Multi-parameter comparison of injection laryngoplasty, medialization laryngoplasty, and arytenoid adduction in an excised larynx model

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Abstract

Objective—Evaluate the effect of injection laryngoplasty (IL), medialization laryngoplasty (ML), and ML combined with arytenoid adduction (ML-AA) on acoustic, aerodynamic, and mucosal wave measurements in an excised larynx setup.

Methods—Measurements were recorded for eight excised canine larynges with simulated unilateral vocal fold paralysis (UVFP) before and after vocal fold injection with Cymetra. A second set of eight larynges was used to evaluate medialization laryngoplasty using a Silastic implant without and with arytenoid adduction.

Results—IL and ML led to comparable decreases in phonation threshold flow (PTF), phonation threshold pressure (PTP), and phonation threshold power (PTW). ML-AA led to significant decreases in PTF (p=0.008), PTP (p=0.008), and PTW (p=0.008). IL and ML led to approximately equal decreases in percent jitter and percent shimmer. ML-AA caused the greatest increase in signal to noise ratio (SNR). ML-AA discernibly decreased frequency (p=0.059); a clear trend was not observed for IL or ML. IL significantly reduced mucosal wave amplitude (p=0.002), while both ML and ML-AA increased it. All procedures significantly decreased glottal gap, with the most dramatic effects observed after ML-AA (p=0.004).

Conclusions—ML-AA led to the greatest improvements in phonatory parameters. IL was comparable to ML aerodynamically and acoustically, but caused detrimental changes to the mucosal wave. Incremental improvements in parameters recorded from the same larynx were observed after ML and ML-AA. To ensure optimal acoustic outcome, the arytenoid must be correctly rotated. This study provides objective support for the combined ML-AA procedure in tolerant patients.

Evidence based medicine level—Not applicable – animal study.

Keywords

vocal fold paralysis; vocal fold injection; medialization laryngoplasty; arytenoid adduction
INTRODUCTION

The recurrent laryngeal nerve (RLN) innervates the intrinsic laryngeal muscles necessary for vocal fold adduction. Injury to the RLN, typically traumatic or iatrogenic, can cause unilateral vocal fold paralysis (UVFP) which impairs voice, swallowing, and breathing function. Numerous surgical interventions are used to medialize the affected fold with the aim of improving laryngeal function by decreasing glottal gap. Two common approaches include injection laryngoplasty (IL) and laryngeal framework surgery.

Vocal fold injection using fat, collagen, micronized dermis, or hydroxyapatite mediates a paralytic vocal fold by increasing vocal fold volume. Injections are less invasive than laryngeal framework surgery and can be performed as an outpatient procedure under local anesthesia. Though convenient, there are several limitations to IL including possible decreased mucosal wave amplitude if vocal fold stiffness is increased, gradual absorption of the injected material into surrounding tissues, and irreversibility. Early injections used Teflon which has a tendency to form granulomas and is difficult to remove once injected. Fat, which has a viscosity close to that of vocal fold tissue, was later developed as an injection material. Currently, micronized dermis is a widely used material that has been reported to improve voice to the same degree as type I thyroplasty. Pearl et al. reported significant improvements in jitter and shimmer as well as habitual phonation time and airflow after injection of micronized AlloDerm; however, Cymetra is a temporary injection agent and its small particles are susceptible to phagocytosis, leading to reabsorption and the need for repeat injections.

Medialization laryngoplasty (ML), introduced by Isshiki, improves vocal quality in patients affected by UVFP and has several reported advantages over IL. Insertion of an implant allows for preservation of the mucosal wave. Implants also retain their size over time and do not carry the risk of reabsorption into tissues. An implant can also be removed easier than injected material, though implant insertion can lead to permanent fibrosis in the paraglottic space as well as changes to the cricoarytenoid joint. However, thyroplasty has several disadvantages not encountered by IL. Submucosal hemorrhage as well as implant extrusion can occur. Silicone implants must be carved during surgery, increasing operation time. Suboptimal shaping can hinder potential improvements in voicing, breathing, and swallowing.

Though thyroplasty is an effective treatment for UVFP, it is limited by an inability to close a wide posterior glottal gap or correct a difference in the horizontal plane of the two vocal folds. Coupling medialization laryngoplasty with arytenoid adduction (ML-AA) has been shown to overcome these limitations and improve vocal outcomes. Despite the benefit of the procedure, AA is performed less frequently than it should due to increased technical demands and time. Overrotation of the arytenoid, apparent shortening of the vocal fold, or a failure to achieve vertical alignment between the folds can occur, mitigating procedural efficacy and compromising voice quality. The benefits of ML-AA are also not well defined. Many studies evaluating the benefit of arytenoid adduction have compared post-procedure outcomes to patients only receiving thyroplasty, rather than analyzing the added benefit of performing arytenoid adduction after thyroplasty in the same patient.

Determining the optimal treatment for UVFP remains a clinical challenge. Damrose et al. observed excellent and persistent voice improvement at three months in 88% of patients injected with Cymetra. Sclafani et al. reported on the molecular benefits of Cymetra injection, including fibroblast proliferation with collagen deposition. As IL is a less invasive and less expensive procedure to perform than ML, it is important to reassess which procedure is most beneficial for patients with UVFP.
This study quantified the effects of IL, ML, and ML-AA on mucosal wave, acoustic, and aerodynamic properties of phonation in an excised larynx. This controlled setting allowed for precise and repeatable measurements of all parameters, as well as analysis of the additive benefits offered by AA.

**MATERIALS AND METHODS**

**Larynges**

Sixteen larynges were excised postmortem from canines sacrificed for non-research purposes according to the protocol described by Jiang and Titze. Canine larynges are much more widely available at our institution than human larynges. As the size and histological properties of the canine and human larynx are similar, it is an appropriate model for studying human laryngeal physiology. Larynges were examined for evidence of trauma or disorders; any larynges exhibiting trauma or disorders were excluded. Following visual inspection, larynges were frozen in 0.9% saline solution. The sixteen larynges were divided into two groups: (1) injection laryngoplasty (IL) and (2) medialization laryngoplasty (ML) without and with arytenoid adduction (ML-AA).

**Apparatus**

Prior to the experiment, the epiglottis, corniculate cartilages, cuneiform cartilages, and ventricular folds of the larynx were dissected away to expose the true vocal folds. The superior cornu and posterosuperior part of the thyroid cartilage ipsilateral to the normal vocal fold were also dissected away to facilitate insertion of a lateral 3-pronged micrometer into the arytenoid cartilage. The larynx was mounted on the apparatus (figure 1) as specified by Jiang and Titze. A metal pull clamp was used to stabilize the trachea to a tube connected to a pseudolung which served as a constant pressure source. Insertion of one 3-pronged micrometer in the arytenoid cartilage ipsilateral to the dissected thyroid cartilage (figures 2A, 3A) allowed for adduction of one vocal fold, simulating UVFP in the unadducted vocal fold as in Czerwonka et al. and Inagi et al.. Methodological consistency was maintained by always adducting the contralateral arytenoid (simulated normal) to the midline. Micrometer positioning remained across sets of trials within the same larynx. Tension on the vocal folds and control of vocal fold elongation was accomplished by attaching the superior anteromedial thyroid cartilage, just inferior to the thyroid notch, to an anterior micrometer. Vocal fold elongation and adduction remained constant for all trials.

The pseudolung used to initiate and sustain phonation in these trials was designed to simulate the human respiratory system. Pressurized airflow was passed through two Concha Therm III humidifiers (Fisher & Paykel Healthcare Inc., Laguna Hills, California) in series to humidify and warm the air. The potential for dehydration was further decreased by frequent application of 0.9% saline solution between trials. Airflow was controlled manually and was measured using an Omega airflow meter (model FMA-1601A, Omega Engineering Inc., Stamford, Connecticut). Pressure measurements were taken immediately before the air passed into the larynx using a Heise digital pressure meter (901 series, Ashcroft Inc., Stratford, Connecticut).

Acoustic data were collected using a Sony microphone (model ECM-88, Sony Electronics Inc., New York, New York) positioned at a 45° angle to the vertical axis of the vocal tract. The microphone was placed approximately 10 cm from the glottis to minimize acoustic noise produced by turbulent airflow. Acoustic signals were subsequently amplified by a Symetrix preamplifier (model 302, Symetrix Inc., Mountlake Terrace, Washington). A National Instruments data acquisition board (model AT-MIO-16; National Instruments Corp, Austin, Texas) and customized LabVIEW 8.5 software were used to record airflow, pressure, and acoustic signals on a personal computer. Aerodynamic data were recorded at a sampling rate...
of 200 Hz and acoustic data at 40,000 Hz. Experiments were conducted in a triple-walled, sound-proof room to reduce background noise and stabilize humidity levels and temperature.

The vocal fold mucosal wave was recorded for approximately 200 milliseconds per trial using a high-speed digital camera (model Fastcam-ultima APX; Photron, San Diego, CA). Videos were recorded with a resolution of 512 × 256 pixels at a rate of 4000 Hz.

**Experimental Methods**

Trials were conducted as a sequence of 5 second periods of phonation, followed by 5 second periods of rest. Five trials were performed for each condition. During each trial, airflow passing through the larynx was increased gradually and consistently until the onset of phonation. All procedures were performed by the same author (MRH) under the supervision of the senior author (TMM). Total experiment time was fifteen minutes for the larynges receiving IL and thirty minutes for the larynges receiving ML-AA. Larynges were thoroughly hydrated with saline solution between trials and between sets of trials to eliminate any potentially confounding effects of dehydration.

IL was performed using Cymetra micronized AlloDerm (LifeCell Corporation, Branchburg, NJ). Cymetra was prepared according to manufacturer specifications by diluting the micronized dermis with 1.7 cc saline. The solution was mixed in two syringes by pushing the syringe plungers back and forth in a continuous motion. Prior to injection, air was expelled from the solution. Approximately 0.3 - 0.5 cc were injected at each of two sites on the vocal fold: just lateral to the vocal process and the lateral aspect of the midpoint of the vocal fold. A 1.5 inch 23-gauge needle (Becton, Dickinson, and Company, Franklin Lakes, NJ) was used at a depth of 2-4 mm. The amount injected was dependent upon the size of the larynx and was determined by minimizing the aerodynamic power necessary to initiate phonation. Caution was taken to avoid overinjection which could lead to vocal fold bowing or excessive medialization at the anterior commissure.

ML was performed using a Silastic implant (Dow Corning Corporation, Midland, MI). The implant was inserted through a 6 × 11 mm thyroplasty window in the thyroid cartilage ipsilateral to the paralyzed vocal fold. Optimal degree of medialization was determined empirically by minimizing the aerodynamic power necessary to initiate phonation.

AA was performed after a set of trials was conducted analyzing the effect of ML. The procedure was performed according to the clinical descriptions by Isshiki. One suture was passed with a needle from the muscular process of the arytenoid anteriorly through the paraglottic space through the thyroid cartilage just lateral to the anterior commissure and the second inferior to the cartilage was tightened to rotate the arytenoid and adduct the simulated paralyzed fold. The optimal degree of rotation was determined by minimizing the aerodynamic power needed to initiate phonation while preserving acoustic quality.

**Data Analysis**

Phonation was evaluated before and after each procedure. Airflow and pressure at the phonation onset were recorded as the phonation threshold flow (PTF) and phonation threshold pressure (PTP), respectively. Phonation threshold power (PTW) was calculated as the product of these values. PTF, PTP, and PTW were determined manually using customized LabVIEW 8.5 software. Phonation onset was determined spectrographically; airflow and pressure at this time were recorded as PTF and PTP.

Measured acoustic parameters included frequency, signal-to-noise ratio (SNR), percent jitter, and percent shimmer. Acoustic signals were trimmed using GoldWave 5.1.2600.0 (GoldWave
Inc., St. John’s, Canada) and analyzed using Computerized Speech Lab (CSpeech) software (Madison, WI).

High speed video recordings of the mucosal wave were analyzed using a customized MATLAB program (The MathWorks, Natick, MA). Vibratory properties of each of the four vocal fold lips (right-upper, right-lower, left-upper, left-lower) were quantified via digital videokymography (VKG). Threshold-based edge detection, manual wave segment extraction, and non-linear least squares curve fitting using the Fourier Series equation were applied to determine the most closely fitting sinusoidal curve. This curve was used to derive the amplitude and phase difference of the mucosal wave of each vocal fold lip, both before and after treatment. Phase difference was calculated as the phase difference between the right upper and left upper vocal fold lips. Mucosal wave amplitude was calculated as the average of the amplitudes of the upper and lower paralyzed vocal fold lips. While only relative rather than absolute values could be obtained due to current technological limitations, this was sufficient for pre-/post-treatment comparisons.

Statistical analysis

Paired t-tests were performed to determine if IL, ML, and ML-AA had significant effects on the parameters of interest compared to the larynx with simulated UVFP. A paired t-test was also performed to determine if AA had a significant effect after ML. If data were not normal according to a Shapiro-Wilk test or did not display equal variance according to a Levene’s test, a Wilcoxon-Mann-Whitney paired rank sum test was performed. Tests were two-tailed and a significance level of $\alpha=0.05$ was used.

To analyze the comparative efficacy of the procedures, percent change of each parameter after treatment was calculated. These percent changes were then compared using a nonpaired t-test. If data did not meet the assumptions for parametric testing, a Wilcoxon-Mann-Whitney rank sum test was performed. Tests were two-tailed with a significance level of $\alpha=0.05$.

RESULTS

Aerodynamics

Summary aerodynamic data and statistics are presented in tables I and II. IL, ML, and ML-AA significantly decreased PTF ($p=0.007$; $p=0.003$; $p=0.008$), while only ML-AA had a significant effect on PTP ($p=0.008$) and PTW ($p=0.008$).

No significant differences were observed in percent change between injection and thyroplasty (PTF: $p=0.766$; PTP: $p=0.773$; PTW: $p=0.639$). The addition of AA led to significantly greater changes as compared to IL in PTF ($p=0.008$), PTP ($p=0.008$), and PTW ($p=0.008$) (figure 4).

Acoustics

Summary acoustic data and statistics are presented in tables III and IV. IL, ML, and ML-AA decreased perturbation measures of percent jitter and percent shimmer. IL decreased percent jitter significantly ($p=0.017$); the decrease in percent shimmer approached significance ($p=0.079$). ML significantly decreased percent jitter ($p=0.03$) and shimmer ($p=0.043$). The addition of AA further decreased percent jitter, though not significantly ($p=0.085$). IL, ML, and ML-AA also increased SNR ($p=0.078$; $p=0.338$; $p=0.042$). Neither IL ($p=0.912$) nor ML ($p=0.747$) had an effect on frequency. There was a discernible decrease in frequency after ML-AA ($p=0.059$).

IL and ML had comparable effects on both percent jitter ($p=0.853$) and percent shimmer ($p=0.670$). A discernible difference was observed, however, for SNR ($p=0.161$). Adding AA
led to greater improvement in perturbation measures compared to IL, though neither reached significance (percent jitter: p=0.105; percent shimmer: p=0.130) (figure 4).

**Mucosal wave**

Summary mucosal wave data and statistics are presented in tables V and VI. All procedures significantly decreased glottal gap (p<0.001). IL also decreased inter-fold phase difference (p=0.042) and amplitude of the paralytic fold (p=0.002). Decreased amplitude can be observed on the videokymogram derived from the high speed video recording (figure 5). ML did not have significant effects on either amplitude (p=0.879) or phase difference (p=0.116). The addition of AA to ML significantly decreased glottal gap (p=0.004) and discernibly increased amplitude (p=0.128).

ML improved mucosal wave amplitude (p=0.006) and phase difference (p=0.05) significantly more than IL. ML-AA decreased phase difference between the right and left vocal folds significantly more than ML alone (p = 0.008). Incremental increases in mucosal wave amplitude can be seen in videokymograms after ML and after ML-AA (figure 6) (figure 4).

**DISCUSSION**

This study performed a quantitative evaluation of IL, ML, and ML-AA to determine the effect of each on various parameters of phonation. Doing so in a controlled excised larynx setting with easily repeatable conditions decreased potential variability which may arise when conducting research on human subjects. Both ex vivo and in vivo canine larynges have been used previously to study interventions for vocal fold paralysis\(^{35,36,38}\). There are several anatomical differences between the human and canine larynx. The thyroid and cricoid cartilages and more angulated and not as tall in the canine larynx, and there is no well-defined vocal ligament\(^{35}\). These differences did not negatively impact the procedures that were evaluated.

IL and ML had significant effects on PTF, but not PTP. The added aerodynamic benefit of AA was evident, significantly decreasing PTF, PTP, and PTW. This change could be attributed to the decreased posterior glottal gap following adduction. Such a decrease would decrease PTF, a parameter dependent upon the cube of neutral glottal half-width\(^{39}\), and increase ease of phonation, promoting more efficient voicing. Though previous studies have measured the effect of different procedures on PTP, this is the first comparison using PTF. Measuring PTF offers an improved method of analyzing aerodynamic improvement after treatment for UVFP, as PTF is more sensitive than PTP to changes in glottal abduction\(^{40}\). This is also the first study analyzing PTW, a comprehensive threshold aerodynamic parameter proposed by Jiang and Tao\(^{39}\). This may be the optimal parameter to use when evaluating post-treatment laryngeal aerodynamics at the phonation threshold, as it encompasses both pressure and airflow.

As phonation tokens were taken at the phonation threshold, differences in frequency can be attributed to differences in PTP. ML-AA had the lowest PTP; accordingly, it also had the lowest frequency. Restoration of vocal fold contact via medialization of the paralyzed fold led to decreases in both percent jitter and shimmer. Interestingly, the addition of AA led to an additional significant decrease in percent jitter, while slightly increasing percent shimmer. Three of the eight larynges undergoing ML-AA had much higher percent jitter (4.47 ± 2.27) and percent shimmer (34.91 ± 10.87) than the other five (percent jitter: 1.18 ± 0.89; percent shimmer: 4.83 ± 3.69). Improper rotation of the arytenoid leading to vocal fold hypo- or hyperadduction in these larynges could have potentially led to this increase in perturbation measures and compromised acoustic quality. Posterior glottal gap in these larynges was higher (48.20 ± 22.97) than in the five larynges achieving superior acoustic outcomes (15.6 ± 9.58), indicating possible hypoadduction. Increased SNR across treatments can be attributed to

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decreased flow, or noise, required for phonation as well as improved acoustic sound, the signal. Improvement could be seen most dramatically with ML-AA, which resulted in an average increase greater than 300%. The three larynges exhibiting increased perturbation parameters also had the lowest SNR, likely due to decreased signal power rather than increased noise power.

Though IL is an effective clinical procedure that has benefits of convenience and relatively minimal invasiveness, its effect on the mucosal wave is detrimental (figure 5). IL led to improvements comparable to ML in acoustics and aerodynamics, but ML increased mucosal wave amplitude of the paralyzed fold while IL significantly decreased it. Analyzing other injection materials, such as fat which has the same viscosity as the vocal fold\textsuperscript{11}, may yield different results; however, a portion of these substances may be absorbed by the surrounding tissue over time\textsuperscript{38,41,42}.

Though ML-AA produced the best results in this study and is often beneficial for patients with posterior glottal chink, it is not appropriate for all patients. Not all patients are amenable to the increased operative time required to perform AA, such as those with iatrogenic UVFP stemming from complications during cardiothoracic surgery or those with severe aspiration that may not be able to remain supine for extended periods of time\textsuperscript{26}. Patients with aspiration that can tolerate the procedure, however, may experience improvement after AA\textsuperscript{43}. Selecting the proper procedure requires consideration beyond voice improvement. Incremental improvements in phonatory parameters after ML and ML-AA provide support, however, for the added procedure in patients who can tolerate it.

The experimental design allowed for easy and direct evaluation of the added benefits of performing AA. The benefits on aerodynamics and SNR, attributable to a decreased posterior glottal gap, were significant. As could be expected, ML-AA produced the most dramatic improvement in phonatory parameters (figure 4). Restoration of a normal mucosal wave, indicated by symmetric and periodic sinusoidal functions with sustained inter-vocal fold contact between cycles\textsuperscript{44}, also occurred in some larynges after AA (figure 6). However, as demonstrated by acoustic parameters, under- or overrotation of the arytenoid, common complications of AA, can sacrifice procedural outcome and should be avoided. Real-time intraoperative voice analysis may provide one means of preventing improper rotation and ensuring optimal voice quality. Future studies could use quantifiable acoustic and mucosal wave parameters to optimize degree of arytenoid rotation while performing AA.

An excised larynx setting offers the benefits of repeatable conditions, but confirming the results of this study in humans would be beneficial. Applying the aerodynamic and mucosal wave analyses used in this study may be of interest, as they have not been applied to UVFP patients previously. Measuring parameters at baseline, after ML, and after AA in the same patient would be advantageous, allowing clinicians to determine the degree to which AA improves laryngeal function after ML. Testing other implant types and injection materials using the excised larynx setup may allow for a convenient determination of optimal treatment methods.

**CONCLUSION**

IL, ML, and ML-AA improved aerodynamic and acoustic phonatory parameters. High speed video recordings revealed significantly decreased amplitude of the mucosal wave after IL, preservation after ML, and even restoration of a normal mucosal wave after ML-AA. Though ML-AA produced the best results, improper rotation of the arytenoid in three larynges demonstrates the need to avoid hypo- and hyperadduction of the paralyzed fold during AA. The excised larynx setup used in this study represents a possible tool which could be used to develop new methods of treating vocal fold paralysis.
Acknowledgments

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References

Figure 1.  
Schematic diagram of excised larynx experimental apparatus.
Figure 2.
Excised canine larynx with simulated right vocal fold paralysis before (A) and after (B) injection laryngoplasty.
Figure 3.
Excised canine larynx with simulated right vocal fold paralysis (A), after medialization laryngoplasty (B), and after medialization laryngoplasty with arytenoid adduction (C).
Figure 4.
Summary percent changes for all parameters tested. Bars represent summary means and error bars represent standard deviation. IL = injection laryngoplasty; ML = medialization laryngoplasty; ML-AA = ML with arytenoid adduction; PTF = phonation threshold flow (units: ml/s); PTP = phonation threshold pressure (units: cmH₂O); PTW = phonation threshold power (units: ml/s*cmH₂O); frequency (units: Hz); SNR = signal to noise ratio.
Figure 5.
Videokymographic images derived from high speed video recordings of vocal fold vibration with simulated vocal fold paralysis (A, lower curve) and after injection laryngoplasty (B).
Figure 6.
Videokymographic images derived from high speed video recording of vocal fold vibration with simulated vocal fold paralysis (A, lower curve), after medialization laryngoplasty (B), and after arytenoid adduction combined with medialization laryngoplasty (C).