Comparison of the efficacy of four endobronchial ablation techniques in dogs

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Abstract. The present study aimed to evaluate the safety and efficacy of four commonly used ablation techniques, namely neodymium-doped yttrium aluminium garnet (Nd:YAG) laser therapy, argon plasma coagulation (APC), high-frequency electrocautery and CO2 cryotherapy. The techniques were performed at various powers or impedance settings, and for various durations, on the trachea of beagle dogs. Pathological changes of the tracheal wall were assessed by bronchoscopy. The endoscopic gross appearance of lesions induced by ablation treatments was consistent with the histopathological changes. The results suggested that cryotherapy was relatively safe, whereas APC induced superficial tissue coagulative necrosis. Furthermore, Nd:YAG laser therapy was the most efficient technique and showed the greatest penetration potential. In general, tissue injury was exacerbated with extended application time, at constant power or impedance. The safest application parameters were 20 W for ≤1 sec for Nd:YAG laser therapy, 40 W for ≤3 sec for electrocautery, 40 W for ≤5 sec for APC and 100 Ω for ≤120 sec for cryotherapy. At the maximum times, these settings resulted in identical pathological changes. Healing of the lesions following ablation was achieved within 3 weeks. The Nd:YAG laser, APC, electrocautery and cryotherapy endobronchial ablation techniques differed according to their potential and limitations for application on the trachea. However, when applied at specific combinations of power or impedance and duration, they exhibited similar efficacies.

Introduction

The diagnosis and treatment of pulmonary diseases has markedly improved with advances in the technologies of interventional pulmonary medicine (1-4). For example endobronchial ablation techniques have been employed in the treatment of intraluminal diseases of the tracheobronchial tree (5,6). The viability of these technologies for the curative treatment of airway obstructions caused by benign lesions, including benign tumors, endobronchial tuberculosis and granulomatosis, has been established (7). They are also important in the palliative treatment of patients with late-stage lung cancers (8). In China, the bronchoscopic ablation techniques commonly used include neodymium-doped yttrium aluminium garnet (Nd:YAG) laser therapy, high-frequency electrocautery, argon plasma coagulation (APC) and CO2 cryotherapy (9-11). These techniques may be performed via rigid or flexible fiberoptic bronchoscopy.

Among the malignant tumors, lung cancer has been ranked first in the world in terms of its morbidity and mortality (12). The majority of patients with lung cancer present at the late stage, when curative surgical resection is not an option, and 30% have obstructions in the trachea or main bronchi (13). For these patients, endobronchial therapy can restore airway patency, alleviate dyspnea, preserve quality of life, improve survival rates and allow further treatments, such as external beam radiation, chemotherapy and surgery (14).

The detection rate for bronchogenic carcinomas in situ and early-stage intraluminal carcinomas has improved with technological developments (15,16). Patients who suffer from superficial lesions, but are inoperable due to an unfit health status, can be treated by bronchoscopic interventions to prevent progression to invasive cancer (17). For these patients, the first choice for palliation or treatment with curative intent is currently photodynamic therapy (18). However, the availability of photodynamic therapy is limited at most institutions in China because of its expense and the cumbersomeness of the equipment. Instead, endobronchial ablation utilizing Nd:YAG laser therapy, electrocautery, APC or cryotherapy are typically used because of their lower cost, portability and comparable efficacy (11). However, long-term observational studies and prospective randomized controlled trials are necessary for definitive verification of these techniques (19).
China initially lagged behind developed countries in adopting interventional pulmonary medicine, and a disparity still exists. In some parts of the country, the availability of ablation technologies remains very limited and there is the question of whether purchasing one or two of these would suffice and be comparable to an entire set of the latest equipment. There is a relative paucity of data to differentiate the various endobronchial ablation technologies according to their biological effects, efficacy and safety with specific applications. In addition, the healing course of lesions induced by endobronchial ablation is not known.

In a preliminary in vitro study, the authors of the present study evaluated several endobronchial coagulation techniques (microwave, APC, electrocautery and cryotherapy) and determined specific values for technical parameters associated with their safety and efficacy (20). In the present study, the efficacy of Nd:YAG laser therapy, electrocautery, APC and CO₂ cryotherapy in dogs was evaluated, to determine their relative merits and the optimal technical parametric values for clinical practice.

Materials and methods

Animals and pre-tracheal ablation procedures. The present study was approved by the Institutional Animal Research Ethics Committee. A total of 6 healthy adult beagle dogs (3 male and 3 female) weighing 10-12 kg were provided by the Laboratory Animal Center at the Second Military Medical University (Shanghai, China). The beagle dogs were bred under normal room conditions at a temperature of 16-26 °C, humidity of 40-70%, noise level <60 dB, and 100-200 lux illumination. Adequate drinking water was provided. The daily quantity of food was approximately 3-5% of the dog weight. The food was divided into two portions; one was provided in the morning, and the other was provided in the afternoon.

General anesthesia was induced using intravenous amobarbital sodium (0.1 ml/kg; Shanghai Xinya Pharmaceutical Co. Ltd., Shanghai, China), and 2% lidocaine (Jincheng Hayes Pharmaceutical Co., Ltd., Jincheng, China) was administered onto the tracheal mucosa. Following anesthetization, the dogs were placed in the supine position with the head and limbs fixed on the operating bench.

Using a laryngoscope blade and an intubation stylet, with the tongue displaced to the left, the blade was introduced with its concave surface directed ventrally and the soft palate was displaced dorsally to reveal the rima glottidis. Subsequently, the tongue was displaced to the left, the blade was introduced with a bite blocker, and the tongue was extended and secured with a strip of gauze tied to the bite blocker.

An Olympus T260 Fiberoptic Bronchoscope (Olympus Corporation, Tokyo, Japan) was introduced into the trachea and the tracheobronchial tree was examined. The middle and lower parts of the trachea, excluding the membranous part, were selected as the target tissue for the ablation treatments. During the bronchoscopy, mucous secretions were removed directly through negative pressure suction when required. Each dog underwent four endobrachial ablation procedures, as described in the following sections.

Nd:YAG laser ablation. A LaserPro 810 Laser Probe (Collin SAS, Bagneux, France) was inserted through the working channel of the bronchoscope, and advanced at least 1 cm beyond the distal end of the bronchoscope. With the aid of a pilot red light, the laser energy was focused on the target tissue; the tip of the probe was directed to the target tissue and was maintained at a distance of 4-10 mm from the tissue surface. Power was set at 20 W, and was applied with a pulse of 1, 2 or 3 sec (setting on the equipment) at three separate sites at 2-cm intervals.

High-frequency electrocautery. An electrocautery probe (VIO® 300 D; Erbe Elektromedizin GmbH, Tübingen, Germany) was passed through the working channel of the bronchoscope, and protruded 1-2 cm beyond the distal end of the bronchoscope. The probe was placed in contact with the target site, and an electric current, with the power set at 40 W, was applied for 1, 3 or 5 sec at three separate sites at 2-cm intervals.

APC ablation. The APC probe (VIO® 300 D) was inserted through the working channel of the bronchoscope, and advanced 1 cm beyond the distal end of the bronchoscope, with the tip of the probe held 4-10 mm from the target tissue. APC was performed with an argon flow rate of 2 l/min and a power of 40 W, with a burst of 1, 3 or 5 sec at three separate sites at 2-cm intervals.

Cryotherapy ablation. Cryotherapy (K300 Cryosurgery Equipment; Beijing Kooland Technology, Co., Ltd., Beijing, China) was performed by passing a cryoprobe through the flexible bronchoscope until it had advanced 1 cm beyond the distal end of the bronchoscope. The tip of the cryoprobe was kept in contact with the tracheal mucosa. Three freeze-thaw cycles, with the impedance set at 100 Ω, were applied at two separate sites (at a 2-cm interval), with each freeze-thaw cycle lasting 60 or 120 sec.

Post-ablation protocol. Two dogs were sacrificed immediately following the endobronchial ablations to evaluate early pathological changes of the tracheal wall. Sacrifice was conducted by the intravenous injection of 0.1 ml/kg pentobarbital sodium, after which the femoral artery and vein in the femoral triangle area were cut out to induce exsanguination. The general health status of the remaining four dogs was monitored daily, including eating and physical activities, with special attention to respiratory complications and accidental death, until they were randomly sacrificed on days 3, 7, 14 or 21 postoperatively. The trachea with the injured sites was removed, divided into tissue blocks (10x10 mm) and rinsed with normal saline. Subsequently, the specimens were fixed in formalin for 24 h, embedded in paraffin, step-sectioned into 3-μm slices and stained with hematoxylin and eosin for analysis under a light microscope.

Evaluation of the biological effects of endobronchial interventions. Assessment of the biological effects of the ablation techniques was based on endoscopic gross findings and histopathological examinations. During the bronchosopic interventions, the gross endoscopic changes within the tracheal lumen were recorded. These involved changes in the local
mucosal color and texture, and the extent of injuries, including necrosis, carbonization, vaporization and perforation.

The tracheal tissue sections were observed under a light microscope to evaluate the histopathological changes of the mucosa, submucosa and cartilage layer. The severity of injuries was defined as mild (+), moderate (++) or severe (+++), according to the extent of the injured area. The maximal depths and dimensions of tissue damage were measured in the central area of the lesions in step sections. The actual measurements were performed using a Vernier caliper (Fig. 1).

Results

Gross appearance of the trachea following endobronchial ablation. Gross alterations of the trachea of the dogs following the four types of endobronchial ablations were observed by bronchoscopy. In general, immediate alterations included desiccation of the mucosal surface, a whitish or charred yellow coagulation spot and hardening of the injured area. Over time, the area of coagulation expanded to become a crater-shaped lesion. The surrounding area and bottom of the lesion were covered with charred eschar, sometimes with perforation.

Laser-induced tracheal injury had a sharp edge (Fig. 2), while injuries induced by high-frequency electrocautery or APC had no sharp boundaries (Fig. 2). The severity of injury positively correlated with the application time. Cryotherapy-induced injury had markedly less obvious tissue defect compared with the other ablation techniques. When 100 Ω impedance was applied for 60 sec, whitish mucosa, edema and hardening of the tissue were observed. When applied for 120 sec, these changes were more obvious and the integrity of the mucosa was destroyed (Fig. 2). Normal tracheal mucosa on gross endoscopic examination showed a smooth mucosal surface, clearly visible vessels and cartilage rings (Fig. 2).

Histopathological alterations of the trachea following endobronchial ablations. Histopathological alterations of the trachea following all four types of endobronchial ablations in the dogs included epithelial and submucosal necrosis and shedding, as well as destruction and perforation of the cartilage layer (Fig. 3). The severity of injuries correlated with the application time of ablation. Notably, the histopathological changes were consistent with the endoscopic gross appearances of the tracheal lumen.

To compare the safety and efficacy of the four types of ablative techniques, the depths and dimensions of tracheal injuries, and the severity of tissue necrosis and cartilage damage, induced by the different methods were examined (Table I). The histopathological changes of the tracheal wall induced by these various ablation techniques varied according to the parametric settings and application times (Table I; Fig. 3). The parametric settings included the power output (W for Nd:YAG laser, APC and electrocautery) or impedance (Ω for cryotherapy).

When the 20-W laser was applied for 1 sec, histopathological changes of the tracheal injury included mucosal necrosis and submucosal coagulative necrosis, which extended deep into the surface of the cartilage (Fig. 3). When the application time was extended to 2 sec, two-thirds of the cartilage layer was destroyed, accompanied by apparent coagulative necrosis in the surrounding tissue (Fig. 3). Furthermore, following application of 20 W for 3 sec, a transmural tracheal defect was observed at the center of the injury and coagulative necrosis in the surrounding tissue was more severe, with perforation of the tracheal wall (Fig. 3). The injuries induced by high-frequency electrocautery and APC were similar to those induced by laser therapy, and the severity of injuries also correlated with the application time of each technique (Fig. 3).

Cryotherapy-induced injury showed no apparent necrosis. However, mucosal and epithelial shedding and thrombembolism formation was observed. The cartilage layer remained intact (Fig. 3). Histological analysis of the normal tracheal wall under an optical microscope showed three layers of structure (from top to bottom): mucosa, submucosa and adventitia (Fig. 3).

Parametric settings and application times that allow equivalent efficacy of the four endobronchial ablation techniques. To identify the settings for the four types of endobronchial ablation techniques that achieved similar efficacies in the dog trachea, the histopathological changes and the depths of injuries induced by each type were compared (Table II). The following settings had equivalent effects on the tracheal wall: 20 W for 1 sec for Nd:YAG laser therapy; 40 W for 1 sec for electrocautery; 40 W for 5 sec for APC; and 100 Ω for 120 sec for CO₂ cryotherapy. The histological characteristics of tracheal injuries under these settings included mucosal necrosis and shedding, submucosal coagulative necrosis and an intact cartilage layer. The depth of injury was <2 mm.

Tissue repair and healing following endobronchial interventions. Changes of tracheal injuries induced by the four endobronchial ablation techniques over time were similar, and could be considered to occur in four phases, including acute injury, inflammation, repair and healing (Fig. 4). The acute phase consisted of pathological changes characterized by desiccation of the mucosal surface, a whitish or charred yellow coagulation spot and hardening of the injured areas (Fig. 3).

The inflammatory phase of injury was evident on day 3 following the procedure, and was characterized by hyperemia.
and edema of the mucosa surrounding the injury, with the presence of tissue defect and necrotic debris. Furthermore, a large number of inflammatory cells were observed to have infiltrated into the injured areas (Fig. 4).

The repair phase was initiated on day 7 following the ablation treatments (Fig. 4). The hyperemia and edema of the mucosa surrounding the lesion gradually subsided. In addition, the tissue defect was replaced by the proliferation of a pseudostratified, columnar epithelium in the mucosa, granulation tissue with the infiltration of inflammatory cells and newly forming capillaries in the submucosa. The gap caused by tissue disruption was filled with granulation tissue.

The healing phase of injuries could be seen 2-3 weeks postoperatively (Fig. 4). The gross appearance of the lesion was similar to that of normal tissue, with no hyperemia and edema. Histopathological analyses showed a well-developed, pseudostratified columnar epithelium and proliferation of fibrous tissue, with fewer inflammatory cells in the submucosa. Fibrous tissue had replaced the damaged submucosa and cartilage to maintain the integrity of the tracheal wall.
Discussion

The present study evaluated the safety and efficacy of four commonly used endobronchial ablation techniques, which were applied via fiberoptic bronchoscopy to beagle dogs. The ablation techniques included Nd:YAG laser therapy, high-frequency electrocautery, APC and CO\textsubscript{2} cryotherapy. The results of the study indicated that the biological effects on the trachea induced by each technique differed according to parametric settings (power or impedance) and application times. However, it was possible to achieve comparable efficacy and safety when these tools were applied at optimal settings and application times, which were specific to each technique. Furthermore, the repair and healing processes in the damaged trachea following these interventions were observed and described.

Ablation may be categorized as hot or cold therapy. The hot therapies, which include Nd:YAG laser therapy, high-frequency electrocautery, APC and CO\textsubscript{2} cryotherapy, and the cold therapy, which is CO\textsubscript{2} cryotherapy. The hot therapies, which include Nd:YAG laser therapy, high-frequency electrocautery, APC and CO\textsubscript{2} cryotherapy, and the cold therapy, which is CO\textsubscript{2} cryotherapy, are effective in treating various respiratory conditions such as tumors, polyps, and strictures. However, the cold therapy, which is CO\textsubscript{2} cryotherapy, has several advantages over the hot therapies. First, CO\textsubscript{2} cryotherapy is a non-invasive procedure that can be performed in the bronchoscopy suite without the need for general anesthesia. Second, the cold therapy, which is CO\textsubscript{2} cryotherapy, has a higher success rate in treating tumors and strictures compared to the hot therapies. Finally, CO\textsubscript{2} cryotherapy has a lower risk of complications such as bleeding and infection compared to the hot therapies.

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Figure 3. Representative images of histopathological changes of the tracheal mucosa of dogs following various endobronchial ablation therapies (hematoxylin and eosin staining, magnification, x40). Nd:YAG laser: 1 sec, mucosal necrosis and defect, with submucosal tissue necrosis at the surface of the cartilage; 2 sec, mucosal/submucosal defect and vaporization deeper into the cartilage, with damage affecting >2/3 of the cartilage, and obvious surrounding tissue necrosis; 3 sec, tracheal wall defect and perforation in the central region, with increased necrosis of the surrounding tissue. Electrocautery: 1 sec, mucosal epithelial necrosis, and submucosal tissue necrosis affecting the cartilage layer; 3 sec, mucosal/submucosal necrosis deeper into the cartilage layer, with 1/2 of the cartilage structure being destroyed; 5 sec, increased tissue necrosis, with damage and fracture of mucosa/submucosa and the cartilage layer, and airway perforation. APC: 1 sec, mucosal shedding and superficial submucosal necrosis; 3 sec, larger coagulation spots, a central depression and surface carbonization; 5 sec, mucosal defect, with submucosal tissue necrosis affecting the surface of the cartilage; the cartilage layer was slightly damaged but retained its integrity. Cryotherapy: 60 sec, epithelial cell shedding and local thrombosis, but no obvious tissue necrosis; 120 sec, mucous layer defect, disordered submucosal structure, and damage reaching to the surface of the cartilage layer; the cartilage structure was intact and local thrombosis was observed. W, watt; Ω, ohm; Nd:YAG, neodymium-doped yttrium aluminium garnet laser therapy; APC, argon plasma coagulation.
electrocautery and APC, produce and apply thermal energy to a tissue, causing immediate coagulative necrosis and tissue vaporization (21,22). Conversely, the effect of cold therapies, including cryotherapy, is relatively delayed, with tissue necrosis appearing 2-3 days following the procedure (23-26). In the present study, the most common histopathological changes of the trachea wall among the various endobronchial ablation techniques were tissue coagulative necrosis and vaporization. However, the extent of tissue damage varied depending on the settings for power output (Nd:YAGlaser, APC and electrocautery) or impedance (cryotherapy), and application time.

Nd:YAG laser therapy was the most efficient ablation technique and resulted in immediate tissue coagulation, penetration, vaporization and transmural tracheal damage during treatment. The effect of high-frequency electrocautery was similar to Nd:YAG laser therapy. Electrocautery applied at 40 W for 3 sec caused tissue vaporization that extended into half of the cartilage layer; after 5 sec of application, the transmural tracheal wall was destroyed. APC induced more superficial coagulative necrosis and less vaporization compared with Nd:YAG laser therapy and electrocautery; when applied at 40 W for 5 sec, the cartilage layer was superficially damaged, but most of the deep structures in the cartilage remained intact. Although cryotherapy was superior in terms of safety and toleration by the bronchial wall, its effect was delayed. Cryotherapy applied at 100 Ω for either 60 or 120 sec did not affect the cartilage layer. These features were consistent with the results of previous studies (5,6,23,24,26-31).

Based on our previous study (20) and clinical experience, the current study chose to assess Nd:YAG laser therapy at 20 W, electrocautery and APC at 40 W, and cryotherapy at 100 Ω. The results indicated that, at any power (or impedance) held constant, tracheal injury was exacerbated with application time. Mucosal coagulative necrosis can infiltrate into the submucosa and subsequently extend into the cartilage layer, resulting in airway perforation. This suggests that ablation may achieve the desired level of tissue damage if the duration of application is adjusted, which is useful, especially when treating carcinomas in situ, to reduce the risk of airway perforation. The results of the present study indicated that the ablation techniques can be applied safely when the following parameters are used: Nd:YAG laser therapy, 20 W for ≤1 sec; electrocautery, 40 W for ≤3 sec; APC, 40 W for ≤5 sec; and cryotherapy, 100 Ω for ≤120 sec.

Nd:YAG laser therapy is the most commonly used endobronchial ablation technique because of the excellent coherence, monochromaticity and collimation of the laser (5,28). However, Nd:YAG laser therapy should be performed with caution in clinical practice. To minimize the risk of airway perforation, the laser beam must always be parallel to the wall of the airway and not perpendicular to it, and the minimum effective power and shortest duration of application should be selected. Lai et al (27) found that the serum levels of interleukin-2 and natural killer cell activity were increased following Nd:YAG laser therapy, suggesting that laser therapy may enhance immunity, in addition to its thermal effect. Long-term studies of patients with late-stage lung cancer have shown that Nd:YAG laser therapy is able to effectively relieve symptoms, improve the quality of life and prolong survival (28,32).
As a thermal ablation technique, high-frequency electrocautery transforms electrical energy into thermal energy to remove lesions. It generally results in coagulative necrosis at low temperatures, and tissue vaporization and carbonization at high temperatures, with the focus on damaged tissue at the surface of contact (29,31). In the present study, the extent of tracheal damage caused by electrocautery applied at 40 W was positively associated with the duration of application. The biological effects of electrocautery on tissue are dependent on various factors, including the nature of lesions, the current waveform, the power output, the duration of contact, the mode of application and the type of probe (29,31,33). Local blood flow and mucosal secretion are also thought to influence the efficacy of electrocautery (34). In the present study, the influence of the local mucosa was excluded, since normal trachea was selected as the target tissue and mucosal secretion could be easily and rapidly removed during the procedure; Our results indicated that the extent of tissue damage was primarily associated with the duration of ablation. Considering the risk of airway perforation as a complication, we recommend that electrocautery be applied with a power setting of 40 W for <3 sec to ensure safety.

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In the present study, it was observed that tissue penetration was limited using APC and, thus, the risk of airway
perforation associated with this technique was less compared with the other techniques. The advantages of APC include the ability to reach lesions located lateral to the probe or around bends and corners that are not suitable for Nd:YAG laser therapy and electrocautery, as well as adequate hemostasis of the lesion (35-37). The complications associated with APC are similar to electrocautery, although a case of fatal air embolism has previously been reported (38). APC is not the best choice for removal of a bulky lesion, since it is less precise compared with other ablation techniques (30).

Cryotherapy causes cellular injury and death by exposing biological tissues to cycles of freezing and thawing. Multiple factors can influence the efficacy of cryotherapy, including the speed of freeze-thawing and the lowest temperature that the cryoprobe can achieve to destroy live tissue. Cryotherapy does not affect cartilage because of its low water content (39). Cryotherapy is used in the treatment of endobronchial tumors. Its advantages include a lower cost, fewer precautions and superior safety. Furthermore, it is less likely to cause complications such as perforation, malacia and cicatrization stricture (25). However, cryotherapy cannot be used to achieve immediate airway patency in patients with severe airway stenosis, owing to its delayed effect (40). In a previous study, the application of cryotherapy was extended by a technique that allowed immediate recanalization of obstructed airways affected by the extraction of large pieces of tumors (38). This involved using a probe that was able to rapidly freeze tissue and remove the entire tissue around the probe before the frozen tissue thawed. However, airway bleeding with the potential for massive hemorrhage is an important consideration, such that the safety of this new technique requires further investigation.

The present study demonstrated that the use of these ablation techniques at specific settings could cause similar biological effects. Nd:YAG laser therapy at 20 W for 1 sec, electrocautery at 40 W for 3 sec, APC at 40 W for 5 sec and cryotherapy at 100 Ω for 120 sec resulted in identical pathological changes in the tracheal wall. These alterations included shedding necrotic mucosa, partial tracheal defects, submucosal coagulative necrosis and destruction of the superficial cartilage layer, with similar infiltration depths of tissue damage. Therefore, we propose that these specific settings are efficacy-equivalent values for these ablation techniques. This is important information for a number of institutes where only one or two endobronchial ablation modalities are available; most endobronchial therapies can be performed to achieve the desired results, even if the available equipment is limited.

It is important to note that fibrous scarring tissue is retractile and, once it loses cartilaginous support, iatrogenic secondary stenosis can develop (30). Verkindt et al (30) found that, after setting electrocautery to deliver 40 or 120 W, coagulative necrosis and intense inflammation of the mucosa of early lesions extended deep into the cartilage layer, followed by formation of transmural fibrosis and the destruction of cartilage, which resulted in iatrogenic secondary stenosis. By mentioning this possibility, the authors do not question the clinical application of ablation techniques, but only want to alert physicians to the potential for iatrogenic secondary stenosis caused by extensive damage to the tracheal wall.

In conclusion, the present study demonstrated that the biological effects of various endobronchial ablation techniques on the trachea were a function of the power or impedance settings and application period, and that these techniques may be equally efficacious when applied using settings and application durations specific to each technique. Specifically, this study determined that the safe parametric values for endobronchial ablation with these techniques were 20 W for ≤1 sec for Nd:YAG laser therapy, 40 W for ≤3 sec for high-frequency electrocautery, 40 W at ≤5 sec for APC and 100 Ω for ≤120 sec for CO₂ cryotherapy. The results of the present study may serve as a reference for the clinical application of endobronchial ablation techniques.

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