The Impact of Freezing Time and Balloon Size on the Thermodynamics and Isolation Efficacy during Pulmonary Vein Isolation Using the 2nd Generation Cryoballoon

Running title: Takami et al.; Freezing Time and Balloon Size of Cryoablation

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Journal Subject Codes: [22] Ablation/ICD/surgery
Abstract:

Background - The differences in ablation characteristics of freezing time and balloon size using 2nd generation cryoballoon (CB) are still unknown.

Methods and Results - Twenty-six dogs underwent pulmonary vein (PV) isolation (PVI). Balloon and tissue temperatures (left atrial (LA)-PV junction, phrenic nerve, and internal esophagus) were monitored. The ablation duration was randomized to either 3 or 4 minutes, which did not show significant differences in temperature profiles, PVI success rate, complications, or histological changes. Twenty dogs underwent cryoablation using 28 mm CB, six dogs were done using the 23 mm CB. Positioning of the 23 mm CB was more distal in the PV, which resulted in better PV occlusion. Temperature profiles showed lower temperatures in the 23 mm CB than in the 28 mm CB (inner balloon, median [range]: -51.5 [-66.0 – -31.0] vs -43.0 [-64.0 – -26.0] °C, p < 0.001; balloon surface: -43.0 [-60.0 – -15.8] vs -6.5 [-46.2 – 28.9] °C, p < 0.001; LA-PV junction: -6.7 [-20.0 – 21.4] vs 15.8 [-14.4 – 35.1] °C, p < 0.001), and trended towards a higher PVI success rate in the 23 mm CB. Histologically, deeper extensions of ablative lesions into the PV were seen with 23 mm CB, and larger ablative lesions were seen in the LA antrum using 28 mm CB.

Conclusions - The efficacy of 3 min ablation was not significantly different from 4 min ablation in dogs. The 23 mm CB had a greater cooling effect than the 28 mm CB for small PVs, but showed narrower ablative lesions in the LA antrum.

Key words: atrial fibrillation, pulmonary vein isolation, cryoballoon, thermodynamics
Background

Recently, pulmonary vein (PV) isolation (PVI) using the 2nd generation cryoballoon (CB), introduced as a therapy for patients with drug-refractory atrial fibrillation (AF), has shown better outcomes than with the 1st generation CB.1-5 However, some questions about the 2nd generation CB remain. Several studies in cryobiology have shown that prolongation of freeze duration produces a greater destructive effect in non-cardiac tissue.6, 7 However, ablation duration to achieve acute and long-term PVI is still debatable. It is also unclear how different sized balloons (23 mm and 28 mm) should be used, as the impact of the balloon size on tissue and balloon temperature profiles and ablative lesion size have not been studied. Information on the effects of ablation duration and balloon size is required to achieve high PVI success rates without severe complications using the 2nd generation CB.

The purpose of the present study was to investigate the impact of ablation duration and balloon size on tissue and balloon thermodynamics, acute and chronic PVI success rates, complications, and chronic histological changes of the PV and left atrium (LA).

Methods

General

This study was designed to compare the ablation effect and safety of different ablation durations (3 min vs 4 min) and different balloon sizes (23 mm vs 28 mm) in an in vivo model. Twenty-six dogs underwent PVI using 2nd generation CB.

Animal Preparation

The protocol was approved by Mayo Foundation Institutional Animal Care and Use Committee. Dogs (30.3±2.5 kg) were anesthetized with intravenous ketamine and diazepam, intubated, and
maintained on 1-3% isoflurane. The surface ECG, body temperature, and blood pressure were monitored.

**Tissue Temperature Monitoring**

Tissue temperatures were monitored by implanted thermocouples (TCs), as reported previously. All dogs underwent left/right thoracotomy for access to the superior/inferior PVs. The pericardium was opened to allow implantation of TCs on the LA-PV junction (3 TCs around each PV). In procedures performed on the right superior PV (RSPV), one or two TCs were implanted along the margin of the phrenic nerve (PN). Esophageal lumen temperature was monitored using a circular catheter with 4 TCs.

**Catheterization**

Sheaths were placed in right external jugular vein and left or right femoral vein and artery. A 6 F decapolar catheter was placed in the coronary sinus. A 10 F 5.5-10 MHz intracardiac echocardiography (ICE) catheter (Acuson-Siemens, Mountain View, CA, USA) was introduced via the external jugular vein. LA size and PV ostium diameter were measured using ICE and PV venography. ICE was used to facilitate contact of the balloon with tissue around the PV orifice and to reduce blood flow leaks around the balloon.

**Cryoballoon Ablation**

Ablation using the 2nd generation CB in a canine model has been reported from our laboratory. After transseptal puncture, a 12 F deflectable sheath (FlexCath, Medtronic CryoCath LP) was introduced. A 3.3 F 15 mm circular catheter (Achieve mapping catheter, Medtronic) was used through the central lumen of a 10.5 F 28 mm/23 mm CB catheter (Arctic Front Advance, Medtronic CryoCath LP). The balloon was placed outside the ostium of the PV, inflated, and advanced to the PV orifice. If a blood flow leak around the balloon was found by ICE, the CB
was manipulated to minimize the leak. After starting ablation, balloon position was maintained throughout the delivery.

Ablation duration in each animal was randomized to 3 min (13 dogs) or 4 min (13 dogs). Ablation was not randomized regarding the balloon size in these animals. This study was conducted in 2 sequential components. In the first 20 consecutive dogs, cryoablation was performed exclusively using the 28 mm CB. Subsequently, cryoablation was performed in 6 consecutive dogs using the 23 mm CB. Balloon size was not based on any animal characteristics, but simply sequential. Ablations were performed until PVI verification after each freeze, with a maximum of 2 ablations. No bonus freeze was applied. During RSPV ablation, the PN was paced at 20 mA for diaphragm monitoring. If PN capture was lost, ablation was stopped. Inner balloon temperatures were monitored via the TC located in the proximal inner balloon. Balloon surface temperatures were recorded using a modified CB with 4 imbedded surface TCs, one in each balloon quadrant. These were placed equidistant between the largest balloon diameter and its tip in both 23 mm and 28 mm CB.

**Distance analysis**

Measurement of distance from balloon surface to TCs was reported previously.8, 9 Distances were calculated from digitally acquired right and left anterior oblique views with the 15 F sheath and balloon diameters serving for calibration.

**Follow-up**

In all dogs, esophageal endoscopy was performed within 3-5 days post ablation. All animals underwent an electrophysiological follow-up study at 30-60 days after ablation to assess chronic PVI using a circular mapping catheter and to rule out PV stenosis. If RSPV ablation was done at first procedure, PN function was evaluated. All dogs were euthanized for histological analysis.
Pathology

Triphenyltetrazolium chloride was used to delineate the cryoablation lesions. Heart, lungs, trachea, and esophagus were removed. Gross pathology was assessed. Each LA-PV junction was cut into 8 quadrants and each of these segments was examined microscopically. The esophagus and PN segments were cut cross sectionally with 2 mm thickness and stained with Hematoxylin-Eosin and Masson Trichrome.

Statistical Analysis

Statistical analysis was performed using JMP 10.0 statistical software (SAS Institute Inc.). Continuous variables which are normally distributed are presented as mean±SD. Continuous variables which are not normally distributed are presented as median [range]. Two-sample t-test was used for group comparisons of normally distributed variables such as animal weight, LA size. Mann-Whitney U test was used for group comparisons of non-normally distributed variables such as balloon and tissue temperatures, thawing time, distance from balloon to thermocouples, and ablation lesion size. Fisher’s exact test was used for comparisons of categorical variables. Whenever multiple measurements had been obtained per animal (PV or ablation level), generalized estimating equation models were used for continuous or categorical variables. Correlation between temperature and distance to each profile was fit using a logarithmic transformation. A p-value of less than 0.05 was used to indicate statistical significance.

Results

Ablation Duration: 3 min vs 4 min

Twenty-six dogs were randomized to 3 min ablation and 4 min ablation. The baseline PV diameter and LA size were not significantly different between the two groups (Table 1).
Ablation and Temperature Data

Sixty-seven cryoablations of 52 PVs were performed. Table 1 shows the comparison of ablation data during PVI between 3 min and 4 min groups. Total ablation duration per PV in the 4 min group was significantly longer than in the 3 min group (310±111 vs 249±89 sec; p < 0.001). Figure 1 shows the representative time-course temperature changes in 3 min and 4 min ablation cases during PVI. Table 1 also shows the comparison of the lowest balloon and tissue temperatures between the 2 groups. There was no significant difference in temperature profiles between the 2 groups. In 4 min groups, temperature drop from 3 min to 4 min was analyzed. The mean temperature drop from 3 min to 4 min was -1.0±1.6 °C in inner balloon, -1.2±3.1 °C at balloon surface, and -1.0±2.0 °C at LA-PV junction. Time to PVI was recorded during 5 of 67 (7%) cryoablations, 2/5 PVs were 3 min freezes and 3/5 PVs were 4 min freezes. Time to PVI was 43 and 70 sec in 3 min freezes, and 37, 45, and 120 sec in 4 min freezes.

Efficacy

Table 2 shows acute and chronic success rates of PVI. Acute PVI success rates were 26/26 (100%) PVs in the 3 min group, and 22/26 (85%) PVs in the 4 min group (p = 0.05). Acute success rate of different balloon size in 3 min group was 20/20 (100%) with 28 mm CB and 6/6 (100%) with 23 mm CB. Success rate in 4 min group was 16/20 (80%) with 28 mm CB and 6/6 (100%) with 23 mm CB (p = 0.75).

A follow-up study was performed after a mean of 46±14 days post-ablation. Chronic PVI success rates were 20/26 (77%) in the 3 min group vs 16/20 (80%) in the 4 min group (p = 0.83). Chronic success rate of different balloon size in 3 min group was 15/20 (75%) with 28 mm CB and 5/6 (83%) with 23 mm CB. Success rate in the 4 min group was 12/16 (80%) with 28 mm CB and 4/4 (100%) with 23 mm CB (p = 1.00).
Analyzing the 5 PVs with recorded time to PVI, two PVs were reconnected and 3 PVs were isolated. Time to PVI in chronic isolated PVs (37, 43, and 45 sec) was relatively shorter than in reconnected PVs (70 and 120 sec). In histology, maximal ablation lesion length was not significantly different between the 2 groups (Table 2). Figure 2 shows representative transmural ablation lesions of the LA-PV junction in both 3 min and 4 min group.

Safety and Complications

Table 1 and Table 2 show temperature profiles of PN and internal esophagus and complication rates.

PV stenosis

Although 2 moderate PV stenoses (60% and 55% stenosis) in the 3 min group and 1 moderate PV stenosis (60% stenosis) in the 4 min group were seen, no severe PV stenosis was observed in either group.

Phrenic Nerve

Lowest PN temperature during ablation was not significantly different between the two groups (Table 1). Figure 3 shows cooling delay in the PN. Tissue temperature continued to decrease after termination of cryoablation. Time to achieve lowest temperature at the PN was 196±11 sec in 3 min ablations and 254±12 sec in 4 min ablations. Neither temporary nor chronic PN palsy was observed in either group (Table 2).

Esophagus

Lowest internal esophageal temperature during ablation was not significantly different between the two groups (Table 1). Cooling delay was also observed at the internal esophagus during ablation (Figure 3). Time to achieve lowest temperature was 212±37 sec for 3 min ablations and 279±35 sec for 4 min ablations. Post-ablation esophageal endoscopy showed an esophageal ulcer.
in 2 of 13 (15%) dogs in the 3 min group and in 1 of 12 (8%) dogs in the 4 min group (p = 1.00, Table 2). No esophageal-LA fistula was observed in either group. Follow-up endoscopy after 30 days showed healing of these lesions in all dogs.

**Balloon Size: 23 mm Cryoballoon vs 28 mm Cryoballoon**

Fourteen cryoablations using the 23 mm CB were performed in 12 PVs (6 left inferior PVs (LIPV) and 6 right inferior PVs (RIPV)) in 6 dogs. Data were compared with that of 24 cryoablations using the 28 mm CB for 20 PVs (10 LIPVs and 10 RIPVs) in 20 dogs. Baseline PV and LA size were not significantly different between the 23 mm and 28 mm CB group (Table 3).

**Balloon Positioning**

Figure 4 shows two representative cases of balloon positioning during PVI of the LIPV using 23 mm and 28 mm CB. Cryoballoon-tissue contact point occurred at a more proximal position (atrial side) in the 28 mm CB than in the 23 mm CB.

Positional relationship between balloon-tissue contact point and LA-PV junction were analyzed by fluoroscopy. In 14 cryoablations using 23 mm CB, 9 of 14 (64%) showed that the balloon-tissue contact point was at the LA-PV junction, and 5 of 14 (36%) showed the contact point in the PV. In 24 cryoablations using 28 mm CB, 14 of 24 (58%) showed the balloon-tissue contact point at the LA-PV junction, 3 of 24 (13%) showed the contact point in the PV, and 7 of 24 (29%) showed the contact point was more atrial than the LA-PV junction.

**Blood Flow Leak around Balloon**

Table 4 shows a comparison of ablation data. Complete PV occlusion during PVI was more often seen in 23 mm CB than 28 mm CB (10/14 (71%) vs 8/24 (33%); p = 0.01), and moderate blood flow leak was more often seen in 28 mm CB than in 23 mm CB.
Temperature Data

Figure 5 shows two representative cases of time-course temperature changes during PVI. Table 4 compares temperature profiles between the two balloon sizes. Lowest inner temperature, balloon surface temperature, and LA-PV tissue temperature in 23 mm CB were significantly lower than those in 28 mm CB. Balloon thawing time (from final temperature to +20 °C) was longer in ablations using 28 mm CB.

Relationship between Temperature and Distance

Figure 6 shows the relationship between lowest tissue temperature and distance from balloon surface. Both balloon sizes showed good correlation between lowest temperature and distance from the balloon surface (23 mm CB: r = 0.90; p < 0.0001, 28 mm CB: r = 0.89; p < 0.0001).

Figure 7 shows a schematic view of lowest temperature distribution in both groups. Lowest temperature distribution was different between 23 mm and 28 mm CB.

Efficacy

Table 5 shows a comparison of the efficacy between 23 mm and 28 mm CB. Acute PVI success rate was 12/12 (100%) in 23 mm CB and 16/20 (80%) in 28 mm CB (p = 0.27). Chronic PVI success rate was 9/10 (90%) in 23 mm CB group and 11/16 (69%) in 28 mm CB (p = 0.21).

Figure 8 shows representative ablation lesions using 23 mm and 28 mm CB. The 28 mm CB created a larger lesion in the LA antrum than 23 mm CB. Table 5 also depicts differences of ablation lesions between the two groups. Maximal ablation lesion extension outside of the PV orifice to the LA endocardial surface was 10.0 [5.0 – 24.8] mm for 28 mm CB and 4.2 [1.5 – 11.9] mm for 23 mm CB (p = 0.001). In contrast, the maximal ablation lesion extension into the PV was 5.3 [0 – 17.0] mm for 28 mm CB and 6.8 [5.6 – 8.5] mm for 23 mm CB (p = 0.006).

Mean length of the remaining myocardial sleeve was 4.7 [0 – 7.0] mm for 28 mm CB, and 1.9 [0
– 4.0] mm for 23 mm CB (p < 0.001). In the 28 mm cryoballoon group, histological gap was observed in 9 PVs. Four of 9 PVs had multiple or wide gaps (> 25% of PV ostium). Analyzing the gap location at the PV ostium, the gap was seen inferiorly for 6 PVs (2 LIPVs, 4 RIPVs), anterior for 4 PVs (1 LIPV, 3 RIPVs), posterior for 3 PVs (2 LIPVs, 1 RIPV), and superior for 3 PVs (1 LIPV, 2 RIPVs). In 23 mm cryoballoon group, histological gap was observed in one PV at the inferior portion of the RIPV.

**Safety and Complications**

**PV stenosis**

Two of 10 PVs showed moderate PV stenosis (60% and 55% stenosis) in the group ablated with 23 mm CB. No PV stenosis was seen in 28 mm CB group (p = 0.10, Table 5). Analyzing ablation data in the two moderately stenotic veins, balloon-tissue contact points during ablation were located inside the PV. Lowest inner balloon temperature was -55.0 ºC and -65.0 ºC respectively. Lowest tissue temperature at LA-PV junction was -17.0 ºC and -12.9 ºC in these cases. Histological analysis revealed that ablation lesion extension from the PV orifice into the PV was 5.8 mm and 5.3 mm respectively.

**Esophageal Complications**

Lowest internal esophageal temperature during ablation was not significantly different between the two groups (Table 4). Esophageal endoscopy showed an ulcer in 3/5 (60%) for 23 CB group, and 1/20 (5%) in the 28 CB group (p = 0.02; [Table 5]). In 3 dogs with esophageal ulcer lesions using 23 mm CB, the PV-diameter/balloon-diameter ratio was 0.62 (14.3/23.0), 0.53 (12.1/23.0), and 0.52 (12.0/23.0) respectively. Lowest internal esophageal temperatures during cryoablation were 21.4 ºC, 9.2 ºC, and 37.1 ºC, respectively. In one dog with an ulcer using 28 mm CB, the PV-diameter/balloon-diameter ratio was 0.56 (15.8/28.0). Lowest internal esophageal
temperature was 15.5 °C. Follow-up esophageal endoscopy at 4 weeks showed healed lesions in all dogs.

Discussion

Major findings

The present study demonstrates the following findings: 1) Balloon and tissue temperature profiles, acute and chronic PVI success rate, complications, and histological changes were not significantly different between 3 min and 4 min ablation durations. 2) Cooling delay after cryoablation termination was observed in PN and internal esophagus. 3) Positioning of 28 mm CB tended to be more proximal at the LA-PV junction than that of 23 mm CB. 4) Better PV occlusion was seen using 23 mm CB. 5) Tissue and balloon temperature profiles revealed significantly lower temperatures in 23 mm CB than in 28 mm CB, with a corresponding trend towards higher PVI success rates in 23 mm CB. 6) Histologically, deeper extension of ablative lesions into the PV was seen in 23 mm CB and larger ablation lesions were seen in the LA antrum in 28 mm CB.

Ablation Duration

Although preset ablation duration using 2nd generation CB is 240 sec by the manufacturer, the adequate ablation duration to achieve chronic PVI remains unknown. In the present study, lowest balloon and tissue temperatures were not significantly different between 3 min and 4 min ablations. During 4 min ablations, additional mean temperature drop in balloon and tissue from 3 min to 4 min was only -1 °C. In addition, ablation lesion size was not significantly different between the 2 groups. Dubuc et al. reported that cryoablation lesion size measured by ICE grew progressively during the first 3 minutes of freezing in atrial myocardium.10 However, they did not find difference in lesion size after 3 minutes. Considering results from previous and present
studies, additional one minute ablation duration (from 3 to 4 min) might not cause additional tissue damage at the LA-PV junction. Therefore, 3 min ablations using 2nd generation CB might be sufficient to achieve acute and chronic PVI. Impact of ablation duration on tissue temperature profiles in PN and internal esophagus was not significantly different between 3 and 4 min ablations. Conductive cooling delay in PN and internal esophagus was observed in both 3 and 4 min ablations. Previously, we showed the spread of conductive cooling for each direction from the balloon-tissue contact point,9 explaining latent cooling that was seen in PN or esophagus even after termination of cryoablation. No severe PN or esophageal complications were observed in this study. Hence, it is not clear whether shorter ablation durations of 3 min reduce complications compared to 4 min ablation. The protocol without bonus freeze in this study was intended to investigate the tissue/balloon thermodynamics, success rates, histological changes, and complication rates with minimum required number of cryoablations. The study showed a chronic PVI rate of 77% in 3 min and 80% in 4 min ablation group in dogs. These rates were similar to a recent clinical study with bonus freeze.11 Therefore, this study suggests that the clinically applied routine bonus freeze for all PVs might have to be reconsidered.

**Cryoballoon Size**

There are two sizes of CBs (23 and 28 mm) which can be used. In this study, the biophysics using the two different balloon sizes were clarified and use of 28 mm CB led to fewer complications.

**Different Thermodynamics between 23 mm and 28 mm Cryoballoons**

Tissue and balloon temperatures were lower using 23 mm CB. There may be several reasons for this. First, balloon surface area is different between the balloons. The number of refrigerant jets within the balloons is the same for both sizes. Therefore, although there is a difference in
refrigerant flow rate between 23 mm CB (6.3 L/min) and 28 mm CB (7.2 L/min), cooling effect of the balloon surface per unit area is still stronger for 23 mm CB. Second, canine PVs are smaller than human PVs. Therefore, relationship between PV size and balloon size might affect thermodynamics. The 28 mm CB tended to position more proximally (atrial side) at LA-PV junction, thus, a larger balloon surface area was exposed to blood flow in the LA. This would cause a warming effect of the inner balloon and surface temperatures. In addition, balloon positioning might affect the distance from balloon surface to TCs at LA-PV junction, which might cause higher tissue temperature at the LA-PV junction in 28 mm CB. Third, better PV occlusion with use of 23 mm CB was seen. We reported that blood flow leaks around the balloon cause a warming effect on balloon and tissue temperatures.9 Higher complete vein occlusion rates with use of 23 mm CB may explain lower balloon and tissue temperatures. On the other hand, we observed a trend towards longer balloon thawing time in 28 mm CB than in 23 mm CB. This might be due to bigger ice formation on the larger surface of 28 mm CB, taking more time to thaw.

Ablation Lesion Characteristics between 23 mm and 28 mm Cryoballoons

This study elucidated different characteristics of ablation lesions. These findings could simply result from differences in positioning of the two sizes of CBs. The bigger balloon might not only be able to ablate for PVI, but could perform PV antral modification. Wide area circumferential ablation has been recommended,12 because ganglionated plexi, non-PV foci, and arrhythmogenic substrate have been observed in the PV antrum. The 28 mm CB might be better to create wide area lesion in LA. Although lower balloon and tissue temperatures, and higher acute and chronic PVI success rates were seen in 23 mm CB, further extension of ablation lesion into the deep PV was observed after 23 mm CB ablation. Previous studies have shown that radiofrequency
application within the vein results in frequent occurrence of PV stenosis. Although the energy source is different, positioning deeper in the PV using 23 mm CB might produce deeper extension of the ablation lesion into the PV, which might cause higher rate of moderate PV stenosis in 23 mm CB group.

Histological gap was most often observed in the inferior portion of PV ostium. In dogs, the angle between the LA and PV is steep; therefore blood flow leaks around cryoballoon were most often seen in inferior portion of PV ostium. Our previous paper showed correlation between location of blood flow leak around the balloon and chronic histological gaps at the PV ostium.

**Esophageal Complication**

Esophageal ulcers were more often observed in 23 mm CB. Distance from balloon surface to esophageal TCs was not significantly different between 23 mm and 28 mm CB. Lower inner balloon and balloon surface temperature profiles in 23 mm CB might cause higher incidence of esophageal ulcer. However, lowest internal esophageal temperature in dogs that had esophageal lesions was different in each dog (from 9.1 to 37.1 °C). Therefore, relationship between internal esophageal temperature and occurrence of esophageal ulcerative lesions could not be delineated in this study.

**Limitations**

This study was done in an *in vivo* canine model. Although dogs are often used as an animal model for catheter ablation studies, anatomy of the PVs is different than that found in humans; the mean ostial diameter of human PVs is larger than that in dogs. Therefore, balloon positioning, balloon and tissue thermodynamics, and ablation lesion size might translate to different results in humans. In this study, cryoablation using 23 mm CB was performed only in the inferior PVs (LIPV and RIPV); temperature data for LSPV, RSPV, and PN between 23 mm
and 28 mm CB could not be compared. Only 3 TCs were implanted at each LA-PV junction, 1 or 2 TCs in PN, and 4 TCs in esophagus. Therefore, we cannot exclude possibility that there might be lower tissue temperatures. Time to PVI during ablation was recorded in only 5 (7%) cryoablations, because positioning of the circular mapping catheter was deep in PV to achieve better PV occlusion. Therefore, correlation between time to PVI and outcomes was not clear in this study. Comparison of complication rates should be viewed with caution as the number of animals ablated with 23 mm CB was small.

**Clinical Implications**

To minimize procedure and fluoroscopy time, ablation duration may need to be reconsidered. The efficacy of 3 min ablation was not significantly different from 4 min ablation. Therefore, 3 min ablation seems to be a very viable option when using the 2nd generation CB. Conductive cooling delay was seen in PN and internal esophageal TCs. Thus, in the clinical arena, continuing to check PN capture and internal esophageal temperature even after stop of cryoablation would be reasonable. Clinically, target PVs are sometimes small, especially the LIPV. In this case, it might be hard to achieve total occlusion using the 28 mm CB and therefore choosing the 23 mm CB for ablation would be an option. However, meticulous balloon positioning and monitoring of temperature achieved should be performed.

**Funding Sources:** This study was funded by Medtronic, Inc.

**Conflict of Interest Disclosures:** Dr. Packer reports participation in research grants supported by the American Heart Association, Biosense Webster, Boston Scientific/EPT, Endosense, EpiEP, EP Advocate, Medtronic, NIH, CardioFocus, Hansen Medical, St. Jude Medical, Siemens Acuson, and Thermedical (EP Limited). He reports serving as a consultant or on the advisory boards of Abiomed, Biosense Webster, Inc., Boston Scientific, CardioDX, CardioFocus,
CardioInsight Technologies, Excerpta Medica, FoxP2 Medica LLC, InfoBionic, Inc., Johnson & Johnson Healthcare Systems, Johnson & Johnson, MediaSphere Medical, LLC, Medtronic CryoCath, OrthoMcNeill, Sanofi-aventis, Siemens, St. Jude Medical, and Siemens AG. Dr. Packer received no personal compensation for these consulting activities. He declares royalties from St. Jude Medical, Blackwell Publishing, and Oxford Royalty. Dr. Lehmann reports reception of a research grant supported by American Heart Association. No other authors declared potential conflicts.

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Table 1: Comparison of ablation data, temperature profiles between 3 min vs 4 min ablation

<table>
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<tr>
<th></th>
<th>3 min ablation (13 dogs)</th>
<th>4 min ablation (13 dogs)</th>
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<td>Total Ablation Time/Vein (sec) *</td>
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<td>Complete PV Occlusion (%)</td>
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<td>Lowest Inner Balloon Temperature (°C) † ‡</td>
<td>-46.5 [-69.0 – 28.0] (n = 36)</td>
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<td>Lowest Balloon Surface Temperature (°C) † ‡</td>
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<td>Lowest LA-PV junction Temperature (°C) † ‡</td>
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<td>Distance from Balloon to LA-PV junction TCs (mm) †</td>
<td>2.6 [0.7 – 16.2] (n = 33)</td>
<td>2.8 [0.5 – 11.5 ] (n = 24)</td>
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<td>Lowest Phrenic Nerve Temperature (°C) † ‡</td>
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<td>Lowest Internal Esophagus Temperature (°C) † ‡</td>
<td>30.7 [18.3 – 38.0] (n = 19)</td>
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<td>Distance from Balloon to Esophagus TCs (mm) †</td>
<td>12.0 [4.5 – 22.9] (n = 19)</td>
<td>12.8 [5.1 – 22.4] (n = 15)</td>
<td>0.56</td>
</tr>
</tbody>
</table>

* Mean ± SD, † Median [range], ‡ Lowest tissue temperature in the all tissue temperatures during each ablation. PV indicates pulmonary vein; LSPV, left superior pulmonary vein; LIPV, left inferior pulmonary vein; RSPV, right superior pulmonary vein; RIPV, right inferior pulmonary vein; LA, left atrium; TCs, thermocouples.
Table 2: Comparison of PVI success rate, complications, and histological changes between 3 min vs 4 min ablation

<table>
<thead>
<tr>
<th></th>
<th>3 min ablation (13 dogs)</th>
<th>4 min ablation (13 dogs)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute PVI (%)</td>
<td>26/26 (100)</td>
<td>22/26 (85)</td>
<td>0.05 ‡</td>
</tr>
<tr>
<td>Chronic PVI (%)</td>
<td>20/26 (77)</td>
<td>16/20 (80) †</td>
<td>0.83</td>
</tr>
<tr>
<td>Esophageal Ulcerated Lesion (%)</td>
<td>2/13 (15)</td>
<td>1/12 (8) †</td>
<td>1.00</td>
</tr>
<tr>
<td>Phrenic Nerve Palsy (%)</td>
<td>0/13 (0)</td>
<td>0/12 (0) †</td>
<td>1.00</td>
</tr>
<tr>
<td>PV Stenosis (moderate) (%)</td>
<td>2/26 (8)</td>
<td>1/24 (4) †</td>
<td>0.59</td>
</tr>
<tr>
<td>Maximal Ablation Lesion Length from to LA (mm) *</td>
<td>10.9 [1.5 – 34.1] (n = 24)</td>
<td>10.5 [2.1 – 29.0] (n = 22)</td>
<td>0.51</td>
</tr>
<tr>
<td>Maximal Ablation Lesion Length to PV Inside (mm) *</td>
<td>5.1 [0 – 12.0] (n = 23)</td>
<td>5.4 [0 – 8.8] (n = 22)</td>
<td>0.61</td>
</tr>
<tr>
<td>Remaining Myocardial Sleeve Length (mm) *</td>
<td>3.3 [0 – 11.1] (n = 24)</td>
<td>4.4 [0 – 8.8] (n = 21)</td>
<td>0.39</td>
</tr>
<tr>
<td>Circumferential transmural lesion (%)</td>
<td>20/26 (77)</td>
<td>16/24 (67) †</td>
<td>0.46</td>
</tr>
</tbody>
</table>

* Median [range], † One dog in 4 min ablation died after first procedure because of the bleeding from the thermocouple suture site, therefore the 12 dogs (24PVs) were done for follow-up study. ‡ Generalized estimating equation model could not be computed for this comparison. PVI indicates pulmonary vein isolation; LA, left atrium.
Table 3: Baseline anatomical characteristics between 23 mm cryoballoon and 28 mm cryoballoon

<table>
<thead>
<tr>
<th></th>
<th>23 mm Cryoballoon (6 dogs)</th>
<th>28 mm Cryoballoon (20 dogs)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg) *</td>
<td>30.4±2.0 (n = 6)</td>
<td>30.3±2.6 (n = 20)</td>
<td>0.91</td>
</tr>
<tr>
<td>LA size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid to Lateral (mm) *</td>
<td>29.2±1.8 (n = 6)</td>
<td>27.3±3.2 (n = 20)</td>
<td>0.18</td>
</tr>
<tr>
<td>Superior to Inferior (mm) *</td>
<td>35.1±1.6 (n = 6)</td>
<td>33.5±4.0 (n = 20)</td>
<td>0.35</td>
</tr>
<tr>
<td>PV size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIPV ostium (mm) *</td>
<td>12.9±0.9 (n = 6)</td>
<td>12.2±1.2 (n = 20)</td>
<td>0.20</td>
</tr>
<tr>
<td>RIPV ostium (mm) *</td>
<td>13.7±0.9 (n = 6)</td>
<td>13.4±1.6 (n = 20)</td>
<td>0.73</td>
</tr>
</tbody>
</table>

* Mean ± SD, LA indicates left atrium; PV, pulmonary vein; LIPV, left inferior pulmonary vein; RIPV, right inferior pulmonary vein.
Table 4: Comparison of ablation data and temperature profiles between the 23 mm cryoballoon and 28 mm cryoballoon ablations.

<table>
<thead>
<tr>
<th></th>
<th>23 mm Cryoballoon (6 dogs)</th>
<th>28 mm Cryoballoon (20 dogs)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veins ablated (n)</td>
<td>12</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Targeted PV: LIPV/RIPV (n)</td>
<td>6/6</td>
<td>10/10</td>
<td>1.00</td>
</tr>
<tr>
<td>Veins ablated with 3 min/4 min ablation (n)</td>
<td>6/6</td>
<td>10/10</td>
<td>1.00</td>
</tr>
<tr>
<td>Ablations Number/Vein (n) *</td>
<td>1.2±0.4 (n = 12)</td>
<td>1.4±0.5 (n = 20)</td>
<td>0.33</td>
</tr>
<tr>
<td>Total Ablation Time/Vein (sec) *</td>
<td>240±63 (n = 12)</td>
<td>282±130 (n = 20)</td>
<td>0.38</td>
</tr>
<tr>
<td>Complete PV Occlusion (%)</td>
<td>10/14 (71)</td>
<td>8/24 (33)</td>
<td>0.01</td>
</tr>
<tr>
<td>Blood Flow Leak: small (%)</td>
<td>3/14 (21)</td>
<td>7/24 (29)</td>
<td>0.55</td>
</tr>
<tr>
<td>Blood Flow Leak: moderate (%)</td>
<td>1/14 (7)</td>
<td>9/24 (38)</td>
<td></td>
</tr>
<tr>
<td>Lowest Inner Balloon Temperature (°C) †‡</td>
<td>-51.5 [-66.0 – -31.0] (n = 14)</td>
<td>-43.0 [-64.0 – 26.0] (n = 24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lowest Balloon Surface Temperature (°C) †‡</td>
<td>-43.0 [-60.0 – -15.8] (n = 14)</td>
<td>-6.5 [-46.2 – 28.9] (n = 23)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lowest LA-PV junction Temperature (°C) †‡</td>
<td>-6.7 [-20.0 – 21.4] (n = 14)</td>
<td>15.8 [-14.4 – 35.1] (n = 24)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Distance from Balloon to LA-PV junction TCs (mm) †</td>
<td>2.3 [0.7 – 8.0] (n = 12)</td>
<td>5.1 [0.5 – 15.2] (n = 22)</td>
<td>0.10</td>
</tr>
<tr>
<td>Lowest Internal Esophagus Temperature (°C) †‡</td>
<td>31.0 [9.2 – 38.0] (n = 14)</td>
<td>34.7 [15.5 – 37.8] (n = 20)</td>
<td>0.73</td>
</tr>
<tr>
<td>Distance from Balloon to Esophageal TCs (mm) †</td>
<td>13.7 [7.5 – 21.5] (n = 14)</td>
<td>13.6 [5.1 – 22.4] (n = 20)</td>
<td>0.75</td>
</tr>
<tr>
<td>Thawing Time (sec) †</td>
<td>24 [4 – 93] (n = 14)</td>
<td>35 [9 – 72] (n = 22)</td>
<td>0.21</td>
</tr>
</tbody>
</table>

* Mean ± SD, † Median [range], ‡ Lowest tissue temperature in the all tissue temperatures during each ablation, LIPV indicates left inferior pulmonary vein; RIPV, right inferior pulmonary vein; PV, pulmonary vein; LA-PV, left atrium – pulmonary vein; TCs, thermocouples.
### Table 5: Comparison of PVI success rate, complications, and lesion characteristics between 23 mm and 28 mm cryoballoon

<table>
<thead>
<tr>
<th></th>
<th>23 mm Cryoballoon (6 dogs)</th>
<th>28 mm Cryoballoon (20 dogs)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veins ablated (LIPV and RIPV) (n)</td>
<td>12</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Acute PVI (%)</td>
<td>12/12 (100)</td>
<td>16/20 (80)</td>
<td>0.27 ‡</td>
</tr>
<tr>
<td>Chronic PVI (%)</td>
<td>9/10 (90) †</td>
<td>11/16 (69)</td>
<td>0.21</td>
</tr>
<tr>
<td>Esophageal Ulcerated Lesion</td>
<td>3/5 (60) †</td>
<td>1/20 (5)</td>
<td>0.02</td>
</tr>
<tr>
<td>PV Stenosis (moderate)</td>
<td>2/10 (20) †</td>
<td>0/20 (0)</td>
<td>0.10 ‡</td>
</tr>
<tr>
<td>Maximal Ablation Lesion Length to LA (mm) *</td>
<td>4.2 [1.5 – 11.9] (n = 10) †</td>
<td>10.0 [5.0 – 24.8] (n = 18)</td>
<td>0.001</td>
</tr>
<tr>
<td>Maximal Ablation Lesion Length to PV Inside (mm) *</td>
<td>6.8 [5.6 – 8.5] (n = 10) †</td>
<td>5.3 [0 – 17.0] (n = 18)</td>
<td>0.006</td>
</tr>
<tr>
<td>Remaining Myocardial Sleeve Length (mm) *</td>
<td>1.9 [0 – 4.0] (n = 10) †</td>
<td>4.7 [0 – 7.0] (n = 18)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Median [range], † One dog in 23 mm cryoballoon group died after first procedure because of the bleeding from the thermocouple suture site, therefore the 5 dogs (10 PVs) were done for follow-up study. ‡ Generalized estimating equation model could not be computed for this comparison. LIPV indicates left inferior pulmonary vein; RIPV, right inferior pulmonary vein; PVI, pulmonary vein isolation; LA, left atrium.
Figure Legends:

Figure 1: Time-course temperature changes in balloon and tissue during cryoablation. Blue line indicates inner balloon temperature. The 4 green lines depict balloon surface temperatures. The 3 red lines show tissue temperatures at the LA-PV junction. Red triangle denotes ablation start and end points. (A) During ablation duration of 3 min. Lowest inner balloon temperature was -45.0 °C, lowest balloon surface temperatures were -43.0 °C, -25.8 °C, -18.0 °C and -15.1 °C. Lowest tissue temperatures at LA-PV junction were -14.6 °C, -6.4 °C, and 15.2 °C. (B) During ablation duration of 4 min. Lowest inner balloon temperature was -43.0 °C, lowest balloon surface temperatures were -41.6 °C, -45.3 °C, -33.4 °C and 12.7 °C. Lowest tissue temperatures at LA-PV junction were -14.4 °C, -8.6 °C, and 11.3 °C. LA-PV indicates left atrium-pulmonary vein; Balloon Inner, inner balloon temperature; Surface, balloon surface temperatures; LA-PV jct, LA-PV junction temperature.

Figure 2: Microscopic histological changes at the LA-PV junction. Upper picture shows a representative ablation lesion of 3 min group, and lower picture shows an ablation lesion of 4 min group. Both pictures depict a transmural lesion at the LA-PV junction. LA indicates left atrium; PV, pulmonary vein.

Figure 3: (A) Representative case of the time course temperature change in internal esophagus probe (ablation duration: 3 min). Temperature at end of cryoablation (180 sec) was 25.7 °C. Temperature continued to decrease to the lowest temperature (24.4 °C) at 208 sec. A cooling delay of 28 sec after termination of cryoablation was seen. (B) Time to achieve the lowest
temperatures at the phrenic nerve (PN) and internal esophagus. In 3 min ablation, time to achieve lowest temperature was 196±11 sec at PN; 212±37 sec at internal esophagus. In 4 min ablation, time to achieve the lowest temperature was 254±12 sec at PN; 279±35 sec at internal esophagus. Cooling delay (*) was observed after cryoablation termination.

**Figure 4**: Differences in positioning at the LA-PV junction between 23 mm cryoballoon (23 mm CB) and 28 mm cryoballoon (28 mm CB) during left inferior PV ablation. Upper pictures illustrate balloon positioning by fluoroscopy. Red circle shows the LA-PV junction (PV orifice). Yellow arrows indicate the balloon-tissue contact points. (A) Balloon-tissue contact points are just at LA-PV junction in 23 mm CB. (B) In 28 mm CB, balloon-tissue contact points are more proximal (atrial side) than the LA-PV junction. Lower pictures display balloon positioning using intracardiac echocardiography (ICE). (C) ICE showed complete PV occlusion using 23 mm CB. (D) Blood flow leak (pink arrow) was seen during cryoablation in 28 mm CB. LA indicates left atrium; PV, pulmonary vein.

**Figure 5**: Two representative cases of time-course temperature changes during PVI (A) Temperature profiles using 23 mm cryoballoon (23 mm CB), and (B) 28 mm cryoballoon (28 mm CB) ablation. Lowest inner temperature and balloon surface temperature in the 23 mm CB ablation were lower than that in 28 mm CB. Tissue temperatures at the LA-PV junction were also lower when using 23 mm CB. Balloon thawing time (from final temperature to +20 °C) was longer when using 28 mm CB. LA-PV indicates left atrium-pulmonary vein; Balloon Inner, inner balloon temperature; Surface, balloon surface temperatures; LA-PV jct, LA-PV junction temperature.
Figure 6: Relationship between the lowest tissue temperature and distance from balloon surface. Lowest tissue temperature was significantly dependent on distance from the balloon. (A) For 23 mm cryoballoon with a correlation: \( r = 0.90, p < 0.0001 \), described by the equation: \( y = 17.242 \ln(x) - 14.157 \). The \( R^2 \) value for this model is 0.81. (B) For 28 mm cryoballoon with a correlation: \( r = 0.89, p < 0.0001 \), described by the equation: \( y = 15.543 \ln(x) - 8.0707 \). The \( R^2 \) value for this model is 0.79. *: \( r \) value derives from the logarithmic transformation.

Figure 7: Schematic view of lowest temperature distribution. The circle indicates the area within 5 mm from the balloon-tissue contact point. (A) Lowest temperature distribution in 23 mm cryoballoon. (B) Lowest temperature distribution in 28 mm cryoballoon.

Figure 8: Ablation lesion characteristics. (A) Measurement of the ablation lesion: a) Maximal ablation lesion length from PV orifice to LA endo surface. b) Maximal ablation lesion length from PV orifice into PV. c) Remaining myocardial sleeve length. (B) Ablation lesion in LA using 23 mm cryoballoon. (C) Ablation lesion in LA using 28 mm cryoballoon. The 28 mm cryoballoon created larger lesion in LA antrum than 23 mm cryoballoon. LA indicates left atrium; PV, pulmonary vein.
A 23 mm Cryoballoon

- Lowest Tissue Temperature (°C)
- Distance from Balloon Surface (mm)

- $R^2 = 0.81$
- $r = 0.90^*$
- $P < 0.0001$

B 28 mm Cryoballoon

- Lowest Tissue Temperature (°C)
- Distance from Balloon Surface (mm)

- $R^2 = 0.79$
- $r = 0.89^*$
- $P < 0.0001$
The Impact of Freezing Time and Balloon Size on the Thermodynamics and Isolation Efficacy during Pulmonary Vein Isolation Using the 2nd Generation Cryoballoon
Mitsuru Takami, H. Immo Lehmann, Juna Misiri, Kay D. Parker, Ray I. Sarmiento, Susan B. Johnson and Douglas L. Packer

_Circ Arrhythm Electrophysiol._ published online June 16, 2015;
_Circulation: Arrhythmia and Electrophysiology_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-3149. Online ISSN: 1941-3084

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