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

FC Wang[1], O Bouquiaux[1], V De Pasqua[1] and PJ Delwaide[2]

University Department of Physical Medicine and Rehabilitation, Hôpital du Sart Tilman, and [2]From the University Department of Neurology, Hôpital de la Citadelle, B-4000 Liège, Belgium

Correspondence:
Dr FC Wang
Department of Physical Medicine and Rehabilitation
CHU Sart Tilman B35
B-4000 Liège, Belgium
Tel: 32/4/366.76.44
Fax: 32/4/366.72.30
E-mail: fc.wang@chu.ulg.ac.be

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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 (T_0) 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 T_4 and at T_{12} ($P < 0.001$).

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 averaging[9] 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

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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.[12] Moreover, patients were screened to exclude any additional pathology affecting peripheral nerves, muscles or neuromuscular junctions. We excluded carpal tunnel entrapment neuropathy on the basis of clinical examination and electrophysiological tests (motor conduction 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 evaluation (T_0).

AMPS was performed on the right side or, if amyotrophy 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:

$$[(T_0 \text{ value-control visit value}) / T_0 \text{ value}] \times 100.$$

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 electrodes 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 compound 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 stigmatic 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 maintained 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 stimulation. 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 estimation. However, to avoid alternation and ensure that any increment of the motor response corresponded to the activation 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 stimulation 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 stimulation 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 compound motor response the preceding one obtained from the same stimulus site with a lower intensity. This reconstruction 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 distinct stimulation points, SMUP which did not fulfil selection 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 ± 9.9 years (range: 36–69); 52.8 ± 10.4 years in group 1; and 56.8 ± 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 ± 9.0 months. In group 1, the mean disease duration was 20.5 ± 9.7 months and in group 2, 21.8 ± 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 ± 25.5 at T_0, 29.8 ± 23.8 at T_4, 23.8 ± 21.1 at T_8 and 15.3 ± 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 ± 162 μV.ms at T_0, 391 ± 204 μV.ms at T_4, 334 ± 202 μV.ms at T_8 and 336 ± 217 μV.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).

Comparison by Mann-Whitney U-testing of percent changes at T_4, T_8 and T_{12} (compared to baseline) did not show any significant difference between group 1 and group 2 (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 ± 7.1 mV.ms at T_0, 9.4 ± 6.5 mV.ms at T_4, 6.7 ± 5.8 mV.ms at T_8 and 4.4 ± 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).

Comparison of group 1 and group 2 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).

In group 1, the percent decline in CMAP size reached on average 95.5% over a one-year period. In group 2, CMAP size remained quite stable till month 8, and at month 12, the percent change in CMAP size was on average 47.3 % (Table 1).

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}.

				TMI			Thenar MUNE							Average SMUP size (μV.ms)							CMAP size (mV.ms)						
Patients	Age (years)	Sex	DD (months)	Amyotrophy T_0	Amyotrophy T_{12}	EMG T_0	T_0	T_4	(V) (%)	T_8	(V) (%)	T_{12}	(V) (%)	T_0	T_4	(V) (%)	T_8	(V) (%)	T_{12}	(V) (%)	T_0	T_4	(V) (%)	T_8	(V) (%)	T_{12}	(V) (%)
1	54	M	12	0	+	CMD	53	28	(−47)	9	(−83)	3	(−94)	423	499	(+18)	600	(+42)	333	(−21)	22.4	14.0	(−38)	5.4	(−76)	1.0	(−96)
2	54	M	20	+	+	SAMD	28	22	(−21)	15	(−46)	4	(−86)	293	268	(−9)	303	(+3)	184	(−37)	8.2	5.9	(−28)	4.5	(−45)	0.7	(−91)
3	36	M	13	+	+	SAMD	19	9	(−53)	6	(−68)	5	(−74)	342	401	(+17)	350	(+2)	453	(+32)	6.5	3.6	(−45)	2.1	(−68)	2.3	(−65)
4	48	F	16	0	+	CMD	32	21	(−34)	18	(−44)	8	(−75)	267	253	(−5)	225	(−16)	215	(−19)	8.5	5.3	(−38)	4.1	(−52)	1.7	(−80)
5	58	F	38	0	+	CMD	40	29	(−28)	25	(−38)	15	(−63)	354	457	(+29)	246	(−31)	373	(+5)	14.2	13.3	(−6)	6.2	(−56)	5.6	(−61)
6	67	M	24	0	+	SAMD	35	21	(−40)	20	(−43)	6	(−83)	341	218	(−36)	164	(−52)	304	(−11)	11.9	4.6	(−61)	3.3	(−72)	1.8	(−85)
7	69	M	9	0	+	Normal	72	67	(−7)	58	(−19)	19	(−74)	170	188	(+11)	209	(+23)	269	(+58)	12.2	12.6	(+3)	12.1	(−1)	5.1	(−58)
8	49	M	20	+	+	CMD	7	6	(−14)	5	(−29)	4	(−43)	345	399	(+16)	71	(−79)	70	(−80)	2.4	2.4	(0)	0.4	(−83)	0.3	(−88)
9	49	M	20	+	+	CMD	22	18	(−18)	14	(−36)	12	(−45)	351	462	(+32)	409	(+17)	333	(−5)	7.7	8.3	(+8)	5.7	(−26)	4.0	(−48)
10	47	M	18	0	0	CMD	74	64	(−14)	53	(−28)	51	(−31)	297	359	(+21)	353	(+19)	296	(0)	22.0	23.0	(+5)	18.7	(−15)	15.1	(−31)
11	59	F	28	+	+	CMD	3	3	(0)	3	(0)	2	(−33)	832	944	(+13)	816	(−2)	953	(+15)	2.5	2.8	(+12)	2.4	(−4)	1.9	(−24)
12	68	F	36	0	0	CMD	77	70	(−9)	60	(−22)	55	(−29)	274	238	(−13)	266	(−3)	251	(−8)	21.1	16.7	(−21)	16.0	(−24)	13.8	(−35)
Patients 1–12		8M, 4F																									
Mean	54.8		21.2				38.5	29.8	(−23.8)	23.8	(−38.1)	15.3	(−60.8)	357	391	(7.8)	334	(−6.4)	336	(−6.0)	11.6	9.4	(−17.4)	6.7	(−43.5)	4.4	(−63.4)
SD	9.9		9.0				25.5	23.8	(16.7)	21.1	(22.0)	18.4	(23.4)	162	204	(19.7)	202	(33.9)	217	(34.4)	7.1	6.5	(24.2)	5.8	(28.9)	5.0	(24.9)
Patients 1–6		4M, 2F																									
Mean	52.8		20.5				34.5	21.7	(−37.2)	15.5	(−53.7)	6.8	(−79.0)	337	349	(2.4)	315	(−8.4)	310	(−8.5)	12.0	7.8	(−35.9)	4.3	(−61.5)	2.2	(−95.5)
SD	10.4		9.7				11.5	7.1	(11.8)	7.1	(17.9)	4.4	(11.1)	54	118	(23.8)	154	(32.3)	100	(24.4)	5.8	4.6	(18.2)	1.5	(12.3)	1.8	(14.2)
Patients 7–12		4M, 2F																									
Mean	56.8		21.8				42.5	38.0	(−10.3)	32.2	(−22.5)	23.8	(−42.5)	378	432	(13.2)	354	(−4.3)	362	(−3.5)	11.3	11.0	(1.1)	9.2	(−25.5)	6.7	(−47.3)
SD	10.0		9.2				35.5	32.2	(6.4)	27.5	(12.5)	23.4	(16.6)	232	271	(14.9)	255	(38.4)	304	(44.7)	8.7	8.1	(11.5)	7.5	(30.1)	6.2	(23.2)

TMI=thenar muscle involvement; MUNE=motor unit number estimate; SMUP=surface motor unit potential; CMAP=compound muscle action potential; V=variation compared to baseline (T_0); T_4, T_8, T_{12} = 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.

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

Variables			Paired Student's t-test
Thenar MUNE	38.5 ± 25.5 (T_0)	29.8 ± 23.8 (T_4)	$p < 0.02$
(n=12)	29.8 ± 23.8 (T_4)	23.8 ± 21.1 (T_8)	$p < 0.02$
	23.8 ± 21.1 (T_8)	15.3 ± 18.4 (T_{12})	$p < 0.02$
Average SMUP size (μV.ms)	357 ± 162 (T_0)	391 ± 204 (T_4)	NS
(n=12)	391 ± 204 (T_4)	334 ± 201 (T_8)	NS
	334 ± 201 (T_8)	336 ± 217 (T_{12})	NS
	357 ± 162 (T_0)	336 ± 217 (T_{12})	NS
CMAP size (mV.ms)	11.6 ± 7.1 (T_0)	9.4 ± 6.5 (T_4)	$p < 0.05$
(n=12)	9.4 ± 6.5 (T_4)	6.7 ± 5.8 (T_8)	$p < 0.05$
	6.7 ± 5.8 (T_8)	4.4 ± 5.0 (T_{12})	$p < 0.05$
	MU loss > 20% at T_4 patients 1–6	**MU loss < 20% at T_4 patients 7–12**	**Mann-Whitney *U*-test**
Thenar MUNE variation	−37.2 ± 11.8 (T_4)	−10.3 ± 6.4 (T_4)	$p < 0.001$
compared to baseline (%)	−53.7 ± 17.9 (T_8)	−22.5 ± 12.5 (T_8)	$p < 0.001$
	−79.0 ± 11.1 (T_{12})	−42.5 ± 16.6 (T_{12})	$p < 0.001$
Average SMUP size variation	2.4 ± 23.8 (T_4)	13.2 ± 14.9 (T_4)	NS
compared to baseline (%)	−8.4 ± 32.3 (T_8)	−4.3 ± 38.4 (T_8)	NS
	−8.5 ± 24.4 (T_{12})	−3.5 ± 44.7 (T_{12})	NS
CMAP size variation	−35.9 ± 18.2 (T_4)	1.1 ± 11.5 (T_4)	$p < 0.002$
compared to baseline (%)	−61.5 ± 12.3 (T_8)	−25.5 ± 30.1 (T_8)	$p < 0.05$
	−95.5 ± 14.2 (T_{12})	−47.3 ± 23.2 (T_{12})	$p < 0.02$

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

Table 2
Statistical comparison of results between 12 patients with amyotrophic lateral sclerosis

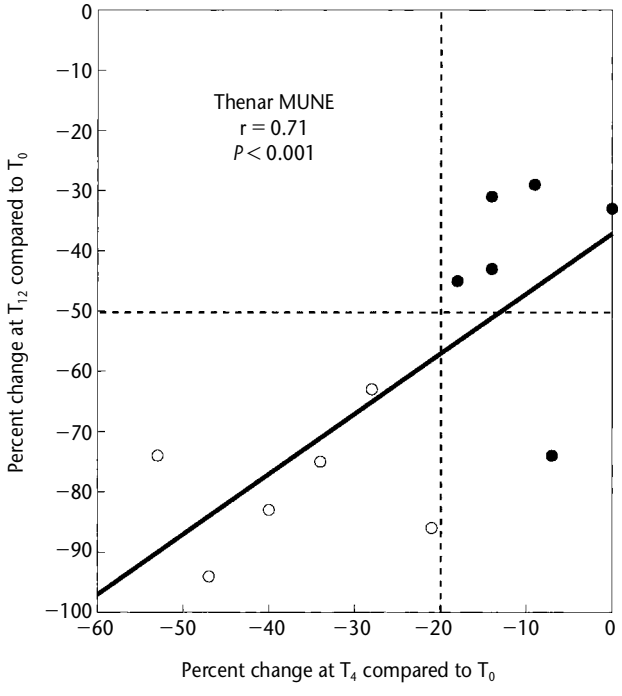

Figure 1
Relationship between the rate of motor unit loss at month 4 and the rate of motor unit loss at month 12 in 12 patients with amyotrophic lateral sclerosis. Percent change (compared to baseline) in thenar motor unit number estimate (MUNE) at T_4 was more than 20% in 6 patients (circles, group 1) and was less than 20% in 6 other patients (dots, group 2).

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 alternation. 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.[14] However, the arguments developed by McComas and colleagues,[5] Galea and colleagues[15] and Doherty and Brown[6] 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,[8] or procedures using a MU recruitment based on voluntary contraction[9] or techniques estimating the MU size by measuring the MU twitch.[16]

Original Research

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.[7] 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.[11]

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,[13] 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,[17] 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.[5]

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

Thenar MUNE

At baseline T_0, 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[7,13] 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.[11,18,19] 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 T_4), and died before T_{12}, and were therefore withdrawn from analysis.

Two subsets of six patients were defined on the basis of the rate of MU loss at T_4, 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 T_0, but in all six at T_{12}, 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 T_0 and in four at T_{12}. 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 T_0, patient 6 at T_8 and patient 8 at T_8 and T_{12} (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,[11,20] 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 (T_0), 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[18] and Daube[21] 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[22] and fibre density,[4] MUNE techniques might be useful for this. Results obtained by AMPS, a simple, reliable, and painless method, suggest

		Thenar MUNE			Average SMUP size			CMAP size		
		T_4	T_8	T_{12}	T_4	T_8	T_{12}	T_4	T_8	T_{12}
Thenar MUNE	T_4	/	0.92^{+++}	0.71^{+}	0.12	0.01	0.03	0.82^{++}	0.76^{+}	0.62°
	T_8	/	/	0.72^{+}	0.03	0.22	0.15	$0.69^{\circ\circ}$	0.72^{+}	$0.69^{\circ\circ}$
	T_{12}	/	/	/	0.28	0.12	0.04	$0.70^{\circ\circ}$	0.51	0.81^{++}
Average SMUP size	T_4	/	/	/	/	0.32	0.16	$0.66^{\circ\circ}$	0.16	0.29
	T_8	/	/	/	/	/	0.54	0.13	0.50	0.28
	T_{12}	/	/	/	/	/	/	0.11	0.58	0.51
CMAP size	T_4	/	/	/	/	/	/	/	0.65°	0.63°
	T_8	/	/	/	/	/	/	/	/	0.81^{++}
	T_{12} /	/	/	/	/	/	/	/	/	/

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.
$^{\circ} = P < 0.05$, $^{\circ\circ} = P < 0.02$, $^{+} = P < 0.01$, $^{++} = P < 0.002$, $^{+++} = P < 0.001$

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.

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