Fasciculation analysis reveals a novel parameter that correlates with predicted survival in amyotrophic lateral sclerosis

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Abstract

Introduction: Prognostic uncertainty in amyotrophic lateral sclerosis (ALS) confounds clinical management planning, patient counseling, and trial stratification. Fasciculations are an early clinical hallmark of disease and can be quantified noninvasively. Using an innovative analytical method, we correlated novel fasciculation parameters with a predictive survival model.

Methods: Using high-density surface electromyography, we collected biceps recordings from ALS patients on their first research visit. By accessing an online survival prediction tool, we provided eight clinical and genetic parameters to estimate individual patient survival. Fasciculation analysis was performed using an automated algorithm (Surface Potential Quantification Engine), with a Cox proportional hazards model to calculate hazard ratios.

Results: The median predicted survival for 31 patients was 41 (interquartile range, 31.5-57) months. Univariate hazard ratios were 1.09 (95% confidence interval [CI], 1.03-1.16) for the rate of change of fasciculation frequency (RoCoFF) and 1.10 (95% CI, 1.01-1.19) for the amplitude dispersion rate. Only the RoCoFF remained significant (P = .04) in a multivariate model.

Discussion: Noninvasive measurement of fasciculations at a single time-point could enhance prognostic models in ALS, where higher RoCoFF values indicate shorter survival.

KEYWORDS
amyotrophic lateral sclerosis, biomarker, fasciculation, high-density surface EMG, survival

1 | INTRODUCTION

Amyotrophic lateral sclerosis (ALS) carries a median survival of 3 to 5 years from symptom onset.1 Amidst significant phenotypic heterogeneity,2 it is challenging to predict individual disease trajectories at diagnosis. An ability to do so would facilitate clinical management planning, patient counseling, and patient stratification in clinical trials.3

The European Network for the Cure of ALS (ENCALS) survival prediction tool is the most comprehensive method to predict an individual...
composite survival endpoint, having been devised and tested in 11,475 patients.\textsuperscript{3,5} It demonstrated predictive accuracy of 78%, requiring eight input parameters that are available near diagnosis.

Several groups have explored the use of biofluid,\textsuperscript{6} neuroimaging,\textsuperscript{7} and neurophysiological\textsuperscript{8} biomarkers as prognostic aids, and it has been suggested that fasciculation potentials on electromyography (EMG) could represent a novel prognostic marker.\textsuperscript{9,10} The Surface Potential Quantification Engine (SPIQE) provides automated analysis of fasciculations captured by high-density surface electromyography (HDSEMG),\textsuperscript{11} a noninvasive method of measuring fasciculation potentials. We postulated that fasciculation frequency rises while muscles remain strong due to increasing hyperexcitability of motor units, before falling in weak muscles secondary to overwhelming motor unit loss.\textsuperscript{12} We also showed that the variability of fasciculation amplitudes steadily increased over time in ALS biceps muscles, reflecting compensatory reinnervation of motor units.

In this study we set out to establish the potential value of these fasciculation measures in predicting survival in ALS.

2 | METHODS

2.1 | Patient recruitment

Ethics approval was obtained (15/NS/0103, 17/EM/0221). Patients with ALS were recruited from the King's College Hospital Motor Nerve Clinic between January 2016 to February 2019 and provided informed written consent before participation. Each patient was diagnosed with probable or definite ALS using the revised El Escorial Criteria.\textsuperscript{13}

2.2 | Data collection and processing

2.2.1 | ENCA LS survival prediction tool

We obtained access to the online ENCA LS survival prediction tool.\textsuperscript{5} Eight clinical and genetic parameters were collected from patient interview or medical notes: site of onset (bulbar vs nonbulbar); age at symptom onset; definite vs probable/possible ALS; diagnostic delay; forced vital capacity (FVC); ALS Functional Rating Scale—Revised (ALSFRS-R) progression rate; presence of frontotemporal dementia; and presence of C9orf72 repeat expansion. The tool produced a predicted median composite survival endpoint (death, tracheostomy, or >23 hours per day of noninvasive ventilation) from time of symptom onset for each patient. These were categorized into survival groups with distinct median predicted survival (in months): very short (17.7); short (25.3); intermediate (32.2); long (43.7); and very long (91.0).

2.2.2 | HDSEMG recordings

At each assessment, 30-minute HDSEMG recordings were taken from biceps brachii (24 bilateral, 7 unilateral). The HDSEMG data collection methods have been reported elsewhere in detail.\textsuperscript{31} Briefly, patients relaxed with forearms prone and elbow angle 90° to 120°. Skin was prepared with lightly abrasive gel and an alcohol wipe. The sensor had 64 circular electrodes (8 × 8 grid) and signals were amplified by an EMG recording system (Refa-64; TMS International BV, Zevenhuizen, The Netherlands). Description and validation of SPIQE's analytical pipeline have been reported elsewhere.\textsuperscript{11} Biceps power scores were rated according to the Medical Research Council (MRC) scale.\textsuperscript{14} All assessments took place at the Academic Neuroscience Centre, King's College Hospital, London, UK.

2.3 | Derivation of rate of change of fasciculation frequency and amplitude dispersion rate

In strong muscles, rate of change of fasciculation frequency (RoCoFF) was calculated by dividing the recorded fasciculation frequency by the time since symptom onset. In weak muscles (MRC <5; 17 of 55 recordings), we predicted the peak fasciculation frequency using previously estimated linear relationships between MRC power score, fasciculation frequency, and time since symptom onset.\textsuperscript{12} Amplitude dispersion rate (ADR) was calculated by dividing the fasciculation amplitude interquartile range (IQR) by time since symptom onset. Calculation of RoCoFF and ADR is demonstrated in Figure 1A,B. Whenever bilateral recordings were available (24 of 31), the maximum RoCoFF and ADR values were chosen, given the asymmetric nature of limb fasciculations in ALS. Extreme RoCoFF and ADR values above 20 (7 of 55 and 1 of 55 values, respectively) were given the value 20 to limit outlier.

2.4 | Computation and statistical analysis

All computation of HDSEMG data was performed in MATLAB (R2014a) using specifically designed scripts. Statistical tests were performed using Prism version 7.0a (GraphPad, Inc, La Jolla, California) or R version 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria). A Cox proportional hazards model was employed in R using the coxph function (survival package). The multivariate model was:

\[
mdl \leftarrow \text{coxph(Surv}(s,e) \sim m + r + a) \]

where Surv created a survival object, s was the predicted survival, e was event type (no censoring was required in this predictive model), m was months since symptom onset, r was the RoCoFF value, and a was the ADR value. Use of the cox.zph function confirmed that the model satisfied the assumption of constant proportionality.

3 | RESULTS

3.1 | Prognostication model

In total, 31 patients were recruited. The eight input parameters for all 31 patients are summarized in Table 1. Ten patients had no ALSFRS-R recorded at diagnosis, and therefore this was recorded at the time of
HDSEMG recording (median, 8 months after diagnosis; IQR, 4-11 months; n = 10). For patients (n = 6) that had their first forced vital capacity (FVC) measurement at least 6 months after diagnosis, FVC at time of diagnosis was estimated using the assumptions that FVC at time of symptom onset would have been 100%, and that there is a linear relationship between time and deterioration of FVC. Median predicted survival was 41 (IQR, 31.5-57) months, categorized into the following survival groups: very short (n = 1); short (4); intermediate (9); long (8); and very long (9).

3.2 | Prognostic ability of RoCoFF and ADR on predicted survival

Patients were assessed with HDSEMG at a median of 11.5 months after diagnosis (IQR, 3-15; n = 31). A total of 55 biceps recordings were analyzed (24 bilateral, 7 unilateral). The median fasciculation frequency was 49.1/min (IQR, 23.4-89.1), median amplitude dispersion 30.9 μV (IQR, 13.9-52.5), and median months since symptom onset...
The median RoCoFF was 4.8/minute per month (IQR, 2.0-8.4) and the median ADR was 2.0 μV/month (IQR, 0.9-2.9). Cox regression curves of predicted survival are shown in Figure 1C,D. Univariate hazard ratios were 1.09 (95% CI, 1.03-1.16) for RoCoFF, 1.10 (95% CI, 1.01-1.19) for ADR and 0.95 (95% CI, 0.91-0.98) for time since symptom onset. Only time since symptom onset (P = .01) and RoCoFF (P = .04) remained significant in the multivariate model.

**4 | DISCUSSION**

Our findings suggest that fasciculation analysis at a single time-point soon after diagnosis reveals a parameter that correlates with predicted survival in ALS. We have devised and tested two new fasciculation parameters, which assume linear rates of change since symptom onset. Higher RoCoFF values (but not ADR) independently predicted a worse prognosis, representing the underlying pathophysiological process of motor unit hyperexcitability. This is consistent with a previous report of the prognostic potential of multiplet discharges, another marker of motor unit hyperexcitability. The derivation of RoCoFF and ADR share similarities with the calculation of disease progression (ALSFRS-R slope) used in the validated prognostic model. Due to the inclusion of a time denominator in the calculations, we adjusted for this in the multivariate proportional hazards model. As expected, patients with a longer duration since symptom onset had an improved prognosis (hazard ratio, 0.95). Upon accounting for this, ADR did not significantly correlate with predicted survival, but RoCoFF remained significant (hazard ratio, 1.09).

We recognize a number of limitations. We compiled clinical and genetic parameters retrospectively, leading to missing data. Sixty-five percent of patients were not tested for C9orf72 and therefore assumed to be negative; however, in the original sensitivity analyses, the survival prediction model provided accurate predictions even in the absence of patient-level information on genetic markers. We also note this cohort was skewed toward a better prognosis than average. Thirty-two percent of patients did not have ALSFRS-R recorded at the time of diagnosis but rather at the time of first HDSEMG recording, at an average of 8 months after diagnosis. This may have falsely enhanced the online tool's predictive ability. As only 10 patients (32%) had HDSEMG recordings within 4 months of diagnosis, future studies should be designed to take measurements prospectively as soon after diagnosis as possible. It would also be optimal to correlate these fasciculation parameters with observed survival endpoints rather than predicted ones.

It is likely that a multimodal approach will be required to predict an individual's disease trajectory with the greatest accuracy, including clinical, genetic, biofluid, neuroimaging, and neurophysiological parameters. HDSEMG is advantageous as it is noninvasive and well tolerated by patients. The automated analytical tool used in this study (SPIQE) minimizes the degree of expert interpretation, making it suitable for large-scale analysis. It also provides a functional assay of motor neurons in different anatomical areas, meaning that combined scores across the body may increase the prognostic value of the test, including muscles known to be affected relatively early in ALS, such as the first dorsal interosseous and tibialis anterior.

In conclusion, we have highlighted the potential utility of fasciculation measurements to aid prognostic modeling in ALS. This noninvasive technique avoids undue distress for patients allowing for future application across multiple muscles, and allows measurement at a single time-point, which would be most readily translatable to a clinical setting. We anticipate that the quantification of fasciculations in this way may complement prognostication models as part of a multimodal approach.

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**CONFLICT OF INTEREST**
The authors declare no potential conflicts of interest.

**ETHICAL PUBLICATION 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.

**DATA AVAILABILITY STATEMENT**
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Motor axonal neuropathy associated with GNE mutations

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Abstract

Background: Mutations in the GNE gene have been so far described as predominantly associated with distal lower-limb myopathies. Recent reports describe mutations in this gene in patients with peripheral neuropathy and motor neuron disease.

Methods: We describe three patients displaying motor neuropathy in association with GNE mutations. Clinical, electrophysiological, imaging, pathological, and genetic data are presented in a retrospective manner.

Results: The three patients had different phenotypes, ranging from mildly progressive lower limb weakness to a rapidly progressive 4-limb weakness. Genetic testing revealed GNE gene mutations in all patients; of those mutations, p.(His186Arg) has not been previously reported. All patients showed evidence of axonal motor nerve involvement on electrodiagnostic examination and/or muscle biopsy.

Abbreviations: ALS, amyotrophic lateral sclerosis; CK, creatine kinase; CMAP, compound muscle action potential; CMT, Charcot-Marie-Tooth; CT, computed tomography; EDX, electrodiagnostic; EMG, electromyography; hIBM2, hereditary inclusion body myopathy type 2; MAF, minor allele frequency; MRI, magnetic resonance imaging; MUAP, motor unit action potential; NA, not available; NGS, next generation sequencing; OMIM, Online Mendelian Inheritance in Man; STIR, short tau inversion recovery.