Left atrial appendage isolation in addition to pulmonary vein isolation in persistent atrial fibrillation: one-year clinical outcome after cryoballoon-based ablation

Hikmet Yorgun, Uğur Canpolat*, Duygu Kocyigit, Cem Çöteli, Banu Evranos, and Kudret Aytemir

Faculty of Medicine, Department of Cardiology, Hacettepe University, Sıhhiye, 06100 Ankara, Turkey

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Aims
In this study, we sought to evaluate the safety and efficacy of cryoballoon (CB) based empirical left atrial appendage (LAA) isolation as an adjunct to pulmonary vein isolation (PVI) compared to the PVI-only strategy in patients with persistent AF.

Objectives
Clinical outcomes of catheter ablation were less beneficial for persistent atrial fibrillation (AF) than paroxysmal AF.

Methods and results
A total of 100 consecutive patients with persistent AF underwent both PVI and additional LAA isolation using CB (Group II). As a control group (Group I), among persistent AF patients, we conducted a retrospective, propensity-score matched cohort, in whom only PVI was performed using CB. Recurrence of atrial tachyarrhythmia (Ata) at the 12th month follow-up was the primary endpoint. Baseline demographic and clinical characteristics were similar between two groups. At the 12th month follow-up, 67 (67%) patients in Group I and 86 (86%) patients in Group II were free of ATa after the index procedure ($P < 0.001$). As a unique complication of LAA isolation, left circumflex artery spasm was observed in 4% of the Group II. After adjusting for several baseline variables, PVI-only strategy was found as a significant predictor for recurrence (HR: 3.37; 95% CI: 1.73–6.56; $P < 0.001$). Transoesophageal echocardiography examination during the follow-up revealed no thrombus in the LAA.

Conclusion
Our findings indicated that LAA isolation as an adjunct to PVI improved 1-year outcomes in persistent AF compared with the PVI-only strategy using CB without an increase in thromboembolic complications.

Keywords
Atrial fibrillation • Cryoballoon • Left atrial appendage isolation

Introduction
Catheter ablation is an established therapeutic option especially in symptomatic patients with drug refractory atrial fibrillation (AF). Pulmonary vein isolation (PVI) is still the cornerstone of ablation technique in AF, which aims the elimination of pulmonary vein triggers. Although the clinical evidence is robust and clear for PVI in paroxysmal AF, the success of PVI-only strategy is limited in persistent AF, mainly due to the role of atrial substrate in the maintenance of AF. To improve the clinical outcomes, additional ablation strategies including linear lesions or complex fractionated atrial electrogram ablation have been implemented; however, the benefit of such approach in persistent AF is inconsistent between studies. Currently, cryoenergy and radiofrequency (RF) energy are the two most common types of ablative methods in the catheter-based treatment of AF. While point-by-point ablation by focal RF energy

* Corresponding author. Tel: +90 312 305 17 80; fax: +90 312 305 41 37. E-mail address: dru_canpolat@yahoo.com

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What’s new?

• Single-shot cryoballoon (CB) technique most commonly used for pulmonary vein isolation (PVI) during AF ablation.
• To the best of our knowledge, this is the first observational study in the literature evaluating the feasibility, efficacy and safety of CB for empirical electrical LAA isolation as an adjunct to PVI.
• Our study findings indicated that LAA isolation with CB besides PVI improved 1-year freedom from AFA compared with the PVI-only strategy in persistent AF without any adverse thromboembolic events.

Methods

Study population

In this observational study, we enrolled patients with persistent AF despite ≥1 antiarrhythmic drug(s) (AAD) who were scheduled for catheter ablation using either second- or third-generation CB in a high-volume referral centre (>150 AF ablations/year since 2010). The study was designed to compare the effectiveness of two different ablation strategies: standard PVI-only (Group I) vs. standard PVI plus empirical LAA-isolation (Group II) using CB technique. Between January and November 2015, we performed LAA isolation in addition to PVI in a consecutive subset of persistent AF patients using CB (Group II). As a control group (Group I), we conducted a retrospective, propensity-score matched cohort analysis in whom only CB based PVI was performed. The definition of persistent AF followed the latest updated AF guidelines.1

Ablation strategy and procedure using CB

Our routine ablation approach to the patients with persistent AF was summarized in Figure 1. In all cases, we prefer to begin in sinus rhythm during ablation; therefore, pharmacologically facilitated electrical cardioversion (ECV) was performed in patients with AF before the procedure. Patients having resistance to cardioversion underwent to the ablation procedure in AF rhythm. If sinus rhythm was not achieved during the procedure, patients underwent ECV at the end of the ablation to restore sinus rhythm.

Pulmonary vein isolation

All procedures were performed under conscious sedation using boluses of midazolam and fentanyl. Invasive arterial blood pressure, oxygen saturation and ECG were continuously monitored throughout the entire procedure. Right femoral vein and left femoral vein/artery punctures were performed with Seldinger technique. A 6 Fr steerable decapolar catheter (Medtronic) was inserted into the LA. Once LA access is obtained, unfractionated heparin boluses were repeatedly administered to maintain the activated clotting time (ACT) of 300–350 s. The sheath was then exchanged with a steerable transseptal sheath (FlexCathMedtronic CryoCath, Minneapolis, MN, USA) over a guidewire (0.032 in., 180 cm Super Stiff, St. Jude Medical, St. Paul, MN, USA). In all patients, either the second- or third-generation 28-mm CB catheter (Arctic Front AdvanceTM and Arctic Front AdvanceTM ST, Medtronic, Minneapolis, MN, USA) was used for PVI. The CB was maneuvered to all PV ostia by using steerable sheath and the AchieveTM (Medtronic, Minneapolis, MN, USA) circular mapping catheter was inserted through the lumen of the balloon catheter. The balloon is inflated in the LA and then directed toward the PV ostia. The assessment of CB occlusion is performed during the injection of 50% diluted contrast through the CB catheter’s central lumen. Ablation procedure was started after the demonstration of optimal vessel occlusion which was defined as the retention of contrast media in the
PVs without backflow into the LA. Baseline potentials of PVs were recorded by using the circular mapping catheter which has been positioned at the PV ostium. The duration of each freezing cycle was 240 s for each targeted PV. After one application, an additional bonus freeze of 240 s duration was applied in case of disappearance of the PV potentials >60 s during the first cycle. The right phrenic nerve was constantly paced from the superior vena cava during freezing at the right sided PVs with a 2000-ms cycle and a 12-mA output in order to detect phrenic nerve palsy (PNP). Intermittent fluoroscopy and direct palpation of the right hemidiaphragmatic excursion was performed during phrenic nerve stimulation. At the end of the procedure, PV conduction was reevaluated by the circular mapping catheter.

Successful PVI was defined as the elimination (or dissociation) of all the PV potentials recorded by the inner lumen circular mapping catheter. Minimum cooling temperature was recorded in all patients, whereas time to PV signal isolation was recorded in case of visible PV signals during the procedure. If a real-time recording of PV potentials was not achieved because of distal positioning of the mapping catheter, mapping catheter was immediately retracted after each completed freeze to re-evaluate the PV activity. Electrical PV isolation was confirmed by entrance and exit block maneuvers by coronary sinus electrode and circular mapping catheter stimulation, respectively.

**Electrical isolation of LAA**

After isolation of all PV potentials, the fluoroscopic position was set at right oblique anterior 30° and cranial 20° angiographic projection and the circular mapping catheter was advanced into the LAA under fluoroscopic guidance. After the inscription of LAA signals, CB catheter was inflated in the LA and positioned at the LAA ostium (Supplementary material online, Video S1). Ablation was begun after optimal LAA occlusion which was defined as the retention of contrast media in the LAA without backflow into the LA (Figure 2A). The duration of CB freeze was 450 s in the first 20 patients and was limited to 300 s in the following cases. If LAA isolation was not achieved in 150 s, a bonus freeze of 300 s was also applied. The left phrenic nerve was constantly paced from the LAA using circular...
mapping catheter throughout the freezing cycle with 2000 ms cycle length and a 12-mA output (Supplementary material online, Video S2). Phrenic nerve capture was assessed by intermittent fluoroscopy and tactile feedback obtained from the patient’s abdomen. Left coronary angiography was performed simultaneously or after the LAA isolation to exclude the left circumflex (Cx) artery vasospasm in all subjects who underwent LAA isolation. All the sheaths were removed at the end of the procedure. Figure-of-eight suture technique was used for 15 Fr venous sheath as described before.19

Successful electrical isolation of LAA was defined as (i) disappearance of the LAA potentials recorded from the circular mapping catheter (entrance block) and (ii) dissociated electrical activity from LA while pacing from LAA using circular mapping catheter (exit block) (Figure 2B and C). Time to isolation, temperature at isolation, nadir temperature, and total freezing time were recorded for all LAA isolation procedures. For the detection of early reconnection, a waiting period of minimum 15 min was considered and re-ablation with CB was performed in case of early reconnection.

**Post-procedural management and follow-up**

TTE was performed immediately after the ablation in order to evaluate possible complications like pericardial effusion. Oral anticoagulation either with warfarin or novel oral anticoagulants was initiated 6 h after the procedure. Routine follow-up visits were performed at 3, 6, and 12 months and every 6 months thereafter or earlier if patients had symptoms consistent with recurrent ATa or procedure-related complications. Physical examination was done at each follow-up visit with the evaluation of arrhythmia-related complaints as well as 12-lead ECG and TTE. A 24-h Holter ECG was recorded at the third month after the procedure, usually on AADs. In the absence of arrhythmia, all AADs were discontinued. Additional 24-h Holter ECG was scheduled at the sixth month and every 6 months thereafter or earlier in case of arrhythmic symptoms. In patients with LAA isolation, TEE was performed at post-ablation 3rd and 12th month visits to assess LAA flow velocity, degree of smoke, or thrombus formation.

Patients remained on the AAD regimen that was prescribed before the ablation in the first 3 months after ablation. Thereafter, the decision for continuation of AADs was given according to the physician’s decision depending on the recurrence of atrial arrhythmias during the follow-up. The need for life-long oral anticoagulation was assessed based on the TEE findings and CHA2DS2VASC score at the third month visit. Patients with CHA2DS2VASC score of ≥2 strictly recommended to use life-long oral anticoagulation. In the LAA isolation group, patients with poor LAA flow velocity (<0.4 m/s) and severe smoke (Grades III and IV) at the third month follow-up maintained on long-term oral anticoagulation.

**Study endpoints**

Acute procedural success was defined as electrical isolation of all PVs (in both groups) and LAA (in Group II). A blanking period of first 3 months after the AF ablation was defined for the study. ATa recurrence was defined as detection of AF, atrial flutter, or atrial tachycardia (>30 s) assessed with ECG. Any recurrence within the first 3 months of ablation was defined as early recurrence, whereas recurrence >3 months following AF ablation was defined as late recurrence. Freedom from ATa recurrence at the 12th month follow-up was the primary endpoint of the study. Safety measures such as complications during index hospitalization, bleeding events, transient ischaemic attack, stroke, and death were also recorded throughout the study period.

**Statistical analysis**

To estimate the propensity score, we used logistic regression including the following co-variates: age, gender, body mass index, history of coronary artery disease, diabetes mellitus, dyslipidaemia, previous history of transient ischaemic attack/cerebrovascular event, hypertension, heart...
failure, smoking, glomerular filtration rate, AF subtypes, CHADS-VASc score, left ventricular ejection fraction, LA diameter, number of failed AADs, and cardiovascular medications. Based on their propensity score, the patients who underwent LAA plus PVI and PVI alone patients were matched on a 1:1 basis with a nearest neighbour algorithm without replacement using a caliper width 1/5 logit of the standard deviation. Matching was done using the nearest neighbour method using a one-to-one (1:1) ratio using R extension pack (R version 2.15.0). The selection process used a P value cut-off of 0.05 for a characteristic to enter and remain in the model. Analyses were conducted in the matched cohorts.

Continuous variables are presented as mean values ± SD or median (minimum–maximum), whereas categorical ones are presented as number (n) and percentage (%). The Kolmogorov–Smirnov criterion was used for the assessment of normality. Comparisons between baseline characteristics were performed by independent Student’s t test, Mann–Whitney rank-sum, Fisher exact, or χ² tests where appropriate. Cox proportional hazards regression was used in order to test the effect of the explanatory variables on AF recurrence, adjusted for other variables. Parameters that are found to be univariately associated with the outcome and those that show an association with the outcome with P < 0.1 are included in the multivariable Cox regression analysis. Time to recurrence of AF was plotted using Kaplan–Meier curves for patients with AF due to study groups (LAA plus PVI vs. PVI-only) separately (with a blanking period of 3 months following CB applied). Repeated measures analysis of variance (rANOVA) was used to analyse differences in continuous variables including LAA flow rates between baseline, 3rd and 12th follow-up. A two-tailed P value <0.05 was considered statistically significant. All analyses were performed, using the SPSS software, version 22.0 (SPSS, Inc., Chicago, IL, USA) and MedCalc 11.4.2 (MedCalc Software, Mariakerke, Belgium).

Results

Baseline characteristics

Among 106 persistent AF patients, we attempted to perform PVI & LAA isolation; however, LAA isolation could not be achieved in 6 (5.6%) of these patients using CB, which were excluded from the analysis. Control group was formed among the propensity-score matched cohort of consecutive persistent AF patients in whom only CB-based PVI has been performed previously in our centre. Finally, a total of 200 patients [n = 100 (91% persistent AF, 9% long-standing persistent AF) in PVI-only (Group I) and n = 100 (90% persistent AF, 10% long-standing persistent AF) in PVI plus LAA isolation (Group II)] were enrolled in our study analyses. The mean age (57 ± 8 vs. 57 ± 10 years, P = 0.896) and gender of the groups (female, 51 vs. 52%, P = 1.000) were similar between groups. The mean duration of AF was median 60 months and CHA2DS2-VASc score was 1.9 ± 1.2 vs. 2.1 ± 1.1, respectively (P = 0.424). Baseline demographic and clinical characteristics of the study and control groups were illustrated in Table 1.

Procedural characteristics

In all patients, either second- or third-generation 28 mm CB was used (72 vs. 28%, P = 0.637). Total procedure and fluoroscopy times were significantly lower in Group I compared with the Group II ([55.4 ± 10.5 min vs. 74.9±10.8 min, P < 0.001] and [6.8 ± 2.4 min vs. 9.1 ± 2.5 min, P < 0.001], respectively). Other intra-procedural characteristics including mean number of freeze–thaw cycles, total number of PVs, PV variants, temperature at isolation, time to isolation, and nadir temperature for each PV of both groups were similar between Groups I and II (P > 0.05) (Table 2). Acute procedural success rates for PV isolation were also similar between each groups (99.7% in Group I vs. 100% in Group II, P = 1.00). CB for LAA isolation was applied for median 300 (180–450) s and time to isolation was median 115.5 (37–370) s in Group II. Temperature at isolation

**Table 1 Baseline characteristics of the study groups (n = 200)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (PVI-only)</th>
<th>Group II (PVI + LAA isolation)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57 ± 8</td>
<td>57 ± 10</td>
<td>0.896</td>
</tr>
<tr>
<td>Sex (female)</td>
<td>51 (51.0%)</td>
<td>52 (52.0%)</td>
<td>1.000</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.5 ± 2.8</td>
<td>24.1 ± 2.9</td>
<td>0.285</td>
</tr>
<tr>
<td>History of CADa</td>
<td>15 (15.0%)</td>
<td>16 (16.0%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>18 (18.0%)</td>
<td>20 (20.0%)</td>
<td>0.857</td>
</tr>
<tr>
<td>Dyslipidaemiab</td>
<td>27 (27.0%)</td>
<td>26 (26.0%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Previous TIA/CVA</td>
<td>9 (9.0%)</td>
<td>10 (10.0%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Hypertension</td>
<td>36 (36.0%)</td>
<td>33 (33.0%)</td>
<td>0.766</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>9 (9.0%)</td>
<td>11 (11.0%)</td>
<td>0.814</td>
</tr>
<tr>
<td>Alcohol intakec</td>
<td>21 (6.8%)</td>
<td>9 (9.5%)</td>
<td>0.258</td>
</tr>
<tr>
<td>Current smoking history</td>
<td>27 (27.0%)</td>
<td>25 (25.0%)</td>
<td>0.872</td>
</tr>
<tr>
<td>CHA2DS2-VASc score</td>
<td>1.9 ± 1.2</td>
<td>2.1 ± 1.1</td>
<td>0.424</td>
</tr>
<tr>
<td>AF type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Persistent</td>
<td>91 (91.0%)</td>
<td>90 (90.0%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Long-standing persistent</td>
<td>9 (9.0%)</td>
<td>10 (10.0%)</td>
<td></td>
</tr>
<tr>
<td>Duration of AF history (months)</td>
<td>60 (12–240)</td>
<td>60 (12–300)</td>
<td>0.438</td>
</tr>
<tr>
<td>LA diameter (mm)</td>
<td>42.8 ± 3.5</td>
<td>43.6 ± 3.7</td>
<td>0.156</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>65.1 ± 3.6</td>
<td>65.3 ± 4.1</td>
<td>0.706</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>74.8 ± 9.9</td>
<td>74.7 ± 10.9</td>
<td>0.971</td>
</tr>
<tr>
<td>No. failed antiarrhythmics</td>
<td>1.7 ± 0.6</td>
<td>1.8 ± 0.6</td>
<td>0.217</td>
</tr>
<tr>
<td>Antiarrhythmic medications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Blockers</td>
<td>78 (78.0%)</td>
<td>75 (75.0%)</td>
<td>0.739</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>30 (30.0%)</td>
<td>30 (30.0%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Dronaderone</td>
<td>10 (10.0%)</td>
<td>9 (9.0%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Propafenone</td>
<td>82 (82.0%)</td>
<td>87 (87.0%)</td>
<td>0.435</td>
</tr>
<tr>
<td>Sotalol</td>
<td>12 (12.0%)</td>
<td>13 (13.0%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Antiplatelet and anticoagulants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>35 (35.0%)</td>
<td>32 (32.0%)</td>
<td>0.765</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>8 (8.0%)</td>
<td>9 (9.0%)</td>
<td>1.000</td>
</tr>
<tr>
<td>Warfarin</td>
<td>13 (13.0%)</td>
<td>22 (22.0%)</td>
<td>0.136</td>
</tr>
<tr>
<td>NOAC</td>
<td>45 (45.0%)</td>
<td>49 (49.0%)</td>
<td>0.671</td>
</tr>
</tbody>
</table>

Data are median (minimum-maximum), means ± SD, or n (%). 
AF, atrial fibrillation; BMI, body mass index; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; EHRHA, European Heart Rhythm Association; LA, left atrium; LDL, low density lipoprotein; LVEF, left ventricular ejection fraction; PVI, pulmonary vein isolation; SD, standard deviation.

aPrevious history for ischaemic heart disease.
bDyslipidaemia is defined as total cholesterol > 200 mg/dL or treatment with a lipid lowering agent.

cAlcohol intake is defined as having up to one drink per day for women and up to two drinks per day for men in which heavy drinkers and abusers were excluded.
and nadir temperature for LAA was mean -42.9 ± 6.2 (-30 to 56)°C and median -50 (-33 to 62)°C. Also mean number of thaw–freeze cycles were 1.15 ± 0.35 (minimum–maximum: 1–2).

### Complications

Procedure-related complications such as vascular access site problems and right PNP were similar between groups (Table 2). We observed left PNP during LAA isolation only in 1 patient (1%) in whom CB freezing immediately terminated and left PNP was resolved on the day after the procedure. Coronary angiography during or immediately after the procedure revealed an asymptomatic left Cx artery spasm in 4% of the LAA isolation group, which was completely resolved after administration of intracoronary nitrate (Figure 3; Supplementary material online, Video S3). During the follow-up, two patients (2%) in Group I and one patient (1%) in Group II had ischaemic stroke. Two patients with CHA2DS2VASc
score of 3 and 4, respectively, experienced ischaemic stroke episode 6 months after the index procedure in Group I, who had sub-therapeutic INR levels. TEE revealed no thrombus in these patients. In the LAA isolation group, only one ischaemic stroke episode was observed 5 months after the procedure in a patient with CHA2DS2VASc score of 5, who discontinued using novel oral anticoagulant therapy for 10 days. Thrombus was also not detected by TEE in this patient. There were two minor gastrointestinal bleeding events in the study population (n = 1 in Group I and n = 1 in Group II). No death among study participants was occurred during the study period.

Baseline and follow-up LAA assessment
Mean LAA flow velocity was similar in both Groups I and II (0.54 ± 0.20 m/s vs. 0.52 ± 0.19 m/s, P = 0.693) and the number of patients with low LAA flow rates (<0.4 m/s) were also same before ablation (25% in both Groups I and II, P = 1.000). During the follow-up, among patients with CHA2DS2VASc score of <2 in Group II, third month TEE control revealed decreased LAA flow velocity (<0.4 m/s) in two patients and high-grade stenosis in two patients in whom long-term oral anticoagulation was recommended. In the remaining 15 patients with CHA2DS2VASc score of <2, oral anticoagulation was stopped after 3 months. In 81 patients with CHA2DS2VASc score of ≥2, we strictly recommended life-long oral anticoagulation. However, TEE examination at baseline and 12th month follow-up in Group II revealed a significant decrease in LAA flow rates without thrombus formation (mean LAA flow rates: 0.52 ± 0.19 m/s at baseline vs. 0.46 ± 0.15 at the 12th month follow-up, P < 0.001). The spontaneous echo contrast grades did not show significant change at the 12th month control compared with the baseline values in the LAA isolation group (P = 0.269) (Table 3). An example of the TEE image and movie of a patient who underwent a second procedure because of PV reconnection with isolated LAA and LAA flow velocity within normal range at 12-month control visit was shown in Supplementary material online, Figure S1 and Video S4.

Procedural outcomes
All of the patients in the analysis completed 12-month follow-up. During the blanking period, 20 patients (20%) in Group I and 11 patients (11%) in Group II had an early AF recurrence (P = 0.117). Among these 31 patients, 10 patients in Group I (10%) and 6 patients in Group II (6%) underwent ECV (P = 0.435). Remaining 15 (15%) patients required no further intervention during the blanking period. At the 12-month follow-up, 67 (67%) patients in Group I and 86 (86%) patients in Group II were free of A.T after the index CB procedure (P < 0.001) (Figure 4). All the recurrences were AF episodes, there was no atrial tachycardia episode during the follow-up among the study population. Among 31 patients with early recurrence, 22 (46.8%) also had late recurrence. When we analysed the results of long-standing persistent AF subgroup, late recurrence was observed in 13/19 (68.4%) of the patients with long-standing persistent AF [n = 6 (60%) in Group I vs. n = 7 (77.8%) in Group II, P = 0.628] which was significantly higher as compared with patients with persistent AF subgroup [n = 34/181 (18.8%)].

There was a total of six patients in whom the CB-based electrical isolation of LAA was unsuccessful despite lower nadir temperatures and adequate freezing time. Second-generation CB was used in four of the six patients (66.7%) and third-generation CB was used in remaining two patients (33.3%). The occlusion of LAA ostium was suboptimal in only one of these six patients (16.7%). During long-term follow-up, late recurrence has been observed among four of the six (66.7%) patients. The second procedure was needed in three of these four patients with late recurrence. During second procedure, PV reconnection was detected in two patients and re-isolated by CB technique. However, in the remaining one patient, there was no PV reconnection, but a severe LA fibrosis has been observed by using 3D electroanatomical voltage mapping and scar homogenization has been performed by RF technique.

Predictors of AF recurrence
In order to evaluate the predictors of recurrence, the whole study population was divided into two groups according to the presence of
late ATa recurrence ($n = 47$ with recurrence vs. $n = 153$ without recurrence). Patients with ATa recurrence showed a greater baseline LA diameter (46.1 ± 2.9 mm vs. 42.3 ± 3.9 mm, $P < 0.001$) and higher LAA flow rate (0.58 ± 0.19 mm vs. 0.52 ± 0.19 mm, $P = 0.057$) compared with the patients without recurrence. Also diabetes mellitus (29.8% vs. 15.7%, $P = 0.054$), hypertension (48.9% vs. 30.1%, $P = 0.022$), congestive heart failure (17.0% vs. 7.8%, $P = 0.092$), use of second-generation CB (87.2% vs. 67.3%, $P = 0.009$), PVI-only strategy (70.2% vs. 43.8%, $P < 0.001$), cardioversion resistant AF before ablation procedure (34 vs. 7.2%, $P < 0.001$) and early recurrence (46.8 vs. 5.9%, $P < 0.001$) were significantly higher among patients with late recurrence. Multivariate Cox regression analysis revealed that baseline LA diameter (HR: 1.22, 95% CI: 1.10–1.35, $P < 0.001$), PVI-only strategy (HR: 3.37, 95% CI: 1.73–6.56, $P < 0.001$) and early recurrence (HR: 3.16, 95% CI: 1.57–6.37, $P = 0.001$) were significant predictors for late recurrence.

Among patients with late recurrence after the index procedure, 8 of 33 (24.2%) in Group I and 3 of 14 (21.4%) in Group II with symptoms on AADs underwent repeat CB-based catheter ablation. During the second procedure, PV reconnection was detected in six patients (75.0%) in Group I and in three patients in Group II (100%). Among nine patients with PV re-connection, only left upper PV re-connection was observed in four patients, only right upper PV re-connection was observed in three patients, and both right upper and right lower PVs re-connection was observed in the remaining two patients. In addition to the isolation of re-connected PVs, successful empirical LAA isolation was also performed in all Group I patients undergoing redo-procedures. No LAA re-connection was detected in Group II patients undergoing redo-procedures. Remaining 25 patients (75.8%) in Group I and 11 patients (78.6%) in Group II with late recurrence were asymptomatic on AADs.

### Discussion

Our study findings indicated that empirical electrical isolation of LAA with CB as an adjunct to PVI improved 1-year freedom from ATa
mainly based on the seminal observation by Di Biase in involving the LAA in the perpetuation of the AF. This approach was initially from the rest of LA, independent of the actual mechanism. LAA isolation using CB aimed to empirically isolate the appendage circumferentially between 60 and 67% in patients with persistent AF. Because of the use of CB ablation for PVI with 1-year freedom from ATa ranging between the follow-up.

Several recent studies revealed encouraging results regarding the use of CB ablation for PVI with 1-year freedom from ATa ranging between 60 and 67% in patients with persistent AF. Because of the moderate success in persistent AF compared with the paroxysmal AF, additional ablation approaches targeting both atrial substrate and non-PV triggers have been developed. Similar to the PVI, CB ablation of LAA aimed to empirically isolate the appendage circumferentially from the rest of LA, independent of the actual mechanism involving the LAA in the perpetuation of the AF. This approach was mainly based on the seminal observation by Di Biase et al., which revealed that LAA was responsible for the recurrence of the atrial arrhythmias in at least 27% of the patients with a previous AF ablation procedure. Moreover, LAA was not only a potential trigger site for AF, localized reentry was also demonstrated in the LAA as an important source of atrial arrhythmias in patients with persistent AF at both index and repeat procedures. In our study, we performed circumferential LAA isolation using CB as an adjunct to PVI which was successful in 100/106 patients (94.4%).

In a recently published trial, empirical circumferential LAA isolation was associated with better outcomes in patients with long-standing persistent AF, both after a single or redo procedure with a success rate of 76% after an average of 1.3 interventions at 24-month follow-up. In a persistent AF population, our findings showed that LAA isolation as an adjunct to PVI improved 1-year outcomes with a success rate of 86% after the index procedure compared with the PVI-only group. Compared with the RF ablation studies, procedure, and fluoroscopy times were shorter using CB in our study. This was mostly due to the less extensive nature of our ablation strategy involving only PVs and LAA compared with the more extensive ablation strategy in RF ablation studies including linear lesions or CFAE ablations as well as additional non-PV targets other than LAA. Moreover, CB isolation of LAA is a straightforward method, which allows single-step circumferential ablation and visualization of effects of ablation on LAA potentials using inner lumen circular map catheter. As a result, addition of LAA isolation to PVI using CB was associated with an acceptable lower procedure and fluoroscopy times compared with the PVI-only strategy in our study population.

An important aspect of LAA isolation using CB procedure is the technical details of the ablation procedure. In this respect, we initially place the inner lumen circular mapping catheter inside the LAA under the guidance of both fluoroscopy and real-time intra-cardiac electrogram recordings. As the LAA is a thin-walled structure, stable and not too deep circular map catheter position with lower tension are important factors in order to prevent perforation while positioning the CB catheter to the LAA orifice. We observed no pericardial effusion or LAA perforation using either second- or third-generation CB in our study population. Furthermore, left PNP is an important concern during the LAA isolation like right-sided PV isolation and stimulation from the circular map catheter provided adequate diaphragmatic contraction in most of the cases. A rare but interesting observation which was not reported during LAA isolation with RF energy was left Cx artery spasm that was observed in 4% of our study group. When the anatomic proximity of Cx to the LAA orifice was considered, injury to the Cx artery should also be kept in mind especially in case of lower nadir temperatures during CB ablation of LAA.

Previous studies demonstrated that lower LAA flow velocity was associated with thrombotic complications after LAA ablation. Given these observations, preserved LAA mechanical functions has paramount importance in preventing thromboembolic complications, when the association between decreased LAA flow velocities and thrombus formation considered. In our study, despite a significant decrease in LAA flow velocity at the 12-month follow-up, mean LAA flow velocity was >0.4 m/s in 66% of the patients. This ratio was higher than a previous study, in which preserved LAA functions was reported in 43.5% of the patients. The relatively high preserved LAA flow velocities in our study might be due to several factors as compared with the PVI-only strategy in persistent AF. Early recurrence, LA diameter and PVI-only strategy were found as independent predictors of late recurrence. Importantly, LAA isolation did not cause any significant procedural complications or adverse thromboembolic events despite the decrease in LAA flow velocities during the follow-up.

**Table 4** Univariate and multivariate Cox proportional Hazard modelling results of the ATa recurrence after CB based AF ablation

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate model</th>
<th>Multivariate model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
</tr>
<tr>
<td>LA diameter (mm)</td>
<td>1.28</td>
<td>1.19–1.38</td>
</tr>
<tr>
<td>Baseline LAA flow velocity (m/s)*</td>
<td>0.35</td>
<td>0.15–0.82</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.94</td>
<td>1.09–3.44</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.93</td>
<td>1.03–3.61</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>2.06</td>
<td>0.96–4.40</td>
</tr>
<tr>
<td>Cryoballoon model*</td>
<td>0.35</td>
<td>0.15–0.82</td>
</tr>
<tr>
<td>PVI only strategy</td>
<td>2.69</td>
<td>1.44–5.04</td>
</tr>
<tr>
<td>Cardioversion resistant AF before procedure</td>
<td>4.05</td>
<td>2.21–7.43</td>
</tr>
<tr>
<td>Early recurrence</td>
<td>7.51</td>
<td>4.20–13.5</td>
</tr>
</tbody>
</table>

AF recurrence is the dependent variable.

AF, atrial fibrillation; CB, cryoballoon; CI, confidence interval; HR, hazard ratio; LA, left atrium; LAA, left atrial appendage; PVI, pulmonary vein isolation.

*Baseline LAA flow velocity <0.4 m/s is used as reference in the model.

*Second generation cryoballoon is used as reference in the model.
following: (i) lower degree of structural remodelling in the LA in our study population, which might increase passive movement of the LAA accompanied to the LA contraction, (ii) electrical reconnection after LAA isolation, which was also reported up to the half of redo procedures.17 Further studies are needed to clarify the effects of electrical isolation on mechanical LAA functions and clinical outcomes.

One of the major concerns regarding the LAA electrical isolation is the potential thromboembolic events related to the impairment in LAA mechanical functions.21 In the recently published BELIEF trial, stroke and thromboembolic events were not increased with empirical LAA isolation in patients with long-standing persistent AF compared with the without LAA isolation group.17 On the other side, Rillig et al.16 reported that electrical isolation of LAA as an adjunct to AF ablation using RF energy was associated with an increased incidence of LAA thrombus and stroke/TIA compared with the without LAA isolation group. The higher incidence of thromboembolic events in this study was mainly attributed to the wide-area LAA isolation including left atrial linear lesions far away from the LAA base, which outweighed the CHA2DS2-VASc-related risk. Despite the inconsistent findings regarding thromboembolic events after LAA isolation using RF energy, there was no study in the literature evaluating the safety of CB-based LAA isolation. In our study, we observed no LAA thrombus during the follow-up. Our results might be due to the several factors including the AF type of the study population, less extensive nature of ablation compared with the aforementioned studies and type of the ablative energy used. Moreover, despite a decrease in LAA flow velocity after CB ablation, it was preserved in most of our study population which might also have protective effects on thrombus formation. Furthermore, optimal anticoagulation regime after LAA isolation has paramount importance in order to prevent future embolic events. In a recent report by Di Biase et al.,22 there was a significant difference in thromboembolic risk after LAA isolation between oral anticoagulation on and off patients (0.08 vs. 2.26%, P < 0.001). These findings emphasize the importance of optimal anticoagulation regime in preventing possible thromboembolic adverse events after LAA isolation even in the presence of disturbed LAA mechanical functions.23 In our study, we continued OACs in all patients with a CHA2DS2Vasc score of ≥2, poor LAA flow velocity (<0.4 m/s) and severe smoke that could also prevent thromboembolic complications. Percutaneous closure of LAA was also a therapeutic option after LAA isolation to prevent thromboembolic events; however, there was limited data about the routine use of this method after LAA isolation.16,17 Given these findings, comparative studies are needed in order to evaluate the exact pathophysiologic processes underlying thrombo-embolic events after LAA isolation as well the optimal treatment strategy to prevent future thromboembolic complications.

Our study has some important clinical implications regarding the safety and efficacy of additional non-PV source ablation in patients with persistent AF. First, LAA isolation in addition to PVI could be achieved using single-step CB technique without a significant increase in the procedure time. Second, CB technique is a straightforward and safe method for LAA isolation with low procedural complication and post-procedural adverse event rate. These safety outcomes were also compatible with recently published LAA isolation studies, in which RF energy was used.11,17 Of note, our study findings might be due to several factors including the high percentage of early persistent AF population and less extensive ablation strategy in our study population. In addition, CB might provide additional benefit beyond LAA isolation, when the effects of CB ablation on cardiac autonomic innervation considered.24 As a result, these findings expand the literature in terms of both the safety concerns regarding the use of CB technique in a such patient population and feasibility of CB technique for the isolation of LAA.

Limitations
Our study has some limitations that should be mentioned. First, despite a relatively high number of enrolled patients, this was a single-centre and non-randomized study. Second, although these findings were representative of the results of a high-volume experienced centre, LAA isolation using CB is not free of potential complications such as LAA perforation, pericardial tamponade, coronary artery spasm, or PNP that should be kept in mind especially in the learning curve period. Third, the number of long-standing persistent AF patients was limited in our study cohort which also limits the extrapolation of these findings in the whole range of non-paroxysmal AF patients that necessitate CB ablation.

Conclusions
In conclusion, we described for the first time that CB based isolation of LAA as an adjunct to PVI improved 1-year freedom from ATa compared with the PVI-only strategy with favorable safety profile in persistent AF. Although the best ablation technique for LAA isolation was still unclear, CB seemed to serve beneficial clinical outcomes without an increase in the major complication rate. Further studies are warranted to assess both safety and long-term efficacy of this novel approach in persistent AF patients undergoing CB ablation.

Supplementary material
Supplementary material is available at Europace online.

Conflict of interest: none declared.

References


23. Di Biase L, Natalie A. Left atrial appendage after electrical isolation: to occlude or not to occlude, that is the question. Circ Arrhythm Electrophysiol 2016;9 pii e004372.