NEUROMUSCULAR ULTRASOUND AS A BIOMARKER OF DISEASE
PROGRESSION IN ALS

BY

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ABBREVIATIONS

ALS   Amyotrophic lateral sclerosis

ALSFRS   Amyotrophic lateral sclerosis functional rating scale

EIM   Electrical impedence myography

FVC   Forced vital capacity

HHD   Hand-held dynamometry

MRC   Medical research council strength testing

MUNE   Motor unit number estimation

NIV   Noninvasive ventilation

PEG   Percutaneous endoscopic gastrotomy

TA   Tibialis anterior

LIST OF TABLES

Table I

ALS Baseline Demographics

Variable	Mean (Range) [Percentage]
Age	63.6 (44-82)
Gender	8 Male [66.7%], 4 Female [33.3%]
Riluzole Use	11 [91.7%]
Race	9 Caucasian [75.0%], 3 African American [25.0%]
Height (cm)	172.7 (157.5-188.0)
Weight (kg)	78.6 (38.5-137.9)
BMI	26.3 (17.7-39.0)
FVC (%)	63.4 (30-94)
ALSFRS	30.5 (17-41)

Table II

Ultrasonographic Parameters in ALS Patients

Part A: Mean Muscle Thickness, Range, and Significance

Variable	Month 0	Month 6	Mean Percent Decrease (%)	p-value
Mean Biceps Thickness (cm)	2.09 (1.4-3.2)	1.74 (1.0-2.2)	23.01 (6.2-59.4)	0.0585
Mean TA Thickness (cm)	2.18 (1.3-3.0)	1.56 (1.0-2.3)	29.16 (5.1-59.4)	0.1157
Mean Geniohyoid Thickness (cm)	0.49 (0.2-0.9)	0.21 (0.2-0.4)	53.00 (25-83)	0.0005
Mean Diaphragm Thickness-Inhalation (cm)	0.29 (0.2-0.6)	0.20 (0.1-0.3)	21.9 (17.6-72.3)	0.0430
Mean Diaphragm Thickness-Exhalation (cm)	0.22 (0.1-0.4)	0.15 (0.1-0.2)	26.37 (21.8-65.2)	0.0130

Part B: Mean Muscle Thickness and Standard Deviation at Baseline and 6 Months

Muscle	Mean ± SD Thickness at Baseline (cm)	Mean ± SD Thickness at 6 Months (cm)	p-value
Right Bicep	2.10 ± 0.565	1.82 ± 0.526	0.2221
Left Bicep	2.04 ± 0.475	1.66 ± 0.565	0.0883
Right TA	2.19 ± 0.518	1.49 ± 0.500	0.0028
Left TA	2.17 ± 0.518	1.69 ± 0.419	0.0206
Geniohyoid	0.49 ± 0.278	0.21 ± 0.081	0.0029
Right Diaphragm at Inhalation	0.31 ± 0.149	0.20 ± 0.058	0.0262
Left Diaphragm at Inhalation	0.27 ± 0.084	0.19 ± 0.062	0.0145
Right Diaphragm at Exhalation	0.21 ± 0.055	0.16 ± 0.565	0.0381
Left Diaphragm at Exhalation	0.22 ± 0.067	0.14 ± 0.047	0.0027

Table III

Correlation between Ultrasonographic Parameter Percent Decrease and FVC and ALSFRS Decrease

Comparison	Correlation Coefficient	p-value
Biceps Percent Decrease vs. FVC Decrease	0.7233	0.0078
Biceps Percent Decrease vs. ALSFRS Decrease	0.6889	0.0132
TA Percent Decrease vs. FVC Decrease	-0.6571	0.0202
TA Percent Decrease vs. ALSFRS Decrease	-0.2115	0.5093
Geniohyoid Percent Decrease vs. FVC Decrease	-0.3319	0.2933
Geniohyoid Percent Decrease vs. ALSFRS Decrease	0.5597	0.0584
Diaphragm Inhalation Percent Decrease vs. FVC Decrease	0.6610	0.0193
Diaphragm Inhalation Percent Decrease vs. ALSFRS Decrease	0.1825	0.5702
Diaphragm Exhalation Percent Decrease vs. FVC Decrease	0.5018	0.0965
Diaphragm Exhalation Percent Decrease vs. ALSFRS Decrease	-0.0325	0.9214

Table IV

Two Ultrasonographer Measurements of Patient and Correlation for Interrater Reliability

Interrater Correlation (p-value)
0.965 (<0.001)

Table V

Summary of Common ALS Biomarker Methods

Biomarker	Methodology	Pros	Cons
Spirometry	Lung assessment	Quantitative; looks at lung efficiency	Limited to lungs, voluntary effort
ALS Functional Rating Scale	Multiple aspect assessment (swallowing, sleep, etc.)	Looks at multiple aspects of patient	Based on physician and patient-assessment
Hand Held Dynamometry	Assessment of muscular strength	Relatively accurate	Voluntary effort, upper limb
Medical Research Council Strength Testing	Grades muscle power from 0-5 relative to muscle	Looks at multiple muscles	Voluntary effort
Electrical Impedence Myography	Looks at electrical impedence of muscles	Accurate, specific plan for ALS	Technical challenges
Motor Unit Number Estimation	Uses electrical myography to determine number of motor units	Specific for muscle groups, shows deterioration of motor units	Time consuming, uncomfortable

ABSTRACT

This study focused on determining whether neuromuscular ultrasound may be a viable option for monitoring ALS progression. Other biomarkers currently available have limiting factors such as subjectivity and invasiveness. 12 participants took part in our study and we measured the thickness of four muscle groups including the biceps brachii, tibialis anterior, geniohyoid, and hemi-diaphragm at both inhalation and exhalation. We measured three times over six months and found significant changes. All muscle groups showed an expected decrease in thickness. When the percentage decline of muscle thickness was correlated to a decline in ALS Functional Rating Scale score (ALSFRS) and Forced Vital Capacity (FVC), the biceps, tibialis anterior, and diaphragm at both inhalation and exhalation showed significant correlations to FVC. The biceps muscle thickness percent decrease also correlated significantly to ALSFRS decline. The ultrasound method provided a strong and accurate measurement for analyzing muscle thickness decrease in ALS patients in this study. The significant findings of this study and success of the experimental method suggest that there is a basis for further such clinical trials on larger groups of ALS patients in the future.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the

deterioration of motor neurons, leading to muscle weakness.[1]The disease has a mean age

of 56 in individuals with no known history and 46 in individuals with ALS affected

family members, but the disease has been shown to have a relatively variable age range.[2]

No cure is available; however, the medication riluzole moderately slows disease

progression.[3] Percutaneous endoscopic gastrostomy (PEG) treats malnutrition associated

with bulbar symptoms causing dysphagia and decreased fluid and food intake. This

method is proven to have a positive impact in 79% of ALS patients, but only in 37.5% in

patients that received PEG in later stages of the disease.[12] Noninvasive ventilation (NIV)

provides ventilatory support without the issues associated with the use of invasive

artificial airways. This particular method prolongs survival by as much as 200 days and

increases the quality of life when compared to standard care.[13] Even though these

methods are incredibly useful, they do not provide more than just a temporary treatment

option.

In addition to these treatments, accommodation options are available to aid the patient in

optimizing their standard of living, which include altered methods of communication and

travel. Methods of diagnosis and monitoring of disease progression are challenging and

limited[4]. Current biomarkers in the diagnosis of ALS include spirometry, the ALS

Functional Rating Scale (ALSFRS), hand-held dynamometry (HHD), Forced Vital

Capacity (FVC), Medical Research Council (MRC) strength testing, electrical impedance

myography (EIM), and motor unit number estimation (MUNE). These diagnostic

methods are subjective, in some cases expensive and limited to only certain regions of the body (Table V).[5]

This study suggests the use of neuromuscular ultrasound as a method of effectively and accurately measuring muscle atrophy in ALS patients. To test this, multiple muscle groups were assessed including the geniohyoid, biceps, tibialis anterior, and the hemidiaphragm in a group of 12 patients with early ALS (diagnosed within one year of the study) every three months, over a course of nine months. Ultrasound methods were adapted from *Neuromuscular Ultrasound* (Cartwright, 2011).[15]

MATERIALS AND METHODS

Participants

Prior to starting the study, approval was given by the Institutional Review Board at Wake Forest University School of Medicine and all participants gave informed consent (reference Appendix section IRB Protocol). 12 participants were recruited and all were adults diagnosed with ALS based on El Escorial Criteria. The date of onset of symptoms, height, weight, and race were recorded in addition to FVC and ALSFRS.

To participate, patients were required to be at least 21 years of age with "Possible", "Probable", "Laboratory-supported probable", or "Definite" ALS based on revised El Escorial Criteria.[19] Exclusion criteria included age under 21 years and/or skin allergy or sensitivity to ultrasound gel.

Ultrasound

Neuromuscular ultrasounds of the biceps brachii, tibialis anterior, geniohyoid, and hemidiaphragms at inhalation and exhalation were performed. A Biosound MyLab 25 (Esaote Group, Genoa, Italy) with an 18 MHz linear array transducer was used for each study. The participants were in a seated position with the ultrasonographer facing the patient. Each muscle group was imaged bilaterally with the exception of the geniohyoid.

To measure biceps thickness, the transducer was placed over the anterior mid-arm at the midpoint between the elbow and the clavicle. The elbow was extended and a measurement was taken from the most superficial point of the muscle to the humerus. The measurements of each side were averaged together to provide the overall bicep measurement of the patient.

Measurements of the tibialis anterior were taken with the knee bent perpendicular to the floor. The transducer was placed 12 cm distal to the fibular head and both sides were averaged for the overall measurement.

To image the geniohyoid, the transducer was placed longitudinally 2 cm from the mandible at the midline. This allowed for the measurement of the thickness of the geniohyoid muscle. Special care was taken for each muscle group to minimize pressure of the transducer to prevent muscle compression.

Finally, to view the hemidiaphragm, the transducer was placed at the anterior axillary line and moved up from the lower costal margin until the intercostal window could be seen.

This was a sagittal view taken at both inhalation and exhalation. Both sides of the diaphragm were averaged for the overall measurement.

Statistical Analyses

Upon completion of the three visits, a mean score was calculated of all muscle group thickness measurements. The absolute and percent decline of this mean score as well as the absolute and percent decline of each individual muscle group and all muscle group percentages averaged together, were calculated and compared to the baseline. T-tests were utilized to determine the significance for the difference between baseline and 6 months for each ultrasonographic parameter. The correlation was investigated between neuromuscular ultrasound mean score and standard biomarkers (FVC and ALSFRS) at baseline through the use of Pearson product-moment correlation coefficients. Correlations with the same parameters and ultrasound measurements of individual muscle groups were also analyzed. Inter-rater reliability between two ultrasonographers was taken with one sample patient to verify the accuracy of the ultrasound measurements through interrater correlation coefficient calculation and determination of a p value for significance. $P < 0.05$ was considered significant.

RESULTS

Twelve ALS patients participated in the study, but, due to extenuating circumstances (including death and drop out), only six were able to complete the three measurements required for the study. Six patients completed the six month visit, 11 completed the three month visit, and 12 participated in the first visit for a baseline measurement. Males comprised the majority of the participant pool at 66.7%. In addition, 75% of the sample consisted of Caucasian individuals and 25% African American. 91.7% of the participants used the ALS drug Riluzole and the average FVC was 63.4% while the mean ALSFRS score was 30.5 at baseline (Table 1).

Muscle thickness showed a relative decline in all muscle groups with significance seen in all muscle group thickness decrease from baseline to six months, excluding the tibialis anterior group. Furthermore, the calculation of the percent decrease of each muscle group over time showed a decrease (Table 2). In comparing the percent decrease of the groups, the geniohyoid muscle displayed the largest average decrease in muscle thickness at approximately 53%. Correlation of the muscle thickness decrease to either FVC or ALSFRS score decrease showed moderate significance (Table 3). The biceps showed the most significant correlation to FVC with a positive correlation and $p < 0.05$, therefore, the result was significant. Table 4 illustrates the correlation between the measurements of two ultrasonographers for a participant. The interrater correlation was 0.965 and it significance with $p < 0.001$.

DISCUSSION

This study focused on determining if a neuromuscular ultrasound model may serve as a tool for monitoring disease progression in ALS patients. The majority of the patients were male (66.7%) which corresponds well to the average demographic of ALS patients (between 56.3% and 69.9% male depending on age group). [6] The average age was 63.6 which correlated well to a typical ALS patient population where the age generally ranges from 40-70.[7, 11] Previous studies have found a difference in the biceps/brachialis muscle complex between ALS patients and healthy controls as well as decrease overtime in muscle width[8]. This study found that muscle atrophy increased and some of the results proved to be significant while the insignificance seen in some of the muscle groups likely stemmed from a small sample size and patient variability.

Similar past studies concluded that there was too much variability between patients and their measures for ultrasound to be an effective mode of monitoring ALS disease progression.[9] However, previous studies finding this result did not analyze thickness in the geniohyoid or diaphragm. This study showed significance between some of the ultrasonographic measurements and FVC and ALSFRS. The bicep had a strong correlation to both the decrease in FVC as well as ALSFRS. In addition, the TA and the diaphragm at both inhalation and exhalation had a significant correlation to FVC exclusively. Interestingly, the geniohyoid showed a limited correlation to both

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measurements. These results urge the need for further study into the use of combined biomarkers and ultrasound to monitor disease progression.

The geniohyoid muscle showed an overall greater percent decrease than the other muscle groups (Table 2). Further investigation into reasoning for faster deterioration of the geniohyoid needs to occur; however, in considering the bulbar symptoms associated with ALS, they generally occur later in progression. The geniohyoid may appear to deteriorate faster due to the late onset by which time patients are weaker. Another possibility may be due to the difference in innervation when comparing to the other muscles discussed in the study since the geniohyoid has a direct cranial nerve innervation from C1 roots of cranial nerve XII. In addition, while limb-onset has been shown to be a prognostic factor for time of progression to bulbar symptom onset, bulbar-onset cannot detect the same for limb symptom onset.[14] Bulbar associated muscles have major involvement in respiration and mastication which are continuous processes necessary for survival, possibly being another factor in the expedient progression observed in the study.[16] Intense exercise has been shown to have a negative impact on the progression of ALS, supporting the possibility that continous use of the geniohyoid may increase the rate of progression of that particular muscle.[17]

To analyze reliability of the ultrasound method, the standard deviations between the measurements of two ultrasonographers were taken of the fourth participant in the study. The overall interrater correlation value of 0.965 proved significant with $p < 0.001$, thus supporting reliable progression analysis of a single patient between different ultrasonographers. Ultrasound measurements lack subjectivity, strengthening the argument in favor of the ultrasound method.

Due to the correlation to other biomarkers as well as the detection of significant muscle

thickness decline, ultrasound stands as an effective biomarker. Ultrasound is noninvasive,

meaning the risk and discomfort to the patient is minimal, a key characteristic of an

effective biomarker.[18] In addition, the time it takes to receive results is generally very

short allowing patients and studies monitoring ALS to be provided with rapid feedback

due to the high level of responsiveness of ultrasound. As determined in our study as well

as in previous studies, the reproducibility of ultrasound results remains very high.

Ultrasonographers are able to obtain similar results due to the lack of variability involved

in the method. Another biomarker, MRC, grades muscle power and, while it has the

capability of looking at multiple muscle sites, it requires voluntary effort by the patient

which can prove difficult for many ALS patients. Another biomarker, EIM, looks at the

electrical impedence of muscles with significant accuracy; however the method is

generally expensive and many technical challenges can be involved. Similarly, MUNE

looks at specific muscle groups and the deterioration of motor units, but it faces the

challenges of expense and discomfort to the patient.[5] Ultrasound is a relatively

inexpensive method with extremely limited discomfort. Another major benefit of

ultrasound is its capability to look at multiple muscle groups throughout the body. On

another note, previous studies found the ultrasound method to be more effective than

other imaging tactics such as Magnetic Resonance Imaging in detecting abnormalities

such as those seen in peripheral nerves.[10] Overall, this method has fewer limitations when

compared to other widely used biomarkers and imaging methods.

This study was the first analyzing the biceps brachii, tibialis anterior, geniohyoid, and

hemi-diaphragm together and correlating them to the ALSFRS and FVC. While a novel

study, underlying issues exist that limited the study. A major limitation of the study was a modest sample size. Further investigation into ultrasonographic correlation to other biomarkers including FVC, ALSFRS, and IL-6 levels should be performed to reach a significant conclusion. In addition, studying ultrasound further may be of benefit to reduce subjectivity in monitoring ALS progression. Future research in ALS with neuromuscular ultrasound may benefit from a focus on determining muscle-nerve connection integrity over time.

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APPENDIX

Institutional Review Board (IRB) Protocol

Authors: Michael Cartwright, MD and Delaney Williams

<u>**RESEARCH PROTOCOL**</u>

<u>**Study title**</u>

Neuromuscular Ultrasound as a Biomarker in ALS

<u>**Key personnel**</u>

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Carolanne E. Milligan, PhD, Professor in Neuroanatomy and Biology

James B. Caress, MD, PhD, Professor of Neurology

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<u>**Research site**</u>

Neurology Department, Wake Forest University School of Medicine, Winston-Salem, NC.

<u>**Project summary**</u>

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease in which motor neurons are lost, ultimately resulting in profound weakness, inability to swallow, respiratory failure, and death.[1] As with other neurodegenerative diseases, ALS biomarkers can assist clinicians and researchers in diagnosis, prognosis, and monitoring of disease progression, and responsive biomarkers can improve clinical care and treatment trials. Current biomarkers include spirometry, the ALS Functional Rating Scale (ALSFRS), hand-held dynamometry (HHD), Medical Research Council (MRC) strength testing, electrical impedance myography (EIM), and motor unit number estimation (MUNE), which assess respiratory capacity, the ability to perform activities of daily living, strength, and the peripheral neurophysiology of affected individuals, to varying degrees.[2] While these biomarkers have proven effective for clinical care and research purposes, they are limited in that they require voluntary effort (spirometry,

HHD, MRC, and MUNE), rely on patient or caregiver self-reporting (ALSFRS), are uncomfortable (MUNE), can be technically challenging (spirometry, HHD, EIM, and MUNE), and, perhaps most importantly, focus only on limited body regions (spirometry and MUNE).[2,3]

Most therapies under development for ALS are systemic, with the goal of preventing, halting, or delaying weakness throughout the body, though a recently approved therapy, the diaphragm pacing system (DPS), focuses specifically on the diaphragm, given its critical association with mortality in those with ALS.[4] Keeping in mind the limitations of currently used ALS biomarkers, as well as the importance of assessing both systemic and diaphragmatic disease involvement, we propose the use of neuromuscular ultrasound as a reliable, responsive, and informative biomarker of disease progression for individuals with ALS.

Neuromuscular ultrasound is a technique in which high-resolution ultrasound is used to image peripheral nerves and muscles.[5] Muscle imaging with ultrasound was initially described in the 1980s, but as image quality and device portability have improved, the field of neuromuscular ultrasound has rapidly expanded and now even very small peripheral nerves and muscles can be imaged and measured with high validity, reliability, and accuracy.[6] Additionally, the cost of ultrasound devices has decreased, making this point-of-care technology common in neurology offices and electrodiagnostic laboratories. We propose that a combination of ultrasonographic muscle measurements will form a reliable, responsive, and informative biomarker for the evaluation of individuals with ALS. The muscles measured will include the geniohyoid, tibialis anterior, biceps brachii, and hemi-diaphragms. This will be conducted in an efficient and reproducible standardized manner, to ensure ease-of-use, generalizability, efficiency, and reliability. The percent change over time of this combination of measurements will be assessed in 50 individuals with ALS over the course of 1 year, and this biomarker will be powerful because it will require minimal voluntary patient effort and will assess multiple body regions (including the tongue and diaphragm).

Project description

Background/ Rationale

The use of a combination of neuromuscular ultrasound measurements as an endpoint for clinical trials is novel, so there is little published on the topic. However, what has been studied and published is promising. There are only 14 studies, in which neuromuscular ultrasound was used to assess extremity muscles, the tongue, and/or the diaphragm in those with ALS, with the first study reported in 2007. More specifically, there are only 3 studies which report serial muscle ultrasound, 1 with serial tongue ultrasound, and none with serial diaphragm ultrasound in individuals with ALS. While limited in number and scope, these studies show that muscle thickness decreases in those with ALS and the

decrease can be quantified over time. Our group in particular has extensive experience with neuromuscular ultrasound and has shown it to be highly reliable in healthy controls and a strong differentiator between controls and individuals with ALS.[7]

Objectives

The objectives of this study are:
- To determine the inter-rater and intra-rater reliability of neuromuscular ultrasound measurements in 50 adults with amyotrophic lateral sclerosis (ALS).
- To determine the mean percent decrease in thickness of four muscle groups (tongue, tibialis anterior, biceps brachii, and hemi-diaphragms) in 50 adults with ALS over the course of 1 year, with measurement intervals of 3 months.
- To determine the correlation of mean percent muscle thickness decrease in 50 adults with ALS over the course of 1 year with maximal inspiratory pressure (MIP), ALS Functional Rating Scale (ALSFRS), standardized strength assessments, and serum biomarkers, with measurement intervals of 3 months.

Methodology

- Research design: Prospective study.

- Participants
  - Adults in the Wake Forest Baptist Medical Center ALSA Center of Excellence will be approached to participate in this study.

  Inclusion criteria
  - Volunteers age 21 or older.
  - "Possible," "Probable," "Laboratory-supported probable," or "Definite" ALS patients based on revised EI Escorial Criteria.[8]

  Exclusion criteria
  - Age less than 21.
  - Skin allergy or sensitivity to ultrasound gel.
  - Individuals who did not consent to the storage of blood samples.

- Procedure

The general approach to this study will be to recruit 50 adults with ALS and perform serial neuromuscular ultrasound, spirometry, ALSFRS, MRC, HHD, and blood draws every 3 months for a total of 1 year. Two investigators with different levels of experience (Dr. Cartwright and Delaney Williams, a student in Dr. Milligan's laboratory) will conduct neuromuscular ultrasound to allow for inter-rater reliability calculations. For each neuromuscular ultrasound evaluation a sum score of all selected muscle

thickness measurements (tongue, tibialis anterior, biceps brachii, and hemi-diaphragms at end-expiration) will be calculated. The absolute and percent decline of this sum score, along with absolute and percent decline of each individual muscle group and mean percent decline of all muscle groups together (averaging the percentages), will be calculated compared to baseline at each visit. Multivariate linear regression models will be built to control for patient demographics and determine the magnitude and rate of change for each ultrasonographic parameter. Correlation between the neuromuscular ultrasound sum score and the standard biomarkers (ALSFRS, FVC, MIP, MRC, and HHD) as well as investigational serum markers (measured in Dr. Milligan's lab) will be assessed through Pearson product-moment correlation coefficients. Similarly, correlations with the same standard parameters and ultrasound thickness measurements of individual muscle groups will be calculated.

Blood samples will be collected from participants by qualified phlebotomists, nurses or physicians. All samples will be stored in a -80 C freezer in the Department of Neurobiology and Anatomy for research project. The blood samples will be handled and measured in the same manner as the ongoing ALS biosample repository study taking place at Wake Forest School of Medicine.

Five (5) tubes of whole blood will be drawn from each subject that consents to having their sample submitted to the Biosample Repository. Each tube will contain 8-10 ml of whole blood (about 2 teaspoons) for a maximum of 50 ml. Two will be yellow cap tubes (containing ACD additive) will be for DNA collection and this blood will be processed on an AutoPure LS DNA robot in the Center for Genomics and Personalized Medicine Research Lab. Purified DNA will be checked spectrophotometrically to determine DNA concentration and assess DNA purity. The samples are then transferred to the Neurology Biosample Repository for storage at -20ºC as concentrated stocks. The remaining tubes will be red or purple top tubes for serum separation used for protein and antibody analysis or for plasma, and sent to Dr. Milligan's laboratory for processing. Depending on the planned projects at the time, other types of tubes may be substituted but the total collection will not exceed 50 ml. Serum will be processed according to the Biosample Repository SOP (attached) that matches national standards for collection. Any samples that deviate from routine collection procedures will labeled as such.

For follow up blood samples, up to 50 ml of blood will be collected in 5 tubes and the processing will be identical. This will occur every 3 months for a total of 5 collections for each participant.

Upon delivery to the lab, the patient ID labels on the specimens will be removed and replaced by a unique repository ID without information that can be directly linked to the

medical record. This unique ID will be linked to the participant medical record number and maintained in a separate file by Dr. Milligan. The key to the code which links the sample to the identifying information will be kept secure and will not be released under any circumstances. The samples are stored indefinitely until they are completely depleted by experiments. Samples may be destroyed by incineration if there is no further research planned, the samples are no longer valid, or at the request of the participant.

Any samples that are to be released to outside investigators (both within and outside the institution) as part of the Biosample Repository will be de-identified (as defined by the HIPAA Privacy Rule Regulations) to protect the privacy and confidentiality of study subjects. Samples will not be released without the approval of the principal investigators and proper documentation of IRB review and approval of the research activities.

- Interventions
  Venipuncture: Standard phlebotomy techniques will be used by qualified personnel. Four tubes of blood will be drawn for this research. Blood drawing can result in minor side effects including pain, bruising and/or bleeding at the needle site. Occasionally, a person feels faint when blood is drawn. Rarely, an infection may develop, which can be treated.

- Observation/outcome
 -Demographics: age, sex, race, height, weight, and medical conditions will be recorded.
 -Mean percent decrease in thickness of four muscle groups (tongue, tibialis anterior, biceps, and hemi-diaphragms).

- Sample size
 - 50 patients with ALS

- Recruitment
  - Potential participants in this study will be approached in the Wake Forest ALSA Center of Excellence Clinic, which is active in both clinical care and research. At any given time, approximately 170 patients with ALS are receiving care in this center, with about 40 patients seen each month. Drs. Cartwright and James Caress are the two physicians in this center, and they will introduce this study to potential participants. ALS Clinic study coordinator, Mozhdeh Marandi, will also assist in identification of potential participants.

- Duration of the study
 - Each participant will have four visits over the course of the year, with measurement intervals of 3 months. We anticipate each visit to last 1 hour.

Data management & analysis

Data will be collected, tabulated and analyzed. The data will be de-identified and stored on a password protected computer. Categorical variables will be reported as frequencies and percentages and continuous variables as means and standard deviations. All data will be analyzed for normality and transformed if necessary.
The following statistical tests will be used:

-Multivariate linear regression models will be built to control for patient demographics and determine the magnitude and rate of change for each ultrasonographic parameter.
-Correlation between the neuromuscular ultrasound sum score and the standard biomarkers (ALSFRS, FVC, MIP, MRC, and HHD) and investigational serum markers (in the Biosample Repository) will be assessed through Pearson product-moment correlation coefficients and/or regression models.

Ethical considerations

Ultrasound is a non-invasive, radiation-free, and painless diagnostic tool, which removes significant risk to patients and study participants. Venipuncture is also a minimally invasive procedure. This leaves coercion to participate as the main ethical concern related to this study. Drs. Cartwright and Caress, and study coordinator Mozhdeh Marandi, all have formal training in the ethical conduct of research and will avoid any undue pressures to enroll participants. Those patients under the clinical care of Drs. Cartwright and Caress will undergo the consent procedure by the physician not directly involved in their care, with the support of Dr. Marandi. Each participant will receive $20 per visit to compensate them for their time, and this modest reimbursement will not unduly influence their decision to participate. The nature of the study and procedure will be fully explained to the participant and informed consent will be obtained from the patients with ALS.

Confidentiality/privacy

Every effort will be made by the investigators to ensure confidentiality and privacy of participants including the following:
- Code names/numbers will be assigned for participants and used on all research documents.
- Information from the research will be used solely for the purpose of the study and any publications that may result from this study.
- Participant date will be kept confidential at all times, the data collected will be kept in secure location, only authorized people will have access to the research records
- All materials including participant information will be destroyed when no longer necessary for research.

References

1   Mitsumoto H, Chad D, Pioro E. Amyotrophic Lateral Sclerosis. Philadelphia: Davis Company; 1988.

2   Rutkove SB. Clinical Measures of Disease Progression in Amyotrophic Lateral Sclerosis. Neurother J Am Soc Exp Neurother 2015;12(2):384–93.

3   Turner MR, Bowser R, Bruijn L, et al. Mechanisms, models and biomarkers in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Front Degener 2013;14 Suppl 1:19–32.

4   Scherer K, Bedlack RS. Diaphragm pacing in amyotrophic lateral sclerosis: a literature review. Muscle Nerve 2012;46(1):1–8.

5   Walker FO, Cartwright M. Neuromuscular Ultrasound. 1st ed. Elsevier; 2012.

6   Cartwright MS, Demar S, Griffin LP, Balakrishnan N, Harris JM, Walker FO. Validity and reliability of nerve and muscle ultrasound. Muscle Nerve 2013;47(4):515–21.

7   Arts IM, van Rooij FG, Overeem S, et al. Quantitative muscle ultrasonography in amyotrophic lateral sclerosis. Ultrasound Med Biol. 2008;34:354–61

8   The ALS Association, "Criteria for the Diagnosis of ALS." ALSA.org2016. Web. 18 Jan. 2016.

CURRICULUM VITAE

DELANEY E. WILLIAMS

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EDUCATION

Wake Forest University Graduate School, Winston-Salem, NC

Master's in Neuroscience, Expected May 2017

Guilford College, Greensboro, NC

Bachelor of Science, Biology with minor in Chemistry, Received May 2015

The Early College at Guilford, Greensboro, NC

National Honor Society & Service Learning Diploma, May 2013

COMPUTER SKILLS

Well--versed in MATLAB programming language

LabPro Vernier Software

ChemDraw

Microsoft Office

RESEARCH EXPERIENCE

ALS Research Group Utilizing Neuromuscular Ultrasound for Disease Biomarker Detection, Wake Forest University School of Medicine, 2015-2017, Winston-Salem, NC

Detected biomarkers in ALS patients over a year using neuromuscular ultrasound techniques. This is an ongoing study for Master's Thesis.

Hyperbaric Oxygen Ambulance for Stroke Patients, Wake Forest University School of Medicine, 2016-2017, Winston-Salem, NC

Worked on the development of hyperbaric oxygen ambulances for stroke patients and performed research to gain funding and support for the project.

Duke University Undergraduate Research Fellowship in Pharmacology, 2014 Durham, NC

Utilized stereotaxic survival surgery, microdialysis, and HPLC methods on a Sprague-Dawley rat model to determine serotonin, dopamine, and norepinephrine levels in rats which were administered MDMA (ecstasy).

Guilford College Epigenetics Research, Guilford College, 2014-

Tested for histone modifications in *Caenorhabditis elegans* model after hookah and e-cigarette administration.

MEMBERSHIPS

Society for Neuroscience

βββ National Biology Honors Society, Sigma Phi, Guilford College

Association of Southeastern Biologists

North Carolina Academy of Science

WORK EXPERIENCE

Early College at Guilford Biology/Environmental Science Teaching Assistant, 2011-2013

Performed laboratory set up, tutored students, and graded assignments.

Assistant Manager at Flintrock Farm Equine Boarding Facility, 2011-present

Aided in daily farm and equine maintenance procedures.

CONFERENCE PRESENTATIONS

Epigenetic Changes Associated with Nicotine-related Products and Effects on Addictiveness to Other Substances

- North Carolina Academy of Science Meeting at Wake Forest, March 27-28, 2015, Winston Salem, NC.
- Southeastern Association of Biologists/βββ Meeting, April 1-4, 2015, Chattanooga, TN.

ADDITIONAL SKILLS

Proficient in German (speaking, writing, and reading)