



Effect of pulmonary vein cryoballoon ablation in dogs with coolant-nitrogen

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Background: The Arctic Front Advance System with nitrous oxide (N₂O) refrigerant is the leading system for the cryoballoon ablation of atrial fibrillation (AF). A novel cryoablation system with nitrogen (N₂) refrigerant was developed with technical improvements seeking to improve outcomes. Cryoballoon ablation with the N₂ refrigerant may be effective and safe for pulmonary vein isolation (PVI).

Methods: In total, 16 dogs were included in the study, of which 13 underwent PVI procedures, and 3 served as baseline controls. Cryoballoons (Cryofocus, Int.) with N₂ refrigerant were used for the study group, which comprised 8 dogs, and second-generation cryoballoons with N₂O refrigerant (Arctic Front Advance; Medtronic, Inc., MN, USA) were used for the control group, which comprised 5 dogs. Three dogs of the study group and 2 dogs of the control group were euthanized on the same day post-ablation. The other 8 dogs of the two groups were euthanized 1 month post-ablation. The removed organs were examined for gross anatomy and histological review.

Results: The average ablation times for each pulmonary vein (PV) in the study group were less than those in the control group (1.1±0.3 vs. 2.0±0.8; P=0.006). The procedure duration of the study group was shorter than that of the control group (379±46 vs. 592±162 s; P=0.013). And the time to isolation (TTI) was similar between the groups. The PVI rate of the single-ablation was higher in the study group than the control group (92.9% vs. 60.0%; P=0.05). In relation to safety, there was no evidence of thrombus, esophageal injury, or pericardial tamponade in any of the dogs. Only 1 incidence of self-limited phrenic nerve paralysis (PNP) was observed in the control group.

Conclusions: The novel cryoablation system with the N₂ refrigerant had better efficacy than and similar safety to that of the system (Medtronic, Int.) with the N₂O refrigerant.

Keywords: Pulmonary vein isolation (PVI); cryoballoon ablation; nitrogen

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Introduction

Atrial fibrillation (AF), which is the most common arrhythmia observed in clinical practice, affects approximately 1–2% of the entire population (1,2). Catheter

ablation to isolate the pulmonary veins (PVs) provides the most effective treatment option. It is performed by either cryoballoon or radiofrequency ablation. A great number of studies have shown that the cryoballoon ablation of PVs is a safe and effective treatment for patients with AF

Table 1 Allocation of animals

Follow-up time	Study group (n)	Control group (n)	Baseline control (n)
Before procedure	8	5	3
Immediately post-ablation	8	5	0
On the same day post-ablation (sacrificed)	3	2	0
1-month post-ablation (sacrificed)	5	3	0

(3-6). Compared to the radiofrequency ablation technique, the cryoballoon ablation technique has several advantages, including a shorter procedure duration, a higher degree of reproducibility, and a shorter learning curve (2,7). Due to its low risk of complications and proven efficacy, the Arctic Front Cardiac Cryoablation Catheter Family (Medtronic, Int.) is the leading system for cryoballoon ablation (4,8,9). Under this system, nitrous oxide (N₂O) refrigerant is injected into the inner balloon via injection tubes to create a cryothermal lesion.

Liquid nitrogen is also widely used in surgery, and can achieve a temperature as low as -196 °C (10). However, its utility in percutaneous cardiac procedures has been limited due to its complex design and potential risks. In recent years, some novel cryoballoon systems have been developed in China, one of which uses nitrogen (N₂) as the refrigerant. Effective innovations have made it possible to achieve pulmonary vein isolation (PVI) safely. This is the first study to use N₂ refrigerant for cryoballoon ablation in dogs, and we evaluated the effectiveness and safety of the new cryoablation system for PVI. We present the following article in accordance with the ARRIVE reporting checklist (available at <https://jtd.amegroups.com/article/view/10.21037/jtd-22-418/rc>).

Methods

Laboratory animal preparation

Experiments were performed under a project license (No. 201821) granted by the Institutional Ethical Committee of Shanghai Putuo District People's Hospital, in compliance with the guidelines of Shanghai Putuo District People's Hospital for the care and use of animals. A protocol was prepared without registration. The use of the dog as a model for cardiac surgical and electrophysiological research is popular. There are subtle differences between hearts of dogs and human, and the model was used in most of the cryoballoon ablation research. In this study,

16 healthy adult Labrador dogs (weighing 25±5 kg), male or female, were enrolled in the study. Of these dogs, 13 underwent PVI procedures with cryoballoons delivering the refrigerant (N₂O or N₂), and the other 3 dogs served as baseline controls (see *Table 1*). For the study group, 26-mm cryoballoons (Cryofocus, Int., MN, USA) with refrigerant (N₂) were used in 8 dogs, and for the control group, 28-mm second-generation cryoballoons with N₂O (Arctic Front Advance; Medtronic, Inc.) were used in 5 dogs. For each experimental group, data of all the animals were included in the analysis. Each animal was fed, monitored and sacrificed in the same way to minimise potential confounders. All the procedures were performed under conscious sedation using intravenous injection of pentobarbital sodium, followed by diazepam and ketamine intramuscular injection. The dogs' surface electrocardiogram and blood pressure was continuously monitored.

The novel cryoballoon catheter system (Cryofocus, Inc.)

The system consists of a steerable catheter with a balloon, a 16-F deflectable delivery sheath, and external equipment that houses the N₂ refrigerant (see *Figure 1*). The cryoablation equipment provides constant pressure and refrigerant based on multi-stage cooling and gradient pressurization technology. The temperature sensor is located at the proximal internal-end of the balloon, and real-time temperature data are shown on the screen of the device. The freezing flow rate is adjusted to maintain the proper temperature. If the temperature drops to the low limit, the equipment will automatically reduce the flow rate to stop any further drop, and vice versa. For security, the cooling output is controlled via a multivariable coupling system (pressure, temperature and flow). Another good design is the cryoballoon of the Cryofocus system has 18 jets (8 jets in Arctic Front AdvanceTM), which results in an increased uniformity of cooling.

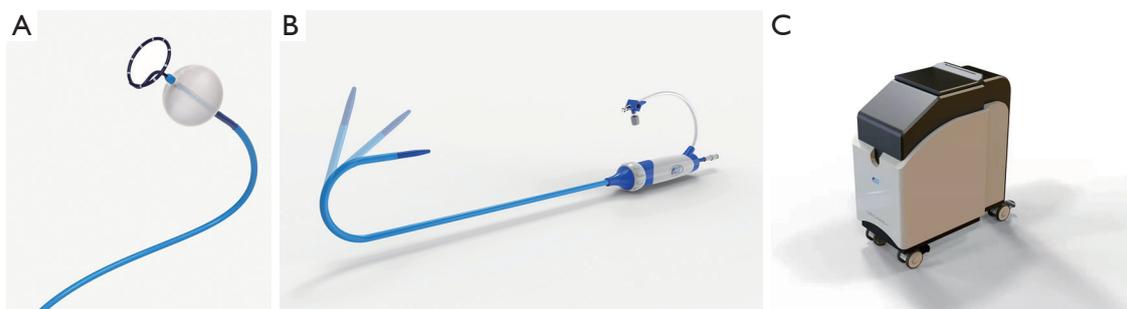


Figure 1 The novel cryoballoon catheter system. (A) Cryoballoon with circular mapping catheter; (B) deflectable sheath; (C) external apparatus.

Ablation strategy

The ablation strategy for the two systems was the same. Specifically, introducer sheaths were placed in both the right and left femoral veins. A 6-F decapolar catheter was inserted into the coronary sinus, and a 6-F quadripolar catheter was positioned at the right ventricle. A Brockenbrough transseptal needle and sheath were inserted through the right femoral venous entrance route. After successful transseptal puncture under fluoroscopy guidance and PV venography, the SL0 sheath was changed over-the-wire for a deflectable delivery sheath, and the cryoballoon catheter was inserted such that the left- and right-superior PVs were targeted for cryoablation. Before the balloon inflation, the circular mapping catheter was placed in the PV to record the potentials. The cryoballoon was then inflated and advanced toward the PV ostium. The vein occlusion was checked with contrast dye injection. After complete occlusion was verified, the freezing began. The duration of each freezing cycle was 180 s for the targeted PV. If time to isolation (TTI) was >60 s, the second cryoablation started. Before the right PVs were ablated, the right phrenic nerve (PN) was paced, and the diaphragmatic movements were closely monitored. In the case of the weakening of the diaphragmatic movements, a freezing stop was immediately triggered. After the ablation, the entrance block was confirmed by the absence of PV potentials, and pace-capture testing was used to assess the exit block with maximum output.

Histologic examination

All the procedures were performed in Tenth People's Hospital (co-construction hospital with the animal laboratory shared), Shanghai, China, bred in single housing

under monitoring and fed with ad-libitum food. Three dogs of the study group and 2 dogs of the control group were euthanized on the same day post-ablation. The other 8 dogs of the two groups were euthanized 1 month post-ablation. The hearts, lungs, trachea, PVs, PNs, and esophagi were removed and examined. For the histological tissue preparation, each sample was fixed in 10% neutral buffer formalin, dehydrated, embedded in paraffin, sliced into 5- μ m serial sections, and mounted onto glass slides. The sections were stained with hematoxylin and eosin (H&E) for general examination. Immunohistochemistry (IHC) was used to check the injury of the PVs, and apoptosis was detected using the terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) method.

Statistical methods

The data were expressed as the mean \pm standard deviation, or as the percentage with count data. The continuous variables were analyzed using the 2-sample *t*-test. The non-parametric variables were analyzed using the Mann-Whitney test. The categorical variables were analyzed using the chi-square test. For all the analyses, the P values were 2-sided, and a P value <0.05 was considered statistically significant. The analyses were conducted with the use of SPSS 20.0 software.

Results

Of the 8 dogs in the study group, 16 PVs were targeted, of which 2 left superior PVs were too small to be ablated. Of the 5 dogs in the control group, 10 PVs were targeted. No interventions were performed in the inferior PVs. Outcome evaluations were based on three criteria, including (I) the acute success of PVI, (II) an assessment of

Table 2 Ablation summary

Group	Mean ablation frequencies of each PV	Mean total ablation time (s)	Mean TTI (s)	Isolation rate of single ablation
Study group	1.1±0.3*	379±46*	48.1±29.1	92.9%
Control group	2.0±0.8	592±162	52.3±51.2	60.0%

All the mean values are presented as the mean ± standard deviation. *, P<0.05, compared to the control group. PV, pulmonary vein; TTI, time to isolation.

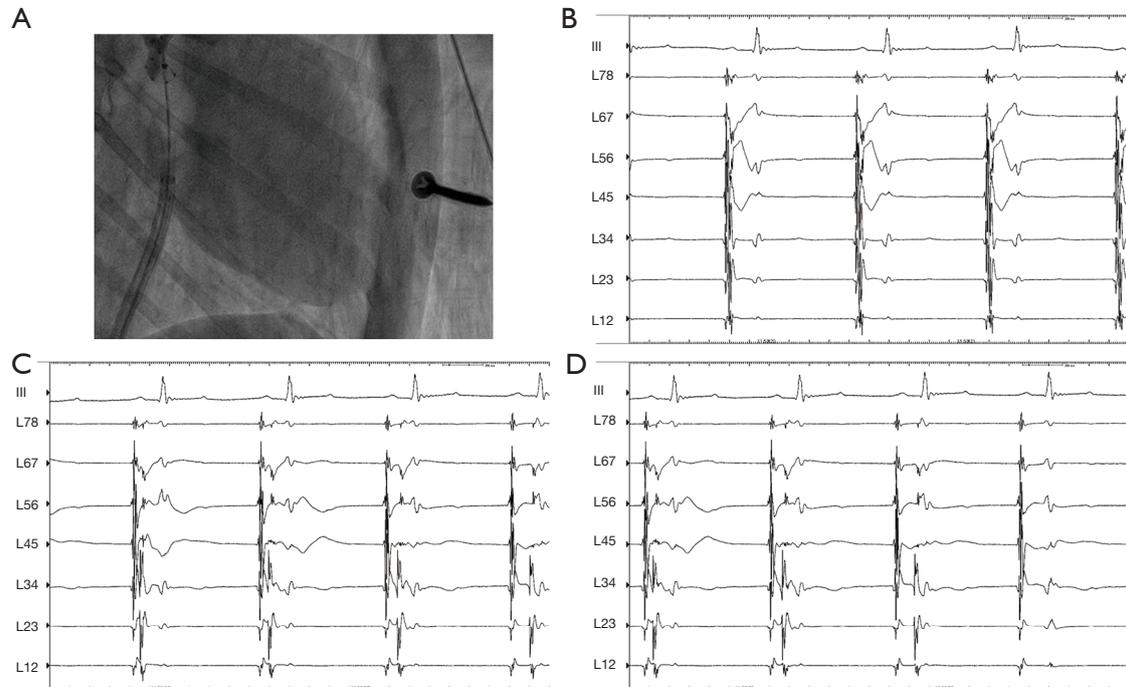


Figure 2 Images of a right-superior PV from the study group. (A) Cryoballoon ablation position; (B) PV potential before ablation; (C) delayed PV potential; (D) isolated right-superior PV. PV, pulmonary vein.

operation complications, and (III) a histopathology review of the lesion area.

The acute success of the PVI was 100% in the study group, and 90% in the control group. The average ablation times of each PV in the study group were less than those in the control group (1.1±0.3 vs. 2.0±0.8; P=0.006). Compared to the Medtronic system, the novel Cryofocus system was associated with a shorter procedure duration (379±46 vs. 592±162 s; P=0.013) and a similar TTI (48.1±29.1 vs. 52.3±51.2 s). The PVI rate of a single ablation was higher in the study group than that in the control group (92.9% vs. 60.0%; P=0.05; see Table 2 and Figure 2).

In relation to the safety, there was no evidence of thrombus, esophageal injury, or pericardial tamponade in

any of the dogs. Only 1 self-limited incidence of phrenic nerve paralysis (PNP) occurred in the control group, which was attributed to a right-superior PV ablation.

A histopathology review was conducted to assess the PV lesions. The pathological manifestations were similar in the two groups. Coagulation necrosis with inflammation was evident at the central part of the lesion (see Figures 3,4). At the peripheral zone, apoptosis became apparent 8 hours post-ablation (see Figure 5). The endothelial layers remained intact, and thrombus formation was not observed. No immediate PN injuries were observed microscopically in the study group; Edema and partial degeneration of PN was observed in 1 dog of the control group (see Figure 6). Esophageal injury was not observed in

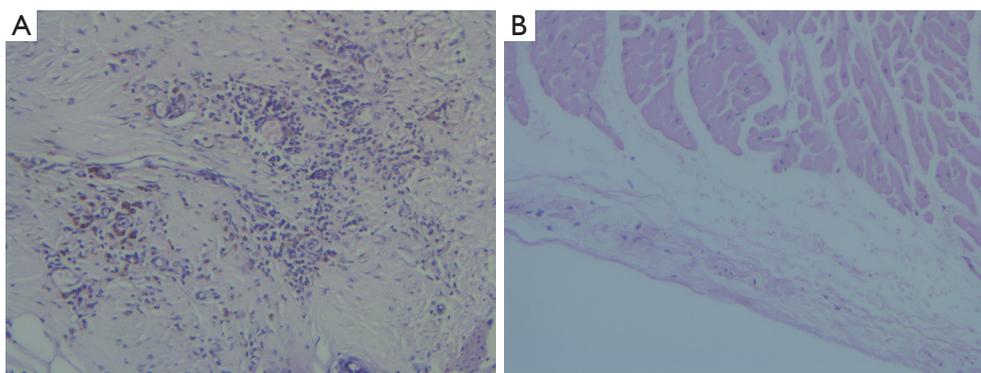


Figure 3 Inflammatory cell reaction (H&E, 200×). (A) Study group: infiltration of neutrophils and macrophages (blue color); (B) baseline control: no obvious infiltration of inflammatory cells. H&E, hematoxylin and eosin.

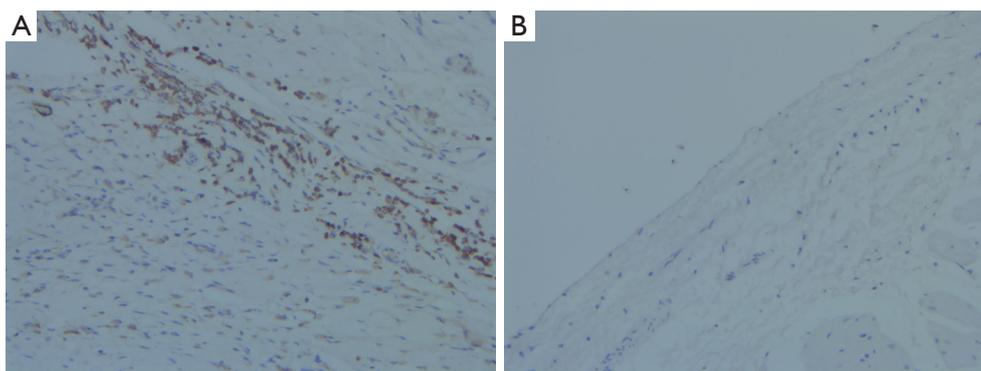


Figure 4 Inflammatory cell reaction (IHC, 200×). (A) Study group: MPO (+); (B) baseline control: MPO (-). IHC, immunohistochemistry. MPO, myeloperoxidase.

either group (see *Figure 7*).

Discussion

The use of a 3-dimensional mapping system, contact force-sensing technology, and irrigated radiofrequency catheter ablation have all been suggested to improve the safety and success of circumferential PVI; however, point-by-point radiofrequency ablation is still challenging due to the need for permanent and continuous transmural lesions (11-13). Various ablation technologies are being developed, among which cryoballoon ablation has unique characteristics that differs from the other types of energy ablation (14). Just a single delivery of cryoenergy has the ability to accomplish PVI, and thus it has been referred to as “one-shot” ablation. Cryoballoon ablation has been demonstrated to have the following 3 major advantages: (I) catheter stability; (II) a lower rate of acute procedural complications; and (III) a

shorter procedure duration (15,16).

The cryoballoon freezes the surrounding tissue via the formation of intracellular ice crystals, which causes cell and tissue damage both during the freezing process and afterwards (17,18). Freezing results in immediate damage, including hypothermic stress and direct cell injury, while vascular-mediated injury and apoptotic cell death result in delayed damage. Factors affecting cryoablation efficacy include the tissue temperature, cooling rate, freezing duration, thawing rate, and blood flow (19). The cooling rate per second is a primary determinant of ablation outcomes. The optimal freeze-thaw cycle requires fast cooling and slow thawing. When rewarming, cell damage becomes more serious due to solution-effect injury and water recrystallization. Cryoablation depends on the elimination of energy and the absorption of heat from the tissue, and the efficacy is determined by the cooling rate.

The currently widely used second-generation cryoballoon

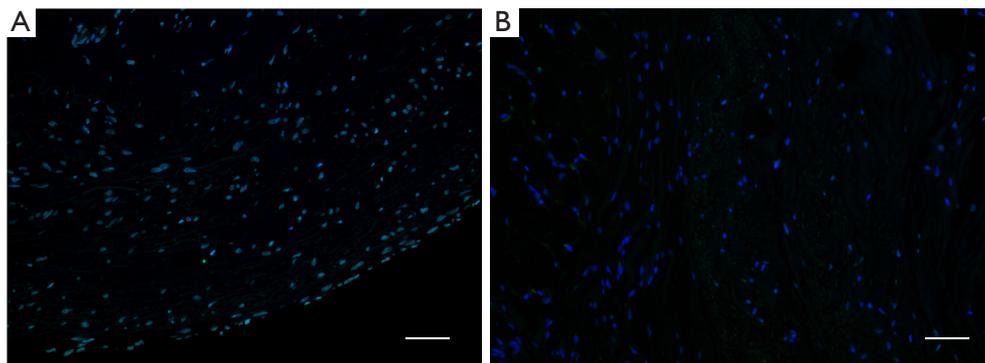


Figure 5 The rate of apoptosis stained by TUNEL (200 \times , green fluorescent nuclei are positive). (A) Study group: 96.997%; (B) baseline control: 4.183%. Scale bar =10 μ m. TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling.

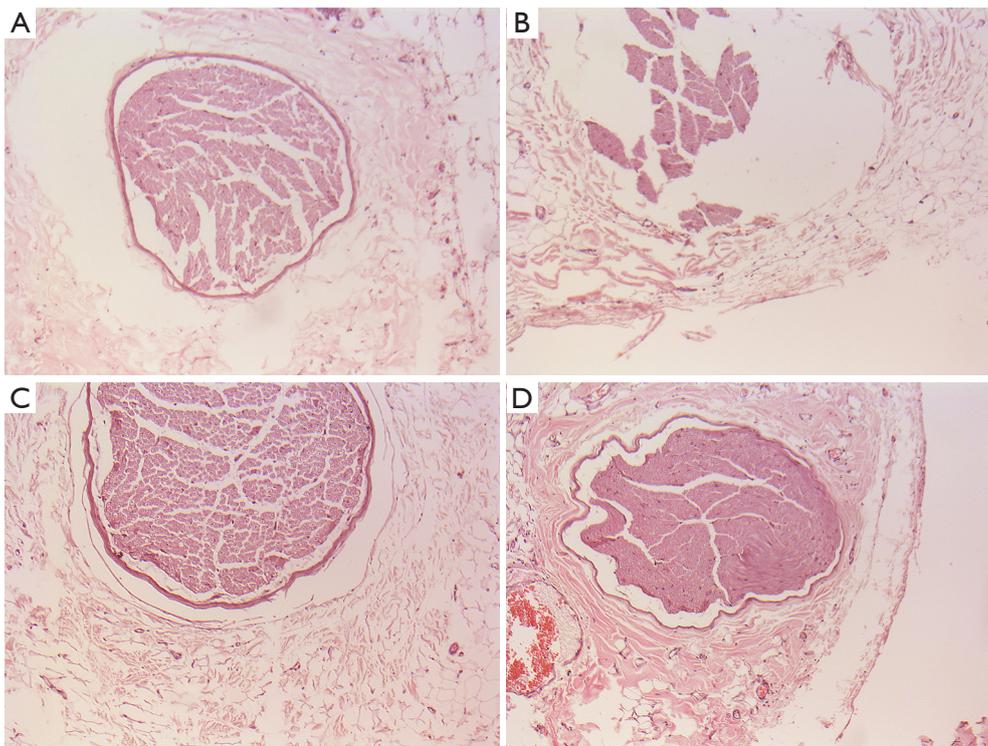


Figure 6 PN sections were stained with H&E (100 \times). (A) Study group: no immediate PN injuries; (B) Control group (PNP was observed in 1 dog): edema and partial degeneration (40–50%); (C) Study group: no PN injuries after 1 month; (D) Control group: no PN injuries after 1 month. PN, phrenic nerve; H&E, hematoxylin and eosin; PNP, phrenic nerve paralysis.

(Arctic Front Advance, Medtronic, Minneapolis, MN, USA) was designed to use N_2O as the refrigerant. While N_2 is used in the novel cryoballoon catheter system (Cryofocus, Inc.), whose faster cooling rate may improve cryoablation efficacy. The boiling point of N_2 ($-196^\circ C$) and the critical temperature ($-147^\circ C$) has a stronger freezing effect than that of N_2O (which has a boiling point of $-88.5^\circ C$ and a

critical temperature of $36.5^\circ C$). The inner-cryoballoon of Cryofocus is equipped with a temperature sensor. Real-time temperature measurement data are fed back to the cryoablation equipment, enabling the freezing flow to be adjusted to ensure that the proper temperature can be maintained.

In this study, there was a slight difference in the immediate

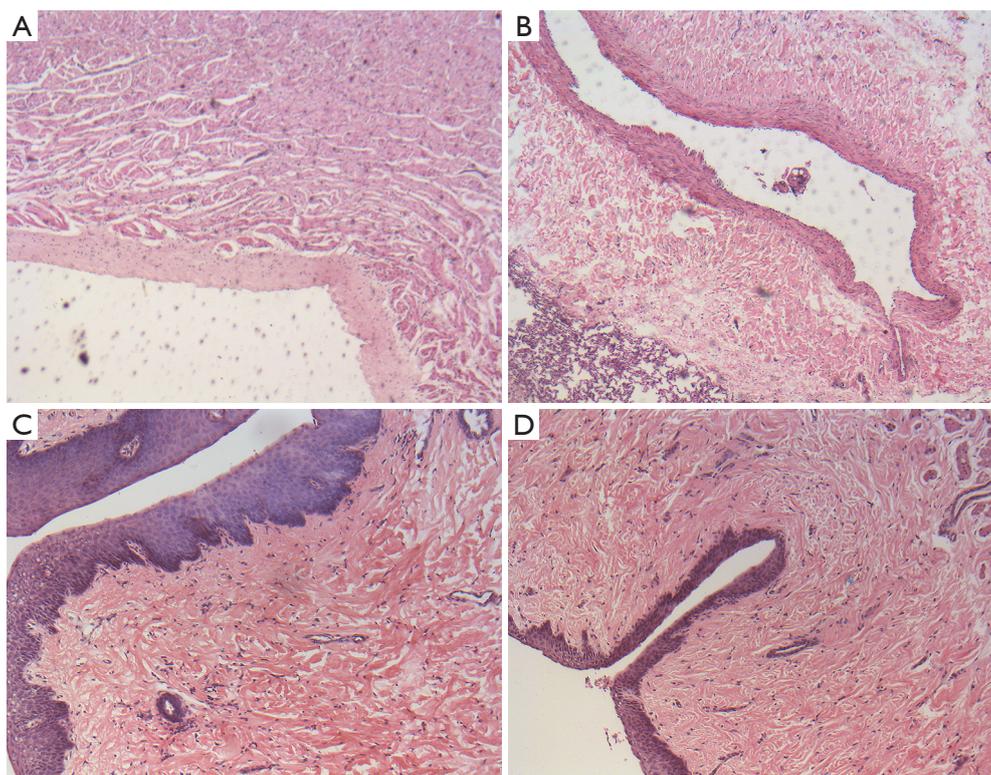


Figure 7 Sections were stained with H&E (100 \times). (A) Study group (immediately after operation): 3 intact histologic layers of atrium (i.e., intima, media, and adventitia); (B) Control group: the same as (A); (C) Study group (1 month after the operation): no injury of the 3 esophageal layers; (D) Control group: the same as (C). H&E, hematoxylin and eosin.

success rate of PVI between the two groups. Additionally, the Cryofocus (N_2) system had a higher PVI rate for single ablation (92.9%), less ablation times of each PV (1.1 ± 0.3) and a shorter procedure duration (379 ± 46 s) than the Medtronic system. This may be related to the rapid cooling rate of the cryoballoon with N_2 refrigerant (3.07 $^{\circ}C/s$), which is faster than that of the cryoballoon with N_2O refrigerant (1.59 $^{\circ}C/s$), and thus more likely to cause irreversible cell death.

TTI is an important parameter and is a strong predictor of longer-term PVI durability if it <60 s (20-24). The TTI was similar between the two groups, but it was a little bit shorter in the study group than in the control group. If the sample size had been larger, it was likely that the difference would have been statistically significant.

In this study, cryoablation caused cellular damage, the necrosis of tissues, and the infiltration of neutrophils. Lesions had well-demarcated boundaries and intact endothelial layers at the PVs in both groups. No thrombus was observed in the two groups. Apoptosis was observed 8 hours after ablation at the peripheral zone of ablation,

where the temperatures and cooling rates achieved were less likely to be immediately lethal to the cells. Cellular injuries might activate caspases, which cleave proteins and cause membrane blebbing, chromatin condensation, genomic fragmentation, and programmed cell death (25).

Except for 1 PNP in the control group, there were no other complications in the two groups. Thus, the Cryofocus system appears to be safe, and the freezing efficacy of the N_2 coolant is strong.

Compared to N_2O refrigerant, the advantage of N_2 refrigerant in PV cryoballoon ablation is stronger freezing effect so that it cost shorter time to complete the procedure. In a word, the novel cryoablation system with N_2 refrigerant might have more efficacy than and a similar safety to that of the Medtronic system.

This study had several limitations. First, as this study was performed on dogs with a different PV anatomy to humans, the conclusions drawn in this study might not necessarily be applicable to humans. Second, an evaluation of 26 PVs in 13 dogs represents a small cohort. Third, we

only measured the acute PVI rate due to the absence of any electrophysiological follow-up. Animal studies may provide useful information for clinical studies, but reliable estimates of human risk require further research.

Conclusions

In summary, the novel cryoablation system with N₂ refrigerant had better efficacy than and a similar safety to that of the Medtronic system with N₂O refrigerant. As this is the first study to evaluate the novel system, future studies need to be conducted to confirm our findings, especially those on safety.

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at <https://jtd.amegroups.com/article/view/10.21037/jtd-22-418/rc>

Data Sharing Statement: Available at <https://jtd.amegroups.com/article/view/10.21037/jtd-22-418/dss>

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://jtd.amegroups.com/article/view/10.21037/jtd-22-418/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Experiments were performed under a project license (No. 201821) granted by the Institutional Ethical Committee of Shanghai Putuo District People's Hospital, in compliance with the guidelines of Shanghai Putuo District People's Hospital for the care and use of animals.

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