Correlations between slow-rate repetitive nerve stimulation and characteristics associated with amyotrophic lateral sclerosis in Chinese patients

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Abstract. [Purpose] To clarify the features associated with decrements in compound muscle action potentials (CMAP) during slow-rate repetitive nerve stimulation (RNS) of muscles involved in amyotrophic lateral sclerosis (ALS) in mainland China. [Subjects and Methods] A retrospective study of decremental responses to slow-rate RNS was performed to compare patients with ALS to those with myasthenia gravis (MG). [Results] A significant decrement (>5%) was observed in at least one muscle in 54% of ALS patients. The trapezius muscle was the most commonly affected (67%). In the ALS group, the CMAP amplitude evoked by the first stimulus was negatively correlated with the CMAP decrement in ulnar but not accessory nerves. Additionally, a positive decrement was associated with disease progression but not gender, age at onset, disease duration, region of onset, ALSFRS-R scores, or ALS diagnostic subgroup in ALS. Furthermore, the incidence of positive decrements and the decremental percentages were significantly higher in myasthenia gravis (MG) than in ALS. [Conclusions] The lower CMAP amplitude by the first RNS stimulus was more likely to induce a positive decrement in the ulnar nerve in ALS patients. The positive decremental responses to RNS observed in ALS indicate the faster progress of the disease, which is helpful for evaluating prognoses.

Key words: Repetitive nerve stimulation, Amyotrophic lateral sclerosis, Prognosis evaluation

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a relentlessly progressive neurodegenerative disorder of unknown etiology that is characterized by selective effects on upper and lower motor neurons1. It is difficult to diagnose ALS because no biological markers are currently available2. Several researchers have described electrophysiological findings that support a diagnosis of ALS. These include studies that have shown decrements in compound muscle action potentials (CMAPs) following repetitive nerve stimulation (RNS) in ALS-affected muscles3–5. RNS was originally described by Harvey et al.6, as a widely-utilized technique for evaluating on the effects of neuromuscular junction defects. RNS is also engaged in routine tests aimed at diagnosing and evaluating patients with myasthenia gravis (MG) and other neuromuscular junction disorders.

In 1959, Mulder et al. first described a decremental response to slow-rate RNS in patients with ALS7. Later, similar reports also demonstrated decremental responses in ALS3–5. Although some studies have been performed to study decremental responses to RNS in ALS patients, the correlations between decrements and the clinical characteristics of ALS patients are not fully understood. Furthermore, no relevant English-language reports describe relationships between ALS and RNS in Chinese populations. This study was performed to investigate the characteristics linked with ALS-associated decrements in
mainland China patients. In addition, we sought to build an experimental foundation with which to evaluate whether RNS-induced CMAP decrements are potential biological markers for diagnosing ALS.

SUBJECTS AND METHODS

A total of 54 ALS patients who were referred to the authors for RNS evaluation at Ren min Hospital of Wuhan University from February 2013 to February 2015 were enrolled. RNS tests were performed on several muscles inpatients suspected of having ALS as part of routine diagnostic procedures in our laboratory. The ALS patients were categorized according to the revised El Escorial criteria\(^8\). Based on these criteria, clinical ALS diagnoses were categorized as clinically suspected, clinically probable, clinically probable laboratory-supported, or clinically definite. The identified ALS patients were then screened for 'mimic syndromes' to exclude any additional pathology that might affect the peripheral nerves, muscles, or neuromuscular junctions.

RNS tests were also performed in 54 MG patients who served as the control group. MG patients were retrospectively enrolled from cases referred to us from February 2013 to February 2015. Adiagnosis of MG was confirmed as a combination of clinical symptoms, a positive anti-acetylcholine antibody test, or reversibility of symptoms with neostigmine\(^9\). Only patients who were examined before any treatments were administered were included. The effects of RNS on the 54 patients with ALS were compared to the effects on MG patients to identify any differences between the effects in CMAP response amplitudes. There was no significant difference in gender or age between the two groups. All subjects provided informed consent to the study design and experimental procedures, and the design of the study was approved by the Ethics Committees of the Ren min Hospital of Wuhan University.

Routine RNS examinations were performed in our laboratory on the following three muscles: abductor pollicis brevis (APB), abductor digitii minimi (ADM) and trapezius (Trap). RNS was performed on the ulnar or median nerve at the wrist by placing surface electrodes over the abductor digitii minimi or abductor pollicis brevis. Additionally, RNS was also performed on the spinal accessory nerve in the posterior triangle of the neck by placing surface electrodes over the trapezius muscle\(^10\). Only patients in whom at least 2 of these three muscles were examined were included. Skin temperature was measured near the examined muscle and maintained above 32 °C during all measurements. Self-adhesive surface electrodes were used to record the belly-tendon CMAP of the muscles. A 3-Hzor 5-Hz train of 10 stimuli was delivered to the nerves, and recordings were obtained using a keypoint workstation (31A06) (Alpine BioMedApS, Denmark). A decrement was defined as a reduction in the peak-to-peak amplitude of the CMAP for the fifth response to the first stimulus. RNS was performed only when no other therapy had been applied to avoid any potential influence of riluzole on decrements. According to the manufacturer and one recent study\(^11\), the symptoms of myasthenia include an adverse reaction to riluzole. Furthermore, all ALS patients underwent routine electromyography (EMG), including routine nerve conduction and needle EMGs to detect 3 or more muscle regions associated with different nerve roots.

In patients with ALS, clinical data were scored using the widely applied revised ALS Functional Rating Scale (ALSFRS-R), in which scores range from 0 to 48. The rate of disease progression was defined using the following equation: disease progression rate\((48-ALSFRS-R\) score\)/duration (months). When the frequency of an incidence was greater than 10\%, it was defined as a definite decrement. Additionally, based on recommendations in the literature\(^12, 13\), a decrement exceeding 5\% was defined as abnormal (borderline decrement). In this study, a borderline decrement (≥5\%) was regarded as a positive CAMP decrement. Descriptive statistics were performed using means and standard deviations. Gender, age at onset, region of onset, diagnostic ALS subgroup and the results for three different muscles (ADM, APB or Trap) were analyzed using \( \chi^2 \) tests to compare the positive decrement (RNS+) and negative decrement (RNS−) groups. Additionally, we examined the impact of the duration of disease, ALSFRS-R scores and the rate of disease progression separately using unpaired t-test in RNS+ and RNS−groups. Spearman’s correlation coefficients were calculated to compare the CMAP amplitudes that were evoked by the first stimulus and the responses of the ulnar and spinal accessory nerves to slow-rate RNS. All results were considered to be significant at \( p<0.05 \). Calculations were performed using SPSS software, version 19 (SPSS Inc., Chicago, IL, USA).

RESULTS

The clinical characteristics of ALS patients were summarized in Table 1. We examined 54 ulnar, 20 median and 38 accessory nerves in 54 patients with ALS. The EMGs of all 54 ALS patients showed extensive neurogenic lesions. There were 29 cases of RNS+ decrement (≥5\%), and of these, 23 cases were definite decrements (≥10\%). We divided the ALS patients into two subgroups: RNS positive decrement and RNS negative decrement, according to decremental percentages of 5\%. The rate of disease progression was statistically faster in the RNS positive decrement subgroup than in the RNS negative decrement subgroup. However, there was no statistical association between the RNS decrement and clinical features of gender, age at onset, disease duration, ALSFRS-R score or region of onset (Table 1).

The incidence of positive decrements was calculated as the ratio between the number of positive decrements and the total number of the group. The relationship between the incidence of positive decrements and diagnostic subgroups is described in Table 2. A positive decrement (≥5\%) was observed in at least one muscle in each of the different diagnostic ALS subgroups as
follows: in 53% of clinically suspected, 57% of clinically possible, 67% of clinically probable laboratory supported, and 57%
of clinically definite cases. There was no significant difference in the incidence of positive decrements between the diagnostic
ALS subgroups according to the R×C χ² test (p=0.217).

The comparison of the frequencies and distributions of decrements in proximal and distal muscles in ALS patients was
carried out. The frequency of positive decrement in each muscle was summarized in Table 3. Both borderline decrements
(>5%) and definite decrements (≥10%) were sorted in the table. In the 54 evaluated cases of ALS, the maximum decremental
percentages in the ulnar, median and accessory nerves were 15.2%, 15.0%, and 35.2%, respectively. Decremental responses
were more frequently observed in proximal muscles (e.g., the trapezius, 67%) than in distal muscles (e.g., ADM, 12%; and
APB, 20%) in ALS patients. Moreover, the decremental responses to RNS were greater in APB (10.3 ± 4%) than in ADM
(8.8 ± 3%).

Table 3 also shows the mean and standard deviations for the decremental percentage in each nerve. We used R ×C
χ² tests to detect the incidence of positive decrements in the three different muscles, and the results were Pearson χ²=35.281,
Asymp.Sig. (2-sided) p<0.001. This indicated that there was a significant difference in the incidence of positive decrements
across the three muscles. The incidence of positive decrements in Trap was higher than the incidence in ADM and APB.

We further explored whether there was a correlation between the decrements and the CMAP amplitudes that were evoked
by the first stimulus in ALS. In the ALS group, there was a negative correlation in ADR (R=−0.484, p<0.001), whereas there
was no relationship in Trap (R=−0.092, p=0.589) (Fig. 1).
Last but not the least, the comparison of the decremental response and CMAP amplitudes in RNS between ALS and MG was conducted. Borderline decrement (>5%) was observed in at least one muscle in 54% of the ALS patients and 81% of the MG patients. The incidence of positive decrements was significantly higher in the MG group than in the ALS group. Table 4 shows the mean and standard deviations for the decremental percentages in the ALS and MG groups. The decremental percentages in the ulnar and accessory nerves were generally higher in the MG groups than in the ALS groups. The frequency of decrements of 10% or more is also shown in Table 4. Even when more conventional criteria (>10% decrement) were applied, a positive decrement was observed in at least one muscle in 43% of ALS patients and 70% of MG patients. This difference was significant.

**DISCUSSION**

In the present study, a decremental response greater than 5% was recorded in 29 (54%) ALS patients, and a decremental response greater than 10% was recorded in 23 (43%) ALS patients. These results are similar to those reported in previous studies of decremental responses to slow-rate RNS in patients with ALS. For example, Denys and Norris reported that 67% of patients showed a decremental response in the thenar muscle\(^{14}\), and James M et al. reported that 52% of patients had borderline decrement (>5%), and 29% of patients had definite decrement (≥10%) during slow-rate stimulation\(^{15}\). Researchers in different regions select different muscles and different criteria for RNS decrements, and this causes the incidence of positive decrements to vary (24–67%). In addition, the maximum decrement percentage was up to 35.2% in Trap in ALS patients.

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**Table 4.** Comparison of RNS decremental response between ALS and MG

| Groups | >5% decrement | ≥10% decrement | Decremental percentage* (%) |
|--------|---------------|---------------|-----------------------------|
|        | ADM           | Del           |                            |
| ALS    | 0.54 (29/54)  | 0.43 (23/54)  | 8.8 ± 3 (5–15.2)           |
| MG     | 0.81 (44/54)  | 0.70 (38/54)  | 29.3 ± 9.2 (5–44.3)        |

RNS: repetitive nerve stimulation; ALS: amyotrophic lateral sclerosis; MG: myasthenia gravis; CMAP: compound muscle action potential; amp: amplitude; Trap: trapezius; ADM: abductor digiti minimi

*Decremental percentage: mean ± standard deviation of the decremental percentage only for patients with a positive (>5%) decrement.
in the present study. The exclusion criteria for a diagnosis of ALS was a decremental percentage in slow-rate RNS that was at most 10% and that could not exceed 20%\(^{15}\). However, recent studies have shown that the decremental percentage can be above 20% in ALS patients, similar to the higher decremental percentages observed in MG\(^{16,17}\). Therefore, a decrement percentages in slow-rate RNS that is greater than 20% should be not regarded as an exclusion criterion for diagnosing ALS.

In the current study, the distributions of decremental responses to ALS included the following: in Trap, 67% were positive decrements, and this was higher than the incidence in distal muscles (ADM, 12%; and APB, 20%). The proximal muscles showed more decrements than the distal muscles, as was previously shown by Tomoko Iwanami et al\(^{18}\). Clinically, the thenar muscles are severely affected in most ALS patients but showed decremental percentages that are lower than those in the proximal muscles in the present and Yamashita’s study\(^{19}\). However, in MG, proximal muscles are more severely affected and show higher decremental percentages than distal muscles\(^{20}\). While these data indicate that the decremental response of thenar muscles is unique to ALS, the reason for this remains unclear. It is probably related to the safety factors associated with different parts of these muscles. Generally, the large and distal limb muscles have higher safety factor and are not subject to show slow-rate decrements in ALS.

Some scholars have proposed that the incidence of positive decrements in RNS in ALS patients with rapidly progressing disease is significantly higher than the incidence in slow progressing patients\(^{21-23}\). Similarly, the present study showed that the rate of disease progression was significantly larger in the RNS positive than in the RNS negative decrement subgroup. Moreover, at a 6 month follow-up, two ALS patients underwent RNS in the present study, and the results showed that in these patients, the CMAP amplitude had decreased with disease progression. Therefore, positive decrements in RNS are an indicator of disease progression and an active period.

The data in this study indicate decremental percentages in RNS are inversely correlated with the CMAP amplitudes evoked by the first stimulus in ADM. This might indicate that the CMAP decrement is more likely to arise from unstable conduction in recently sprouted nerve terminals instead of degenerating axons\(^{17}\). However, in the present study, there was no correlation between the decremental percentage in RNS and the CMAP amplitude evoked by the first stimulus in Trap. This may be partially because the CMAP decrement reflects potential differences in the physics of the two nerves. Baumann et al. demonstrated that the impact of RNS on CMAP amplitudes changed when different nerves were tested in normal subjects\(^{24}\). Several possible factors that might be responsible for changes in CMAP sizes in response to RNS in normal subjects have been proposed, and these include the properties of muscles, changes in neuromuscular transmission and changes in the nerve membrane\(^{19}\). However, more ALS patients are needed to further explore the relationship between RNS decrements and the CMAP amplitudes evoked by the first stimulus.

In the present study, we show that the distribution of decrements is very similar between ALS and MG. Clinically, proximal muscles are more severely engaged in both ALS and MG, which may suggest a lower safety factor for neuromuscular transmission in proximal muscles\(^{25}\). Hence, ALS patients with positive decrements should be distinguished from those with MG. In the present study, for RNS performance, both the incidence of positive decrements and the decremental percentage were greater in the MG than in the ALS group, as previously suggested by Tomoko Iwanami et al.\(^{18}\) This may be because of variation in the participation of different endplates in ALS: some newly re-innervated endplates have low safety factors, while others are normal. Besides, another feature of ALS is that CMAP decrements were more prominent in the APB than the ADM, unlike what is observed in MG. This may reflect the “split-hand” trend in ALS\(^{26,27}\), and our observations further confirm this phenomenon. Moreover, except for their significantly different clinical manifestations on EMG, in ALS, the motor neuron conduction velocity (NCV) is often accompanied by a significant reduction in amplitude, and this is an important difference between ALS and MG\(^{28,29}\).

Mori A et al. proposed that CMAP decrements in the median nerves could be useful, at least when differentiating ALS patients with cervical region onset from controls with active neuropathic diseases\(^{3}\). In the present study, we reveal that the incidence of positive decrements was as high as 43% (definite decrement or≥10%), which generally highlights the characteristics and advantages of RNS in patients with ALS. It is particularly noteworthy that a RNS positive decrement indicates the rapid progression of ALS and may therefore be an indicator of prognosis. Hence, it could be hypothesized that CMAP decrements could be a potential assistant diagnostic index for ALS. Although single-fiber electromyography (SFEMG) is acknowledged as the most sensitive technique for studying neuromuscular transmission defects, this procedure is more invasive, time-consuming, and technically demanding. In contrast, RNS is noninvasive and well tolerated and enables the study of weak muscles and muscles close to the area of maximal weakness\(^{28,30,31}\). Moreover, many primary hospitals are unable to perform SFEMG for technical and financial reasons, whereas RNS is financially and technically feasible and extremely accessible. Because of these advantages, RNS is more suitable for ALS clinical work.

A methodological limitation of the current study is that it is a retrospective study. Data collection may therefore be incomplete. It is also a single-center, small sample study, which can cause bias. Thus, a prospective multicenter study including a
larger population should be performed. Moreover, the present study included a Chinese population, and the results may not be representative of all ALS populations in the world. However, our data at least provide evidence of a relationship between ALS and RNS. Because we documented a high incidence of positive decrements in ALS, RNS can be used as an assistant method to diagnose ALS, as has previously been suggested by many scholars. Further research into the diagnostic role of RNS in ALS would be highly interesting.

In conclusion, decremental responses to RNS were frequent in ALS but not as frequent as in MG. CMAP decrement in RNS may be a potential assistant diagnostic index for ALS. In ALS patients, the lower CMAP amplitude evoked by the first stimulus in RNS is more likely to induce positive decrements in the ulnar nerve. In ALS, a positive decremental response to RNS indicates faster progress of the disease, and this should be helpful when evaluating prognoses.

**Declaration of interest**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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