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Research Article

# The Compound Muscle Action Potential as Neurophysiological Marker for Amyotrophic Lateral Sclerosis

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## Abstract

**Objectives:** To definite the peripheral nervous involvement in ALS through the repeated use of the compound motor action potential (CMAP) to test the progression of disease, to determine different change of phrenic CMAP and forced vital capacity (FVC) in spinal and bulbar onset, and to establish clinical and neurophysiological features of patients with poor prognosis.

**Material & Methods:** CMAP from phrenic, ulnar, and medial plantar nerves, Medical Research Council (MRC) score, revised ALS functional rating scale (ALSFRS-R) and FVC were evaluated in 117 ALS patients every three months in one year-period.

**Results:** Bulbar onset patients had lower FVC but similar amplitude of phrenic CMAP at baseline compared to spinal onset patients. The patients with poor prognosis had lower phrenic CMAP and FVC at baseline. CMAP values, when compared to the rate found in the previous visit, reduced significantly in both poor and good prognosis groups during the entire follow-up period, while the FVC reduced significantly only in the first three months.

**Conclusions:** CMAP is a reproducible sensitive marker for motor neurons loss and collateral reinnervation in ALS also in a short period of time. The changes in CMAP, MRC, FVC and ALSFRS-R score resulted correlated, but CMAP is the only parameter with the advantage to demonstrate objectively the progression of disease in both patients with poor and good prognosis for the entire period of follow-up. It should be used as clinical outcome of ALS in clinical trials, taking advantage of its objectivity and selectivity for peripheral nervous system study.

***Keywords:*** *Amyotrophic Lateral Sclerosis: ALS; Compound Muscle Action Potential: CMAP; Neurophysiological Marker*

## Introduction

Amyotrophic lateral sclerosis (ALS) is the most frequent adult-onset motor neuron disease characterized by the progressive loss of upper and lower motor neurons (MNs). The death occurs usually on few years after the symptoms onset [1].

Given the lack of biomarkers, diagnosis of ALS is supported by clinical and electrophysiological findings after the exclusion of other

diseases with analogous symptoms [2]. An early anticipation of the clinical course is important to plan the best support for both the patient and his family, justifying a continuous interest in methods to monitor disease progression. Besides clinical methods to monitor disease progression, such as the revised ALS functional rating scale-revised (ALSFRS-R) and the Medical Research Council (MRC) scale for muscle strength, several studies have suggested the motor unit number estimation (MUNE) technique and the motor unit number index (MUNIX) as appropriate measurements to study MUs loss [3-6]. Previous studies have also highlighted the importance of the compound muscle action potential (CMAP) as a possible biomarker of progression of ALS [7-9]. A decrement of CMAP during the disease course in ALS subjects is described and attributed to the failure of conduction of repeated stimuli for degenerating motor axons and regenerating nerves secondary to collateral sprouting [10,11]. Furthermore, the phrenic CMAP was indicated as predictor of hypoventilation in ALS [12,13].

The ability of CMAP to progress similarly to other clinical scales for strength evaluation in ALS patients is noted, but a description of a possible different pattern of CMAP change in patients with spinal and bulbar onset or with good or poor prognosis has not yet been illustrated.

The purposes of this study were to determine if the CMAP, systematically repeated every three months, correlates with other clinical parameters in order to monitor objectively the peripheral nervous involvement of ALS disease; to determine a possible different change of phrenic CMAP and forced vital capacity (FVC) in patients with spinal and bulbar onset; to evaluate the clinical and neurophysiological characteristics of the patients with poor prognosis at 12 months.

## Material and Methods

### Patients

One hundred-fifty six consecutively recruited patients from 2009 to 2013 (94 men and 62 women; mean age 63 years with range 24 - 85 years) with definite or probable ALS according to the revised El Escorial criteria were enrolled [14]. The study was approved by the medical ethics committee of the Umberto I Hospital in Rome. Inclusion criteria contained FVC > 25% predicted value, a MRC Scale for muscle strength of at least 3/5 in abductor digiti minimi (ADM) and abductor hallucis (AH), and a clinical follow-up with at least three visits. Patients with cervical spondylosis, diabetes mellitus, severe primary pulmonary diseases and polyneuropathy were excluded.

Nine patients were excluded because they did not respect FVC and/or MRC values defined in inclusion criteria. Thirty patients were excluded because they had only two visits in their follow-up. The data analysis was done on 117 patients (78 with spinal onset, 39 with bulbar onset).

### Study Design

The patients underwent visits every three months. The following data were obtained at each visit (baseline, 3, 6, 9 and 12 months: T0-T4) after the study entry (baseline): *i.* amplitude of CMAP from bilateral phrenic, ulnar, and medial plantar nerves; *ii.* MRC score for upper and lower limbs; *iii*. ALSFRS-R; *iv.* FVC evaluation. The same examiner performed all electrophysiological studies and clinical evaluations.

### Neurophysiological Assessments

Electrophysiological studies were performed with a Micromed Myoquick EMG machine 1400 device (Micromed System Plus Evolution 1.04.0097 S.p.A., Treviso, IT). All measurements were recorded bilaterally. In order to avoid exclusion of a large part of the population which is frequently affected by carpal tunnel syndrome or by compression-caused peroneal neuropathy (often present in ALS patients because of immobility), we decided to study nerves usually fewer subjected to compression. CMAP was recorded from diaphragm, ADM, and AH, respectively with phrenic, ulnar, and medial plantar nerve stimulation.

The amplitudes of registered CMAP in right and left side for each stimulated nerve were summarized. The sum of all CMAP (from phrenic, ulnar and medial plantar nerves) was also evaluated (score abnormality).

### Stimulation technique

Electrical stimulation was delivered to the phrenic, ulnar and medial plantar nerves [15]. The stimulus intensity was set to evoke a maximal CMAP in diaphragmatic, ADM and AH muscles.

### Recording technique

The ENG evaluation was carried out using a pair of Ag/AgCl surface electrodes (Disposable Adhesive Surface Electrodes, Spes Medica S.r.l., Italy) [15]. A recurring henna pinpoint tattoo was placed close to the center of the interested muscle to mark the position of the electrodes on the skin in order to assist in exact repeat placement of electrodes. The amplitudes of the initial negative peaks of the CMAP were measured and the changes in baseline-peak CMAP amplitude (mv) compared with months 3, 6, 9, and 12 after study entry were analyzed.

### Clinical Assessments

Muscle strength of upper and lower limbs were assessed with the MRC Score, an ordinal scale ranging from 0 (absence of movement) to 5 (contraction against full resistance) quantifying muscle weakness in isolated muscles or muscle groups [16]. In this test, eight muscle groups in upper limbs and seven muscle groups in lower limbs are tested. The maximum score is 40 for each upper limb, 35 for each lower limb.

The ALSFRS-R is a validated measure of functional impairment in ALS [17]. It is a questionnaire-based functional scale, containing 12 items rated from 0 (complete dependence for that function) to 4 (normal function), divided into three sub scores (bulbar 12, spinal 24, and respiratory 12), with normal function defined by a score of 48.

### Respiratory Assessments

Spirometry was carried out with participants in a sitting position wearing a nose clip, asked to blow into the mouthpiece of a spirometer (Winspiro PRO 5.8) as forcefully and quickly as possible and to continue blowing until all of the air was expelled from lungs. The FVC (L) values were analyzed and expressed as percentages. The highest value was used in the analysis.

### Statistical Analysis

Demographic, clinical and neurophysiological variables were analyzed using t-test. Comparisons of overall survival were analyzed using the Kaplan-Meier method, and *P* values were calculated using the log-rank test and Gehan Wilcoxon test. The correlation between coefficient measurements were calculated using Spearman test. Chi-square test was used to compare qualitative values. Kruskal-Wallis ANOVA and Wilcoxon Signed-Ranks test were performed for comparison between and within groups.

A $P$ value $< 0.05$ was considered to be statistically significant for all analyses. The SPSS statistical package, v. 13.0 (Chicago, Illinois) was used for all analyses.

## Results

All 117 ALS patients were followed for at least six months from baseline. At baseline, patients with bulbar onset were mainly females (62% vs 22%, $p \leq 0.01$) and older (67.2 ± 10.3 vs 62.6 ± 11.0, $p \leq 0.01$), with a lower duration of disease (14.6 ± 6.3 vs 25.1 ± 20.0, $p \leq 0.01$) and a poorer FVC (67.1 ± 18.2 vs 79.7 ± 21.7, $p \leq 0.01$). Seventy-five patients (88%) had a complete 12-month follow-up period. As

expected in a progressive disease, a gradual reduction in CMAP amplitude, ALSFRS-R, MRC score and FVC during the follow-up period was evidenced. The changes of FVC in the time in bulbar and spinal patients is reported in Figure 1. In bulbar ALS, the levels of FVC was lower than in spinal ALS. Moreover, they showed a similar reduction of amplitude of phrenic CMAP in the time (Figure 2).

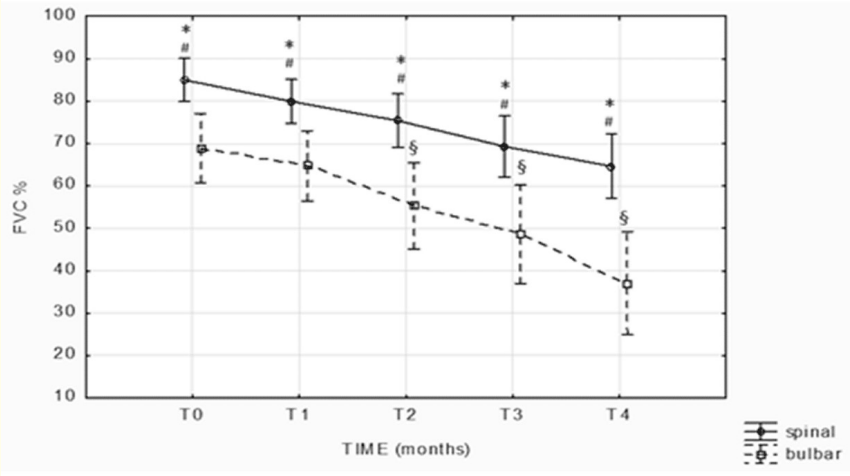


***Figure 1:*** *Time changes of FVC in ALS patients with bulbar and spinal onset.*
** P < 0.05 in the comparison between bulbar and spinal (Mann-Whitney test).*
*# P < 0.05 compared with the basal levels in the spinal group, (Wilcoxon Signed Ranks Test).*
*§ P < 0.05 compared with the basal levels in the bulbar group, (Wilcoxon Signed Ranks Test).*
*Vertical bars denote 0.95 confidence intervals*

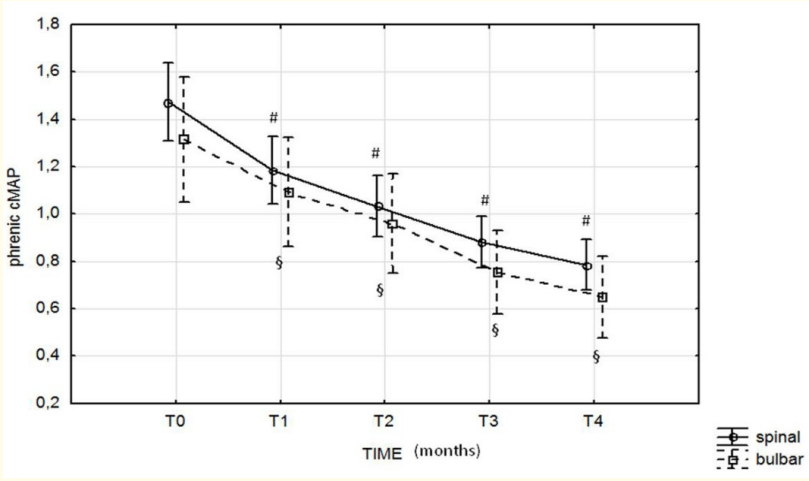


***Figure 2:*** *Time changes of phrenic CMAP in ALS patients with bulbar and spinal onset.*
*# P < 0.05 compared with the basal levels in the spinal group, (Wilcoxon Signed Ranks Test).*
*§ P < 0.05 compared with the basal levels in the bulbar group, (Wilcoxon Signed Ranks Test).*
*Vertical bars denote 0.95 confidence intervals*

## Correlation Results

The phrenic CMAP correlated with FVC in the all visits (T0: $r_1 = 0.28$, $p < 0.05$; T1: $r_2 = 0.33$, $p < 0.05$; T2: $r_3 = 0.36$, $p < 0.05$; T3: $r_4 = 0.41$, $p < 0.05$; T4: $r_5 = 0.45$ $p < 0.05$).

The ulnar CMAP correlated with MRC of upper limb in the all visits (T0: $r_1 = 0.63$, $p < 0.05$; T1: $r_2 = 0.65$, $p < 0.05$; T2: $r_3 = 0.68$, $p < 0.05$; T3: $r_4 = 0.67$, $p < 0.05$; T4: $r_5 = 0.68$, $p < 0.05$).

The medial plantar CMAP correlated with MRC of lower limbs in the all visits (T0: $r_1 = 0.34$, $p < 0.05$; T1: $r_2 = 0.49$ $p < 0.05$; T2: $r_3 = 0.48$, $p < 0.05$; T3: $r_4 = 0.53$, $p < 0.05$; T4: $r_5 = 0.75$, $p < 0.05$).

The summated CMAPs correlated with ALSFRS-R-spinal sub score in the all visits (T0: $r_1 = 0.50$, $p < 0.05$; T1: $r_2 = 0.57$, $p < 0.05$; T2: $r_3 = 0.61$, $p < 0.05$; T3: $r_4 = 0.59$, $p < 0.05$; T4: $r_5 = 0.54$ $p < 0.05$).

## Longitudinal Study in poor prognosis patients

We defined "poor prognosis" death or tracheostomy occurred during the 12 months of the study. Death or tracheostomy happened after six months the first visit in 21 patients. Thirteen patients (11%, seven with spinal onset, six with bulbar onset) died 32 ± 17 months after the start of symptoms, and eight patients (7%, five with spinal onset, three with bulbar onset) underwent a tracheostomy during the follow-up. The analysis of poor prognosis factors was done in 21 patients (13 died patients and 8 trached patients) compared to 75 patients survived in the same 12-month follow-up period. At baseline, patients with poor prognosis were above all females (57% vs 40%, $p < 0.05$), older (67.6 ± 11.1 vs 60.2 ± 10.4, $p < 0.01$), with a lower FVC (60.5 ± 22.3 vs 80.5 ± 20.0, $p < 0.01$) and a smaller phrenic CMAP (1.1 ± 0.4 vs 1.4 ± 0.6, $p = 0.01$). The tracheotomy or death happened 24.3 ± 10.3 and 34.0 ± 19.6 months after the start of symptoms in bulbar and spinal ALS respectively ($p = 0.04$ by the log-rank test; $p = 0.04$ by the Wilcoxon test) (Figure 3).

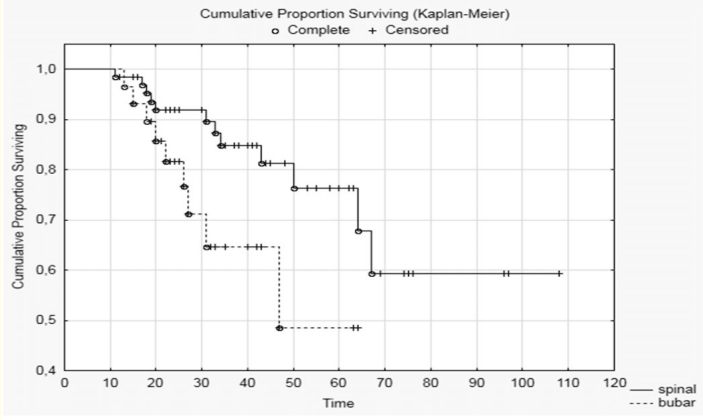


***Figure 3:*** *Death and tracheostomy in spinal and bulbar ALS patients from onset of symptoms during the 12-month follow-up.*

For ALS patients with poor prognosis, the evaluations of FVC and phrenic CMAP were available only at T0, T1, T2, and T3 because after they died or was trached.

In poor prognosis group, comparing each value with that found three months before in the previous visit, the FVC value progressively reduced but with a significant difference only between T1 and T0 ($p < 0.01$) (Figure 4).

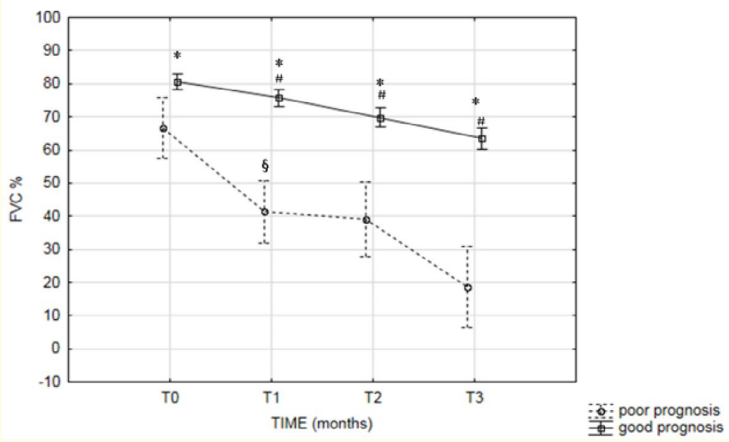


***Figure 4:*** *Figure 4: Time changes of FVC in poor and good prognosis ALS patients.*

** P < 0.05 (Kruskal-Wallis ANOVA) for the comparison between poor and good prognosis ALS patients.*

*# P < 0.05 (Wilcoxon Signed Ranks Test) for the comparison with the previous visit in the good prognosis ALS patients.*

*§ P < 0.05 (Wilcoxon Signed Ranks Test) for the comparison with the previous visit in the poor prognosis ALS patients.*

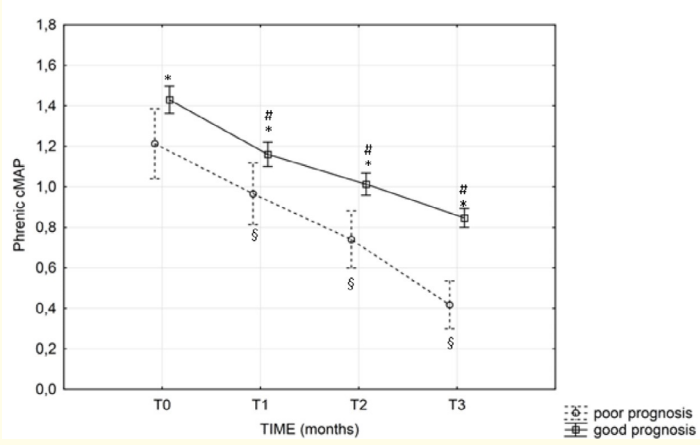


***Figure 5:*** *Time changes of phrenic CMAP in poor and good prognosis ALS patients.*

** P < 0.05 (Kruskal-Wallis ANOVA) for the comparison between poor and good prognosis ALS patients.*

*# P < 0.05 (Wilcoxon Signed Ranks Test) for the comparison with the previous visit in the good prognosis ALS patients.*

*§ P < 0.05 (Wilcoxon Signed Ranks Test) for the comparison with the previous visit in the poor prognosis ALS patients.*

In comparison to the previous visit, the ALSFRS-R respiratory value reduced in poor prognosis group, with a significant difference only between T1 vs T0 and T2 vs T1 ($p < 0.01$), and in good prognosis group between all the evaluations (T1 vs T0, T2 vs T1, T3 vs T2; $p < 0.01$). The ALSFRS-R bulbar values were progressively reduced in both the poor and good prognosis groups in all the times ($p < 0.01$).

The upper limbs MRC values significantly reduced in both good and in poor prognosis groups ($p < 0.01$). Also, the lower MRC value reduced progressively in all the times, in both good ($p < 0.01$) and in poor ($p < 0.05$) prognosis groups in all the times.

| | | T0 Mean (SD) | T1 Mean (SD) | T2 Mean (SD) | T3 Mean (SD) |
|---|---|---|---|---|---|
| FVC% | Good prognosis | 80.5 (20.0)* | 75.7 (20.1)*# | 69.8 (24.9)* | 63.5 (28.0)* |
| | Poor prognosis | 60.5 (22.3)* | 37.6 (24.8)*# | 32.8 (21.5)*# | 18.6 (17.6)* |
| Phrenic CMAP | Good prognosis | 1.4 (0.6)* | 1.2 (0.5)*# | 1.0 (0.5)*# | 0.8 (0.4)*# |
| | Poor prognosis | 1.1 (0.4)* | 0.8 (0.4)*# | 0.6 (0.4)*# | 0.4 (0.3)*# |
| Ulnar CMAP | Good prognosis | 18.9 (9.5) | 16.9 (9.1)* | 15.3 (9.1)* | 13.7 (9.1)* |
| | Poor prognosis | 15.2 (7.1) | 12.1 (7.8)*# | 7.9 (6.1)*# | 5.2 (5.1)*# |
| Medial Plantar CMAP | Good prognosis | 15.9 (11.4)* | 14.7 (11.1)*# | 13.4 (11.0)*# | 11.2 (10.1)*# |
| | Poor prognosis | 14.4 (11.6)* | 9.9 (8.7)*# | 8.0 (7.5) *# | 4.1 (3.9)*# |
| Total CMAP | Good prognosis | 36.2 (16.2) | 32.8 (15.9)*# | 29.7 (15.4)*# | 25.7 (15.0)*# |
| | Poor prognosis | 30.6 (14.6) | 22.9 (13.8)*# | 16.5 (11.5)*# | 9.1 (8.1)*# |
| Respiratory ALSFRS-R | Good prognosis | 11.6 (1.2)* | 911.3 (1.6)* | 10.8 (2.0)* | 10.5 (2.2)* |
| | Poor prognosis | 10.7 (1.6)* | 9.7 (2.3)*# | 8.4 (3.8)*# | 7.7 (2.1)* |
| Bulbar ALSFRS-R | Good prognosis | 10.5 (2.0)* | 10.0 (2.1)*# | 9.5 (2.8)*# | 9.1 (3.2)*# |
| | Poor prognosis | 8.5 (3.6)* | 6.6 (3.4)*# | 5.5 (3.3)*# | 3.4 (2.1)*# |
| Spinal ALSFRS-R | Good prognosis | 18.7 (5.4) | 16.8 (5.8)# | 15.2 (6.1)*# | 13.2 (6.6)*# |
| | Poor prognosis | 18.0 (6.6) | 14.6 (7.1)# | 10.0 (6.4)*# | 5.6 (5.5)*# |
| Upper limbs MRC | Good prognosis | 67.3 (13.9) | 63.6 (16.2)# | 59.7 (17.6)# | 54.9 (18.2)*# |
| | Poor prognosis | 60.1 (17.5) | 57.8 (17.7)# | 51.8 (18.1)# | 28.9 (27.1)*# |
| Lower limbs MRC | Good prognosis | 59.1 (14.7) | 56 (17.0)# | 52.9 (18.2)*# | 49.4 (19.7)*# |
| | Poor prognosis | 56.8 (13.7) | 50.7 (16.7)# | 45.3 (16.6)*# | 24.9 (23.8)*# |
| Total MRC | Good prognosis | 126.4 (25.3) | 119.7 (28.5)# | 112.6 (31.0)# | 104.3 (32.5)*# |
| | Poor prognosis | 116.8 (27.2) | 108.5 (32.6) | 97.1 (32.6)# | 82.3 (38.0)*# |

***Table 1:*** *Changes of clinical and neurophysiological variables in the "poor" and "good" prognosis ALS patients. The results are expressed as mean and standard deviation.*

** P < 0.05 (Kruskal-Wallis ANOVA) in the comparison between poor and good prognosis;*

*# P < 0.05 (Wilcoxon Signed Ranks Test) in the comparison between the previous levels within groups (poor and good prognosis)*

## Discussion

### Correlation between CMAP and other clinical tools in ALS progression

The phrenic, ulnar and plantar medial CMAPs appear a valid and useful tool for monitoring the progression of the ALS disease. Our results demonstrates that the CMAP from phrenic stimulation is related to ALSFRS-R respiratory subscale, the CMAP from ulnar stimulation

to MRC for upper limbs, and the CMAP from plantar medial stimulation to MRC for lower limbs. In our knowledge, longitudinal studies of CMAP applied on phrenic, upper and lower limbs nerves together, compared to clinical parameters evaluating the disease progression on a so large number of ALS patients, were not published before [7,18]. Monitoring correctly ALS disease progression with a sensitive, accessible and reproducible neurophysiological method is particularly relevant, having the advantage of greater objectivity compared to other tools as the MRC and ALSFRS-R scales [19-23]. We demonstrated that FVC worsened significantly only in the first three months of follow-up in patients with poor prognosis, while the CMAPs worsened significantly in the entire follow-up period, also in the advanced phase of ALS disease. Similarly, the ALSFRS-R respiratory did not worsen significantly in the last period of follow-up in the poor prognosis group, because probably the clinical scales are not sufficiently sensitive in the advanced phases of disease and more dependent on patients. Also, the MRC score, reducing similarly in both groups with poor and good prognosis, could not be able to differentiate timely the two groups with different prognosis because of their low sensitivity.

As previously confirmed, CMAP represents the early loss of MU, considering also that more than 50% of MUs can be lost before strength is reduced [8,9,11,24]. Certainly, methods like MUNE are more sensitive than CMAP but unfortunately, it is not practicable in large clinical centers needing of long time for analysis, and obtainable from only a limited number of muscles, excluding also the diaphragm [21]. Moreover, phrenic CMAP study does not depend on patient strength and collaboration, and therefore it provides objective information also when the spirometry is not possible.

### Clinical and neurophysiological characteristics of the ALS patients with bulbar and spinal onset

Our data have highlighted no significant difference between spinal and bulbar phenotypes in terms of CMAP, resulting this last probably more effective to differentiate better the patients with good and poor prognosis. Similarly to previous data, also in the present study no difference in phrenic CMAP was evidenced between patients with spinal and bulbar onset [13]. The patients with bulbar onset showed a significant decline of FVC values during the 12-month follow up despite of a not essential concomitant decrease of amplitude of phrenic CMAP. In the advanced phase of the disease, ALS patients exhibit rapid declines in pulmonary performances, because the loss of respiratory motor neurons seems too severe for compensation, resulting in inevitable ventilatory failure [25-28]. The phrenic CMAP allows to study selectively the peripheral drive, and not the complex respiratory capacity as FVC does, depending this last not only by both central and peripheral neurological drive control, but also by a possible secondary impairment of pulmonary system and the cooperation of patient [29]. Our analysis confirm conclusions of other studies regarding to changes in the central drive control as pertinent reason for the abnormal respiratory tests in patients with bulbar onset [30]. Moreover, in ALS patients with spinal onset, the ventilatory insufficiency could be closely related to diaphragmatic impairment related to a direct profound degeneration of phrenic motor neurons [12,31-34].

### Clinical and neurophysiological characteristics of the ALS patients with poor prognosis

In our casuistry, death or tracheostomy happened between six and twelve months after the first visit in 18% of patients. In this subgroup of patients, a more rapid progression of parameters such as FVC, ALSFRS-R score, and CMAPs from stimulation of phrenic, ulnar, and medial plantar nerves, was highlighted during the 12 month-follow up. Interestingly at baseline the FVC, but not phrenic CMAP, was significantly lower in patients with poor prognosis. Moreover, only CMAP worsened significantly during the entire follow-up period in both patients with good and poor prognosis, while FVC, though progressively more lower in patients with poor prognosis in all visits, resulted significantly reduced in the entire period of follow-up only in patients with good prognosis. According to this data, the phrenic CMAP should be considered a relevant criterion for prognostic purposes. Probably in our study the different change of FVC and phrenic CMAP in patients with poor prognosis could indicate a different impairment of both central and peripheral neurological drive control. Neurophysiological study of phrenic nerve can contribute to understanding respiratory disorders in ALS when used in combination with conventional tests of pulmonary function, permitting to specify the alteration of the peripheral and central control drive, to predict severe complications, and to plan therapeutic interventions in a hospital or in the patient's home, preventing emergency measures. In this

context, also a small motor response to phrenic nerve stimulation should suggest imminent respiratory failure and the need for a more complete respiratory evaluation. Furthermore, in ALS a complementary CMAP study should be always taken in consideration.

## Conclusion

In conclusion, we confirm that CMAP is a reproducible sensitive marker able to highlight MNs loss and collateral reinnervation in ALS also in a short period of time. The changes in MRC, FVC and ALSFRS-R score resulted correlated, but they provide information indistinctly on damage of first and second motor neuron, central and peripheral nervous system. The CMAP should be used as outcome of progression of the disease in clinical trials, taking advantage of its objectivity, and its ability to prove progression of disease in both patients with poor and good prognosis for a long period of follow-up.

## Acknowledgements

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### Conflicts of Interests

None of the authors have potential conflicts of interest to be disclosed.

### Funding Sources

None.

### Footnotes

Poor prognosis is defined as tracheostomy or death occurred during the 12 months of the study; good prognosis is defined as the absence of death or tracheostomy during the 12 months of the study.

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