in thenar MUNE compared to baseline indicated a significant difference between both groups at each 4-monthly interval (Table 2). In group 1, with a rapid rate of progression, there was thenar wasting in two patients at T₄, but in all six at T₁₂, and EMG recordings at baseline in the six patients revealed thenar motor denervation which was chronic (n=3) or subacute (n=3). In group 2, with a slower rate of progression, there was thenar wasting in three patients at T₄ and in four at T₁₂. Thenar muscle EMG data at baseline remained normal in one patient and indicated chronic motor denervation in five (Table 1).

Average SMUP size

Associated with the reductions in thenar MUNE was the enlargement of surviving units, as indicated by the increase in average SMUP sizes measured in all 12 patients, with the exception of patient 7 at T₄, patient 6 at T₈, and patient 8 at T₈ and T₁₂ (Table 1). The smallest SMUP recorded in the 12 patients with ALS was 35 µV.ms and the largest was 2738 µV.ms. No difference was observed between average SMUP size results estimated in all 12 patients with ALS by repeated AMPS testing at 4-monthly intervals. In addition, there was no statistical difference between percent changes compared to baseline calculated from both subsets of six patients (Table 2). These data were probably indicative of the fact that the patients were not at the same stage of evolution and that several parameters influenced the MU size in different ways. Among these parameters, the following ones had to be considered: 1. collateral reinnervation, which was probably more effective in the early stages, and small MU loss increased the average SMUP size; 2. the disappearance of some large units and the MU ‘disintegration’ phenomenon, which might occur in terminal stages, decreased the mean MU size.

CMAP Size

Values of CMAP size confirmed that collateral reinnervation is able, from an electrophysiological point of view, to compensate for motor denervation. In fact, at the time of the first examination (T₀), seven of 12 patients kept their thenar CMAP size within normal limits. However, among
the four patients from group 1 (rate of MU loss more than 20% at $T_4$) in whom CMAP size remained within normal limits at $T_0$, only two kept this variable within the normal range at $T_4$ and none at $T_8$ and $T_{12}$. Conversely, in the three patients from group 2 (rate of MU loss at $T_4$ less than 20%), in whom CMAP size was within normal limits at $T_0$, the variable remained quite unchanged at $T_4$ and $T_8$, and even up to one year after diagnosis in two patients (Table 1). Consequently, Mann-Whitney U-testing indicated that percent changes, compared to baseline, were significantly different between groups 1 and 2 at $T_4$, $T_8$ and $T_{12}$ (Table 2).

Relationships between variables

CMAP size is the product of MUNE and average SMUP size. Correlations between changes in thenar MUNE and CMAP size (Table 3) were therefore expected. However, change in average SMUP size was correlated with change in CMAP size only at $T_4$ (Table 3). We can thus postulate that CMAP size variations over time were more influenced by MUNE than by average SMUP size variations. On the other hand, CMAP size variations over time might be due to a change in the placement of recording electrodes, which should induce similar changes in average SMUP size. It seems that it was not the case in the present study, since both variables were poorly correlated. As indicated under ‘Patients and Methods’, in this longitudinal study a meticulous replication of recording electrode placement was sought by measuring in each patient the precise distance between the stigmatic electrode and the tip of the thumb.

The relationship between different values from the same variable, measured over time, might be a way to determine the prognostic value of the variable under analysis. From this point of view, the best correlation coefficient, between results at $T_4$ and $T_{12}$ (the most distant percent change evaluations), was found for change in thenar MUNE ($r = 0.71$) (Table 3). This correlation was indicative of a quite stable rate of MU loss over the one-year period; hence, the percent change in MUNE at $T_4$ might be considered a prognostic value of the rate of MU loss at $T_{12}$. Figure 1 shows that in group 1, the rate of MU loss at $T_{12}$ compared to baseline was more than 50%, while five of six patients from group 2 presented a rate of MU loss less than 50%. These data give substance to the prognostic value of percent change in thenar MUNE at $T_4$ over a short follow-up period. Nevertheless, in longer follow-up, Dantes and McComas and Daube found that the initial, usually rapid, loss of MU was followed by a slower loss of the remaining MU. In the present study, patients with ALS were not at the same stage of evolution. In fact, some patients were in the initial phase of the disease (i.e. patient 7 at $T_0$: thenar MUNE = 72, disease duration = 9 months) and some others in a later phase (i.e. patient 11 at $T_0$: thenar MUNE = 3, disease duration = 28 months).

Conclusion

With a view to clinical trials, it should be useful to be able to distinguish patients with ALS according to a rapid or a slow rate of progression. Besides fibrillation abundance, shape variability of MU potentials, decremental response to repetitive nerve stimulation and fibre density, MUNE techniques might be useful for this. Results obtained by AMPS, a simple, reliable, and painless method, suggest

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<th>Thenar MUNE</th>
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<th>CMAP size</th>
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MUNE= Motor unit number estimate; SMUP=surface motor unit potential; CMAP= compound muscle action potential; $T_4$, $T_8$, $T_{12}$ = evaluation by using the adapted multiple point stimulation method after 4, 8 and 12 months of riluzole therapy.

$^*$ = $P < 0.05$, $^{**} = P < 0.02$, $^{***} = P < 0.01$, $^{****} = P < 0.002$, $^{*****} = P < 0.001$

Table 3

Correlation coefficients ($r$) between percent changes (compared to baseline) in AMPS variables.
that slow progression is characterized by a rate of MU loss of less than 20% every four months and stable CMAP size, and conversely for rapid progression.

Acknowledgements

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References