Article

The Impact of Laser Surgery for Vocal Cord Cysts on Patients' Voice Acoustic Analysis Parameters and Inflammatory Response

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AIM: Vocal cord cyst is a common benign proliferative lesion of the vocal cords, usually resulting from excessive use of the voice, environmental pollution, and inflammatory infections. This study aims to explore the differences in the effectiveness of carbon dioxide (CO_2) laser or surgical treatment for vocal cord cyst patients.
METHODS: This retrospective study analyzed 81 patients with vocal cord cysts who underwent surgical treatment at Ruijin Hospital, Shanghai Jiao Tong University School of Medicine between July 2021 and July 2024. Among them, 40 patients were treated with CO_2 laser surgery (the laser group), and 41 underwent traditional surgical treatment (the surgery group). The study compared the pre- and post-treatment voice acoustic parameters, aerodynamic parameters, levels of inflammatory factors, stress response indicators, and surgical complications between the two groups.
RESULTS: Before surgery, there were no significant differences between the laser group and the surgery group regarding jitter, shimmer, harmonic-to-noise ratio (HNR), maximum phonation time (MPT), mean airflow rate, and subglottic pressure ($p > 0.05$). However, at 4 weeks post-surgery, the laser group showed lower values for jitter, shimmer, and mean airflow compared to the surgery group ($p < 0.05$), while the laser group demonstrated a longer MPT compared to the surgery group ($p < 0.05$). Before surgery, there were no significant differences between the laser and surgery groups regarding the levels of interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) ($p > 0.05$). However, at 4 weeks post-surgery, the IL-1β and TNF-α levels in the laser group were lower than those in the surgery group ($p < 0.05$). Before surgery, there were no significant differences between the laser and surgery groups in Voice Handicap Index (VHI-10) scores and the Grade, Roughness, Breathiness, Asthenia, Strain (GRBAS) scores ($p > 0.05$). Moreover, the VHI-10 and GRBAS scores were lower in the laser group compared to the surgery group at 4 weeks post-surgery ($p < 0.05$). Additionally, the complication rates in the laser group were 12.50%, compared to 24.39% in the surgery group, indicating no significant differences ($p > 0.05$).
CONCLUSIONS: Both CO_2 laser and conventional surgery for treating vocal cord cysts show favorable outcomes. However, CO_2 laser surgery offers faster recovery of vocal function and is more effective at reducing the inflammatory response.

Keywords: vocal cord cysts; carbon dioxide laser surgery; traditional surgery; voice acoustic parameters; aerodynamic parameters; inflammatory factors

Introduction

Vocal cord cysts are a common benign proliferative lesion of the vocal cords, typically caused by excessive vocal use, environmental pollutants, and inflammatory infections [1]. Epidemiological study has shown that vocal cord cysts account for approximately 20% of all benign proliferative vocal cord diseases [2]. They are more common in adults, usually occurring unilaterally, though they may sometimes lead to contralateral vocal cord contact nodules. The causes of vocal cord cysts include vocal cord gland duct obstruction, mucus retention, trauma, embryonic dislocation, upper respiratory tract infections, and exposure to productive dust or harmful gases. The "body-cover" theory divides the vocal cords into five layers, with only the "cover layer"—comprising the mucosal epithelium and the superficial layer of the lamina propria—capable of vibrating and modulating pitch [3]. Due to the delicate structure and critical function of the vocal cords, treatment for vocal cord cysts prioritizes preserving normal tissue and maintaining functionality. In recent years, advances in laryngeal microsurgery techniques have led to minimally invasive approaches becoming the main approach for treating vocal cord cysts.

Currently, two surgical techniques commonly used in clinical practice for treating vocal cord cysts are traditional laryngeal microsurgery and laryngeal laser surgery [4]. Traditional laryngeal microsurgery involves the use of cold instruments, such as laryngeal knives, scissors, and forceps, to excise the cyst. It offers precise removal of the affected

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tissue while minimizing damage to surrounding normal tissue [5]. In contrast, laryngeal laser surgery, primarily using carbon dioxide (CO_2) lasers, offers precise cutting combined with hemostatic capabilities, substantially reducing intraoperative bleeding. Additionally, it provides high tissue selectivity, allowing for delicate handling of the affected vocal cord areas [6].

Despite the widespread use of CO_2 laser surgery and traditional microsurgical procedures, comparative investigations on their efficacy, postoperative recovery, inflammatory response, and complication rates remain limited. The study aims to address this gap by comparing the differences in vocal parameters, aerodynamic parameters, and biochemical indicators between two approaches, providing a new perspective on exploring the optimal treatment plan. Furthermore, utilizing modern phonetics and histopathology, this study evaluates the impact of surgical methods on tissue repair and stress responses. These findings not only provide clinicians with evidence-based data for treatment selection but also deepen the theoretical understanding of the pathological and physiological changes after vocal cord cyst surgery. Considering this, the study compares the clinical outcomes of CO_2 laser surgery and surgical microsurgery for vocal cord cysts, aiming to provide robust references for clinical practice, help optimize treatment strategies, and ultimately improve patients' outcomes and quality of life.

Materials and Methods

Recruitment of Study Participants

This retrospective study recruited 81 vocal cord cyst patients who underwent surgical treatment at Ruijin Hospital, Shanghai Jiao Tong University School of Medicine between July 2021 and July 2024. The sample size of cases was calculated using the formula as follows: $N = Z^2 \times \sigma^2/d^2$, where Z is the confidence interval, N is the sample size, d is the sampling error margin, and σ is the standard deviation, usually assumed to be 0.5. Out of the total patients, 40 received CO_2 laser surgery (the laser group), and 41 underwent traditional surgery (the surgery group). The treatment method was selected by the patients and their families.

This study was a retrospective study and was exempted by the Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine. The study was conducted in accordance with the Declaration of Helsinki, and informed consent was obtained from each participant.

Inclusion and Exclusion Criteria

Patients were diagnosed following the guidelines outlined in the 9th edition of Otorhinolaryngology-Head and Neck Surgery [7].

Inclusion criteria: (1) Individuals aged 18 years or older; (2) those diagnosed with vocal cord cyst or polyp using laryngoscopy, presenting clinical symptoms such as dysphonia or vocal cord abduction disorder; (3) and those who did not respond to at least 3 months of conservative treatment; (4) patients treated by the same group of surgeons.

Exclusion criteria: (1) Patients diagnosed with laryngeal tuberculosis, (2) throat malignant tumor; (3) patients with a history of throat trauma or surgery, (4) Reinke's edema; (5) patients coexisting neuromuscular paralysis disorders; (6) patients with a history of glucocorticoid use within the past 2 months.

Surgical Approaches

Patients in the laser group received CO_2 laser surgery. During this procedure, a laser-safe endotracheal tube was inserted, and strict laser safety protocols were followed. Surrounding tissues were protected by covering them in gauze soaked in saline solution to prevent damage. After saline irrigation, the cyst was excised using a micro-focused CO_2 laser. The laser was operated with the following settings: a spot diameter of 0.25 mm, a duration of 0.05 seconds, and a power of 3 W in repeat super-pulse mode.

Furthermore, the patients in the surgery group received traditional surgical interventions. During cyst excision, saline irrigation was initially applied. The cyst was then excised using micro forceps and scissors. For larger cysts, microflap surgery techniques were employed to preserve the integrity of the lamina propria and muscle layer.

Observational Indicators and Detection Method

Collect patients' gender, age, BMI, smoking history, alcohol consumption history, disease course, and affected side information from the medical record system.

The study assessed and compared the voice acoustic parameters and aerodynamic parameters [jitter, shimmer, harmonic-to-noise ratio (HNR), maximum phonation time (MPT), mean airflow rate, and subglottic pressure], along with levels of inflammatory factors [interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α)] between the two groups both before treatment and 4 weeks post-surgery. Furthermore, the rate of surgical complications was also evaluated and compared between the two groups.

Voice impairment was evaluated using the Voice Handicap Index (VHI-10) [8]. This index assesses patients across three dimensions—physical, functional, and emotional—using 10 questions. Each question is scored on a scale from 0 to 4, with higher scores indicating severe voice impairment.

Furthermore, the Grade, Roughness, Breathiness, Asthenia, Strain (GRBAS) scale [9] is a subjective tool used to evaluate a patient's voice. This scale assesses five parameters: G (overall grade of hoarseness), R (roughness), B (breathiness), A (asthenia or weakness of voice), and S (strain in the voice). Each parameter is scored on a scale ranging from 0 to 3, where 0 shows normal, 1 indicates mild abnormality, 2 denotes moderate abnormality, and 3 shows severe abnormality.

The study assessed and compared voice acoustic and aerodynamic parameters between the two groups. To ensure the accuracy of measurements, all of the vocal and aerodynamic parameter evaluations in this study were performed under the guidance of the same professionally trained researcher. Before measurement, patients were advised to maintain consistent conditions, such as being in the same physical state and undergoing assessment at the same time of day to reduce changes caused by physiological fluctuations. Furthermore, patients were instructed to maintain the same frequency of voice to avoid vigorous use or prolonged vocal rest before testing. The evaluations were performed using the Divas voice analysis software (version 2.7, XION, Berlin, German). Patients were seated in a soundproof room with ambient noise of <45 dBA, approximately 30 cm from the microphone. During the test, patients were instructed to take a deep breath and sustain the vowel sound "/a/" for as long as possible, repeating the task three times. The longest segment was selected for voice analysis to determine the MPT.

For voice analysis, patients were asked to produce a natural, sustained "/a/" vowel, and a stable 3-second sample was used to measure jitter, shimmer, and the harmonic-to-noise ratio (HNR). Mean airflow and subglottic pressure were evaluated using the Phonatory Aerodynamic System Model 6600 (PENTAX Medical, Tokyo, Japan).

Furthermore, fasting peripheral venous blood samples (5 mL) were collected from patients both before and after treatment. IL-6 (Catalog No.: RM17601), IL-1β (Catalog NO.: RK05046), and TNF-α (Catalog NO.: RK05051) levels were determined using enzyme-linked immunosorbent assay (ELISA). The ELISA assay kits were obtained from ABclonal (Woburn, MA, USA).

Statistical Analysis

Data were analyzed using SPSS statistical software (version 21.0, IBM, New York, NY, USA). Continuous variables such as jitter, shimmer, HNR, MPT, mean airflow and subglottic pressure were examined for normality. Shapiro-Wilktest test was used for normality testing. Normal distributed variables were expressed as mean $\pm$ standard deviation ($\bar{x} \pm$ s), and comparisons between groups were performed using the *t*-test. Categorical data, such as affected side distribution and complication rates, were expressed as percentages (%), with group comparisons performed using the chi-square χ^2 test. A *p*-value of <0.05 was considered statistically significant.

Results

Comparison of General Data Between the Two Groups

As shown in Table 1, the baseline data were comparable between the laser and surgery groups, revealing no significant differences ($p > 0.05$).

Comparison of Voice Acoustic and Aerodynamic Parameters Between the Two Groups

Before surgery, there were no statistically significant differences between the laser and surgery groups in terms of jitter, shimmer, HNR, MPT, mean airflow, and subglottic pressure ($p > 0.05$). However, at 4 weeks post-surgery, both groups showed significant reductions in jitter, shimmer, mean airflow, and subglottic pressure ($p < 0.05$), while demonstrating a substantial increase in HNR and MPT ($p < 0.05$). Furthermore, at 4 weeks post-surgery, the laser group showed lower values of jitter, shimmer, and mean airflow compared to the surgery group ($p < 0.05$), and patients in the laser surgery group exhibited a longer MPT than those in the surgery group ($p < 0.05$, Table 2).

Comparison of Serum Inflammatory Indexes Between the Two Groups

Before operation, IL-6, IL-1β and TNF-α levels were comparable between the laser and operation groups ($p > 0.05$). However, 4 weeks after operation, IL-6, IL-1β and TNF-α levels were significantly decreased in both groups ($p < 0.05$). Furthermore, 4 weeks after surgery, IL-1β and TNF-α levels were reduced in the laser group than in the surgery group ($p < 0.05$, Table 3).

Comparison of VHI-10 and GRBAS Scores Between the Two Groups

Before surgery, there were no significant differences in VHI-10 and GRBAS scores between the laser and surgery groups ($p > 0.05$). However, 4 weeks post-surgery, both groups showed a significant reduction in VHI-10 and GRBAS scores ($p < 0.05$). Additionally, at 4 weeks post-surgery, the VHI-10 and GRBAS scores were reduced in the laser group than in the surgery group ($p < 0.05$, Table 4).

Comparison of Complications Between the Two Groups

After surgery, the complication rates were comparable between the laser (12.50%) and surgery (24.39%) groups, indicating no significant differences ($p > 0.05$, Table 5).

Discussion

Vocal cord cysts can lead to hoarseness, reduced voice quality, and other voice-related symptoms, significantly affecting patients' quality of life [10]. Due to the delicate structure of the vocal cords, which is essential for their function, the primary goal in treating vocal cord cysts is to remove the cyst effectively while preserving as much normal vocal cord tissue and functionality as possible. Traditional surgery, known for its direct and thorough approach, is the preferred choice for many otolaryngologists in treating vocal cord cysts. However, it has certain limitations, such as longer recovery times, a relatively higher risk of bleeding, and the need for advanced technical expertise during the procedure [11]. With advances in laryngeal microsurgical techniques, treatment methods for vocal cord cysts have su-

Table 1. Comparison of general data between the two groups.

Experimental groups	n	Age (years old)	BMI (kg/m^2)	Gender (%)		Smoking (%)	Drinking (%)	Disease duration (month)	Distribution of affected side (%)	
				Male	Female				Left side	Right side
Laser group	40	34.8 $\pm$ 8.2	24.10 $\pm$ 2.00	12 (30.00)	28 (70.00)	7 (17.50)	10 (25.00)	6.47 $\pm$ 2.61	20 (50.00)	20 (50.00)
Surgery group	41	36.4 $\pm$ 9.0	23.70 $\pm$ 2.24	18 (43.9)	23 (56.1)	12 (29.27)	15 (36.59)	6.90 $\pm$ 2.44	27 (65.85)	14 (34.15)
t/χ^2		–0.836	0.847	1.678		1.562	1.274	–0.766	2.089	
p-value		0.406	0.400	0.195		0.211	0.259	0.446	0.148	

BMI, body mass index.

Table 2. Comparison of voice acoustic and aerodynamic parameters between the two groups ($\bar{x} \pm s$).

Experimental groups	n	Jitter (%)		Shimmer (%)		Harmonic-to-noise ratio (dB)	
		Before surgery	4 weeks after surgery	Before surgery	4 weeks after surgery	Before surgery	4 weeks after surgery
Laser group	40	5.38 $\pm$ 1.40	1.80 $\pm$ 0.43[a]	8.40 $\pm$ 1.66	3.20 $\pm$ 0.83[a]	18.33 $\pm$ 2.40	22.67 $\pm$ 2.54[a]
Surgery group	41	5.11 $\pm$ 1.53	2.21 $\pm$ 0.56[a]	8.04 $\pm$ 1.74	4.16 $\pm$ 1.20[a]	18.96 $\pm$ 2.71	23.50 $\pm$ 2.85[a]
t		0.828	–3.689	0.952	–4.178	–1.107	–1.383
p-value		0.410	$<$0.001	0.344	$<$0.001	0.272	0.171
Experimental groups	n	Maximum phonation time (s)		Mean airflow (L/s)		Subglottic pressure (cmH$_2$O)	
		Before surgery	4 weeks after surgery	Before surgery	4 weeks after surgery	Before surgery	4 weeks after surgery
Laser group	40	4.81 $\pm$ 1.30	8.63 $\pm$ 2.36[a]	0.76 $\pm$ 0.18	0.46 $\pm$ 0.15[a]	11.40 $\pm$ 2.84	6.93 $\pm$ 1.88[a]
Surgery group	41	5.10 $\pm$ 1.46	6.94 $\pm$ 2.18[a]	0.72 $\pm$ 0.17	0.54 $\pm$ 0.17[a]	10.96 $\pm$ 2.92	7.55 $\pm$ 2.03[a]
t		–0.943	3.349	1.028	–2.244	0.687	–1.425
p-value		0.348	0.001	0.307	0.028	0.494	0.158

[a], compared to the preoperative, $p < 0.05$.

Table 3. Comparison of serum inflammatory indexes between the two groups ($\bar{x} \pm s$).

Experimental groups	n	IL-6 (ng/L)		IL-1β (ng/L)		TNF-α (ng/L)	
		Before surgery	4 weeks after surgery	Before surgery	4 weeks after surgery	Before surgery	4 weeks after surgery
Laser group	40	160.41 $\pm$ 19.20	109.37 $\pm$ 10.53[a]	274.12 $\pm$ 26.81	148.34 $\pm$ 18.59[a]	168.29 $\pm$ 27.10	115.04 $\pm$ 17.36[a]
Surgery group	41	157.09 $\pm$ 16.78	113.76 $\pm$ 12.12[a]	269.58 $\pm$ 28.21	157.03 $\pm$ 20.27[a]	164.54 $\pm$ 25.58	124.32 $\pm$ 19.77[a]
t		0.824	–1.746	0.736	–2.010	0.649	–2.243
p-value		0.413	0.085	0.464	0.048	0.518	0.028

IL-6, interleukin-6; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; [a], compared to the preoperative, $p < 0.05$.

Table 4. Comparison of VHI-10 and GRBAS scores between the two groups ($\bar{x} \pm s$, score).

Experimental groups	n	VHI-10 score (points)		GRBAS score (points)	
		Before surgery	4 weeks after surgery	Before surgery	4 weeks after surgery
Laser group	40	13.20 ± 3.29	2.83 ± 0.73[a]	2.43 ± 0.61	0.86 ± 0.25[a]
Surgery group	41	12.73 ± 3.81	3.40 ± 0.96[a]	2.21 ± 0.73	1.15 ± 0.28[a]
t		0.594	–3.002	1.470	–4.913
p-value		0.554	0.004	0.146	<0.001

VHI-10, Voice Handicap Index; GRBAS, the Grade, Roughness, Breathiness, Asthenia, Strain; [a], compared to the preoperative, $p < 0.05$.

Table 5. Comparison of complication rates between two groups ($\bar{x} \pm s$).

Experimental groups	n	Vocal cord edema	Vocal cord granuloma	Vocal cord adhesion	Complication rate (%)
Laser group	40	4	1	0	5 (12.50)
Surgery group	41	7	2	1	10 (24.39)
χ^2					1.897
p-value					0.168

bstantially improved. CO_2 laser surgery has become increasingly popular due to its high precision and minimal thermal damage. This approach allows for precise cutting and coagulation, reducing intraoperative bleeding, shortening surgery time, and minimizing damage to surrounding healthy tissue due to selective tissue targeting [12]. This method is widely used in the treatment of early laryngeal cancer. This study analyzes its application and effectiveness in treating vocal cord cysts.

In this study, both groups showed significant postoperative improvements in voice acoustic and aerodynamic parameters. Notably, the CO_2 laser group demonstrated more significant enhancements in jitter, shimmer, and mean airflow values compared to the traditional surgery group, suggesting that CO_2 laser surgery offers more benefits for restoring vocal cord function. Compared to traditional laryngeal microsurgery, the high precision of CO_2 laser surgery allows for smoother vocal cord edges after the removal of carbonized tissue. Additionally, the concentrated energy and high temperature of the laser enable efficient cyst removal while quickly sealing microvessels less than 0.5 mm in diameter, thereby accelerating the hemostasis process [13]. Similarly, Liang *et al.* [14] reported that transoral CO_2 laser surgery for early glottic carcinoma shows pronounced outcomes, promoting the recovery of voice and swallowing functions. This further validates the effectiveness of CO_2 laser treatment. Moreover, this technique reduces damage to the superficial lamina propria of the vocal cords, alleviating the risk of postoperative adhesion between the vocal cord mucosa and the vocal ligament, which is crucial for optimal functional recovery [15–17]. A previous study by Remacle *et al.* [18] compared the efficacy of CO_2 laser surgery and traditional laryngeal microsurgery in treating vocal cord cysts. They found that CO_2 laser laryngeal microsurgery significantly improves the quality of speech. These observations validate the effectiveness and safety of CO_2 laser surgery in treating vocal cord cysts. This study chose the 4-week post-surgery period as the observation time point is because it typically marks the end of the acute healing phase. At this time, tissue repair and the inflammatory response generally stabilize, providing a reliable and balanced evaluation during the observation window.

Further analysis revealed that the MPT in the CO_2 laser surgery group was better than in the traditional surgery group, primarily due to the reduced tissue damage and faster healing time associated with laser surgery. Specifically, the precise operation of CO_2 laser surgery reduces the instability often caused by the long-distance manipulation of traditional "cold instruments" such as laryngeal knives, scissors, and forceps. Furthermore, the laser beam effectively seals small blood vessels on the surface of the vocal cords, ensuring a clear surgical field and enabling precise cyst removal, thereby elevating the success rate of the treatment. Similar finding has been reported in domestic study [18]. A meta-analysis and systematic review by Gandhi *et al.* [19] showed that CO_2 laser surgery has similar therapeutic effectiveness to conventional laryngeal microsurgery for vocal cord cysts. However, CO_2 laser surgery allows for a quicker postoperative recovery and improved voice quality. These findings align with the results of this study, further validating the effectiveness and safety of CO_2 laser surgery in treating vocal cord cysts.

Regarding inflammatory markers, IL-6, IL-1β, and TNF-α levels were significantly decreased in both groups after surgery, which aligns with the expected reduction in the inflammatory response following treatment. However, at 4 weeks post-surgery, the levels of these markers were lower in the laser group than in the surgery group, indicating that CO_2 laser surgery significantly reduces the inflammatory response associated with vocal cord cysts. This outcome is due to the minimally invasive nature of laser surgery. Conversely, traditional laryngeal microsurgery, being more invasive, often triggers a pronounced stress response, as evidenced by elevated postoperative levels of inflammatory factors, such as IL-6, IL-1β, and TNF-α.

In contrast, during CO_2 laser surgery, the high-energy laser beam is primarily absorbed by water molecules, resulting in minimal energy penetration into surrounding tissues. The radiation depth is typically limited to 0.3 mm, enabling the laser to precisely target the affected area while reducing thermal damage to surrounding healthy tissues. This precision significantly reduces inflammatory response induced by surgery [2,20,21]. A previous study by Ostrander *et al.* [22] has also shown that laser surgery reduces thermal damage to surrounding tissues by precisely controlling energy transfer, thereby lowering inflammatory responses. These findings are consistent with the results of our study.

This study showed significant postoperative improvements in both VHI-10 and GRBAS scores across both patient groups following surgery, with the laser group revealing more pronounced outcomes. These observations further underscore the advantage of CO_2 laser surgery in enhancing voice quality. The improved outcomes are attributed to the precision of laser technology, which, when operated at the correct focal distance, confines tissue damage to a radius of 5–100 micrometers and affects only 5–10 cells, typically affecting 1–5 cells. This precision shows that the surrounding normal tissue remains intact, preserving vocal function [12]. In terms of complications, there was no significant difference in complication rates between the two groups, indicating that the safety of CO_2 laser surgery is comparable to that of traditional surgical methods. This safety is attributed to the CO_2 laser's limited area of effect and its adjustable intensity and brightness, making it a reliable and highly safe surgical option [23].

The limitation of this study is that lifestyle habits, such as excessive use of voice, smoking, and alcohol consumption, and patient treatment compliance, may have affected treatment efficacy and the recovery process. These confounding factors were not fully controlled for this study. Additionally, as this was a retrospective analysis, the risks of selection bias and recall bias cannot be excluded. Future research should address this gap by incorporating these factors and validating the results through multicenter, large-scale, prospective study designs.

Conclusions

In conclusion, both CO_2 laser surgery and traditional microsurgery for vocal cord cysts demonstrate favorable outcomes. However, CO_2 laser surgery results in better recovery of vocal function and is more effective at reducing the inflammation.

Availability of Data and Materials

The data analyzed was available on the request for the corresponding author.

Author Contributions

NJC designed the research study. ZHS and XMS performed the research. XMS provided help and advice on the ELISA experiments. ZHS and NJC analyzed the data. NJC drafted this manuscript. All authors contributed to important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was a retrospective study and the ethic approval was exempted by the Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine. The study was conducted in accordance with the Declaration of Helsinki, and informed consent was obtained from each participant.

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Conflict of Interest

The authors declare no conflict of interest.

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