

Evaluation of Surgical Strategies for Bilateral Vocal Fold Paralysis Using Excised Canine Larynges

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Summary: Objective. The objective of this study was to provide a theoretical basis for the selection of optimal surgical procedures in *ex vivo* simulated bilateral vocal fold paralysis (BVFP).

Study Design. Four surgical stages were sequentially performed on 10 excised canine larynges with simulated BVFP: (1) transverse cordotomy, (2) medial arytenoidectomy, (3) subtotal arytenoidectomy, and (4) total arytenoidectomy.

Materials and Methods. The sound pressure level, the signal-to-noise ratio, the glottal resistance, the glottal airflow (GF), the maximal glottal area (MGA), and spectrograms were measured after each stage. For comparative analysis of variance, a randomized block design and the Student-Newman-Keuls test were performed.

Results. Under stable phonation, the sound pressure level showed no significant differences among the four stages. The signal-to-noise ratio was significantly different between the preoperative period and stage 1, as well as between stages 2 and 3. Glottal resistance was significantly different between the preoperative period and stage 1 and between stages 1 and 2. GF and MGA were significantly different among all stages, compared with those between stages 3 and 4 for GF and the preoperative period and stage 1 for MGA. The spectrograms indicated that the degree of disorder in the acoustic signals gradually increased.

Conclusions. Based on a comprehensive analysis of GF and voice quality in excised canine larynges, which simulated BVFP, our results suggest that the optimal surgical choice for BVFP is either medial or subtotal arytenoidectomy.

Key Words: Bilateral vocal fold paralysis—Acoustics—Aerodynamics—Arytenoidectomy—Excised canine larynges.

INTRODUCTION

Because of failure of bilateral vocal fold abduction and loss of the normal protective reflex in the glottis, patients with bilateral vocal fold paralysis (BVFP) often present with potentially life-threatening breathing difficulties and pulmonary aspiration. Currently, common surgical treatments for patients with this condition include laser vocal fold surgery, laser arytenoid surgery, arytenoid abduction, laryngeal nerve transplantation, and tracheotomy. In 1984, Ossoff et al¹ successfully used CO₂ endoscopic laser arytenoidectomy to treat patients with BVFP. Because this surgical approach has fewer complications, preserves phonation, and involves less hospitalization and patient suffering, it has become the preferred treatment.

Still, remaining controversy remains over the different approaches, and the scope of laser arytenoidectomy has led to extensive research and comparisons.^{2–10} Despite the variability in approaches, the underlying purpose of this research has been to effectively resolve breathing difficulties while preserving adequate voice quality. Thus, current discussion focuses on evaluation of the selection and efficacy of endoscopic laser

surgical methods. Given that different surgical methods cannot be performed on the same patient for *in vivo* comparison, the present study used an excised canine laryngeal model to simulate human BVFP. Given its frequent use in clinical applications, standard laser surgery was selected and the four surgical stages were performed sequentially to collect pre- and postoperative acoustic, aerodynamic, and maximal glottal area (MGA) parameters for each stage. Subsequent calculation and analysis of parametric variations for each parameter facilitated the evaluation of the effect of the surgical stages on voice quality and airflow, which can go through the open glottal area.

MATERIALS AND METHODS

Preparation of models

Ten excised canine larynges were obtained after cardiovascular research experiments at the Medical College of Xiamen University. The larynges (bilateral vocal folds with normal morphology and symmetrical positions) were selected; those with vocal fold diseases or injuries were excluded; vocal fold length was maintained within 15 ± 2 mm) were removed from the animals after death and immediately stored in a 0.9% saline solution at -25°C for rapid cryopreservation. Before the experiment, which was completed within 1 week, the larynges were placed in physiological saline for slow thawing at 4°C . After complete thawing, the extrinsic laryngeal muscle and surrounding tissue anterior to the thyroid cartilage were removed, along with the false vocal folds and epiglottis, preserving the integrity of the entire vocal folds and arytenoid cartilage. The larynx was then fixed to a phonation experimental platform.¹¹ Each vocal fold was fixed using two steel needles in the medial position to create the laryngeal model to simulate BVFP. All experiments were conducted within 24 hours of establishing the model. All experiments were conducted within 24 hours of establishing the model.

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Surgical procedures

The four surgical stages were performed sequentially on the same larynges (Figure 1): stage 1: transverse cordotomy (TC); stage 2: medial arytenoidectomy (MA); stage 3: subtotal arytenoidectomy (SA); and stage 4: total arytenoidectomy (TA).

Based on the current mainstream trends of laser arytenoidectomy, the procedures in stages 1 and 2 were conducted according to the method described by Rosen and Simpson.¹² The procedure in stage 3 was based on the technique described by Remacle et al.³ The procedure in stage 4 was based on the technique described by Ossoff et al.¹

Experimental process

In an anechoic chamber, the model was fixed to the experimental platform. A microphone (ECM-678; Sony, Tokyo, Japan) was placed 15 cm away from the vocal folds, with its midline aligning with the glottal opening. To visualize the vocal fold vibration process, a high-speed camera (Phantom MIRO M110; Ametek, Tokyo, Japan) was placed 30 cm above the vocal folds. A specified airflow pressure was produced by an air compressor (Model 2530; Xiamen Taixing Mechanical and Electrical Co., Ltd., Xiamen, China) placed beyond the experimental platform. The airflow was heated and humidified (approximate temperature

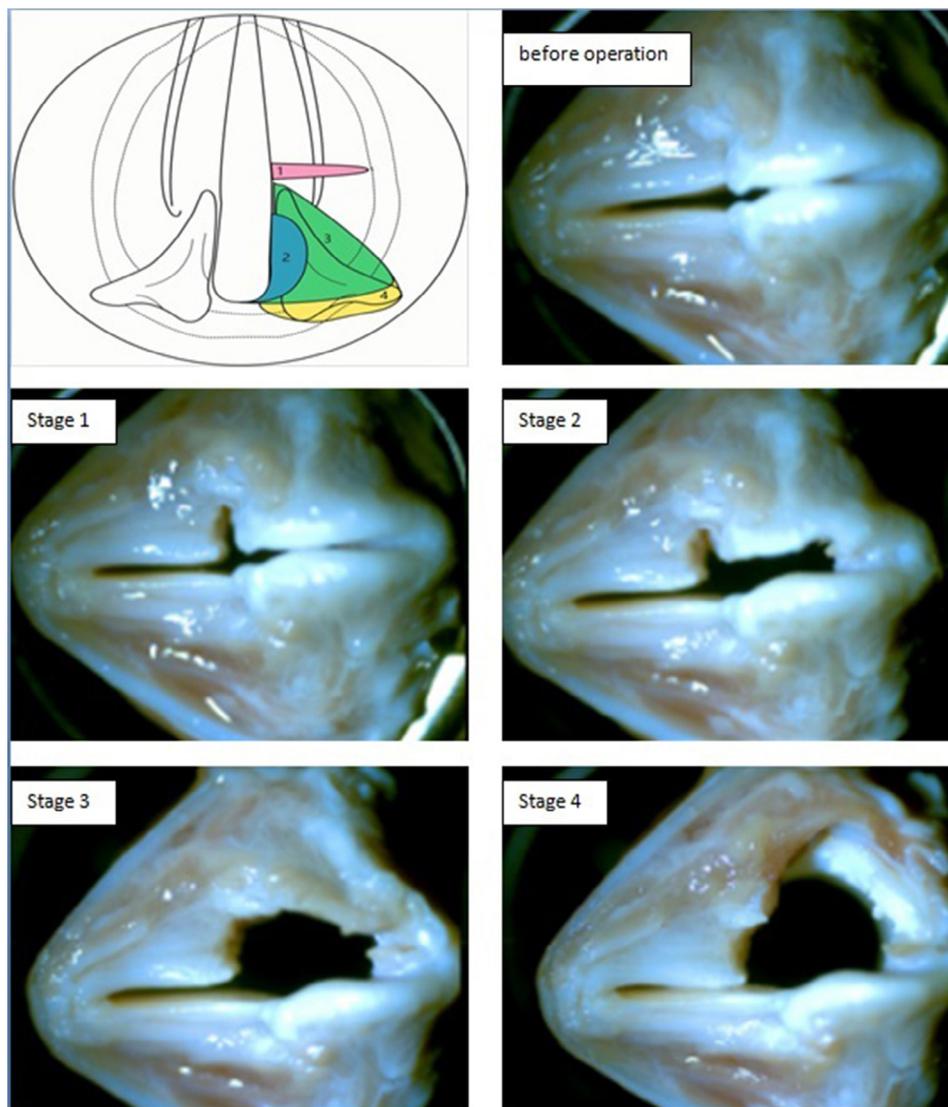


FIGURE 1. Schematic diagram and real images of the scope of surgical resection at each stage. *Notes:* Stage 1 (*pink*): The entire vocal fold just anterior to vocal process was transected laterally. A complete cordotomy extends laterally 3–4 mm into the false vocal fold tissue. Stage 2 (*blue*): Based on stage 1, the anterior–posterior dimensions of this area of obliteration should be posterior to the tip of the vocal process and should not extend to the posterior arytenoid tissue, with preservation of the vocal process and adjacent mucosa in the intra-arytenoid area. Stage 3 (*green*): Based on stage 2, the majority of the arytenoid cartilages including the vocal process were removed with preservation of the posterior shell. Stage 4 (*yellow*): Based on stage 3, the entire arytenoid cartilage was removed. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

37°C, humidity >90%) using a modified atomizing humidifier (022G877S; PARI GmbH, Starnberg, Germany). An artificial lung (custom-made cylinders [radius 8 cm, height 16 cm]) was used to drive vocal fold vibration. Airflow pressure changes were measured using a custom-made diaphragm pressure gauge (YE-100B; Shanghai Huanhong Automatic Instrument Science & Technology Co., Ltd., Shanghai, China) below the artificial lung. The ventilation tube was connected to an electronic flow meter (MF-5706-N-10; Nanning Kongxin Instruments Ltd., Nanning, China). A sound level meter (WS1361, accuracy 1.0 dB; Shenzhen Wanshengtong Co., Ltd., Shenzhen, China) was placed 15 cm from the vocal folds.

Collection and analysis of acoustic signals and aerodynamic parameters

The electronic flow meter and the diaphragm pressure gauge were used to measure glottal airflow (GF) and subglottal pressure, respectively, during stable phonation after each of the four surgical stages. Glottal resistance (GR) was calculated using the equation $GR = \frac{P_{sub}}{GF}$. Acoustic measurement tools included a microphone, an electret condenser microphone, and a sound card. *Cool Edit* audio software (Adobe Systems, San Jose, CA) was used to collect 3 seconds of acoustic signals after stable phonation at a sampling frequency of 44.1 kHz. Analysis to obtain the spectrograms was performed using *Cool Edit*, and the *CSpeech* software (Milenkovic, 2001, Madison, WI) was used to obtain the signal-to-noise ratio (SNR). Measurement of the sound pressure level (SPL) was performed using the sound level meter.

Collection and analysis of high-speed digital images

Phantom Camera Control software (AMETEK, Berwyn, PA) was used for the analysis of the high-speed images. Based on previously described methods,¹³ Lagrange interpolation and Canny edge detection were applied to calculate the MGA (maximum

value of the glottal open area) after each of the four surgical stages. The conversion between image pixel glottal area was measured by placing a grid paper (1 cm²) on the vocal fold plane (the area of the 200 × 200 pixels was 1 cm²).

Statistics

SPSS 13.0 for Windows (SPSS Inc., Chicago, IL) was used to perform analysis of variance for the randomized block design, as well as the Student-Newman-Keuls test. The significance level was $\alpha = 0.05$.

RESULTS

Comparison of parameters after the four surgical stages

For the comparison of GR, GF, SPL, SNR, and MGA among the four surgical stages, the analysis of variance results of the randomized block design showed that all parameters had statistical significance. The results of pairwise comparisons between adjacent groups for these five parameters are shown in [Table 1](#).

SPL was not significantly different among the four surgical stages.

The SNR increased after stage 1, followed by a decrease after stage 3. The SNR between the preoperative period and stage 1 and between stages 2 and 3 was significantly different, but not between stages 1 and 2 or between stages 3 and 4.

GF gradually increased; the differences between all of the stages were statistically significant with the exception of the difference between stages 3 and 4.

GR gradually decreased; between the preoperative period and stage 1, as well as between stages 1 and 2, the differences were significantly different, but not among the remaining stages.

MGA gradually increased; whereas the difference between the preoperative period and stage 1 was not significantly different, the differences among the remaining stages were statistically significant.

TABLE 1.

Pairwise Comparisons Between Adjacent Groups for GR, GF, SPL, SNR, and MGA Values From Excised Canine Laryngeal Models Among the Four Surgical Stages

Stage Parameter	Preoperative	1	2	3	4
GR	0.78 ± 0.18	0.62 ± 0.12	0.46 ± 0.07	0.32 ± 0.07	0.23 ± 0.05
P _{GR}		0.018*	0.013*	0.075	0.779
GF	1668.59 ± 389.58	2029.34 ± 319.19	2406.89 ± 243.72	2713.65 ± 299.52	2902.43 ± 113.98
P _{GF}		0.001†	0.000†	0.003†	0.059
SPL	76.28 ± 2.19	75.84 ± 3.21	76.58 ± 3.42	80.49 ± 3.46	81.5 ± 3.78
P _{SPL}		0.999	0.999	0.102	0.999
SNR	2.93 ± 1.13	7.73 ± 3.96	6.95 ± 4.89	2.91 ± 2.13	2.07 ± 0.38
P _{SNR}		0.001†	0.571	0.005†	0.541
MGA	35.53 ± 0.08	41.56 ± 0.86	52.78 ± 0.13	63.97 ± 0.16	72.76 ± 0.20
P _{MGA}		0.151	0.010*	0.010*	0.039*

Notes: The *P* values listed in the third column refer to the comparisons between preoperative and stage 1. Those in the fourth column refer to the comparisons between stages 1 and 2. Those in the fifth column refer to the comparisons between stages 2 and 3. Those in the sixth column refer to comparisons between stages 3 and 4.

* Indicates $P < 0.05$.

† Indicates $e < 0.01$.

Abbreviations: GF, glottal airflow; GR, glottal resistance; MGA, maximal glottal area; SNR, signal-to-noise ratio; SPL, sound pressure level; P_{GR}, P_{GF}, P_{SPL}, P_{SNR}, and P_{MGA}, *P* values for GR, GF, SPL, SNR, and MGA, respectively.

Figure 2A–E shows the spectrograms of acoustic signals collected from larynx no. 3; the degree of disorder of the acoustic signals increased gradually.

Relationships between MGA and GF among the four surgical stages

When subglottal pressure was controlled within a specific range, the increase in MGA and changes in GF showed a nonlinear relationship (Figure 3).

DISCUSSION

The invasiveness of experimentation in human subject studies is limited because of many considerations. An example of these limitations lies within the inability to perform different *in vivo* surgical procedures sequentially on the same patient. These shortcomings have been overcome in part by the excised animal model, particularly, the canine model. It is widely acknowledged that canine larynges can be used experimentally to simulate human laryngeal activity,^{14–17} given that the general morphology of canine vocal folds is very similar to that of human vocal folds, with comparable vibration characteristics and highly similar microstructures and anatomic characteristics. Therefore, canine vocal folds have been proven as indispensable pathologic models in the simulation of human voice disorders and the exploration of treatment methods.^{18,19} Additionally, Chan and Titze²⁰ demonstrated no major changes in the viscoelasticity of canine vocal folds that were cryopreserved for 1 day, 1 month, or even longer, making them ideal for use in the laboratory. Given these advantages, an excised canine model was used in our study to overcome the limitations of *in vivo* human subjects by allowing us to perform sequentially staged operations on an individual larynx.

Many studies have evaluated the efficacy of laser surgical methods for patients with BVFP. However, analysis of the advantages and disadvantages among different surgical procedures has rarely been conducted. Furthermore, comparison of different procedures in the same patient has also been proven to be impossible because of the aforementioned obstacles. Because of a lack of information regarding patient characterization, comparison of individual differences between patients has also been neglected, contributing to the limitations and the one-sidedness of such research results. The canine model allowed us to perform horizontal analyses among different surgical procedures to determine the effects of each stage of surgery on voice quality, glottal open area, and GF, which cannot be accomplished in humans.

In this experiment, because the vocal folds were transected perioperatively and only the arytenoid cartilage was fixed to obtain glottal closure, under the effect of airflow, the upward and downward movements of the vocal fold became the main sound source. Given the fixed positioning of the folds, the GF during phonation is equal to that of the GF during breathing in the simulated BVFP experimental model. The acoustic signals became more chaotic with increased noise. Given that these acoustic signals were not type 1, we analyzed them based on SPL, SNR, and spectrograms to evaluate voice quality, but not jitter and shimmer. When measured in the human body,^{5,8} however, it was found that postoperative jitter and shimmer increased, but the values

remained within the normal range. This finding may be due to the measurements being acquired 3–6 months postoperatively and the fact that the human body has self-healing processes. Furthermore, GR, GF, and MGA were used to evaluate the level of respiratory difficulty and thus may provide more insight into the effects of these procedures in comparison with SNR and SPL.

Khalifa²¹ found that TC effectively eliminated breathing difficulties, and this claim is well supported by our results. TC did not markedly enhance MGA, but significantly increased GF and led to a corresponding decrease in GR, indicating that breathing difficulties were partially alleviated. In addition, SNR increased significantly because of poor glottal closure, resulting in increased breathing sounds and vocal fold segment participation in phonation. Furthermore, the spectrograms show that interruptions were observed in the formants after stage 1; sound energy distribution and frequency changes were irregular; mixed frequency components were observed; and noise values increased gradually. This finding indicates that TC had an impact on phonation, but only partially.

In theory, MA only involves concave resection of the arytenoid body along the glottal edge and does not involve the vocal process and the posterior arytenoid tissue. Hence, MA should not affect the normal anatomic position of the vocal folds and should spare adjacent mucosa in the intra-arytenoid area. Theoretically, this has a relatively minor impact on voice quality and significantly increases the breathing area, thus markedly enhancing GF. Regarding the advantages and disadvantages of TC and MA, Bosley et al⁶ compared phonation and breathing conditions among 11 cases of TC and 6 of MA. A double-blinded audio perceptual analysis indicated no significant changes in voice quality between the two surgical methods. However, 4 of 6 of MA patients and 2 of 11 of TC patients had increased Voice Handicap Index-10 scores. Moreover, scores were significantly higher in MA than in TC patients. After the two procedures, 62.5% of patients reported significantly better breathing, whereas 25% reported partial improvement. Based on these results, Bosley et al claimed that both procedures alleviated breathing difficulties while also reducing the occurrence of postoperative voice disorders and swallowing difficulties. Hence, Bosley et al believed that both procedures were suitable for patients with BVFP. However, Bosley et al's study compared the advantages and disadvantages between different patients, whereas the differences between individuals could not be measured. Therefore, interpreting these results has limitations. The present study performed different procedures on the same canine larynx, the results indicating that when compared with TC, MA produced higher GF and MGA, a markedly reduced GR, and improvement in the degree of respiratory obstruction. Further, the absence of a significant intergroup difference in SNR is similar to the results reported by Bosley et al,⁶ where the spectrogram showed that the acoustic signals indicated an insignificant increase in the degree of disorder. Therefore, our results indicate that the advantages of MA are more significant than those of TC.

Remacle et al³ modified the technique of arytenoidectomy described by Ossoff et al,¹ termed "SA." The principal modification involves preservation of a thin (2 mm) posterior shell, thereby providing good postoperative fixation of the arytenoid region.

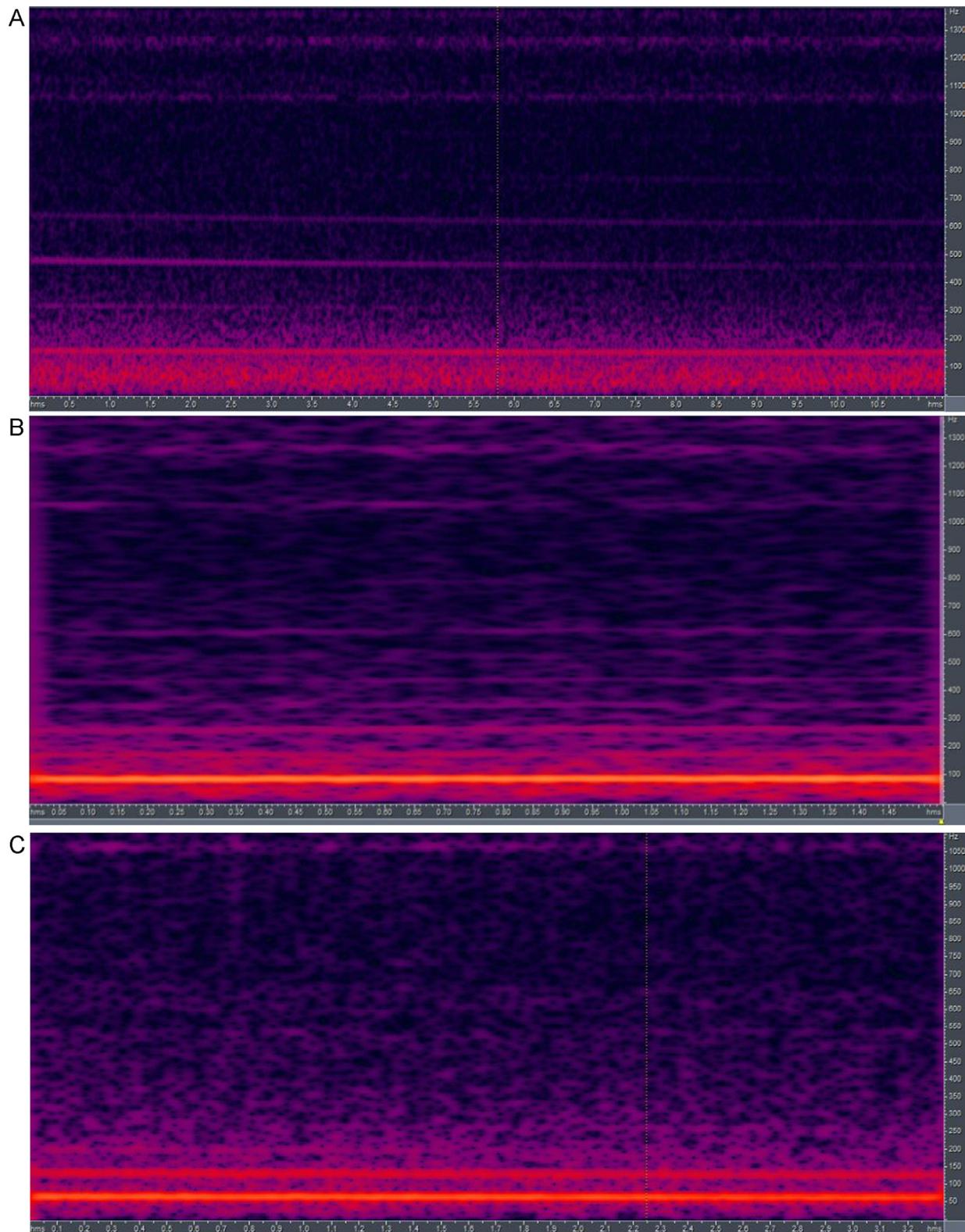


FIGURE 2. (A) The spectrogram of the excised canine laryngeal model before operation. (B) The spectrogram of the excised canine laryngeal model after stage 1. (C) The spectrogram of the excised canine laryngeal model after stage 2. (D) The spectrogram of the excised canine laryngeal model after stage 3. (E) The spectrogram of the excised canine laryngeal model after stage 4. *Notes:* During the preoperative period, the spectrogram was clear, energy distribution was uniform, formants were clearly visible, and the patterns of sound energy distribution and frequency changes did not show signs of disorder. After stages 1 and 2, the spectrogram showed interruptions, and sound energy distribution and frequency changes were irregular; however, the formants were still clearly visible. After stages 3 and 4, sound energy distribution and frequency changes became disordered and formants could not be observed.

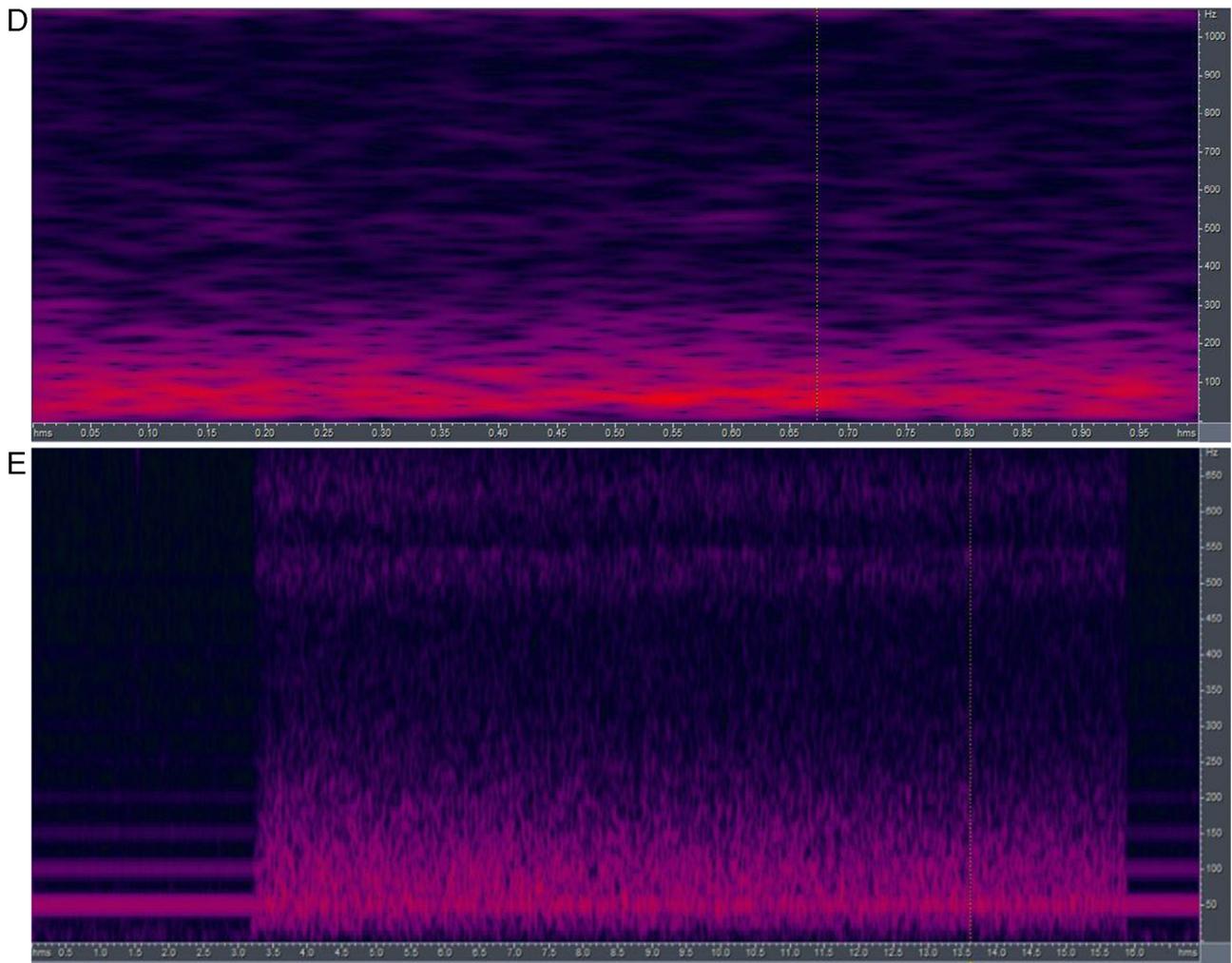


Figure 2. (Continued)

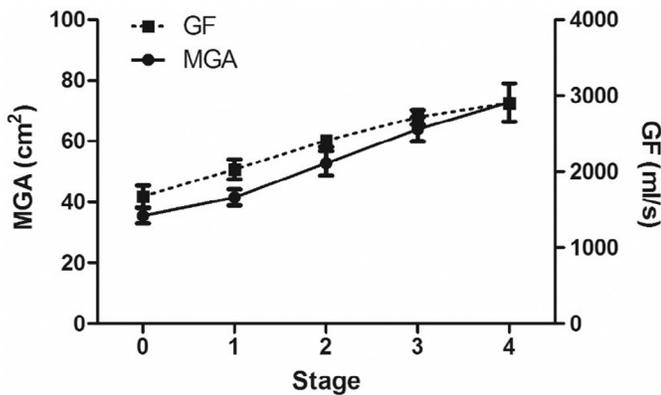


FIGURE 3. Relationship between MGA and GF in the excised canine laryngeal models among the four surgical stages. *Notes:* After stage 1, MGA increased by 6.01 cm², whereas GF increased by 360.57 mL/s. After stage 2, MGA increased by 11.22 cm², whereas GF increased by 377.55 mL/s. After stage 3, MGA increased by 11.19 cm², whereas GF increased by 306.76 mL/s. After stage 4, MGA increased by 8.79 cm², whereas GF increased by 188.78 mL/s.

The risk of aspiration is thus averted and the collapse of arytenoid mucosa into the larynx during inspiration is prevented. Gandhi⁷ also verified the importance of SA in reducing pulmonary aspiration and other complications. The present study found that, compared with MA, SA further enhanced GF and MGA. Although the difference in GR was not significant, there was decreased GR and marked alleviation of breathing difficulties. Although SNR decreased significantly after SA, acoustic signals were extremely weak and virtually could not achieve phonation, whereas breathing sounds were obvious. This could be a result of the glottal opening being artificially large in the experimental model. The spectrograms indicated an increased degree of disorder in acoustic signals, chaotic sound energy distribution, and frequency changes. These results imply that caution should be exercised when selecting SA in patients with high requirements for sound quality.

The major difference between total arytenoidectomy and SA is retention of part of the arytenoid cartilage. Two studies^{3,9} claimed SA to be beneficial for improving voice quality and to be effective in preventing pulmonary aspiration. However, as SA retains part of the arytenoid cartilage, in theory, it would be less effective in alleviating breathing difficulties than TA. The present

study examined this problem and found that, although TA significantly increased MGA compared with SA, the former did not increase GF and the changes in GR were small. Thus, TA provided limited improvement in breathing. The nonlinear relationship between MGA and GF was observed in the present study, which suggests that GF cannot be surgically increased simply by increasing MGA. For example, after stage 1, MGA increased by 6.01 cm², whereas GF increased by 360.57 mL/s. After stage 4, MGA increased by 8.79 cm², whereas GF increased only by 188.78 mL/s. Comparisons of phonation indicate that, although the difference in SNR was not statistically significant between TA and SA, this was still markedly reduced after TA and phonation was nearly impossible. Analysis of the spectrograms also indicated that TA exacerbated voice quality without further alleviating breathing difficulties while also markedly increasing the probability of complications.^{3,9} Hence, SA should be selected in the clinical setting, which will significantly improve patients' quality of life.

Using excised canine larynges, Fariborz and Michael found that SPL did not appear to be impacted by the adjustments in ventricular gap.²² The present study also found that SPL appears to be similar in all surgical stages with no significant differences. SPL did not appear to be impacted by the adjustments in glottal size.

One advantage of our study is that all four surgeries were sequentially performed on the same excised canine larynx, thus resolving the problem of not being able to perform different surgical procedures on the same patient. The limitation of the present study is that excised canine larynges cannot be used to simulate the postoperative self-healing processes and the scar tissue formation processes of the human body; therefore, the impact of the variation in postoperative healing and the scar tissue on surgical efficacy could not be examined. The scar tissue is always presented in these cases in the clinical setting, so the clinical guidance provided by the present study still has limitations. In future studies, we will test the results *in vivo* using canine larynges and hope to provide a fundamental theoretical basis for selecting the optimal surgical approach for human patients.

CONCLUSIONS

Compared with TC, MA enhanced GF more effectively without exacerbating voice quality. In comparison with MA, SA alleviated dyspnea more effectively but significantly worsened voice quality. Finally, when compared with SA, TA did not significantly worsen voice quality but did not contribute substantially to increasing GF, merely increasing MGA during breathing. Based on a comprehensive analysis of GF and voice quality in excised canine larynges, which simulated BVFP, our results suggest that the optimal surgical choice for BVFP is either MA or SA.

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