Assessment of Laryngopharyngeal Sensation in Adductor Spasmodic Dysphonia (SD TT)

NCT05158179

Document date: 10/21/21

PI's: Yue Ma MD and VyVy Young MD

Study Protocol and Statistical Analysis Plan

Participant Recruitment

Subjects with adductor type LD (AdLD) were identified and recruited by advertisements and by study clinicians at their institution. Advertisements were physically placed in the UCSF Voice and Swallowing Center clinic and were delivered electronically through the website of the National Spasmodic Dysphonia Association and to area LD ("spasmodic dysphonia") support groups.

Adults greater than 18 years old with AdLD were included if they received a diagnosis established by a multi-disciplinary evaluation by a fellowship-trained laryngologist and voice-specialized speech-language pathologist. Subjects diagnosed by a physician outside of the study facility were evaluated in-person by a fellowship-trained laryngologist to confirm their eligibility for this study. All patients were required to have history of favorable response to intra-laryngeal botulinum toxin A (BtxA) treatment, as further demonstration of the correct diagnosis of ADLD. This was especially helpful for patients initially diagnosed outside of our center. Subjects with abductor or mixed type LD were not included in the present study due to their low incidence, but concurrent essential tremor (ET) of the vocal tract was accepted.

Patients were also excluded if they had a history of: concurrent laryngeal diseases or conditions other than AdLD+/-ET; bleeding disorder or current anticoagulation use; head and neck radiation; active tobacco use; or drinking more than two alcoholic beverages per day. If laryngopharyngeal lesions and/or masses, impeding abnormal laryngopharyngeal structure(s), or excessive post-nasal drip were noted on laryngoscopy, then examination ceased, and patients were excluded. Prior to testing, subjects also completed symptom-specific patient reported outcome measures (PROMs), including: Reflux Symptom Index (RSI),[18] Voice Handicap Index-10 (VHI-10),[19] Dyspnea Index (DI),[20] Cough Severity Index (CSI),[21] and Eating Assessment Tool-10 (EAT-10).[22] These PROMs are standardly collected as part of the routine clinical are for all patients undergoing evaluation at this center and are reported herein for holistic

characterization of these subjects *but not* intended as a reflection or measure of the severity of their AdLD. Lastly, to avoid any potentially confounding physical effects from the injection (e.g., vocal fold edema), no testing was performed in the two weeks immediately following BtxA treatment. For patient convenience and to facilitate maximal enrollment, patients were allowed to undergo testing at any point outside of this post-injection window, although the majority pursued testing immediately prior to a BtxA injection on the same day.

Healthy controls were defined as adults between 18 and 85 years of age without laryngopharyngeal disease and were subject to the same screening and exclusion criteria.[23] Additionally, controls were excluded if they had abnormal patient reported outcome measures (i.e. Reflux Symptom Index (RSI) score> 13, Eating Assessment Tool-10 (EAT-10) score>2, or Voice Handicap Index-10 (VHI-10) score> 11).

Aesthesiometer Device

As outlined in previous work, modified nylon monofilaments fixed to the end of 5-French open lumen catheters were utilized to deliver tactile stimuli to laryngopharyngeal subsites.[12] The Food and Drug Administration (FDA) has previously ruled the aesthesiometer to be a Nonsignificant Risk (NSR) Device Study (Q190371/S001). Three nylon monofilament sizes (6-0, 5-0, 4-0) were cut to a calibrated length of 30 mm to deliver three distinct tactile stimuli of increasing strength, known as buckling-force. The 6-0, 5-0, and 4-0 monofilaments deliver an increasing mean force of 0.03 g, 0.11 g, and 0.30 g, respectively.[12] An intermediary strength “4.5-0” monofilament was created by using a 5-0 monofilament of 25mm length, which has been demonstrated to have a mean buckling force of 0.19 g.[23]

During testing, the monofilaments were passed through the working channel of a flexible ENF-VT2 laryngoscope (Olympus America, Inc., Center Valley, PA) to allow for direct visualization and concurrent stimulation of laryngopharyngeal subsites.

Laryngopharyngeal Sensory Evaluation

All subjects underwent a standard protocol for laryngopharyngeal sensory evaluation. This has been described in detail previously but briefly reviewed again here.[12,13] Prior to testing, subjects were screened for any recent changes in conditions affecting voice and swallowing to ascertain subjects-maintained inclusion criteria. Three investigators executed laryngopharyngeal testing: 1) one to operate the laryngoscope; 2) one to maneuver the aesthesiometer monofilament; and 3) one to monitor testing, record subjective patient response, and observe cough/gag/swallow responses.

An investigator then examined both nasal passageways with a nasal speculum, selecting the more patent pathway for laryngoscope insertion. The more patent nasal passageway was topically anesthetized with cottonoid pledgets soaked in a 50/50 mixture of 4% lidocaine hydrochloride and neosynephrine. The cottonoid pledgets were compressed to remove excess liquid to avoid unintentional spillage posteriorly to the nasopharynx and potentially larynx, and left in place for five minutes.

The channeled laryngoscope was then inserted along the inferior meatus towards the posterior nasopharyngeal wall. The 6-0 monofilament was pressed against the posterior nasopharyngeal wall and swiftly removed to establish a perceptual strength of “1”. If subjects were insensate to this stimulation, monofilament size was incrementally increased (i.e., 5-0, 4.5-0, and 4-0) until the stimulus was perceived, to establish this internal anchor. As per the validated, standardized protocol, all AdLD participants were stimulated on the left, allowing maximal visualization of the vocal folds during testing due to camera configuration of the flexible laryngoscope. Only four controls were stimulated on the right side, during earliest phases of testing.

Starting with the 6-0 monofilament, the lateral pyriform sinus (LPS) was presented with the planned stimulus, followed by the aryepiglottic fold (AEF) and the false vocal folds (FVF). (Figure 1) Participants were instructed to raise their hand when the stimulus was detected, and then were asked

by the study team to report a perceptual strength "score," in comparison to the nasopharyngeal anchor strength of 1. LAR response was observed and recorded by the study team. A negative response was defined as a lack of LAR to two appropriate stimuli. Gag or cough response to stimuli delivery were also recorded, assuming a positive LAR during these observations.

The LPS and AEF were tested in order of increasing stimulus: 5-0, 4.5-0 and 4-0 monofilaments. The false vocal folds (FVF) were tested last, in the same order of increasing monofilament strength. Testing of FVF terminated after the first observed LAR given site sensitivity, assuming positive responses would also be present for increased monofilament strength.

LAR Assessment

Confirmation of LAR was determined using post-hoc frame-by-frame analysis of video recordings. Unilateral or bilateral vocal fold adduction following stimulus delivery was recorded as a positive response. Upon review, stimuli were excluded if they did not produce 10-30% monofilament buckling (as observed by the reviewer), were entrapped by saliva, or could not compress orthogonally to the mucosal surface. Additionally, if line of sight of visualization of vocal fold adduction was obstructed or confounded by phonation, the stimuli were excluded. All stimuli were reviewed by one study investigator with 30% of stimuli reviewed by a blinded second reviewer. Disagreements were reconciled by a blinded third reviewer.

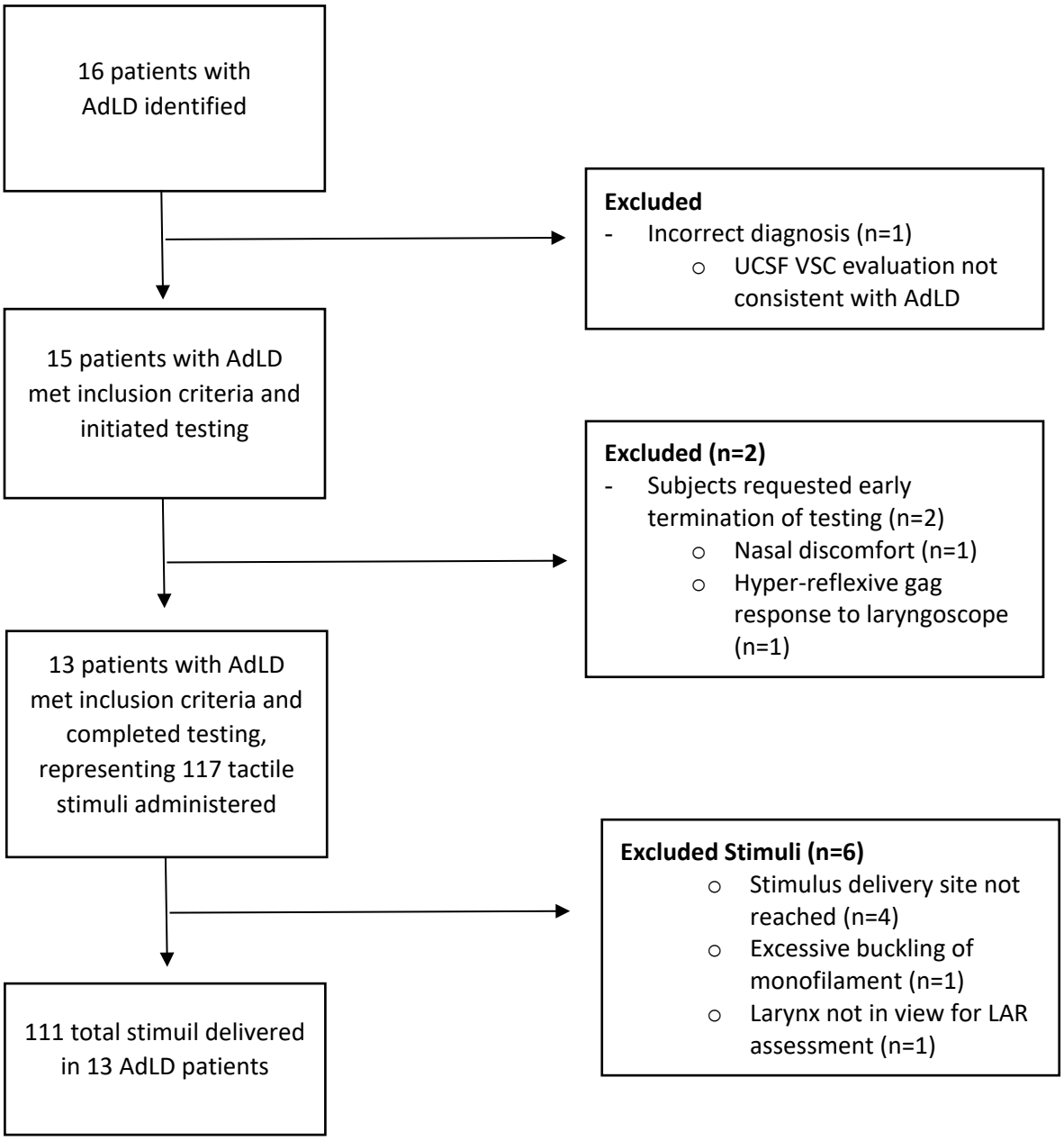
Statistical Methods

Patient demographics, clinical characteristics, and sensory outcomes were summarized using descriptive statistics (means, standard deviations (SD), counts, and percentages). Two-sample Mann-Whitney and Fisher exact tests were used to compare strength ratings and LAR rates between AdLD and control groups at specific sites and filament sizes. To assess predictors of LAR, subjective detection, and

gag/cough responses, a mixed-effects logistic regression model was implemented. The unit of analysis was binary LAR response (Yes or No) to a tactile stimulus at a specific site with a specific monofilament. Univariable and multivariable models included the following fixed effects: tactile force, stimulation site, age, gender, and laryngeal dystonia diagnosis (AdLD+/-ET) as five predictors. In the model, a random intercept for each subject accommodated correlation of the repeated binary responses within subjects. Maximum likelihood estimation was used for model fitting. Odds ratios, 95% confidence intervals, and Wald test p-values were reported for logistic regression models. No formal adjustment was made for multiple hypothesis testing. In the regression model, the reference levels for the categorical predictors were 6-0 for tactile force, LPS for site, male for gender, and control for cohort group. To assess mean differences in perceptual strength ratings, linear regression modeling was utilized including the same predictors. Stata (Ver 14.1, StataCorp LLC, College Station, TX) was utilized for statistical analysis, with a p-value < 0.05 considered statistically significant.

Summary data

1. **Participant Flow** (basically a CONSORT flowchart of the number of subjects enrolled, completed, not completed, and reason not completed)



Abbreviations: UCSF VSC: University of California San Francisco Voice and Swallowing Center

2. **Baseline Characteristics** (age, sex, race/ethnicity, other study specific baseline measures)

Subject	Sex	Age	Concurrent Essential Tremor	VHI-10 at time of testing	BtxA injection location	Months post injection
1	M	42	No	37	Unilateral	9 months
2	F	71	No	16	Bilateral	3 months
3	F	29	No	25	Unknown	1 month
4	F	69	Yes	7	Unilateral	6 months
5	F	62	No	18	Unilateral	6 months
6	M	68	No	15	Bilateral	3 months
7	M	45	No	24	Bilateral	3 months
8	F	84	Yes	25	Unilateral	24 months
9	F	53	No	18	Unilateral	4 months
10	F	56	No	30	Bilateral	3 months
11	F	76	No	20	Unknown	1 month
12	F	70	No	33	Unilateral	4 months
13	M	61	No	40	Bilateral	3 months

Abbreviations: VHI-10: Voice Handicap Index-10; BtxA: botulinum toxin A

Sixteen subjects with AdLD were identified and recruited; 3 subjects could not complete laryngopharyngeal testing. Thirteen total subjects were included in analysis (mean age 60+/-15.2 years, 9 women). Patient demographics are outlined in Table above. The healthy control cohort comprised 33 individuals, 36% female (n=12) with a mean age of 52 +/- 18.1 years.

3. **Outcome Measures** (as specified in the study record)

Primary outcome measure was the presence or absence of the Laryngeal Adductor Reflex (LAR) as observed via flexible laryngoscopy. A previously validated, standardized protocol was utilized to

stimulate the larynx in 3 distinct anatomic sites (lateral pyriform sinus (LPS), aryepiglottic fold (AEF), and false vocal folds (FVF)), with a series of monofilaments of increasing size/force: 6-0, 5-0, 4.5-0 and 4-0 monofilaments.

Additional outcome measure included subjective perceptual strength rating, as reported by the patient, on a scale of 1-10.

4. **Adverse Events**: none

5. **Limitations and Caveats**

Results of this study showed statistically significant differences in laryngeal responsiveness between AdLD and normal cohorts. However, these results may have limited generalizability beyond AdLD (i.e., to abductor-type LD, mixed LD, or isolated essential tremor). The impact of concurrent essential tremor on results could not be assessed in this small study. This study is also limited by the non-randomized nature of testing protocol (although the rationale for this protocol has been described previously in the literature) and the unknown effects of BtxA dose or timing on laryngeal sensation testing results. Future, prospective, larger-scale, ideally multi-institutional studies are needed to explore the findings of this study further.