On the Quest for the Best Freeze: Predictors of Late Pulmonary Vein Reconnections Following Second-Generation Cryoballoon Ablation

Running title: Ciconte et al.; Late PV Reconnection after Cryoballoon Ablation

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Abstract:

**Background** - The second-generation cryoballoon (CB-Adv) is effective in achieving acute pulmonary vein isolation (PVI) and favorable clinical outcome. To date, no data is available regarding factors affecting late PV reconnection following CB-Adv ablation.

**Methods and Results** - A total of 29 consecutive patients (25 male, 86.2%; mean age 57.8±13.8 years) underwent a repeat procedure, after a mean 11.6±4.5 months (range 3.5-19.7 months), following index ablation using the 28-mm CB-Adv. All repeat ablations were performed using a 3-dimensional electroanatomical mapping system. Among all 115 PVs, including 1 LCOs, 25 (21.7%) showed a PV reconnection in 20 patients (1.25 per patient). Persistent PVI could be documented in 90/115 PVs (78.2%). In 9/29 patients (31%) all PVs were electrically isolated. In the multivariable analysis, time to PVI (p=0.03) and failure to achieve -40°C within 60 seconds (p=0.05) independently predicted late PV reconnection. At receiver operator curve analysis, time to PVI <60 seconds identified absence of PV reconnection (sensitivity 86.7%, specificity 86.2%, positive predictive value 59.1% and negative predictive value 96.4%; area under the curve 0.85, CI 0.73-0.97; p<0.001).

**Conclusions** - The rate of late PV reconnection following second-generation CB ablation is low (1.25 PVs/patient). Faster time to isolation and achievement of -40°C within 60 seconds independently predict durable PVI. In addition, 60-seconds cut off for time to PVI indicates persistent isolation with 96.4% negative predictive value. These parameters might guide the operator whether to perform further applications in order to ensure a long-lasting PVI.

**Key words:** atrial fibrillation; pulmonary vein isolation; ablation; cryoballoon ablation; second generation cryoballoon; pulmonary vein reconnection
Introduction

Pulmonary vein isolation (PVI) represents the cornerstone treatment for atrial fibrillation (AF)\(^1\). Data available in the literature indicate that second-generation Cryoballoon (CB-Adv; Arctic Front Advance, Medtronic, Minnesota, USA) is a safe and effective tool in achieving both acute PVI and favorable clinical outcome\(^2-5\). Despite encouraging results, arrhythmic recurrences after the index procedure remain relatively frequent which, in most of cases, might be related to PV reconnections, potentially reflecting the lack of efficacy in achieving transmurality and homogeneous long-lasting lesions\(^2-7\).

To date, little information is available regarding occurrence of late PV reconnection following CB-Adv ablation\(^6\). Although, recently published data indicates that roughly \(90\%\) of PVs are still isolated 3 months after CB-Adv ablation\(^7\), to the best of our knowledge, predictors of late PV reconnections following second-generation CB ablation for the treatment of AF have not been identified yet.

Methods

Aim of the study

The aim of the study was to assess predictors of late PV reconnections following PVI initially achieved using CB-Adv. Electrical reconduction rate and gap localization were also taken into consideration as an end-point.

Study population

In this study population, all index CB-Adv procedures were performed as from October 2012 (Table 1). All patients having undergone a repeat procedure for atrial tachyarrhythmia (ATa) recurrence following PVI initially achieved with CB-Adv were consecutively included in this
study and clinically followed-up according with our standard clinical practice (Table 2).

Exclusion criteria were long-standing persistent AF\textsuperscript{1}, presence of an intracavitary thrombus, uncontrolled heart failure, moderate or severe valvular disease, previous PVI procedure, left atrium (LA) diameter $\geq$ 55 mm and contraindications to general anesthesia. The study was approved by the institutional ethics committee on human research of our Institution.

**Pre-procedural management**

All patients provided written informed consent to the procedure. Structural heart disease was defined as: Coronary Artery Disease (CAD), impaired left ventricular ejection fraction (LVEF) $<40\%$, LV hypertrophy $>15$ mm, valvular insufficiency $>2/4$, significant valvular stenosis and prior valve replacements. All antiarrhythmic drugs were discontinued at least 3 days before ablation, except amiodarone, which was interrupted 1 month before. For patients under novel anticoagulant agents our practice is to stop anti-coagulation as follows: a) the last dose of dabigatran was given the morning 1 day prior to the procedure; b) and the last dose of rivaroxaban was given the evening 2 days prior. For warfarin uninterrupted administration is performed\textsuperscript{5}. No patient was receiving apixaban. A transthoracic echocardiogram (TTE) was performed within 1 week prior to ablation. To exclude the presence of intracavitary thrombi, all patients underwent trans-esophageal echocardiography (TEE) the day before the procedure. All patients underwent a pre-procedural CT-scan to assess detailed LA and PV anatomy.

**Cryoballoon ablation as index procedure**

Our standard ablation procedure has been previously reported in detail\textsuperscript{3,5}. All CB-Adv ablation were performed under general anesthesia. Briefly, after obtaining LA access, through a steerable 15 Fr sheath (FlexCath Advance\textsuperscript{®}, Medtronic\textsuperscript{©}), inner lumen mapping catheter (MC; Achieve\textsuperscript{®}, Medtronic\textsuperscript{©}) was advanced in each PV ostium. A 28 mm CB-Adv (Arctic Front Advance\textsuperscript{TM},
Medtronic©) was advanced inflated and positioned in each PV ostium. Optimal vessel occlusion was considered as achieved when selective contrast injection showed total contrast retention with no backflow to the atrium. Once occlusion was documented, cryothermal energy was started. Cryo-energy applications lasted at least 180 seconds. PV activity was recorded with the MC at a proximal site in the ostium prior to ablation in each vein. During ablation, if PV potentials (PVPs) were visible during energy delivery, time to isolation was recorded when PVPs completely disappeared or were dissociated from LA activity. Durable PVI was assessed at least 20 minutes after cryoenergy application. In order to avoid phrenic nerve palsy (PNP) a decapolar catheter was inserted in the superior vena cava and diaphragmatic stimulation was achieved by pacing the ipsilateral phrenic nerve with a 1200 ms cycle and a 20 mA output. During the whole procedure, activated clotting time was maintained over 250 seconds by supplementing heparin infusion as required. Total procedure duration was considered as from having obtained femoral venous access to catheter removal.

**Repeat ablation procedure**

A repeat ablation procedure using a RF irrigated-tip CF catheter was offered to all patients experiencing atrial tachyarrhythmia (ATa) recurrences, except to those who became responsive to previously ineffective drugs or with short-lasting arrhythmic episodes. All patients agreed to undergo a repeat ablation. Briefly, after double transseptal puncture, 3D LA reconstruction geometry was performed with CARTO3 (Biosense-Webster). The circular mapping catheter was positioned in each proximal PV ostium to assess potential reconnection and to facilitate localization of conduction gaps. Late PV reconnection was defined as a LA-PV electrical reconnection observed at the time of the repeat procedure. The location of gaps along previously deployed circumferential lesions was defined as the site of late PV reconnection in four different
anatomical regions of the PV antrum: antero-superior (AS), antero-inferior (AI), postero-inferior (PI), postero-superior (PS). Once localized, irrigated RF applications were applied on the conduction gaps until documented isolation, which was also evaluated 30 min after. Contact-force data were continuously monitored throughout the entire procedure, with the aim to achieve at least 10 grams (mean) with a vector perpendicular to the tissue and with an upper limit of 50 grams. Patients experiencing PV reconnection underwent re-PVI. In case of atrial tachycardia (AT), the 3D electroanatomical mapping system allowed to identify the underlying mechanism. After AT termination, if necessary, re-isolation of the PVs was also performed in sinus rhythm.

**Follow-up**

After discharge, patients were scheduled for follow-up visits with baseline ECG and 24h Holter recordings at 1, 3, 6 and 12 months. Any symptoms following ablation were deemed as deserving a Holter monitoring. All reports of Holter or ECG recordings having been performed in referring centers were sent to our centre for confirmation of the diagnosis of ATa recurrence. Furthermore telephone calls were performed during the follow-up. All documented ATa episodes >30 s after the index procedure, with standard ECG or 24h ECG Holter monitoring and during both planned and symptom driven consultation, were considered as a recurrence.

**Statistical analysis**

Categorical variables are expressed as absolute and relative frequencies, while continuous variables are expressed as mean ± SD. Comparisons of continuous variables were done with a Student t test and binomial variables with chi-square or Fisher test as appropriate. Factors predicting PV reconnections were identified by univariate and multivariable analyses using the Cox proportional hazards regression model. To avoid over-fitting of the model, the convention of limiting the number of independent variables entered was followed. In our analysis, independent
variables for entry into the model were selected according to their weight on univariate testing (p
values and shorter 95% confidence intervals); consequently, 2 variables were eligible for this
analysis: time to PVI and achievement of -40°C within 60 seconds (no/yes = 0/1). Receiver-
operator characteristic (ROC) curve was constructed to evaluate the performance of time to PVI
in predicting arrhythmia recurrence. Procedural parameters were analyzed using generalized
estimated equations methods (logit models, Table 3) or robust sandwich covariance estimators
(Cox models, Table 4) to account for multiple observations per patient (i.e., 4 PVs). A 2-tailed
probability value of <0.05 was deemed significant. Statistical analyses were conducted using the
SPSS software (SPSS v22, Chicago, IL, USA).

Results
Study population
Among a total of 212 patients having undergone CB-Adv ablation from October 2012 to
December 2013, 171 did not show arrhythmic episodes during the follow-up, whereas 41
continued to experience arrhythmic recurrences (Table 1). Nadir and mean temperature at 60
seconds during cryoenergy application were significantly higher in those experiencing
arrhythmic recurrences as compared to those who did not (Table 1). In addition, time to isolation
was significantly shorter in patients who did not experience ATa recurrences (Table 1). No other
differences could be observed between the groups (Table 1). A repeat ablation procedure was
only offered to patients experiencing ATa recurrences, except to those responsive to previously
ineffective drugs or with short-lasting arrhythmic episodes. Among 41 patients having
arrhythmia recurrences, 29 (25 male, 86.2%; mean age 57.8±13.8 years) underwent a repeat
ablation, after a mean 11.6±4.5 months (range 3.5-19.7 months) following index PVI procedure
carried out with CB-Adv technology for paroxysmal and early persistent AF (PAF and earlyPersAF). Table 2 shows baseline clinical characteristics of the study population. At the pre-procedural CT scan, a discrete left common ostium (LCOs) could be observed just in one patient, whilst a distinct 4-PV pattern was present in the remaining 28 patients (96.5%).

**Index ablation procedural characteristics**

Among all 115 identified PVs, including LCOs, acute isolation was achieved in 105 veins (91.3%) during the first freeze. PV potentials during cryo-applications could be observed in 81 PVs (70.4%): 21/28 LSPVs (75%), 19/28 LIPVs (67.8%), 22/29 RSPVs (75.8%) and 19/29 RIPVs (65.5%). After a single application, PVI was achieved in 27/28 LSPVs (96.4%), 26/28 LIPVs (92.8%), RIPVs 25/29 (86.2%), 27/29 RIPVs (93.1%). Additional cryoballoon applications were necessary to achieve PVI in the remaining cases. At the end of the procedure all veins were isolated with 1.4±0.6 mean number of freeze-thaw cycles. All cryo- ablations were performed with the large 28-mm balloon allowing successful isolation in all veins without the need of additional focal catheter applications.

**Late PV reconnection following second-generation cryoballoon ablation**

Among all 115 PVs, including 1 LCOs, 25 (21.7%) showed a late PV reconnection in 20 patients (1.25 per patient), at the time of repeat ablation procedure (Figure 1). Overall, persistent PVI could be documented in 90/115 PVs (78.2%) (Figure 2). In 9/29 patients (31%) persistent isolation could be demonstrated in all PVs, whilst PV reconnection could be documented in 20/29 patients (69%). In addition none of the patients exhibited reconnection in all 4 veins.

According to the PV location, different rates of persistent isolation could be documented: 26 LSPVs (92.9%), 22 LIPVs (78.6%), 21 RSPV (72.4%) and 21 RIPVs (72.4%). In the patient with LCOs, a conduction gap could be observed in the inferior aspect of the vein (Figure 3, blue
star). Among all 25 reconnecting veins, 16 (64%) were in right-sided PVs, while 9 (36%) were left-sided PVs (p=0.17). Only 2 out of 25 (8%) reconnections were located in the LSPV, therefore representing the less frequent site of conduction gap. Out of 25 PV reconnections, a single freeze was applied at the time of index procedure in 14/25 PVs (56%), whereas the remaining 11/25 veins (44%) received a bonus freeze (p=0.23). Of note, only 1 patient experienced a transient PNP during RSPV treatment, determining a premature freeze termination (total application time was 120 seconds). At the time of repeat procedure, both right-sided PVs were persistently isolated. Spatial distribution of late conduction gaps per PV-region is shown in Figure 3.

**Predictors of late PV reconnections**

Late PV reconnection occurrence was associated with warmer nadir temperature (-48.7±4.7 vs -51.5±4.7 °C; p=0.009), longer time-to-isolation (71.4±18.8 vs 42.3±27.2 seconds; p<0.001) and failure to achieve -40°C within the first 60 seconds of application (20/25, 80% vs 22/90, 24.4%; p<0.001) during the index procedure (Table 3). However, no difference could be observed in mean rewarming time among veins showing or not PV reconnections (p=0.08). No statistically significant difference could be also observed in the other procedural aspects such as mean number of application, total duration of application (single vs bonus freeze), real-time PVI recording and degree of occlusion (Table 3). In the univariate analysis, time to PVI, -40°C achievement within 60 seconds and nadir temperature were significantly associated with LA-PV reconduction (Table 4). Multivariable analysis confirmed time to PVI and failure to achieve -40°C within 60 seconds as independent predictors of late PV reconnections (Table 4). ROC curve was constructed from time to PVI (Figure 4). Predictive performance increased with faster time to isolation showing an area under the curve (AUC) of 0.83 (CI 0.734-0.937, p<0.01). A
time to PVI <60 seconds presented 86.7% sensitivity and 86.2% specificity, with a 59.1% of positive predictive value (PPV) and 96.4% negative predictive value (NPV).

**Repeat procedure electrophysiological findings**

A total of 29 patients underwent a redo procedure, which was performed with a RF irrigated-tip catheter guided by CF monitoring using a 3D electroanatomical mapping system. The repeat procedure was performed because of AF recurrence in 25/29 (86.2%), while a regular AT was documented in 4/29 (13.8%) (Figure 5). Atrial tachycardias presented as roof-dependent left flutter in 1 patient, mitral-isthmus-dependent flutter in another 1 and 2/5 showed PV reconnections in the LIPV and RIPV respectively. Re-PVIIs and substrate ablations were successfully performed in all patients. A mean number of 3.0±1.6 RF applications was needed to achieve PV re-isolation. Cryolesions were located at the PV antrum and point-by-point RF applications focused at the gaps without the need to ablate the whole quadrant. Among 7 patients receiving additional RF applications (Figure 5), 4 were treated with a cavo-tricuspid line, 2 with a roof line and 1 with a mitral isthmus line. None of patients died or experienced cerebrovascular events in the peri-procedural period. After a mean follow up of 10.5±6.1 months, 5/29 (17.2%) continued to experience symptomatic AF episodes. None of those presenting with AT experienced further arrhythmic recurrences.

**Discussion**

To the best of our knowledge this is the first study reporting predictors of late PV reconnection following second-generation CB ablation. The main findings of our study are: 1) PV reconnection rate is 22% (1.25 PVs/patient); 2) time to isolation and -40°C achievement within 60 seconds are independent predictors of PV reconnections; 3) 60-seconds cut-off for time to
PVI indicates persistent isolation with 96.4% NPV.

**Recovered PV conduction following PVI**

Although recent developments in catheters design aiming at ablation lesion improvement, the incidence of clinical recurrences following PVI is still not negligible. Recovery of conduction has proven to be the cause of the vast majority of ATa recurrences following PVI and conduction gaps ablation represents the cornerstone in the management of patients undergoing a repeat procedure. In our study, electrical conduction gaps could be identified in 68.9% of patients in at least 1 PV. In addition, the rate of persistent PVI is considerably high as observed in our series (78.3%), and this finding is in line with recently published data, reporting 77-91% rate of durable PVI roughly 6 and 3 months after initial CB-Adv ablation, respectively. The low incidence of late reconnections following CB ablation, as observed in our study, might be due to the novel catheter design, which results in a larger and more uniform zone of freezing on the balloon surface. This technology might offer better catheter stability in particular LA structures, such as the appendage-LSPV ridge, if compared with a focal RF catheter. In fact when using cryoenergy, the balloon adheres to the atrial wall when reaching lower temperatures, thus resulting in a better catheter–tissue contact and leading to more transmural lesions in such critical anatomical regions. The latter may also explain the lower reconnection rate in LSPV following CB ablation as compared to the point-by-point RF approach.

**Predictors of late PV reconnections**

Cryoballoon temperature is automatically monitored overtime during freezing. It gives reliable information about balloon-tissue contact explaining the association of low temperatures with ablation efficiency. As demonstrated in our series, a late PV reconnection was more frequent in those veins with a warmer nadir temperature and a delayed time to isolation. Interestingly,
these findings are in agreement with previous studies, showing that a minimal temperature ≤51°C predicted successful isolation and no acute resumption of PV conduction\textsuperscript{13,15}. However, the multivariable analysis showed that time to PVI and failure to achieve -40°C within the first minute of cryoenergy application are independent predictors of late PV reconnections. In addition, a faster time to PVI is usually achieved with the CB-Adv as compared to its predecessor\textsuperscript{3}. As observed in our series, a longer time to isolation was associated with an increased incidence of late reconnection. Conversely, a shorter time to isolation and a lower nadir temperature seem to be more frequently observed in patients who do not experience arrhythmic recurrences after ablation, as occurred in our cohort. This appears to be in agreement with previously published observations describing that a longer time to effect is associated with acute PV reconnection and clinical arrhythmic recurrences\textsuperscript{5,15}. Of note, according to our data, if isolation is achieved within 60 seconds, a late PV reconnection might be ruled out with a 96.4% NPV. For the first time, this finding shows a direct link between fast isolation times (i.e. less than 1 minute) and durable PV isolation. Moreover, as demonstrated in the current study for the first time, the achievement of -40°C within 60 seconds significantly predicted long term persistency of PVI. This new parameter might be a reliable indicator of the correlation between the slope of the freezing curve and the durability of isolation. The abovementioned cut-off was selected for various reasons. First, lesion formation with cryo-energy is based around the generation of hypothermia at the catheter-tissue interface. Progressive cooling below -40°C results in the formation of intracellular ice crystals, which is the first step in ensuring adherence of the catheter to the tissue during the cryo-lesion\textsuperscript{16}. In addition, full-flow cryo-refrigerant is usually achieved within 1 minute, and at that time the slope of the curve starts to plateau. Therefore, during hypothermia the catheter adheres to the tissue affording greater stability,
therefore eliminating the “brushing effect” that occurs during beat-to-beat rocking heart motions and with respiratory variations\textsuperscript{17}.

Limitations

The study was a non-prospective, non-randomized, single-centre trial conducted in a relatively limited number of patients. Future larger studies with longer follow-up are needed to confirm these data. A further limitation of this study consists in the fact that only patients with documented ATa recurrences after the index procedures have been included. Therefore, the rate of late PV recovery in our patient cohort might represent an overestimation of the real incidence of late PV reconnections in individuals having undergone second generation CB ablation. The parameter ‘failure to achieve -40° within 60 seconds’ has been derived from a retrospective analysis of the data. For this reason, a prospective study is necessary to confirm its usefulness. Nor systematical esophageal probe neither esophagastroduodenoscopy were used, thus the incidence of esophageal lesions following ablation might have been underestimated\textsuperscript{18}. Finally, diaphragmatic electromyography (CMAP) is valuable in predicting and potentially preventing PNP during cryoballoon ablation\textsuperscript{19}. However, CMAP has not been performed in this study. Some observations might be relevant for further statistical analysis. Despite independent variables for entry into the multivariable model have been selected according to their weight on univariate testing to avoid potential over fitting, we cannot exclude that nadir temperature might be an independent predictor as well. Further prospective and larger studies are needed to assess this issue.

Conclusions

The rate of late PV reconnection following second-generation CB ablation is low (1.25 PVs/patient). Faster time to isolation and achievement of -40°C within 60 seconds independently
predicted durable PVI. In addition, 60-seconds cut-off for time to PVI indicates persistent
isolation with 96.4% NPV. These parameters may not only be a marker of an effective ablation,
but they can also guide the operator whether to perform further applications in order to ensure a
long-lasting PVI.

Conflict of Interest Disclosures: CdA and GBC receive compensation for teaching and
proctoring purposes from AF solution Medronic. PB, CdA and GBC receive research grants on
behalf of the centre from Biotronik, Medtronic, St Jude Medical, Sorin, Boston Scientific and
speakers fees from Biosense-Webster, Biotronik, Medtronic and Boston Scientific.

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   Single 3-minute freeze for second-generation cryoballoon ablation: One-year follow-up after


Table 1: Clinical characteristics of the entire cohort having undergone Cryoballoon ablation

<table>
<thead>
<tr>
<th></th>
<th>Overall (n = 212)</th>
<th>No Arrhythmia Recurrences (n = 171)</th>
<th>Arrhythmia Recurrences (n = 41)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>60.5±9.5</td>
<td>61.3±12.7</td>
<td>59.7±10.2</td>
<td>0.45</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>169 (80)</td>
<td>139 (80)</td>
<td>32 (78)</td>
<td>0.66</td>
</tr>
<tr>
<td>LA dimensions, mm</td>
<td>45.9±4.2</td>
<td>46.7±5.1</td>
<td>45.3±7.8</td>
<td>0.16</td>
</tr>
<tr>
<td>CHA2DS2-Vasc score, n</td>
<td>1.3±1.2</td>
<td>1.2±0.5</td>
<td>1.3±1.7</td>
<td>0.51</td>
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<tr>
<td>Paroxysmal AF, n (%)</td>
<td>175 (83)</td>
<td>142 (83)</td>
<td>33 (81)</td>
<td>0.65</td>
</tr>
<tr>
<td>Time to PVI, seconds</td>
<td>52.3±15.1</td>
<td>40.2±18.7</td>
<td>65.7±25.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nadir Temperature, °C</td>
<td>-50.6±7.5</td>
<td>-53.5±5.8</td>
<td>-49.3±9.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean Temperature at 60 seconds, °C</td>
<td>-43.7±10.5</td>
<td>-46.3±5.2</td>
<td>-39.5±6.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean total number of freezes, n</td>
<td>1.5±0.9</td>
<td>1.3±0.5</td>
<td>1.4±0.8</td>
<td>0.31</td>
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<tr>
<td>Mean LSPV freeze duration, seconds</td>
<td>280±144</td>
<td>279±130</td>
<td>299±115</td>
<td>0.36</td>
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<tr>
<td>Mean LIPV freeze duration, seconds</td>
<td>303±141</td>
<td>286±139</td>
<td>311±141</td>
<td>0.29</td>
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<tr>
<td>Mean RIPV freeze duration, seconds</td>
<td>292±146</td>
<td>289±143</td>
<td>306±145</td>
<td>0.49</td>
</tr>
<tr>
<td>Mean RSPV freeze duration, seconds</td>
<td>269±122</td>
<td>255±126</td>
<td>284±130</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Data are expressed in mean ± standard deviation or number and percentage as appropriate.
AF: atrial fibrillation, LA: left atrium, LSPV: left superior pulmonary vein, LIPV: left inferior pulmonary vein, PVI: pulmonary vein isolation, RSPV: right superior pulmonary vein, RIPV: right inferior pulmonary vein
Table 2: Clinical characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Overall (n = 29)</th>
<th>Persistent Isolation (n = 9)</th>
<th>Late PV reconnection (n = 20)</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>57.8±13.8</td>
<td>61.6±8.8</td>
<td>56.7±15.4</td>
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<td>Male, n (%)</td>
<td>25 (86)</td>
<td>8 (89)</td>
<td>17 (85)</td>
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<tr>
<td>BMI</td>
<td>27.3±4.1</td>
<td>26.6±4.2</td>
<td>27.9±3.8</td>
<td>0.42</td>
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<tr>
<td>Hypertension, n (%)</td>
<td>10 (3)</td>
<td>3 (33)</td>
<td>7 (35)</td>
<td>1.00</td>
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<tr>
<td>Dyslipidemia, n (%)</td>
<td>9 (31)</td>
<td>2 (22)</td>
<td>7 (35)</td>
<td>0.67</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>5 (17)</td>
<td>3 (33)</td>
<td>2 (10)</td>
<td>0.28</td>
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<tr>
<td>CAD, n (%)</td>
<td>2 (7)</td>
<td>1 (11)</td>
<td>1 (5)</td>
<td>0.53</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>57.7±11.3</td>
<td>59.2±16.4</td>
<td>55.7±9.1</td>
<td>0.46</td>
</tr>
<tr>
<td>LA size, mm</td>
<td>44.7±8.6</td>
<td>46.6±6.5</td>
<td>42.8±8.6</td>
<td>0.26</td>
</tr>
<tr>
<td>CHA2DS2-Vasc score, n</td>
<td>1.2±1.1</td>
<td>1.0±1.3</td>
<td>1.3±0.7</td>
<td>0.60</td>
</tr>
<tr>
<td>Paroxysmal AF, n (%)</td>
<td>20 (69)</td>
<td>6 (67)</td>
<td>15 (75)</td>
<td>0.67</td>
</tr>
<tr>
<td>Procedure duration, minutes*</td>
<td>88.7±23.4</td>
<td>86.8±14.4</td>
<td>89.3±26.3</td>
<td>0.75</td>
</tr>
<tr>
<td>Fluoroscopy duration, minutes*</td>
<td>14.3±8.2</td>
<td>13.3±9.4</td>
<td>15.8±8.1</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Data are expressed in mean ± standard deviation or number and percentage as appropriate.
*Procedure and Fluoroscopy duration refer to the index procedure.
AF: atrial fibrillation, BMI: body mass index, CAD: coronary artery disease, LA: left atrium, LVEF: left ventricular ejection fraction.
Table 3: Index procedure characteristics according to PV isolation persistency

<table>
<thead>
<tr>
<th></th>
<th>Persistent PVI</th>
<th>Late PVR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CB-AdvA (29 pts; 115 PVs)</strong></td>
<td>(n=90)</td>
<td>(n=25)</td>
<td></td>
</tr>
<tr>
<td>Mean number of applications, n</td>
<td>1.3±0.6</td>
<td>1.5±0.5</td>
<td>0.13</td>
</tr>
<tr>
<td>Single freeze, n</td>
<td>64 (71.1)</td>
<td>14 (56)</td>
<td>0.22</td>
</tr>
<tr>
<td>Nadir temperature, °C</td>
<td>-51.5±4.7</td>
<td>-48.7±4.7</td>
<td>0.03</td>
</tr>
<tr>
<td>Time to PVI, seconds</td>
<td>42.3±27.2</td>
<td>71.4±18.8</td>
<td>0.05</td>
</tr>
<tr>
<td>-40°C in 60 seconds, n</td>
<td>68 (75.5)</td>
<td>5 (20)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean occlusion grade, n</td>
<td>3.8±0.4</td>
<td>3.7±0.8</td>
<td>0.39</td>
</tr>
<tr>
<td>Real-Time PVI, n (%)</td>
<td>65 (72.2)</td>
<td>16 (64)</td>
<td>0.48</td>
</tr>
<tr>
<td>Rewarming time, seconds</td>
<td>30.6±3.8</td>
<td>28.9±4.8</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Data are expressed in mean ± standard deviation or absolute number (percentage). PVI: pulmonary vein isolation, PVR: pulmonary vein reconnection, CB-AdvA: Second-Generation Cryoballoon Ablation.
**Table 4:** Univariate and Multivariable Cox regression analysis indicating factors predicting late PV reconnection

<table>
<thead>
<tr>
<th>Variables</th>
<th>β Coefficient</th>
<th>Hazard Ratio (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to PVI</td>
<td>0.03</td>
<td>1.03 (1.02-1.05)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>-40°C in 60 seconds</td>
<td>-1.79</td>
<td>0.17 (0.05-0.53)</td>
<td>0.003</td>
</tr>
<tr>
<td>Nadir Temperature</td>
<td>0.14</td>
<td>1.16 (0.96-1.39)</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>Multivariable analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to PVI</td>
<td>0.03</td>
<td>1.03 (1.01-1.04)</td>
<td>0.01</td>
</tr>
<tr>
<td>-40°C in 60 seconds</td>
<td>-1.13</td>
<td>0.32 (0.15-0.94)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

The hazard ratio for Time to PVI considers every second increase.
The hazard ratio for Nadir Temperature considers every centigrade decrease.
PVI: pulmonary vein isolation
Figure Legends:

**Figure 1:** Carto3 voltage map showing cryoballoon lesion demarcation on the posterior LA wall and complete PVI at the time of redo procedure (PA-view). The scale was set from 0.15 to 1.2 mV: purple color identifies LA areas with a voltage >1.2 mV, whereas red color < 0.2 mV.

**Figure 2:** Carto3 AP-view voltage map showing RSPV reconnection (left panel; before ablation) and re-isolation (right panel; after ablation) at the time of redo procedure. In the right panel, the red dots represent ablation points needed to achieve re-isolation.

**Figure 3:** Distribution of reconducting gaps (red stars) following CB-Adv ablation. The blue star indicates the reconnection occurring at the inferior aspect of the left common ostium.

**Figure 4:** Receiver-operating characteristic (ROC) curve analyzing sensitivity and specificity of time to PVI in predicting late PV reconnections following second-generation cryoballoon ablation. This analysis accounts for multiple observations per patient.

**Figure 5:** Flow chart demonstrating arrhythmia outcome of patients. CFCA indicates contact-force guided catheter ablation.
Area under curve = 0.8356  se(area) = 0.0516
Study Population
CB-Adv Ablation
n=29

AF
n=25

AT
n=4

Redo with CFCA

Re-PVI
n=18

Additional RF
n=7

Re-PVI
n=2

Additional RF
n=2
On the Quest for the Best Freeze: Predictors of Late Pulmonary Vein Reconnections Following Second-Generation Cryoballoon Ablation

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