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Multi-dimensional electrical impedance myography of the tongue as a potential biomarker for amyotrophic lateral sclerosis

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Abstract

Objective

In amyotrophic lateral sclerosis (ALS) bulbar disease biomarkers are lacking. We evaluated a novel tongue electrical impedance myography (EIM) system, utilising both 2D and 3D electrode configurations for detection of tongue pathology.

Methods

Longitudinal multi-frequency phase angle spectra were recorded from 41 patients with ALS (baseline, 3 and 6 months) and 30 healthy volunteers (baseline and 6 months). . ALS functional rating scale-revised (ALSFRS-R) data and quantitative tongue strength measurements were collected. EIM data were analysed for reliability (intra-class correlation coefficient; ICC) and differences between patients and volunteers ascertained using both univariate (Mann-Whitney U test) and multivariate techniques (feature selection and L2 norm).

Results

The device produced highly reliable data (pooled ICC: 0.836). Significant EIM differences were apparent between ALS patients and healthy volunteers ($P<0.001$). EIM data demonstrated a significant relationship to tongue strength and bulbar ALSFRS-R scores ($P<0.015$). The EIM recordings revealed a group level longitudinal change over 6 months and consistently identified patients in whom symptoms or tongue strength changed.

Conclusions

The novel EIM tongue system produces reliable data and can differentiate between healthy muscle and ALS-related disease.

Significance

Tongue EIM utilising multiple frequencies and electrode configurations has potential as a bulbar disease biomarker in ALS.

Highlights

Novel electrical impedance myography system uses electrodes on both surfaces of the tongue.

The device produces reliable data, even in inexperienced hands.

The results differentiate between healthy and ALS muscle and reveal change over time.

Keywords

Amyotrophic lateral sclerosis, electrical impedance myography, motor neuron

1. Introduction

Clinical practice and research in amyotrophic lateral sclerosis (ALS) is hampered by the limitations of available disease biomarkers, with bulbar biomarkers particularly scarce (Kiernan et al. , 2011, Simon et al. , 2014, Benatar et al. , 2016). Bulbar-onset ALS makes up approximately 25% of the total caseload and nearly all patients exhibit bulbar impairment during their disease course (Haverkamp et al. , 1995). Impaired bulbar function significantly impacts on quality of life and is associated with a poor prognosis(del Aguila et al. , 2003), making new tools to identify and monitor bulbar disease highly desirable (Chiò et al. , 2009, Fujimura-Kiyono et al. , 2011). Despite this need there are few methods offering an objective measurement of bulbar disease. Quantitative tongue strength assessments have found reduced tongue strength in ALS (Weikamp et al. , 2012), but such tests are highly dependent on patient effort. The standard means of assessing ALS disease progression, the ALS functional rating scale-revised (ALSFRS-R), comprises a bulbar sub-score, which is subjective and may be affected by symptomatic interventions (Giess et al. , 2000, Pinto et al. , 2017). Imaging modalities have shown promise, particularly ultrasound through detection of fasciculations (Misawa et al. , 2011). Changes in tongue thickness have also been reported, with early evidence to suggest a relationship with functional measures (Nakamori et al. , 2016); the test is relatively easy to perform, although further work is required to standardise the methodology.

Electrical impedance-based approaches, pioneered by Dr S Rutkove and termed electrical impedance myography (EIM), show enormous promise as a potential biomarker for ALS and other neuromuscular conditions (Rutkove et al. , 2014, Sanchez et al. , 2017b, Mul et al. , 2018, Shefner et al. , 2018, Kapur et al. , 2019). Using surface electrodes placed on a muscle of interest, low intensity alternating current (AC) is applied across a range of frequencies and the resulting surface voltages measured. From this, a transfer impedance is derived comprising resistive and reactive (capacitive) components that can provide information on muscle structure. For example, changes to myocyte size and fat content that occur after denervation alter the way current passes through and is stored by the tissue (Sanchez et al. , 2017a). As the amplitude of the applied AC is below the threshold for depolarisation of nerve and muscle, the technique is painless and non-invasive. The approach is simple to use and has been applied to bulbar ALS via recordings on the tongue; however, the optimal configuration of electrodes and input frequencies are unknown (Shellikeri et al. , 2015, McIlduff et al. , 2016, Pacheck et al. , 2016, McIlduff et al. , 2017).

The tongue has arguably the most complicated muscle fibre arrangement of any muscle in the human body, with fibres running in longitudinal, transverse and vertical alignments (Gilbert et al. , 2005, Gaige et al. , 2007). Previous electrical impedance work in both muscle (Narayanaswami et al. , 2012,

Rutkove et al. , 2014, Schwartz et al. , 2016) and mucosal surfaces (Brown et al. , 2000, Murdoch et al. , 2013, Tidy et al. , 2013) has utilised 2D planar electrode arrays. Given the unique myoarchitecture of the tongue, we hypothesised that a multi-dimensional approach to recording, comprising both 3D (i.e. electrodes on both the superior and inferior tongue surfaces) and 2D (all electrodes on one surface) recording arrangements would be needed to provide a sensitive assessment of disease status. In order to test this, we developed a novel EIM device capable of multi-dimensional electrode configurations. We assessed the reliability of the device, its ability to detect ALS tongue pathology and disease progression, and the correlation to symptom questionnaire scores and tongue strength.

2. Methods

2.1 Participant recruitment

We recruited patients with a diagnosis of ALS from the Sheffield ALS clinic between 2015 and 2018. All patients were required to meet the minimum diagnostic category of the Awaji-Shima criteria for ALS (de Carvalho et al. , 2008). We included both patients with and without clinical and/or EMG (via the Awaji-Shima criteria) evidence of lower motor neurone (LMN) bulbar dysfunction. Healthy volunteers with no history of neurological illness were recruited. Exclusion criteria for both groups included a history of oral malignancy, previous tongue biopsy, concurrent oral ulceration/infection, and active implanted electrical medical devices such as pacemakers, vagal nerve stimulators, and diaphragmatic pacing devices. Informed consent was obtained from all participants and the study was approved by a local research ethics committee (reference 15/YH/0121).

2.2 Probe design

A bespoke, handheld bioimpedance device was designed and constructed specifically for use on the tongue (figure 1). At the tip of the tweezer-like design a stop prevented undue compression of the tongue and defined the inter-electrode plate distance (figure 1). Four gold electrodes arranged in a square with an inter-electrode distance of 5 mm on both the upper and lower electrode plates. The plate separation distance was 7 mm, which was arrived at through the testing of 3D printed models on the participants of the study, assessing for comfort and electrode plate contact. Development was undertaken by TJH and JJPA. The physical compliance of the tongue muscle enables the instrument to investigate a fixed volume of tissue, reducing possible sources of inter-subject variation. Eight electrode configurations were chosen in order to study tissue in both 2-dimensional and 3-dimensional arrangements (figure 1). A further four were initially utilised but dropped from analysis as recordings were not reproducible (supplementary figure 1). A sub-sensory threshold sinusoidal

current of 5 μA root mean square was injected across 14 frequencies, starting at 76 Hz and then doubling with each step increase, to a maximum of 625000 Hz.

2.3 EIM recording procedure

Recordings were performed with the participants sitting upright and the probe placed centrally on the tongue. Recording time was 10 – 20 seconds. At completion of the recording, the real and imaginary components of bioimpedance for each electrode configuration were visually inspected on custom written software. If unusable data were observed on inspection of the spectra due to, for example, patient movement during recording, the recording was re-attempted, providing the patient was happy to proceed. In contrast to previous studies (Shellikeri et al. , 2015), initial recordings of satisfactory quality were not discarded. Each recording session included 2 separate measurements, made 5-10 minutes apart. EIM recordings were undertaken initially by JJPA (a consultant clinical neurophysiologist, total recordings = 185). Approximately 6 months into the study JJPA was joined in recording by SJF and, subsequently, HEM (medical students, 35 and 55 recordings, respectively), neither of whom had any prior experience of ALS patients, clinical research or electrophysiological recordings. Their training consisted of watching JJPA complete one recording and brief instruction on the recognition of poor-quality data (e.g. negative real part, see below). They then performed examinations unsupervised and all their data were included in the analysis. Patient participants underwent recordings every 3 months for 6 months, healthy volunteers underwent two recordings at baseline and 6 months. Before and after each recording the re-usable probe was cleaned using the Tristel Wipes, a system used in clinical environments for flexible endoscopes and ultrasound probes.

2.4 Non-impedance data capture

Clinical examination was documented by an experienced clinician (JJPA, PJS or CJM). ALSFRS-R data were collected, as were the results of the most recent EMG. Following EIM recordings, evaluation of tongue strength was undertaken with quantitative muscle testing (QMT) system (Averil Medical), coupled to the Iowa Oral Performance Instrument (IOPI)(Easterling et al. , 2013, Shellikeri et al. , 2015). Participants were asked to maximally push the small rubber balloon of the IOPI against their hard palate for 10 seconds, with the maximum force recorded (Newtons, N). Two trials were conducted with a short rest period between measurements; the trial with the highest recorded value was used for analysis.

2.5 Statistical analyses

Following data download, real and imaginary components of complex impedance were exported and phase angle ($\mathrm{PhA} = \arctan2(\mathrm{X}, \mathrm{R})$, and magnitude $|Z| = \sqrt{R^2 + X^2}$ calculated by custom software. These data were then analysed using either IBM SPSS statistics (version 24), GraphPad (version 8), or custom code in Python. Prior to analyses, any recordings comprising negative resistivity (real part) values at any frequency were excluded (this likely indicates poor electrode contact). Thus, a single negative value at any frequency resulted in all data for that spectra being excluded. A Root Mean Squared Standard Deviation-based outlier detection algorithm was then used to remove spurious data, e.g. due to movement (see supplementary methods). As two recordings were available for analysis, if an initial recording was removed by the post hoc quality control, the second was used in its place. If both runs were available, the first was used as default. If no data were available, then that electrode configuration (for that particular recording) was excluded from the analysis, in keeping with previous studies (Shefner et al. , 2018). Data imputation was not used. As our impedance data are non-normally distributed, univariate comparisons of phase angle between patients and volunteers were undertaken with Mann-Whitney U testing, using the Benjamini-Hochberg false discovery rate (FDR) correction for multiple comparisons (Q-value of 0.05). Analysis of group demographics were undertaken using t-tests and Fisher's exact test, as appropriate. Intra-class correlation coefficient (ICC) analysis was undertaken using a single measure, two-way random effects model. Presented reference lines correspond to guidance for determining ICC performance (Cicchetti, 1994): Poor <0.4; Fair 0.4-0.59; Good 0.6-0.74; Excellent 0.75+.

Multivariate analyses were undertaken using custom code written in Python (see supplementary methods). Robust scaling was used in order to standardise the contributions of data from each input frequency. The spectral features most useful in both identifying disease (by comparing patients and volunteers) and in detecting longitudinal change (by comparing baseline and 6-month data) were identified using a wrapper algorithm with an exhaustive search approach (Guyon et al. , 2003) and 3-nearest neighbour and 4-fold cross-validation (supplementary methods). Pareto ranking was subsequently undertaken to identify the best feature (input frequency) combination. In order to assess the performance of individual electrode configurations, sensitivity, specificity and the area under the receiver operating curve (AUROC) were calculated.

To facilitate exploration of the relationship between tongue EIM and bulbar symptoms/tongue strength, and longitudinal EIM analysis, we reduced the features identified in the wrapper analyses to a single value, the L2 norm (supplementary methods). This is a commonly applied mathematical function useful for normalising observations and represents the distance of the vector coordinate from the origin of the vector space. It was calculated for each electrode configuration and applied to symptom/strength correlation using Pearson correlation, as it is normally distributed. P values were

corrected for multiple comparisons using the Benjamini-Hochberg false discovery rate (FDR) correction.

For longitudinal EIM analysis in the patient group (baseline, 3 months, 6 months) the L2 norm for each electrode configuration, ALSFRS-R bulbar subscore and tongue strength data were first normalised to the baseline visit. Percentage change over time for EIM data, ALSFRS-R bulbar subscore and tongue strength were analysed with a mixed model using a compound symmetry covariance matrix, fitted using Restricted Maximum Likelihood and a Geisser-Greenhouse correction. Tukey's correction for multiple comparisons was used. For healthy volunteers, 2 visits (baseline and 6 months) were available, this analysis was performed using paired t-tests. The standardised response mean (SRM; mean of paired scores change over time/standard deviation of the measured change), was used to assess outcome responsiveness between baseline and 6-month time-points. Longitudinal change at an individual patient level was assessed by using the maximum healthy volunteer L2 norm as a threshold for disease-related change in the patient group.

3. Results

3.1 Clinical characteristics of the study subjects

41 ALS patients (22 men, 19 women; mean age 62) and 30 healthy volunteers (14 men, 16 women; mean age 56) were recruited (figure 2). At baseline, 3 patients were unable to perform the EIM recordings: 2 had advanced/end-stage disease and complete tongue paresis, 1 was unable to tolerate removal of intermittent ventilatory support for enough time to allow recording. All volunteers were able to undertake the recordings successfully. The groups were matched for age and gender (table 1). 17 patients had bulbar-onset disease, 24 had limb-onset disease. Median disease duration was 24 months (range 5 to 204). At the time of recruitment, the Awaji-Shima diagnostic criteria classified 10 patients as definite, 17 as probable, and 14 as possible. At the end of the 6-month follow up period all participants continued to have a clinical diagnosis of ALS. A total of 30 patients were determined to have clinical evidence of LMN bulbar disease at their baseline visit, as judged by the presence of tongue wasting, fasciculations or EMG abnormalities fulfilling the Awaji-Shima criteria. The average ALSFRS-R bulbar sub-score at the time of recruitment was 8. Average tongue strength was significantly lower in the patient group, compared to healthy volunteers (8.7 N vs. 16.2 N, $P<0.0001$).

Measurement	Patients N=41	Healthy volunteers N=30	P value (Statistical test)
Mean age in years (range)	62 (30-83)	56 (22-86)	P=0.12 (t-test)
Male: Female, n (%)	22:19 (54:46)	14:16 (47:53)	P=0.63 (Fisher's test)
Site of disease onset, n (%)	Limb: 24 (59%) Bulbar: 17 (41%)		
Median symptom duration in months (range)	24 (5-204)		
Awaji-Shima classification at time of enrolment, n	Possible: 14 Probable: 17 Definite: 10		
Clinical examination, n (%): Dysarthria Tongue atrophy Tongue fasciculations Normal	28 (68%) 21 (51%) 28 (68%) 11 (27%)		
Clinical evidence of LMN tongue pathology, n (%)	30 (73%)		
Mean total ALSFRS-R score (range)	31 (4-45)		
Mean bulbar ALSFRS-R sub-score	8 (0-12)		
Maximum tongue strength in N (SD)	8.7 (4.8)	16.2 (2.4)	P<0.0001 (unpaired t-test)

Table 1. Demographic, clinical, electrophysiological and tongue strength characteristics.

3.2 Reliability of EIM recordings

Intra-class correlation coefficients (ICCs) were calculated across participant groups, examiners, and electrode configurations. Using all available recordings, the overall ICC was 0.836, with both 3D and 2D electrode configurations demonstrating similar test-retest reliability (ICC: 0.86 and 0.81, respectively; figure 3). The patient group demonstrated a slightly better performance (ICC: 0.885, vs. ICC: 0.788 for healthy volunteers). Test-retest reliability was consistent across visits in both groups (baseline patient ICC: 0.877, 3-month patient ICC: 0.855, 6-month patient ICC: 0.896; baseline healthy volunteer ICC: 0.774, 6-month healthy volunteer ICC: 0.805). The inter-rater ICC was 0.788, with intra-rater reliability slightly higher at 0.854. The experience of the examiner in clinical research and neurophysiological recordings did not appear to impact on reliability, with both experienced and inexperienced examiners achieving similar ICC values (figure 3). To complement the ICC analysis,

coefficient of variation was calculated, and Bland-Altman plots were constructed, the latter demonstrating high agreement and a mean difference (indicating the estimation of systemic bias in the recordings) near zero (supplementary table 1 and supplementary figure 2).

3.3 Comparison of EIM between ALS patients and healthy volunteers

Comparison of ALS patients and healthy volunteers at baseline revealed significant differences across all electrode configurations and at multiple frequencies, with a clear trend towards improved discrimination at the higher frequency range (figure 4; a phase angle plot for each electrode configuration can be found in supplemental figure 3). To further evaluate the performance of different electrode configurations, frequency-based feature selection was performed using the wrapper algorithm, followed by pareto ranking to identify the best frequency combination for each electrode configuration. The performance of each configuration is shown using AUROC, sensitivity and specificity (figure 4; individual ROCs with 95% confidence intervals shown in supplementary figure 4). A strong discrimination performance was seen across both 2D and 3D electrode configurations with the top performing arrangements being 3D.

3.4 Relationship of tongue EIM to bulbar symptoms and tongue strength

For each electrode configuration, the correlation of the EIM data to the ALSFRS-R bulbar subscore and tongue strength was assessed (figure 5 and table 2). A significant linear correlation between tongue EIM and bulbar ALSFRS-R sub-score was observed in three of the four 3D electrode configurations and two of the four 2D arrangements. Similar data were also obtained for each of the ALSFRS-R subscore components (supplemental figure 5 and supplemental table 2). A significant linear correlation was also observed for tongue strength (figure 5 and table 2). A potential relationship between EIM data and age was also examined using healthy volunteer data and the L2 norm (supplemental figure 5). A comparison of EIM data in healthy volunteers aged <55 and ≥55 years also failed to indicate any significant age effect (supplemental figure 6).

Electrode configuration	ALFRS-R bulbar subscore		Tongue Strength	
	r	P	r	P
3D:1	-0.133	0.361	-0.068	0.653
3D:2	**-0.519**	**0.000**	**-0.397**	**0.004**
3D:3	**-0.313**	**0.014**	**-0.342**	**0.008**
3D: 4	**-0.526**	**0.000**	**-0.454**	**0.000**
2D: 1	-0.175	0.163	-0.037	0.770
2D: 2	**-0.464**	**0.000**	**-0.403**	**0.001**
2D:3	-0.075	0.654	-0.202	0.224
2D:4	**0.434**	**0.003**	-0.044	0.077

Table 2. Summary table for the correlation analyses between EIM data (using the L2 norm) and bulbar symptoms/tongue strength for all electrode configurations. The values meeting the FDR-adjusted P value (P= 0.014) are show in bold/shaded.

3.5 Longitudinal changes

We first undertook a group level analysis to ascertain the change over time relative to the baseline visit by taking an average of all the electrode configuration data. We found a significant change in EIM phase angle at a group level in the patients (figure 6; P=0.04 between month 0 and month 3, 95% confidence interval = -70.75 to -0.13 and P=0.002 between month 0 and month 6, 95% confidence interval -149.9 to -10.64) but not in healthy volunteers (P=0.14, 95% confidence interval = -3.63 to 24.33). No significant change occurred in the ALSFRS-R bulbar subscore over the 6-month study timeframe (p=0.16). Tongue strength in patients demonstrated a non-significant group level reduction (P=0.05), there was no change in healthy volunteers (P=0.7).

We next explored the utility of the tongue EIM system in identifying change at an individual patient level (figure 6 D &E). Using the maximum change in the volunteer group as the threshold for disease-

related progression, the EIM data consistently identified patients displaying marked deterioration in bulbar symptoms or tongue strength (table 3). One patient with a 4-point change in bulbar ALS-FRS was not identified in this analysis; however, this patient had deteriorated to the point that EIM recording was not possible.

Patient	ALSFRS-R bulbar subscore change	% change in tongue strength	% change in L2 norm
1	-1	+12	77.9
3	+1	-38	82.7
6	-1	-33	15.6
8	-1	-13	71.9
16	+2	-43	33
19	+2	-13	203
20	+1	-13	19
21	-5	-51	195.4
24	-1	-58	304
30	-2	-5	26.6
31	-1	-9	28.5
32	-3	-36	23.6
36	-4	-45	123.7
39	-2	-76	213.2
Patients not identified with ≥2 point change in ALSFRS-R		**Patients not identified with ≥10% tongue strength drop**	
n=1 (-4; severe tongue atrophy/paresis prevented EIS recording)		n=4 (changes: -14%, -15%, -24%, -25%)	

Table 3. Change in bulbar symptoms and tongue strength for patients in which a change in EIM was observed.

4. Conclusion

In this study we have successfully tested a novel EIM system for evaluation of tongue muscle in ALS. The results show that our device is reliable, even in inexperienced hands, and highly sensitive in detecting ALS-related tongue pathology. The ease of recording makes the system suitable for further investigation.

Our data suggest that interrogation of muscle in multiple tissue planes holds promise as means to maximise the utility of EIM. Despite a heterogenous patient group the EIM data could distinguish between patients and healthy volunteers. The pioneering work of the Rutkove group has shown the immense potential of EIM as a biomarker in ALS (Vucic et al. , 2018). So far, these studies have utilised 2D electrode configurations in which all electrodes are on the same surface, with some studies rotating electrodes, or using arrays which permit the flow of current in more than one direction (Garmirian et al. , 2009, Shefner et al. , 2018). Notwithstanding such differences, as well as variations in electrode size and composition, the studies of the Rutkove group also found differences in tongue EIM in ALS compared to healthy volunteers which, like the data we present here, varied with frequency (McIlduff et al. , 2016, McIlduff et al. , 2017).

The complexity of the tongue blade myoarchitecture necessitates our multi-dimensional approach as a uniform pathological change throughout the muscle and across all patients seems unlikely. In keeping with this, limb biopsy specimens often reveal small groups of atrophic muscle fibres distributed heterogeneously across different samples (Baloh et al. , 2007, Al-Sarraj et al. , 2014, Jokela et al. , 2016). We are only aware of tongue biopsy in ALS following development of macroglossia (McKee et al. , 2013), but it is not uncommon to have to manipulate the EMG needle during examination of the tongue (and other muscles) in order to find abnormalities. While 3D electrode configurations outperformed 2D arrangements in disease identification and symptom/strength correlation, it seems rational that multiple electrode configurations, exploring different regions/planes of tissue are required to maximise the potential of the technique.

We chose to examine phase angle, reported as the most disease-sensitive parameter (Rutkove et al. , 2007) and the focus of most recent studies (e.g. (Shefner et al. , 2018)). As a ratio of resistance and reactance, it is more resilient to the potentially confounding effects of tissue volume (Rutkove et al. , 2007, Rutkove et al. , 2016). Our device provides a fixed volume of tissue between the electrodes and utilised a small distance between electrodes. The modelling work of Pacheck et al., indicates tongue volume has little impact on impedance data and we were recording in the midline of the tongue blade and hence not near the muscle edge. (Pacheck et al. , 2016). We therefore consider that it is unlikely that our results are explained by differences in tongue volume.

It is theoretically possible that differences in saliva could impact on the mucosa-electrode interface e.g. due to saliva pooling. While we did observe a significant linear relationship between EIM data and the ALSFRS-R saliva subscore, similar results were observed for the other components (supplementary figure 4) and there was a high correlation between the saliva score and the bulbar subscore total (r^2 = 0.625; data not shown). Furthermore, studies on the effects of conductive fluid on tissue impedance measurements indicate that if the fluid film is thin and the resistivity of the tissue is less than 100 times the resistivity of the fluid (if higher it may act as a short-circuit), then the effect is minimal (Finkelstein et al. , 1984, Jones et al. , 2001). Our system was constructed with electrode faces polished flat in order to ensure saliva film thickness was <10μm and almost all measurements (~99%) were within the resistivity limit. Any potential effect will also be reduced by using a tetrapolar measurement approach.

Impedance spectra produce a high dimensional dataset for the interrogation of muscle health. Consequently, we used a machine learning approach to select the most relevant information and reduce the dimensionality of the results. Previous approaches to impedance data analysis have included focusing on one (Tarulli et al. , 2009) or two (Roy et al. , 2019) input frequencies, ratios of two frequencies (Schwartz et al. , 2015), or the slope between two ratios (Rutkove et al. , 2017). Less frequently, machine learning, in conjunction with other clinical parameters (Srivastava et al. , 2012), or in combination with mixed models (Kapur et al. , 2019), has been used. Here, we employed a data driven approach to identify and utilise key spectral features. While previous reports have found that different frequencies may be useful for different muscles (McIlduff et al. , 2017), we took this a stage further and found that, interestingly, different features were selected for different tasks, e.g. patient/volunteer classification and disease related change. In our analyses we chose a feature selection approach in order to both maintain the original data projection and remove redundant data. The wrapper approach used is computationally expensive, but has the advantage of optimising classifier performance through examining learning results (Hsu et al. , 2011). There are, however, a range of other machine learning techniques that could be trialled. Once optimised the analysis can be streamlined, with the potential for real-time, bedside results.

Both univariate and multivariate analyses identified mid-to-high range input frequencies as most discriminatory (figure 4). The majority of our patients had clinically evident disease in which one might expect the underlying ultrastructure of the tongue to demonstrate grouped atrophy (Baloh et al. , 2007, Al-Sarraj et al. , 2014). We would therefore anticipate patient/healthy differences in EIM phase angle to manifest at frequencies in which the current crosses the capacitance of the sarcolemma, which was indeed the case. Some recent studies have indicated that EIM may be able to detect muscle changes secondary to UMN dysfunction (Li et al. , 2017, Zong et al. , 2018). While a mixed UMN/LMN

dysarthria is the most common pattern encountered in ALS (Aronson et al. , 1992), it remains to be determined if dual pathology can be separated or quantified, either in the tongue or limb muscles.

Our study population had a relatively broad mix of patient phenotypes. At a group level we saw little change in bulbar symptoms/tongue strength over the 6-month period, possibly due to the attrition of patients with the most pronounced disease progression. We also included several patients with atypical disease durations of up to 204 months; such patients are now included in many trials and are found in all specialist ALS clinics. A greater understanding of the potential of the device would come from larger numbers of patients, particularly those with no overt clinical signs of bulbar disease who go on to develop such symptoms.. Despite the challenges inherent in monitoring ALS patients, our simple change metric detected a change in the patient group over time.We were also able to identify change at an individual patient level, consistently identifying patients with symptom progression/loss of tongue strength. This suggests that the machine learning approach was capturing clinically relevant phenomena, although we acknowledge that our data represent only a first step in ascertaining the utility of this.

A further limitation is the lack of an ALS-mimic group. While we have previously shown that bulbar EMG abnormalities are highly specific to ALS (Jenkins et al. , 2016), evaluation of the present device in patients with conditions such as spinobulbar muscular atrophy would provide insight into whether tongue impedance measurements are specific to ALS. In limb muscles early results suggest that impedance have limited efficacy in distinguishing ALS from mimic conditions (Sanchez et al. , 2017b). An additional limitation, not yet addressed in EIM studies, is the effect of fasciculations. In theory, florid, repetitive fasciculations that disrupt electrode contact could pose difficulties, but we are not aware of any studies examining this. Studies on the effect of muscle contractions on impedance recording have shown changes in various parameters, which have been argued to reflect physiological, rather than morphological effects (Shiffman et al. , 2003, Li et al. , 2016). Whether similar changes occur when muscle activity is limited to the level of the motor unit is unknown.

Our work provides evidence that our EIM probe can be rapidly applied to ALS patients. We adopted an approach advocated recently for its similarity to typical clinical trials – brief instruction, then independent recordings (Geisbush et al. , 2015, McIlduff et al. , 2016). We included two inexperienced examiners, both of whom achieved test-retest reliability on par with that of a more experienced rater. ICC results were similar to those reported for other EIM devices (e.g. (McIlduff et al. , 2016)) and coefficient of variation results comparable to those reported for other neurophysiological techniques such as the motor unit number/size indices (Neuwirth et al. , 2016, Alix et al. , 2019).

A small number of patients were not able to perform the recordings due to end-stage bulbar disease or respiratory difficulties. Identifying bulbar pathology in end-stage disease is not a significant issue and monitoring such advanced disease may only reveal a plateau. At the time of recruitment, the patient with respiratory failure did not have access to nasal non-invasive positive pressure ventilation, which may have facilitated recording. In contrast to previous studies we experienced no difficulties with eliciting a gag reflex (Shellikeri et al. , 2015), most likely as our device does not need placement along a large portion of the tongue.

In conclusion, we present a novel EIM device that produces reliable data which can be used to identify tongue muscle pathology in ALS. It is easy to use and is well tolerated. Our work illustrates the potential of multi-dimensional tongue EIM as a biomarker for bulbar disease in ALS.

FIGURE LEGENDS

Figure 1. The novel EIM device.

(A). The novel EIM device with 4 electrodes on the upper and lower arms. The vertical bars provide the compression width.

(B). The electrode configurations used, separated into 3D and 2D groups, utilising the key employed in (A).

(C). Example of the recording in an ALS patient (consent provided).

Figure 2. Flow chart of the study.

Figure 3. **Reproducibility assessments for patients, volunteers and different examiners.**

ICC plots, including 95% confidence intervals, of 3D and 2D electrode configurations (A), patients and volunteers (B) and comparison of ICC at baseline and 6 months in both participant groups (C). Inter- and intra-rater analyses (D) and comparisons of different examiner combinations (E, F) were also undertaken.

Figure 4. Differences in the phase angle data of patients and healthy volunteers.

(A). Median phase angle (+95% confidence interval) for the 3D:2 electrode configuration for both patients and healthy volunteers.

(B). Summary of differences between patients and healthy volunteers at baseline using a Mann-Whitney U test and FDR correction (target P value for significance $P<0.02$). Values represent P values, shaded boxes denote significance following the FDR correction.

(C). Summary of performance of the wrapper algorithm using AUROC, sensitivity and specificity. A particularly high performance was apparent for 3D electrode configurations.

Figure 5. Relationship of EIM data to bulbar symptoms and tongue strength in ALS patients.

(A-B). Scatter plots of the L2 norm and the ALSFRS-R bulbar subscore (A) and tongue strength (B) for the electrode configuration 3D:4.

(C-D). Scatter plots of the L2 norm and the ALSFRS-R bulbar subscore (A) and tongue strength (B) for the electrode configuration 2D:2.

Figure 6. Change over time in EIM, bulbar ALSFRS-R and tongue strength.

(A). Change over time in the L2 norm for both patients and healthy volunteers (mixed model, significant difference between baseline and month 6). The standardised response mean, calculated using the patient data, is shown.

(B). Change in bulbar ALSFRS-R subscore over time. No significant change was seen (mixed model), the associated SRM is shown.

(C). Change in tongue strength over time. No significant change was seen (mixed model), the associated SRM is shown.

(D & E). L2 norm for the absolute differences in phase angle for patients and volunteers across 3D (D) and 2D (E) electrode configurations. The shaded box denotes the threshold for accepting change in patients and was taken from the greatest value seen in healthy volunteers.

Supplementary figure 1. Additional 3D electrode configurations tested.

(A). Summary of four additional electrode configurations tested during the study. These comprised an additional 3D group, with current and voltage electrodes on opposing surface of the tongue.

(B). Intra-class correlation coefficient plot demonstrating poor performance of this group. Due to the lack of reliable data it was excluded from the main analysis.

Supplementary figure 2. Bland-Altman plots and data.

(A-B). Bland-Altman plots for patients and healthy volunteers.

(C). Summary table.

Supplementary figure 3. Median phase angle spectra for all electrode configurations.

Supplemental figure 4. ROC curves for each electrode configuration and selected features.

ROC curves for each electrode configuration using the features (input frequencies) selected through the exhaustive wrapper algorithm and pareto ranking. 4-fold cross validation was undertaken and the results for each fold are shown, together with the mean and 95% confidence interval.

Supplementary figure 5. Relationship of EIM data to ALSFRS-R bulbar subscore components.

(A-C). Scatter plots of the L2 norm and the ALSFRS-R bulbar subscore components for the electrode configuration 3D:4.

(D-F). Scatter plots of the L2 norm and the ALSFRS-R bulbar subscore components for the electrode configuration 2D:2.

FDR-adjusted p-value = 0.014.

Supplementary figure 6. Effect of age.

(A). Scatter plot of L2 norm and age for healthy volunteers.

(B). Summary of differences between healthy volunteers aged <55 years and ≥55 years. using a Mann-Whitney U test and FDR correction. Values represent P values.

Supplementary table 1. Coefficient of variation for the different participant groups, electrode configurations and examiners.

Supplementary table 2. Summary table for correlation analyses between EIM data (L2 norm) and bulbar symptoms/tongue strength.

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Conflict of interest

The authors declare no conflicting interests.

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