Reinnervation as measured by the motor unit size index is associated with preservation of muscle strength in amyotrophic lateral sclerosis but not all muscles reinnervate

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Conflict of interest

CN and MW have received honoraria from Hänseler AG, Switzerland, as advisory board members. CN and MW have received advisory board honoraria from Biogen Idec and MW from Merz Pharma, CN from Sanofi Genzyme, Roche and Biogen Switzerland, Switzerland. Dr. Nandedkar is an employee of Natus Medical Inc.

Ethical statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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ABSTRACT

Introduction: The motor unit size index (MUSIX) may provide insight into reinnervation patterns in diseases such as amyotrophic lateral sclerosis (ALS). However, it is not known if MUSIX detects clinically relevant changes in reinnervation, or if all muscles manifest changes in MUSIX in response reinnervation following motor unit loss.

Methods: 57 patients with ALS were assessed at 3-monthly intervals for 12 months in 4 centres. Muscles examined were abductor pollicis brevis, abductor digiti minimi, biceps brachii and tibialis anterior. Results were split into two groups: muscles with increases in MUSIX and those without. Longitudinal changes in MUSIX, motor unit number index (MUNIX), compound muscle action potential (CMAP) amplitude and MRC strength score were investigated.

Results: 133 muscles were examined. 59% of muscles exhibited an increase in MUSIX during the study. Muscles with MUSIX increases lost more motor units (as measured by MUNIX: decline at 12 months -58%, $p<0.001$) than muscles that did not increase MUSIX (MUNIX decline at 12 months -34.6%, $P<0.001$). Despite this, longitudinal changes in muscle strength were similar between the two groups. When motor unit loss was similar, an absence of MUSIX increase was associated with a significantly greater loss of muscle strength ($P = 0.002$).

Discussion: MUSIX increases are associated with greater motor unit loss but relative preservation of muscle strength. Thus, MUSIX appears to be measuring a clinically relevant response that can provide a quantitative outcome measure of reinnervation in clinical trials. Furthermore, MUSIX suggests that reinnervation may play an important role in determining the progression of weakness.

Key words:

Amyotrophic lateral sclerosis, motor unit number index, motor unit size index, reinnervation, strength

INTRODUCTION

In amyotrophic lateral sclerosis (ALS), compensatory reinnervation at the distal motor axon following lower motor neuron (LMN) loss is an important aspect of disease pathophysiology. For example, chronic neurogenic findings on electromyography (EMG) mirroring collateral reinnervation form part of all proposed diagnostic criteria[1-3]. As reinnervation ensures a greater number of muscle fibres continue to be functional, it is considered that reinnervation plays a crucial role in maintaining muscle power and hence function[4]. A quantitative measure of the reinnervation response would therefore be of value to ALS research.

To achieve this, an effective means to quantify the reinnervation is required. While the motor unit number index (MUNIX) technique is an established technique for the quantification of functional motor units in human subjects with diseases such as ALS[5,6], less well studied is the motor unit size index (MUSIX), a quantity derived by the division of the compound muscle action potential (CMAP) amplitude by the MUNIX value. We have previously shown that MUSIX is a reliable biomarker in a healthy volunteer study across a range of commonly examined muscles, highlighting its potential as a biomarker for reinnervation[9]. MUSIX was also recently included as a secondary endpoint in the phase 2 RESCUE-ALS trial[10]. Several reports have documented the more rapid decline in MUNIX relative to CMAP in ALS, an observation attributed to collateral reinnervation partially compensating for CMAP amplitude decay[5,11-13]. However, the relationship between MUSIX and muscle strength is currently unexplored.

The aim of this study was to assess whether increases in MUSIX were associated with clinically detectable preservation of muscle strength. We identified muscles that manifested increases in MUSIX and compared electrophysiological parameters and muscle strength to muscles that did not. We then assessed the effect this had on clinically determined muscle strength.

METHODS

Patients fulfilling possible, probable, probable-laboratory supported and definite categories of the revised El Escorial criteria for ALS were recruited from specialist centres in St. Gallen, Lisbon, Milwaukee and Sheffield. Written consent was provided by all participants and the study protocol was approved by local ethics committees. Exclusion criteria included co-existent conditions that could impact upon the recordings such as carpal tunnel syndrome and peripheral neuropathy. Time from symptom onset, defined as one of weakness, dysarthria, dysphagia, dyspnoea, or gait impairment, had to be <24 months. Subjects were assessed at 3 monthly intervals (+/- 2 weeks).

A detailed description of the MUNIX/MUSIX procedure is previously reported[14]. Briefly, a CMAP was recorded and electrode position optimised to achieve the greatest possible amplitude. A CMAP amplitude greater than 0.5mV at baseline was required for the muscle to be included in the study. Isometric contraction of the muscle under assessment was then performed at varying levels of force. MUNIX/MUSIX recordings were made from the following muscles: abductor pollicis brevis (APB), abductor digiti minimi (ADM), biceps brachii (BB) and tibialis anterior (TA). Recordings were made from a single side, which was studied at all subsequent visits. The clinically stronger side was selected. Alongside MUNIX/MUSIX, manual muscle testing was performed by an experienced neurologist/clinical neurophysiologist using the MRC grading scale.

Muscles were separated into two groups on the basis of their MUSIX change over the course of the study period. In the first group ("MUSIX-UP"), data from muscles demonstrating a >20% increase in MUSIX relative to their baseline (i.e. first) visit at any point during the 12-month follow-up were aggregated. Thus, a muscle with a 20% increase at only one visit across the 12-month period would be counted in the MUSIX-UP group. This threshold was chosen as it is above published values for test-retest variability of MUSIX[9], thus ensuring that MUSIX changes were related to pathological change, rather than inherent variability of the test. The other group ("MUSIX-STABLE") demonstrated ≤20% change in MUSIX across all visits.

To assess change in electrophysiological parameters over time, percentage changes relative to the baseline visit were calculated using a nested model design, in which muscles were nested within participants. Linear mixed-effects models were established to investigate longitudinal changes. Fixed factors were time and MUSIX group (MUSIX-UP or MUSIX-STABLE). P-values of <0.05 were considered significant. Tukey's correction was used to adjust for multiple comparisons between groups of muscles/patients. As in previous studies, standard error of the mean (SEM) was applied for time series data[5,11]. Changes in MRC strength score were investigated using binary logistic mixed-effects models, where the MRC grading scale was dichotomised into no clinically evident weakness (MRC = 5) and clinically detectable weakness (MRC < 5). Individual models were fitted for each subset of patients, again with a nested model design and time as the fixed factor. Patient groups were also added as a fixed factor in models where patients are pooled together. Lasagna plots were used to visualise the longitudinal changes of the distribution of the strength scores[15]. Rates of MRC strength change were calculated as:

$$MRC\ progression\ rate = \frac{(MRC_{baseline} - MRC_{last\ visit})}{Time\ between\ visits\ (months)}$$

A mean MRC progression rate of $\geq$ 0.1 was used to dichotomise fast/slow MRC progression. Categorical analyses were undertaken using Chi-squared test. Analyses were performed using RStudio or GraphPad Prism (version 9).

RESULTS

A total of 57 participants (44 men, 13 women) entered the study with a total of 133 muscles studied longitudinally. Of these, 35 participants reached follow-up month 12 (73 muscles). Mean age was 60 years and there was a slight preponderance of limb onset disease (58%; table 1). The clinical and demographic details of the cohort are provided in table 1. In addition, the breakdown of participants from each centre are presented in supplemental table 1. While the ages of participants varied slightly across centres (P=0.036), other demographics and disease-related measures did not.

Over the course of the study, 59% of muscles (n=78) exhibited a >20% increase in MUSIX over the 12-month study period; these are termed the MUSIX-UP group. Those that did not manifest such an increase are grouped into the MUSIX-STABLE group. Comparison of the baseline electrophysiological parameters of the MUSIX-UP and MUSIX-STABLE groups for each muscle demonstrated no significant differences between the two groups (table 2).

For the whole cohort (i.e. all muscles included in the study), longitudinal analysis demonstrated a decline in the MUNIX (decline at 12 months, -49.2%). This was accompanied by an increase in MUSIX (increase at 12 months, +46.3%) and decreases in CMAP amplitude (decline at 12 months, -37.5%) and muscle strength (decline at 12 months, -22%, figure 1A; the corresponding values are provided in supplemental table 2).

The MUSIX-UP group followed a similar pattern with the MUNIX decreasing by -58% by 12 months and MUSIX increasing by +73.9% (figure 1B, corresponding values provided in supplemental table 3). The MUNIX-STABLE group also demonstrated a decline in MUNIX but to a lesser degree: -34.6.% at 12 months, with a MUSIX change of -0.2% (figure 1C, corresponding figures provided in supplementary table 4).

When analysing changes in muscle strength, the proportion of muscles with no clinical evidence of weakness significantly reduced over time in the whole cohort, as well as in both MUSIX-UP and STABLE groups (figure 1D-F).

Analysis of the presence/absence of MUSIX change for each muscle revealed significantly more ADM muscles were allocated as MUSIX-UP (77%), while more BB muscles were found in MUSIX-STABLE (68%; supplemental figure 1). No significant differences in the proportion of APB and TA muscles allocated to the MUSIX-UP and MUSIX-STABLE groups was observed. The patterns of change in MUNIX, MUSIX, CMAP and MRC score in MUSIX-UP and MUSIX-STABLE groups for individual muscles are shown in supplementary figure 2 and 3. These demonstrate particularly marked MUSIX changes in the hand muscles (APB/ADM) in the MUSIX-UP group. In the MUSIX-STABLE group, the longitudinal changes of both the electrophysiological parameters were broadly similar. In both the MUSIX-UP and MUSIX-STABLE groups, muscle strength appeared relatively more preserved in tibialis anterior and less well preserved in APB (supplemental figure 3).

When longitudinal changes between MUSIX-UP and MUSIX-STABLE groups were directly compared, significantly greater loss of MUNIX was seen in the MUSIX-UP group (figure 2A). However, no significant change in the log odds of having clinically detectable muscle weakness (i.e. MRC score < 5, logit = 0.403, p = 0.816). Thus, it would appear that MUSIX increases were representing a functionally relevant reinnervation response that was maintaining muscle strength.

To further investigate the relationship between reinnervation as measured by MUSIX and the preservation of muscle strength, muscles were stratified into fast and slow progressors on the rate of MRC strength loss (see methods). This was then combined with the MUSIX groups to create two subsets of muscles. One subset demonstrated fast MRC progression but no MUSIX increase, while the second subset manifested slow MRC progression and an increase in MUSIX. The groups “fast MRC progressing MUSIX-STABLE” group (n = 16 muscles) and “slow MRC progressing MUNIX-UP” group (n = 44 muscles) were then compared. Despite markedly different MUSIX changes, the MUNIX

changes of these two groups were very similar (figure 3A). However, even though motor unit loss was similar, MRC strength changes were significantly different (log odds = 2.69, p = 0.002**, figure 3B-C).

DISCUSSION

There are two key observations from the present study. Firstly, MUSIX increases, which we infer to mean physiologically functional reinnervation, were associated with relative preservation of muscle strength, despite prominent motor unit loss in ALS patients. Secondly, not all muscles generated a reinnervation response, despite loss of motor units.

In the context of chronic partial denervation, the reinnervation of muscle fibres via collateral sprouting from survival motor axons, and its effect upon muscle strength has been appreciated for over 70 years[16,17] . The clinical importance of these observations was quickly recognised and studied over several decades in a range of disorders, including ALS[18-23], polio[24,20,25] , spinal muscular atrophy[26] and neuropathies[27]. Our finding of similar strength changes in the MUSIX-UP and MUSIX-STABLE groups, despite significantly different levels of motor unit loss, is thus in agreement with a longstanding literature and indicate that MUSIX is detecting a clinically detectable/relevant reinnervation response.

At a group level, our results demonstrated early changes in both MUNIX and MUSIX. Several studies have documented similar early findings in neurophysiological assessments regarding motor unit number (e.g. using motor unit estimation and MUNIX), as well as motor unit potential parameters and fibre density on needle EMG[11,28-31]. Previous work has also suggested that compensatory reinnervation may not last in ALS[22,32], while animal studies raise the possibility that neuromuscular junction remodelling is a highly dynamic process[33]. MUSIX increases were, on average, maintained in our cohort, although participant attrition raises the possibility that those with falling motor unit sizes could have been the ones to drop out.

Muscles that did not manifest a MUSIX increase, despite motor unit loss measured by MUNIX, were associated with fast, progressive loss of muscle strength. This would suggest that in such muscles, the capacity to reinnervate may be just as important as the degree of motor unit loss. Questions remain on how even the most widely used drugs in ALS, such as riluzole, alter the pathophysiology of the

motor unit. Thus, MUNIX and MUSIX could be valuable biomarkers in phase 1 and 2 clinical trials, providing insights into how new drugs are altering pathophysiology.

The finding of limited reinnervation in around 40% of muscles is interesting and has been previously reported in pathological studies of muscle in ALS. Al-Sarrajet al., found that, while evidence of denervation was present in all samples, only half manifested evidence of reinnervation[34]. This failure of reinnervation is a product of the poor health of the motor neuron pool, distal axon, or both, is presently unknown. Motor units tend only to be reinnervated by overlapping motor units i.e. those arising from the same nerve fascicle[35]. Thus, a rapid or synchronous loss of motor units from a single fascicle may not trigger reinnervation. Our data suggest that the rate of motor unit loss may be a more important factor in determining a widespread reinnervation response in ALS as measured by MUSIX, although further work is required to establish this.

Potential technical issues also need consideration. For example, it could be that limited reinnervation of a small number of units, as might be detected during needle EMG, was being missed by the limited pick-up area of surface EMG recordings (radius <20mm)[36], although concurrent changes in both have been reported previously[37,38]. If so, this would suggest that in the MUSIX-STABLE group, even if reinnervation was occurring, there were insufficient numbers of larger motor unit potentials to alter MUSIX or, more importantly, to maintain strength. To clarify this, future studies might undertake a direct comparison of MUSIX to needle EMG.

A simple question one might ask, is whether there is an absolute threshold of motor neurone loss that is required before a reinnervation response is seen with MUSIX. The present results, and those of previous studies[39], would suggest that this is not the case. For example, in the MUSIX-STABLE group, a 34.6% decrease in MUNIX was seen at 12 months, with no associated MUSIX increase. By contrast, a 36.4% decrease in MUNIX was seen at 6 months in the MUSIX-UP group, this time accompanied by a statistically significant 35% increase in MUSIX. Thus, MUSIX-STABLE muscles were losing sufficient numbers of motor units to trigger reinnervation. Similar baseline electrophysiological characteristics

preclude absolute differences in the size of the motor unit pool at the start of the recording period as an alternative reason for these differing reinnervation responses.

There are several limitations to our study. Only muscles on one side were recorded, the stronger side was selected to try and avoid early drop out of already weak muscles (i.e. an early floor effect). In addition, our cohort comprised a relatively high proportion of bulbar-onset patients (40%). However, previous work has demonstrated that MUNIX decline is evident in the limbs of such patients[5]. Assessment of all four limbs and measurements from other easily accessible muscles may provide further insights into reinnervation patterns and their relationship to disease progression. As with any longitudinal study in ALS, our cohort was subject to attrition and this factor, together with the variability of the MUNIX/MUSIX test, may mean that small effect sizes are missed. In addition, while the MRC muscle strength score remains a mainstay of clinical examination, it is susceptible to inter-rater variation[40]. Although all examiners were experienced physicians, differences in the determination and grading of weakness could have affected the results. Furthermore, the MRC scale is not linear with regard to absolute muscle power, thus subtle changes in strength may be missed. Future work with quantitative measurements of muscles strength, such as handheld dynamometry, may help overcome issues regarding inter-observer variation and provide greater insight into more subtle, earlier changes in muscle strength. Lastly, some authors have suggested that MUNIX is too heavily reliant on CMAP amplitude to be considered a measure of functional motor neurones[41], although this analysis did not take full account of the effects of low contraction data[42]. In our data, the MUNIX declined more than CMAP amplitude in the MUSIX-UP group (table 2), which is consistent with both prior reports and the expected effect of reinnervation[5,11,12,43].

In summary, this study suggests that a robust MUSIX increase is associated with a relative preservation of muscle strength. Thus, MUSIX is measuring a clinically relevant reinnervation effect. However, some muscles did not manifest MUSIX increases, despite significant loss of motor units and muscle strength. As boosting reinnervation in these muscles might provide considerable therapeutic benefit, future

studies to determine the reasons for this apparent failure of reinnervation, and how it may be overcome, are necessary.

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Figure 1. Longitudinal changes in MUNIX, MUSIX and distribution of MRC muscle strength score.

A. Longitudinal changes for all participants showing group level decreases in MUNIX/CMAP, with concomitant increases in MUSIX.

B. Longitudinal changes in the MUSIX-UP group.

C. Longitudinal changes in the MUSIX-STABLE group. Here, MUSIX does not increase and, as expected CMAP and MUNIX are largely identical.

D. Longitudinal changes of the proportion of MRC scores for all participants.

E. Longitudinal changes of the proportion of MRC scores for MUSIX-UP group.

F. Longitudinal changes of the proportion of MRC scores for MUSIX-STABLE group.

Brackets denote SEM. Asterisks denote p-values for MUSIX (upper half) and MUNIX (lower half P value represents MUSIX-UP vs. MUSIX-STABLE. *p<0.05, **p<0.01, ***p<0.001). The term logit refers to the log odds of having an MRC score < 5. See supplemental tables for further details of CMAP and MRC score statistics.

Figure 2. Reinnervation is associated with greater motor unit loss.

A. Significant differences in MUNIX are seen between the MUSIX-UP and MUSIX-STABLE groups.

B. Significant differences in MUSIX are seen between the MUSIX-UP and MUSIX-STABLE groups.

Asterisks denote p-values: *p<0.05, **p<0.01, ***p<0.001.

Figure 3. Lack of MUSIX is associated with a more rapid loss of muscle strength.

A. Muscles in the fast MRC progression MUSIX-STABLE group lose motor units (MUNIX) at a similar rate to the slow MRC progression MUSIX-UP group. Asterisks denote P-values for MUSIX (upper half). *P<0.05, **P<0.01, ***P<0.001.

B. Longitudinal changes of the proportion of MRC scores for slow MRC progression MUSIX-UP group.

C. Longitudinal changes of the proportion of MRC scores for fast MRC progression MUSIX-STABLE group.

Supplementary figure 1.

Distribution of MUSIX change by individual muscles. ADM had significantly more muscles allocated to the MUSIX-UP group, while biceps had significantly more allocated to the MUSIX-STABLE group. No significant difference in the proportion of muscles allocated to the MUSIX-UP and MUSIX-STABLE groups was seen in APB or BB. *p<0.05, **p<0.01.

Supplemental figure 2. Longitudinal data for individual muscles.

A-D. Longitudinal plots for MUNIX/MUSIX/CMAP for individual muscles in the MUSIX-UP group. Particularly prominent MUSIX increases are seen in the hand muscles (APB/ADM).

E-H. Longitudinal plots for MUNIX/MUSIX/CMAP for individual muscles in the MUSIX-STABLE group. The change in parameters was largely consistent across the different muscles.

Supplemental figure 3. Longitudinal MRC score distributions for individual muscles

A-D. Lasagna plots for longitudinal change in the proportion of MRC scores for individual muscles in the MUSIX-UP group.

E-H. Lasagna plots for longitudinal change in the proportion of MRC scores for individual muscles in the MUSIX-STABLE group.

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