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Long-Term Evolution of Patients Treated for Paroxysmal Atrial Fibrillation with First and Second Generation Cryoballoon Catheter Ablation with a Prospective Protocol Guided by Complete Bidirectional Left Atrium-Pulmonary Veins Disconnection after Adenosine as Main Target end Point to achieved. Seven Years Follow-up of Patients with a rough estimation profile of Low ALARMEc Score. A Single Center Report

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Abstract

Introduction: Cryoballoon ablation (CB) has proven effective for treating patients with paroxysmal atrial fibrillation (PAF). We analyzed our seven year follow-up of patients, treated for PAF with first (CB1) and second generation (CB2), with demonstration of LA-PV disconnection with bidirectional block (BB) after adenosine (AD).

Methods: Since November 2008 to May 2015, 128 patients, 97 male (58±7 years), without heart disease, highly symptomatic, refractory to antiarrhythmic drugs (AAD) were treated, and follow-up (1411 ±727 days). Left atrial size: 37±6 mm.

Results: A total of 439 PV were successfully isolated (91.9%). Acute reconduction: 44 PV (9%): 16 after CB; 16 unmasked by AD; 12 extrapulmonary muscular connections (EMC). Main complication was phrenic nerve palsy (PNP): 9 (7 %). On follow-up, 114 patients (89%) remain asymptomatic in sinus rhythm (SR), free of medication. Fourteen patients (11%) had arrhythmia recurrence: 12 male (52±8 years). Early recurrences occurred in 9 male. Late recurrences presented 3 male at 24, 27 and 60 months, and 2 female at 7 and 40 months respectively. All recurrence patients were Redo, and remain in SR without medication during follow-up.

Conclusions: CB alone is very effective and safe for the definitive treatment of patients suffering PAF with 72.6% success rate, increasing up to 89.1% when this protocol is applied in a single procedure. After Redo, all population group (100%), remain in sinus rhythm, freedom of arrhythmia, without AAD, in this very long term follow-up. Checking for BB, AD protocol, and ruling out EMC allowed-us to identified 14.8% of patients with underlying substrate for potential arrhythmia recurrence. CB2 applications entail a highest risk of PNP. Patients with a rough estimated profile of low ALARMEc score (≤ 1) have an excellent long term outcome, being this series the largest follow-up described so far, for patients treated for PAF with CB.

Introduction

Complete electrical isolation of pulmonary veins (PVI) from the left atrium (LA) is crucial to cure patients (pts) with Atrial Fibrillation (AF).[1-4]

The Cryoballoon catheter ablation technique (CB) has proven effective to achieve this electrical disconnection of pulmonary veins (PV) from LA, resulting in a demonstrated effectiveness to treat pts suffering from PAF.[5-11]

However, some observations[12] have shown, at least when first generation CB was used that cryoenergy CB application doesn't produce a homogeneous circumferential lesion in all PV, which is related to their anatomical shape, thickness and size with a non-uniform distribution of the atrial muscle around them.

Key Words:

Cryoballoon, Paroxysmal Atrial Fibrillation, Ablation.

Disclosures:
None.

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Table 1: Demographic and clinical pts/features

128 pts (mean age 53±13)	
Male/female	97 (75.8%) / 31 (24.2%)
Mean age (male/female)	58±7 / 61±10 years
Mean years/ suffering PAF	5±5 years (1-5)
Mean number/ episodes PAF/ year	54±67 (2-200)
Hypertension	36 (28%)
Diabetes	6 (4.7%)
Structural heart disease	NONE

The more elliptic rather than circular variable form at the PV-LA junction level where cryoenergy is delivered can result in a non-uniform and persistent cellular lesion which, as is generally accepted, is the principal cause of PV reconnection after CB ablation.[13] A better quantification of the Cryoablation and the anatomical extent of PV have been better clarify recently.[14] Incomplete lesions with dormant tissue despite a "perfect" occlusion can occur leading to a residual conduction (RC) gaps causing, or responsible for PV reconduction which is the main underlying anatomical substrate for clinical arrhythmia recurrence.[15, 16]

Adenosine has been used to "unmask" RC in apparently isolated PV with RF[17] and the routine use of AD after acute CB-PVI allows to identify incomplete lesions with dormant tissue not evident in basal conditions [18-20] and focal RF applications [21] or freeze "touch-up" [18-20] eliminate such RC.

The only no evidence of PV/ electrical activity on the circular-mapping-catheter at the LA-PV junction level after CB-PVI is not enough to assure complete PV-LA electrical disconnection and checking for entry and exit block is mandatory to confirm it.[22-24]

We analyzed the seven year follow-up experience of our pts, initially treated with CB for PAF, with a prospective protocol with demonstration of complete BB electrical PV-LA-PV block post-cryo and after AD as the main target end point to achieve in all cases.

Methods

Since November 2008 to November 2015, a total cohort of 128 pts (mean age 56±13 years), highly symptomatic, suffering from recurrent PAF, refractory to medical treatment (Table 1), were treated with the "CB" and followed-up.

Prior to CB, all pts were previously treated with membrane active antiarrhythmic drugs: Class IC (88.2%); Class III (2.3%); Beta Blockers (BtB) (84.3%) and BtB +1C: 76.5%.

None with structural heart disease. Morphological and structural data can be showed on Table 2:

Exclusion Criteria:

- Prior Stroke, TIA or thromboembolism.
- Cryoglobulinemia and hematological or coagulation disorders.

Table 2: Morphological and structural LA/PV/LV data

DIAMETERS (mm)	LA	PV (483)	LCT (26)	RCT (3)	Mean LVEF 67±5% (59-79)
AP	37±6 (21-50)	18±5 (8-32)	26±6 (18-35)	28±1 (27-29)	
SI	53±8 (40-75)	20±4 (10-28)	26±5 (17-31)	28±5 (23-33)	Mean LA/ AREA (cm^2) 22±4 (11-32)
TR	46±7 (35-61)				

LA: Left Atrium. PV: Pulmonary Vein. LCT: Left Common Trunk. RCT: Right Common Trunk. LVEF: Left Ventricular Ejection Fraction. AP: Antero-Posterior (parasternal long axis). TR: Transversal. SI: Supero-Inferior.

- Presence of intracavitary thrombi as well as clinically- significant associated comorbidity

Previous Studies And Anatomical Approach: 2D-Transtoracic echocardiogram (TTE) as well as, same day, transesophageal echocardiogram was performed in all cases, to assess cardiac anatomy and to rule out intramural thrombi.

3D/ high resolution/64- slice Multidetector CT scan (Toshiba Aquilion 64, TSX-101A, Tokyo, Japan), and in some alternative cases, RMN (1.5T/ Magnetom Symphony, Siemens, Germany) were used for typification and better definition of cardiac anatomy, morphology, number, caliber and size of PV in addition to internal endoluminal navigation analysis to assess the thickness of the interpulmonary ridge and the morphological shape and size of PV ostium to choose the optimal CB size and the best orientation to address the balloon wedging at the LA-PV junction in an attempt to induce the biggest cryo lesion at the most proximal antral location including the interpulmonary ridge at the carina level in a sort of different morphological anatomical variants, as showed in Figure 1.

Procedure: All pts provided informed consent prior to the procedure. The procedure was approved by hospital's clinical ethics committee. Prior to the procedure, all antiarrhythmic drugs (AAD) were discontinued at least 5 times their half-life; 48 hours for beta blockers and at least 10 days for Amiodarone.

All procedures were performed under general anesthesia with orotracheal intubation under propofol for anesthesia induction, cisatracurium for neuromuscular relaxation (only at the time of intubation), continuous perfusion of remifentanil for analgesia and mechanical ventilation maintained with Sevoflurane gas.

Transeptal Approach: Seldinger technique was used for all vascular access. A decapolar 6 French electrocatheter through an antecubital vein was positioned into the coronary sinus (CS) for pacing and anatomical reference purposes. Cuatripolar/6French catheter was positioned at the A-V-nodal-his bundle junction through left femoral vein, for the same anatomical reference purpose, being moved later to superior vena cava (SVC) for pacing during CB applications at the right sided PV.

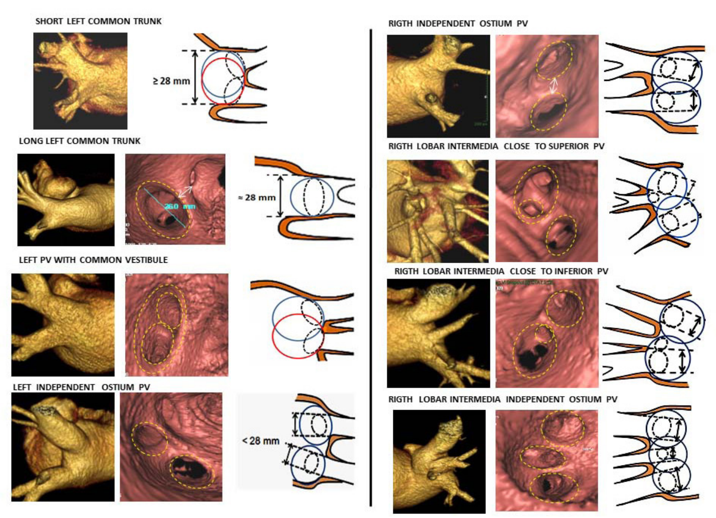

Figure 1: Endoluminal and CT Scan reconstruction anatomical approach, to assess diameter/ shape and sizes of PV/LA-PV junction level and interpulmonary ridge, in relation to the size of CB to be used and the orientation for better PV-LA wedging

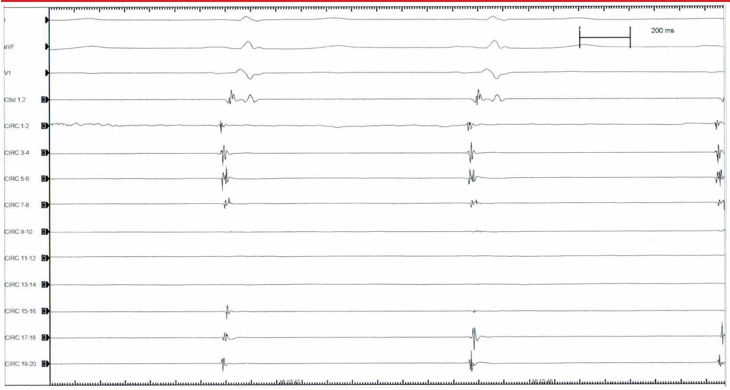

Figure 2A: Atrial far-field and synchronous PV electrical activity as recorded with the circular catheter mapping at the PV-LA junction antral level

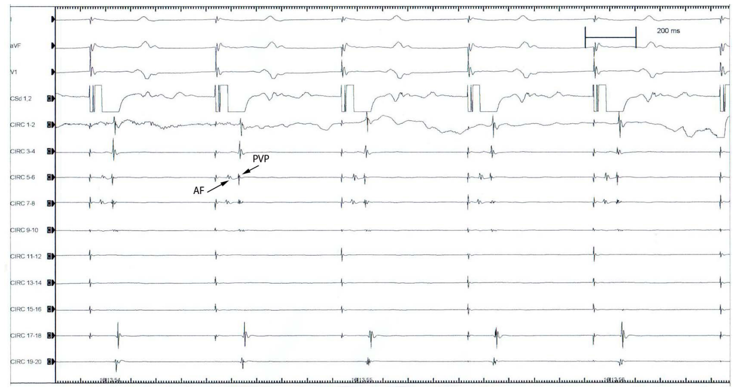

Figure 2B: Asynchronous atrial far-field (Af) and PV electrical potential (PVP) as recorded on circular catheter mapping by pacing CS

Through right femoral vein, an introducer and fast-cath 8.5 French sheet SLO, (Saint Jude medical, Minnesota, USA), was advanced over a 0.32 mm J typed shape guide wire to the SVC. Then, the guide wire is withdrawal, and a modified Brockenbrough needle (BRKO 71 cm beveled cut 30º/ Saint Jude Medical, MN, USA) is advance through the SLO sheet, and descending the whole transeptal assembly to embed fossa ovalis.

After gaining left atrial access, a bolus of 10.000 IU of sodium heparin was administered, followed by continuous perfusion as needed to maintain the activation clotting time ≥300 sec, as previously described.[25] At the end of the procedure, anticoagulation is reversed with protamine and 1grm. of lysine acetylsalicylate given i.v, along with low molecular weight heparine depending on patient's body surface (1 mgr/Kg body weight) given subcutaneously, in addition to 100 mg of flecainide given intravenously in 10 minutes. Continuous intravenous perfusion of sodium heparine adjusted to patient's body weight is started 4 hours later after removing all catheters from the vascular bed. Twenty-four hours later, oral anticoagulation with Vitamin K antagonist dicumarol is started targeting an international normalized ratio (INR) in the range of 2.0 to 3.0, plus additional platelet inhibition with 100 mg of ASA.

PV/Cartography/ Mapping: Once in the LA chamber, the long 0.32mm guide-wire is advanced into the left superior PV (LSPV) and selective PV angiogram is performed, and in the same manner for the remaining veins, Left Inferior (LIPV), Right Superior (RSPV) and Right Inferior (RIPV).

After removing the entire transeptal assembly, keeping the guide-wire in the LSPV, a steerable 15F over-the wire sheath (Flex Cath, Cryocath, Medtronic,USA) is advanced and positioned in the LA. Then basal electrical cartography of the veins is obtained (Figure 2A) with a circular duodecapolar mapping catheter with adjustable diameter (Reflexion spiral, Saint Jude Medical, MN, USA) positioned at the PV-LA junction antrum level, starting on LSPV and followed by LIPV, RSPV and RIPV respectively. We used a 20 pole circular mapping catheter to achieve sharper signals and better recognition between PV potentials and far-field atrial activity. This variable catheter adjustable in diameter is more useful when varying PV size or common ostium encountered, and also, allows for better contact and stability at the ostium of the PV when the circular catheter is fully expanded, leading to relative oversizing. Although, when fully expanded electrobipoles overlap and could cause contact signal artifact and repetition of recorded signals.

After recording the LA- PV junction electrical activity we pace CS to separate atrial far-field electrograms from PV electrical activity (Figure 2.B), as in sinus rhythm it is difficult to distinguish because they are activated synchronously. After 30 minutes of CB applications all PVs were mapping again to assess electrical PV-LA isolation.

Cryoballoon: After withdrawing the circular catheter mapping, a 28 or 23 mm double walled CB catheter (Artic Front, Medtronic, USA) is advanced over the wire up to the LA, inflated and positioned in the PV ostium of each vein and gently pushed against the PV-LA antrum to get a perfect occlusion achieved when selective contrast medium injected (50% ratio with 0.9% saline solution) is full retained into the vein with no evidence of contrast leakage back to the atrium (grade IV) according to the degree of occlusion classification proposed and used by Neumann et al [8] to grade I with poor occlusion leading to an immediate rapid outflow contrast medium back to the LA. Until the second generation CB (CB2) was commercially available (April, 2013) patients were treated with the first generation CB (CB1).

Bidirectional LA-PV-LA Block Protocol

Exit Block: By pacing PV from all 20 poles of the circular catheter mapping at high amplitude voltage (20 mA) with consistent 1:1 PV capture and no evidence whatsoever of any atrial response.

Entry Block: By pacing LA from the CS-Catheter at three different cycle lengths (600, 500, 400 ms) with consistent 1:1 LA capture and no evidence whatsoever of any PV electrical activity in any of the 20 poles of circular-catheter mapping positioned at the LA-PV junction antral level.

AD Protocol: Included bolus i.v administration of increasing doses (12-18-24… mgrs.), and pacing PV/LA when A-V nodal conduction block occurred.

Extrapulmonary Muscular Connections (Emc)/ Rule-Out Protocol: Included pacing distal vein from the circular catheter mapping after complete BB demonstrated at the LA-PV junction antral level (Figure 4.A) and the demonstration of 1:1 PV-LA conduction resumed. (Figure 4.B)

RF Protocol: Focal RF applications were used for eliminating RC gaps when evident after single CB application or after checking for BB Block, post -AD, or when EMC was demonstrated. (Figure 5).

Sixty second "touch-up" of focal RF was used to eliminate all residual gaps only when evident in no more than 2 pairs of the circular catheter mapping. Otherwise, when more a repeated new CB application was performed.

Phrenic Nerve Physiology Control: Phrenic nerve physiology was

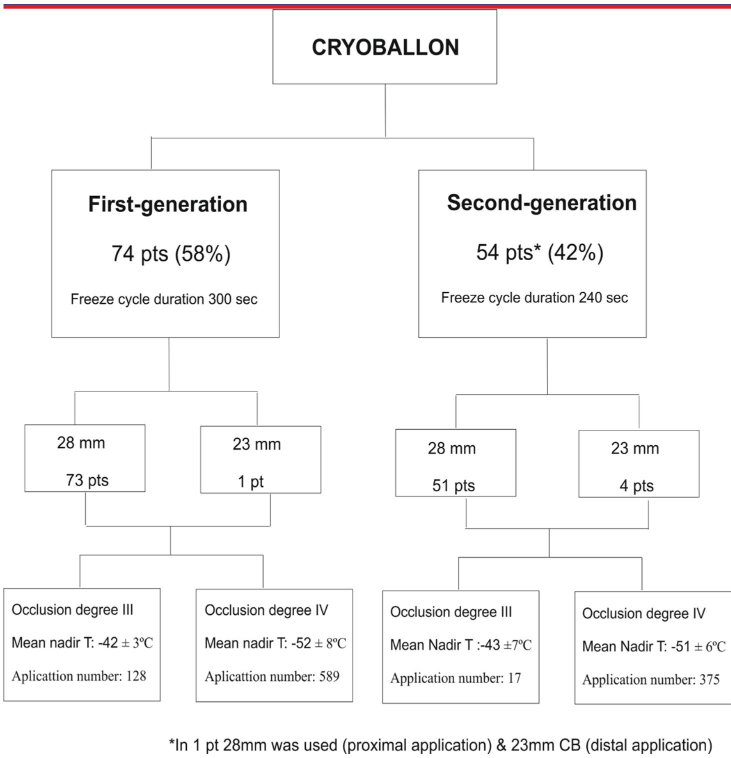

Figure 3: Diagram flow showing the type of balloon used for different group of pts, occlusion degree, and temperature reached

monitored in all cases during right-sided PV/CB applications, by placing the cuatripolar electrocatheter in SVC and pacing at 2.000 ms cycle length, checking the intensity of diaphragm contractions by intermittent fluoroscopy and tactile feedback placing the operator's hand on the patient´s abdomen, and immediately stop freezing when intensity of the diaphragm contraction weakens or is suddenly stopped.

Follow-Up Protocol: Before discharge the hospital, TTE was performed in all cases to rule out pericardial effusion and chest-X-ray taked in a deep breath, upright position, to confirm normal phrenic nerve physiology.

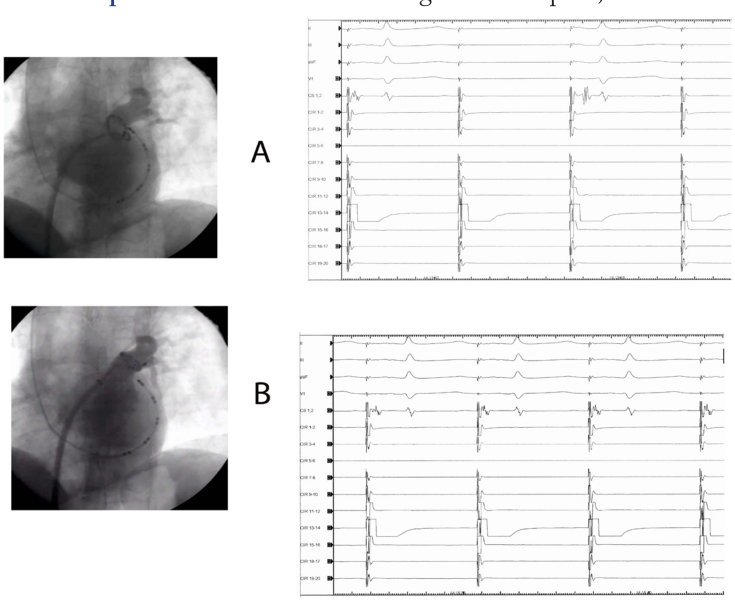

Figure 4: A.Upper panel: Left side: pacing proximal antrum (circular 13-14) showing exit block (right side). B. Lower panel: Left side (same patient): pacing distal vein (circular 13-14), 1:1 PV/LA conduction resumed (right side)

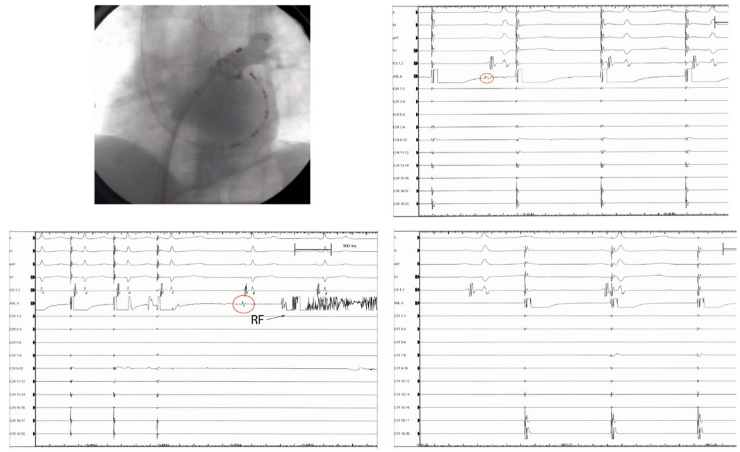

Figure 5: Same patient as figure 4 A, B. Upper panel right side: pacing gap (red circle) distal vein with RF catheter (left side) with: 1:1 PV/LA conduction (third and fourth paced beat) demonstration at the right side recording. Lower panel (left side): pacing gap RF catheter, 1:1 PV/LA conduction is evident (three paced beat). After stop pacing, RC gap is evident (red circle), followed by RF application. After focal RF, exit block is demonstrated (right side)

The immediate follow-up included holter monitoring at 7, 15,30,45,60 and 90 days respectively, and thorax CT-Scan at 30 and 90 days. All pts received AAD, mostly Class IC+ BtB, and oral anticoagulation with vitamin K antagonist dicumarol is started 1 day after PVI, targeting an INR within 2.0 to 3.0 range for at least three months, along with additional platelet inhibition agent (ASA, 100 mgrs/daily).

After a three-month blanking period on medication, all AAD were discontinued, and follow-up started to count. All pts were monitored by continuous daily trans telephonic information in case of symptoms, and monthly ECG holter monitoring was routinely done over 1411±727 days (46.6±24.2 months) of follow-up.

Results

Acute PVI And LA-PV Reconduction

A total of 483 PV including 29 CT (26 Left/3Right), were treated with CB and complete PVI demonstrated in 439 (91.9%). Acute reconduction post CB showed 44 PV (9%) 16 PV out of 483 (3.3%) after single CB. In 16 PV out of 483 (3.3%), RC was unmasked after AD, in 10 patients. In 12 PV out of 483 (2.7%), EMC could be demonstrated in 9 pts.

In six out of 16 of the acute reconnected PVs, RC appear after incomplete CB occlusion (degree III), (Figure 7) and in the same proportional rate after AD, (Figure 8.A,B), all eliminated by focal RF applications (Figure 8C).

Interestingly, all acute PV-LA RC (44 PV) occurred only with CB1.

Follow-Up: Follow-up of 1411±727 days started to count after three month blanking-period when AAD was discontinued.

Arrhythmia Recurrences: On follow-up, 14 pts (10.9%) out of 128 experienced clinical recurrence of the arrhythmia: 12 male (52±8) and 2 female (63±13) years respectively. Early recurrence occurred immediately at the early stage of follow-up in 9 males (mean age

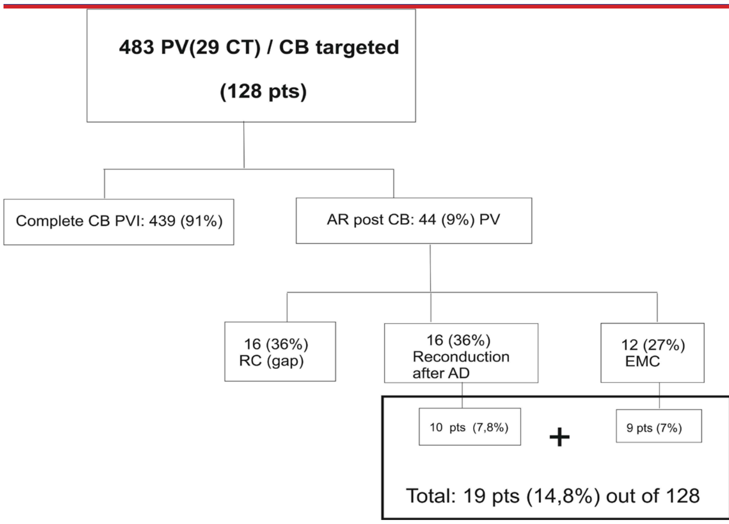

Figure 6: Diagram flow showing total acute reconnected PV and number of patients. AR: acute reconnection

50±7 years), when medication was stopped after the three-month blanking-period. Late recurrences occurred in 3 males (mean age 55±9 years) at 24, 27 and 60 months, and 2 female (mean age 63±13 years) at 7 and 40 months respectively.

All 14 recurrence pts allow for a second procedure (Redo).

In a Redo follow-up of 41±16 months, all 14 pts remain in sinus rhythm without medication.

The remaining 114 pts (89.1%) followed-up 1411±727 days, are asymptomatic, free of drugs, in sinus rhythm.

Seven pts (5.4%) had aphonia. Transient PNP: 7 (5.4%). Permanent PNP: 2 (1.5%), Pulmonary infiltrates (Figure 9): 5 (3.9%).Mild dyspepsia: 2 (1.5%), Severe intraprocedural bronchospasm: 2 (1.5%), Discrete hemoptoic sputum: 2 (1.5%).

No major side effects or complications occurred in our pts treated with CB, with no mortality, none atrioesophageal fistula, and none pulmonary vein stenosis.

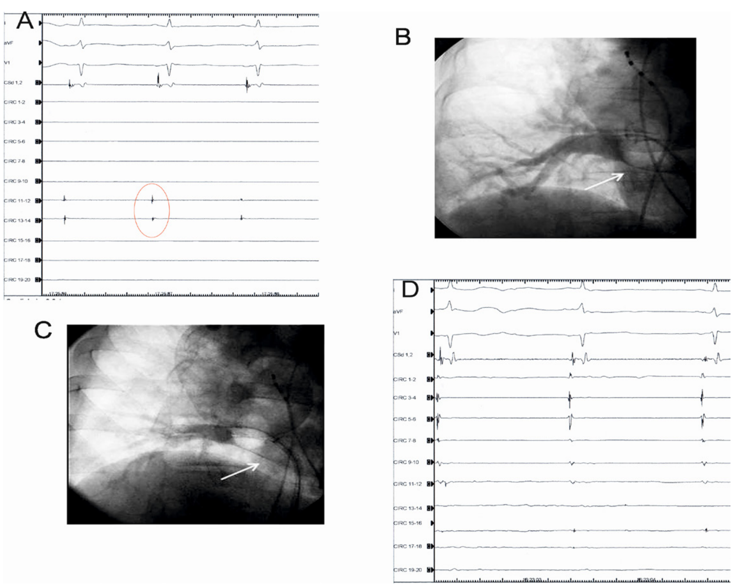

Figure 7: A, Residual conduction gap (red circle) evident after incomplete CB occlusion (B, C) (degree III) with contrast leakage evident (arrow) as compare with PV/LA electrical activity recording in the same patient before the incomplete CB application (D)

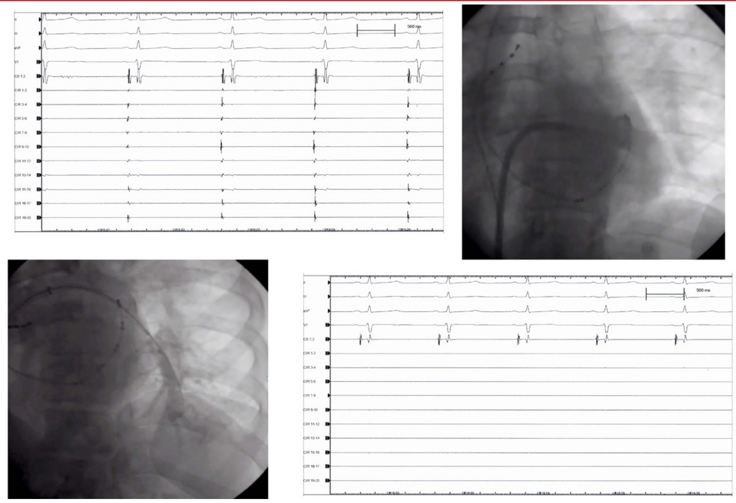

Figure 8A: Upper panel: showing PV electrical activity recorded at the 10 bipoles of the circular catheter mapping (left side) placed at the PV-LA junction antral level (right side). Lower panel: after CB application occlusion degree IV (left side), PV electrical activity is no more recording at circular catheter mapping (right side)

Side Effects And Complications Follow-Up: Aphonia: lasting ≤ 72 hours. Transient PNP: full complete recovery during the procedure. Permanent PNP: still evident on follow-up (1-3 years). Pulmonary infiltrates: In asymptomatic pts were shown at first month's CT-Scan control performed, there having been no evidence 2 months later on another CT-Scan routinely performed. Mild dyspepsia: quick complete resolution ≤ 72 hours on omeprazole and protective gastric diet. Discrete hemoptoic sputum: lasting ≤ 72 hours. Severe intraprocedural Bronchospasm: requiring 48 hours of treatment in the ICU.

Discussion

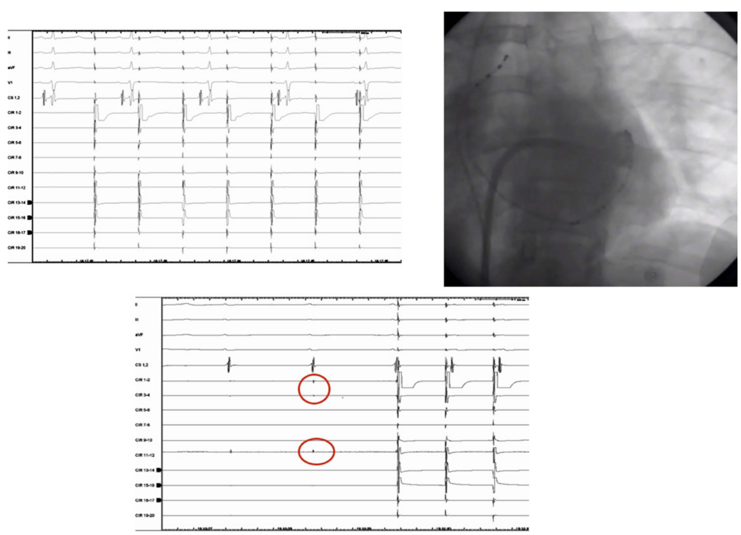

Figure 8B: Same patient as in Figure A. Upper panel: (left side): pacing (circular 1-2) at PV-LA junction antral level (right side), demonstrated exit block. Lower panel: dormant tissue unmasked by AD (red circle), at the time of complete A-V conduction block, and 1:1 PV-LA conduction demonstrated (second and third paced beats), by pacing gap

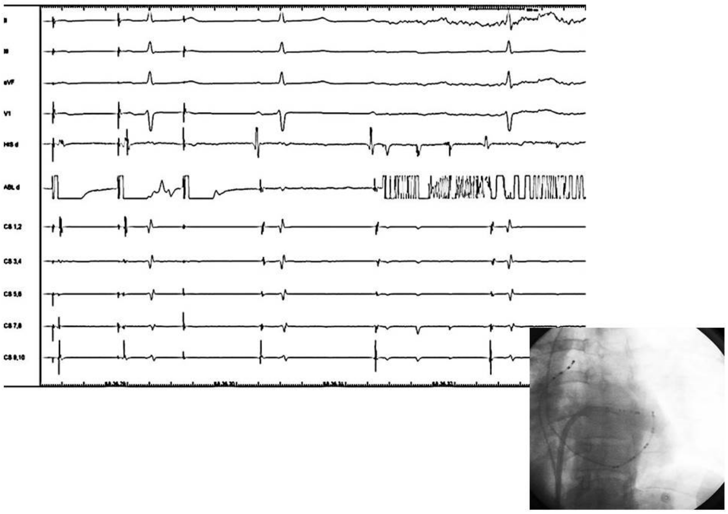

Figure 8(C): Same patient as in Figure 8A and B. By placing RF catheter (lower right side figure) on PV dormant tissue location unmasked by AD, and pacing gap from RF catheter (upper side recording) 1:1 PV-LA conduction showed on first and second (left side) paced beats during AD effect, as evident with completed A-V conduction block, followed by RF application

Over the last few years, CB ablation technology has emerged into the arrhythmia arena as an useful and safe tool to treat pts suffering from atrial fibrillation by achieving through its applications an acute electrical disconnection of the PV from the LA in a range of (90-100 %) in the majority of the series already published,[26] which is the main key to cure this arrhythmia.

Although in the majority of the clinical and randomized studies published, the results of the CB technique do not significantly differ in the short, medium and long-term outcome from those using RF as an energy source,[27-30] this "point to point" technique, can be more tedious, and time-consuming , most likely requiring better operator skill and involving an inherent clinically-significant risk of major complications, sometimes difficult to manage and treat, such as reentry left atrial tachyarrhythmia, thromboembolic events, pericardial effusion, PV stenosis or atrioesophageal fistula [31-34] which can be avoided or minimizing their incident by a "single shot" CB technique.

Since the first human experience published by Van Belle et al [5] treating pts suffering PAF with CB ablation, the technique has become widely-used as useful and safe tool to face the definitive treatment of this disturbing arrhythmia, by achieving ≥ 95% of acute electrical PVI in the majority of the series already published.[26]

Side Effects And Complications:

Aphonia: We cannot say for certain that this complication was

Table 3: Side effects and complications

TYPE	Pts
Aphonia	7 (5.4)%
Transient Phrenic nerve palsy	7 (5.4)%
Phrenic nerve paralysis	2 (1.5)%
Pulmonary infiltrates	5 (3.9)%
Dyspepsia	2 (1.5)%
Bronchospam	2 (1.5)%
Hemoptoic sputum	2 (1.5)%

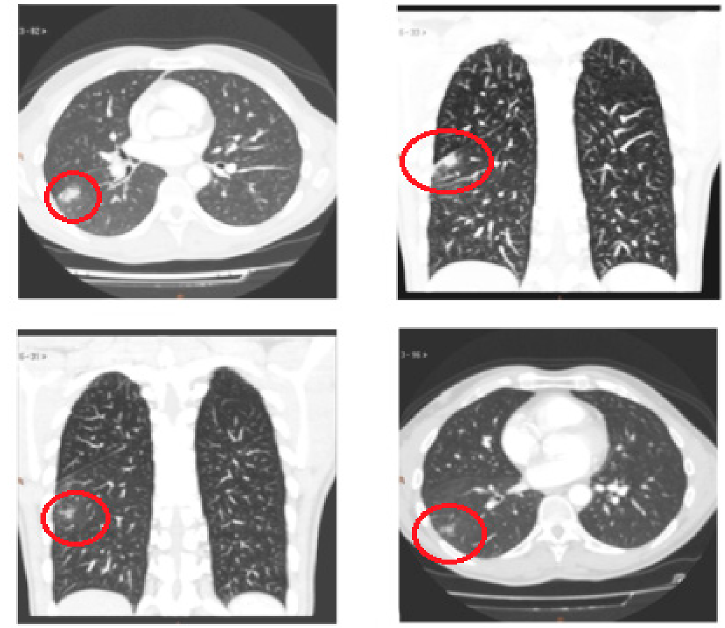

Figure 9: CT Scan slides showing pulmonary infiltrate (red circles)

strictly CB-related, first of all, because as far as we know, it has not been previously described, which is hardly surprising, especially after the findings described in the largest survey published so far,[35] focusing this complications topic in 500 consecutive pts. Although we can argue the possibility that this complication has been associated with endotracheal intubation maneuvers during general anesthesia, as orotracheal intubation, when difficult, can cause some laryngeal or vocal chord trauma, this however was not the case regarding our 7 patients affected in whom the orotracheal intubation, was smoothly performed and non-traumatic. Hence the most logical explanation for this symptom does not seem to have likely had anything to do with this orotracheal maneuvers. We raised the question, of a possible transient injury of the left recurrent laryngeal nerve, as has been warned by Cabrera and colleagues [36] in an unpublished abstract presentation at Hearth Rhythm 2011meeting, especially when CB applications take place deeper into the LSPV, along with the structural displacement towards the anatomical left recurrent laryngeal nerve bed, by strongly wedging the balloon in the venous ostium for a better occlusion.

Moreover, all 7 pts who experienced aphonia were treated with the CB2 which had been designed with a new technological implementation resulting in a more homogeneous intake and distribution of the refrigerant flow around the balloon sphere, increasing the surface contact cooling that might induce deeper lesions with greater likelihood of affecting more extracardiac structures.[35, 39]

PNP occurred in 9 pts (7 %). Only 2 are still permanent after 1 and 3 years follow-up respectively, with not clinical compromise, doing a normal life and completely asymptomatic. This incidental complication rate is consistent with the majority of the largest CB series already published: 4.7%,[10-37] 7%,[5] 7.2%,[35] 7.5%,[8] with some discrete higher incidence 11.1% [9] and 11.2%.[11]

In our series, the common characteristic of this complication was the lower nadir temperature level reached, of ≤ 60º C in the majority of cases (78%) (Table 4). As the majority of pts were treated with the CB1 (74 out of 128), the PNP balloon-related rate was: 5.4% CB1 vs 9.2% CB2 (5 pts out of 54). This higher PNP rate occurring with CB2 as compared to CB1 has been showed by others.[38, 39] In 340 consecutive pts treated by Aryana et al [38] with CB1 (140) and 200 with CB2, PNP occurred with CB1 in 12.1% pts vs 16.2% when CB2 was used. In a similar difference percentage rates Fürnkranz et al [39] reported 8.7 % of PNP with CB2 vs 5.7% when CB1 was employed.

Important to remark in our study that the 2 pts with persistent PNP after 1 and 3 years of follow-up, PNP occurred suddenly at 100 and 156 seconds of CB1 applications, when the lowest temperatures of -70 and -68ºC were respectively reached. In the other 7 pts with transient PNP, the CB applications were immediately stopped as soon as weakness of the diaphragm intensity contraction was adverted.

Table 4: Ocurrence of PNP related to the CB used, time of application and nadir temperature reached

TRANSIENT PHRENIC NERVE PALSY					
	TºC	Seconds	CB mm	CB Generation	78% Mean TºC≥ -60ºC
1	-68	122	28	FIRST	
2	-73	222	28	FIRST	
3	-55	89	28	SECOND	
4	-56	165	23	SECOND	First Gen CB: 55.5%
5	-60	115	28	SECOND	
6	-68	100	28	SECOND	
7	-65	190	28	SECOND	
PERMANENT PHRENIC NERVE PARALYSIS					Second Gen CB: 45.5%
1	-70	100	28	FIRST	
2	-68	156	28	FIRST	

The highest level of PNP (19%) reached with the CB2 observed by Cherchia et al [40] related with the lower CB temperature reached, has move to this group to stop CB applications when -60 º C nadir temperature level is reached, in addition to limiting the freeze application time to 180 seconds, in an attempt to avoid major complications.[41,42,35] At the same time, these authors have proposed a modification technique to prevent PNP, consisting after tight wedging of the inflated CB inside the RSPV ostium, to withdraw it until a small leak of contrast is observed, since the CB volume increases slightly at the onset of CB application. This technical maneuvers described by Casado-Arroyo et al [43] from the same Brussel's group, offers the advantage of a more proximal CB application and it has been suggested to use by others.[44] Martins et al suggest the use of Casado-Arroyo technique, particularly when the vertical projection of the PN reaches the distal part of the CB (Zone B1 in their study) with a 98% of negative predictive value, and Ströker et al [45] had recently emphasized the need to perform a preprocedural anatomic assessment, in order to evaluate the risk of PN injury, such PV orientation, larger PV dimensions, shorter distance to SVC, the presence of early branches originating from the main ostium, and right –sided long CT; anatomical variations which were associated with PN injury.

Pulmonary Infiltrates: Of unknown origin, mostly showed on the right side in distal pleural location, found in 5 asymptomatic pts (3.9%) on CT-Scan control routinely performed 1 month after the procedure, which were no longer evident at the 3-month control CT-Scan performed, at follow-up. Those pulmonary infiltrates, radiologicaly in appearance of inflammatory aspect, producing no clinical impact on pts, strongly suggest a probably origin related with the transmission of cold into the lung parenchyma during CB application, as it has been experimentally demonstrated in dogs [46] as small subtle foci of ablated –related superficial pleural fibrosis.[47]

Bronchospasm: Severe intraprocedural bronchospasm occurred in two pts who had a past medical history of mild chronic bronchitis whom required medical treatment for ≤ 48 hours in the ICU. This complication might have a difficult explanation and could have been due to a combination of several factors working together, such as prior bronchial damage in pts with chronic bronchitis, the possibility of major injury due to ice formation inside the bronchial lumen [48] as well as the possible trigger effect of AD which, although anecdotic, has been described.[49]

Hemoptoic Sputum: Two pts presented discrete hemoptoic sputum on the immediate post- procedure, being otherwise on oral anticoagulation treatment regime and completely asymptomatic. This type of complication might also be due to several factors working together or may even have a different origin. Firstly, as it has been experimentally demonstrated,[50, 51] the expansion of ice within the fragile microvasculature leads to the interruption of vascular integrity, which is the reason for the intramyocardial hemorrhage, as well as the hemoptysis associate with cryo injury to the lung tissues, and secondly, the possibility of bronchial erosion as has been demonstrated [52] as a cause of hemoptysis.

Dyspepsia: Two pts complain of mild dyspepsia. As no esophagogastroduodenal endoscopy study was performed, we cannot assure it was related or not, with some reversible esophageal ulceration.[53]

Epicardial PV-LA muscular connections: Electrically functioning EMC with PV-LA 1:1 conduction (Figure 4 A,B) demonstrated, was found in our pts using this protocol in 12 PV (2.5% of total 483 PV), totaling 27.2% of the all post CB- PV reconducted (44 PV), in 9 pts (7%).

Since the first human demonstration of the presence of electrical conduction between PV was made,[54] other investigators had demonstrated the incidence of the interpulmonary vein electrical connections as being responsible of the maintenance of the arrhythmia in a single pt with PAF,[55, 56] and Takahashi et al demonstrated in 49 consecutive pts, the presence of electrical connections between contiguous PV in 14% of the pts underwent atrial RF catheter ablation to treat their drug-resistant AF.[57]

Perez- Castellano et al [58] using RF catheter ablation for ostial PVI in 100 consecutive pts with drug-refractory atrial fibrillation, found in 3% of the veins, venoatrial epicardial connections inserted at distance from the venous ostium, and 10% with epicardial connections between the ipsilateral PV in 20% of pts resistant to atrial ablation, suggesting a different disconnection approach for PV showing those extrapulmonary epicardial connections associated with an increased rate of early recurrence of conduction.

The morphological evidence of these muscular connections between contiguous veins has been demonstrated by Cabrera et al,[59] confirming the anatomical underlying substrate of such electrically-functioning connections. More recently, Squara et al [60] have demonstrated the prevalence of those electrical connections between ipsilateral pulmonary veins and their implications for ablation and AD testing in 30 pts submitted to RF catheter ablation. They found a high presence of ipsilateral PV connections after antral PVI in up to 65.6% of total PV sets without carina ablation, lowered to 17.7% when the carina ablation was performed, emphasizing the need for carina ablation. Squara et al [60] also described acute reconnection of at least 1 of the PV to the LA, in 18% of the PV sets.

In our work 44 PV (9.1%) of the total PV faced (483) showed acute reconduction after single CB (Figure 6).As we have not conducted any previous study to assess the prevalence of these possible direct connections between ipsilateral pulmonary veins, and quantifying the incidence rate that the electrical impulse originating from 1 PV would propagate to the adjacent ipsilateral vein, as well as the indirect connections LA muscular sleeves, the only way to demonstrated some EMC in a practical clinical setting is the demonstration of complete BB at the PV-LA junction antral level by pacing from all 10 pairs of poles of the duodecapolar circular catheter mapping, following by demonstration that 1:1 PV-LA conduction resumed by pacing distal vein (Figure 4.A,B) no further than 5-10mm from the endocardium of the interpulmonary isthmus, as Cabrera et al [59] have demonstrated as the limit of distance where the insertion of the muscular connections, can be founded.

By doing so, we found electrically -functioning EMC in 12 (2.5%) of the total PV-LA reconnected after CB-PVI, totaling 27% of the all early- reconducted veins, which is consistent with the figures published by others,[58] having carried out the same protocol, to rule-out EMC, by pacing distal vein after PVI-atrial isolation. Another interesting aspect to be taken in account is that, in the majority of CB-applications the interpulmonary ridge of the PV isthmus at the carina level is, affected, regardless of PV anatomy, (except for long CT) affected by cryo lesion at the endocardial superior and inferior aspects during CB applications at the superior and inferior PV.

Interestingly, those 12 EMC were demonstrated after acute BB was achieved in 9 pts, totaling a 7% of the total population of pts treated.

AD Protocol And Acute Early PV-LA Reconnection: A total of 483 PV including 29 CT were treated with CB and complete CB-PVI demonstrated in 439 (90.9%). Acute reconduction post CB was shown 44 PV (9.1%) (Figure 6): Sixteen PV (3.3% of the total PV; 36% of the reconducted ones), show acute reconnection due to incomplete lesion with “dormant tissue” unmasked by AD,[17,18] by inducing hyperpolarization to restore excitability by activating AD-sensitive potassium channels restoring conduction of dormant PV as it has been better clarified and demonstrated by Datino et al[61]

Arrhythmia Recurrence And Reconduction: For analysis of the location of conduction gaps, the ipsilateral LA-PV junction was divided into four segments in a clockwise sense, starting at 10 o’clock

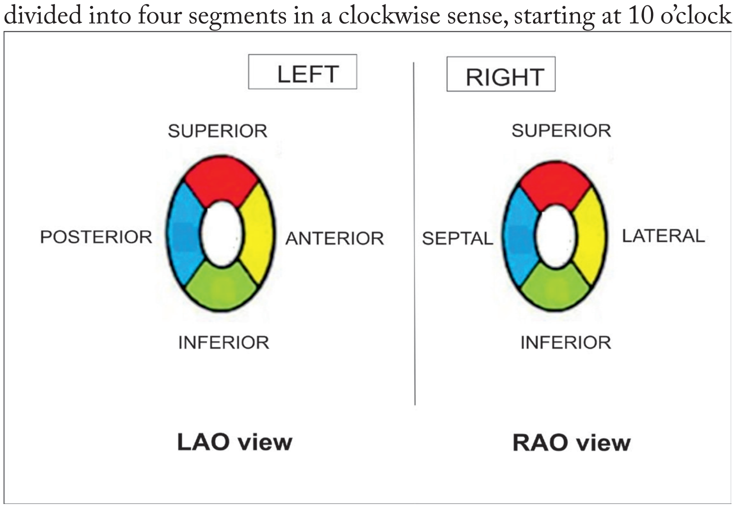

Figure 10: Diagram representation for the different number of RC found in the different segment location

(Superior, inferior, anterior, and posterior, for left PVs, and superior, inferior, septal, and lateral, for right PVs) (Figure 10).

Forteen pts (10.9%) had clinical recurrence of the arrhythmia and allow for a Redo. Fifty-four PV including 2 CT were newly CB treated in a Redo with CB2. Reconduction was encountered in 29 PV (53.7%) in different segment locations (Figure 10.A) revealing the almost even distribution of RC in the superior segments, with greater number of reconductions shown at the inferior aspect of the LSPV. Also the PV showing the largest number of reconduction was the LSPV (37.9%) (Table 5).

Finally, the RC segment distribution on Redo cases was random, being unrelated to those shown at first procedure (Figure 10.B).

Fürnkranz et al [15] find the inferior segments of the LA-PV junction most often affected by reconduction in addition to the LSPV having shown the high rate of reconduction (63%), suggesting that the superior ridge may have contributed to this relatively high rate of reconduction in this LSPV.

In addition to the above, Fürnkranz et al hypothesized in their aforementioned study that the high incidence of inferior conduction gaps might be due to different causes, related to the difficulty sometimes involves in deflecting the sheath/balloon system in order to reach the inferior aspect of PV, resulting in incomplete balloon-time contact. Conversely when approaching superior PV, both sheaths and balloon can be used to create a strong push onto the PV ostium to occlude the blood flow, achieving better occlusion and more permanent tissue cryolesion.

In the original study conducted by Chierchia et al,[19] enrolling 39 pts treated for PAF with CB1, AD testing after CB induced a LA-PV reconnection only in 7 (4.6%) PV which often occurred in the inferior aspect of the lower veins, especially of the right inferior. All these RC gaps being eliminated by further CB applications or focal cryo “Touch-up”. Chierchia et al,[16] have also shown a 2.8% early spontaneous reconnection after 30 minutes of CB applications in a cohort of 26 pts treated for PAF with CB1.More recentlyin a study conducted by Ciconte et al [20] in 50 consecutive pts treated for PAF or early persistent AF ≤ 6 months, with CB2, spontaneous (4 veins) and AD-induced (4 veins) PV reconnections occurred in the 4% of initially isolated veins (8 veins) in 6 pts (12%).

Our results are consistent with the aforementioned studies, entailing 36% totally reconnected PV showing spontaneous reconduction, totaling 3.3% of the total 483PV-CB treated. Beside the highest reconduction gaps on the inferior aspect of the LSPV, conversely to the segment location reconduction showed by Fürnkranz et al [15] in 26 pts refered for RF PV ablation after CB first procedure failed, inferior segments showed gaps in 85% and 77% at the lateral and septal location respectively and 42% and 31% respectively at the

Table 5: Number of PV showing reconduction and their percentages of the total PV reconnected

14 pts: 54 PV (2CT)

29 PV reconnected (53.7%)

PV	nº	%
CT	2	6,8%
LSPV	11	37,9%
LIPV	7	24,1%
RSPV	4	13,7%
RIPV	5	17,2%

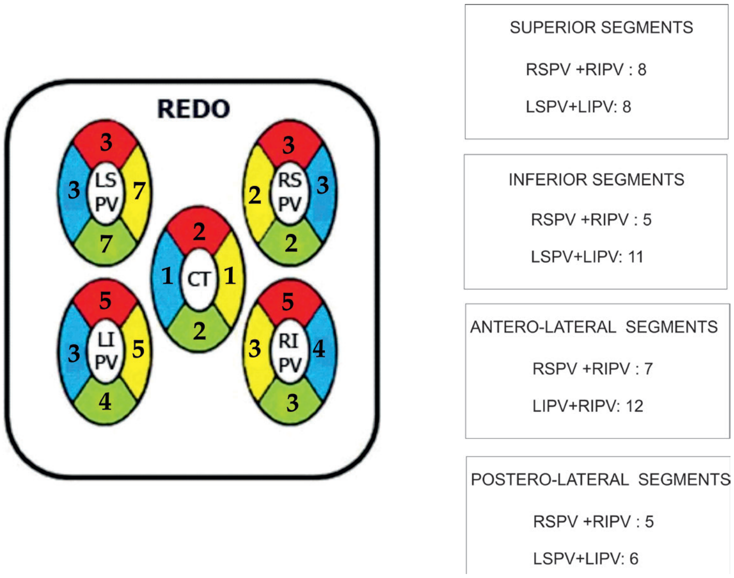

Figure 10A: Segment distribution appearance of RC gaps

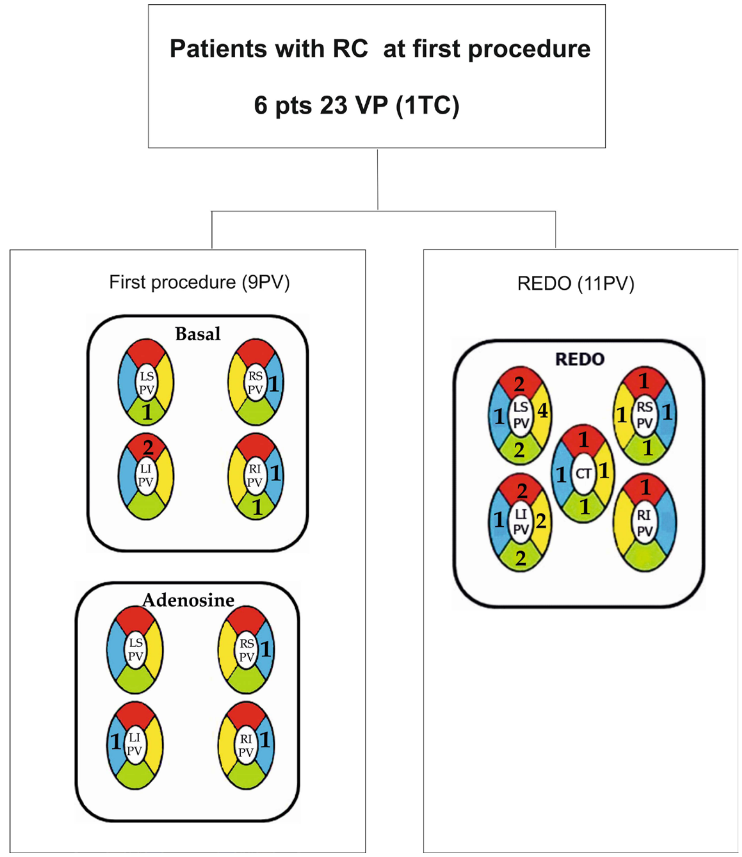

Figure 10B: Reconduction was shown at first procedure in 6 pts with 23 PV including 1 TC. Left side upper figure showed number of gaps after CB, and unmasked by AD (lower left figure), as compared to number and distribution of gaps showing at Redo (right figure) on the same pts

lateral and septal aspect of the superior segments, our segment reconduction locations showed a most uniform distribution.

One possible underlying explanation for this, perhaps being the fact that in our pts all procedures were performed by the same operator (JMP) and were evaluated individually case by case, based not only the size of balloon to be used, but also on the orientation to be applied [15] according to PV anatomy, morphology, and angle direction, previously assessed with CT-PV slide reconstructions, in conjunction with the aforementioned endoluminal anatomical approach (Figure 1). All of these factors combined might play an important role toward achieving better occlusions and more uniform lesions adding to minimize possibilities of PV-LA reconduction, which is the principal cause of clinical arrhythmia recurrences.

We have not done any protocol to rule out non PV-Foci, as a potential cause of arrhythmia recurrence,[62] given that all recurrences were Redo, and PV-LA reconduction was evidenced in all cases.

Patient's Risk Profile Of Recurrent Arrhythmia

We have not calculated the individual risk of recurrence of the arrhythmia based on the clinical patient data profile to assess the ALARMEc score, proposed by Neumann's group,[63, 64] but rather a rough estimate for our entire pt population treated, without atrial enlargement, suffering PAF, having normal renal function, with glomerular filtration rate ≥ 68 mL/min, none with structural heart disease, and only 33% of them with some metabolic disorder. We can approximate and estimate an ALARMEc score ≤ 1, according to the most favorable outcome of these patients, in whom the arrhythmia substrate mostly underlying on PV triggers.

Duration Of The Procedure

We experience a rapidly important decrease on the total duration time of the procedure in relation with the learning curve.[65] For the first 10 cases performed the mean time duration of procedure (since the transeptal approach to withrawal LA catheters) was 361 minutes and the mean fluoroscopy time: 86 minutes. For the last 20 pts treated, the mean duration time was 150 ±39 minutes, and fluoroscopy time 35±10 minutes.

Statistical Analysis

Continuous variables are expressed as mean ± SD.

Categorical variables are expressed as percentages.

Study Limitations

This study has some limitations. First, this is a single center study. Second, because of the large interval of time (monthly) between holter monitoring recording on follow up, to detect arrhythmia events, along with patients whom eventually do not feeling symptoms, the success rate may have been overestimated, as none of the patient population included in the study were monitored with and implantable loop recorded.

Conclusions

The results and follow-up of our series of 128 pts treated for PAF over 7 years, allow us to conclude: 1. Cryoenergy PV applications doesn't induce a homogeneous circumferential lesion in all PV which dependents on the PV anatomy, shape, size and thickness as well as the uniform distribution of cold and temperature reached at the PV-LA junction level which is the main cause of spontaneous reconnection or incomplete lesions with dormant tissue. 2. Routine use of AD after acute CB-PVI allowed-us to identify incomplete lesions with dormant tissue in 7.8% of pts. 3. Electrically functioning EMC might be identified in 7% of pts by pacing distal vein after complete antral PVI. 4. In summary, checking for BB, AD protocol and, pacing distal vein after PVI to rule out EMC allowed-us to identified 14.8% of pts with underlying tissue substrate for potential arrhythmia recurrence. 5. All residual gaps can be eliminated by further CB shots or focal RF applications. 6. All RC gaps occurred only with CB1 applications. 7. CB2 applications by inducing a wider and deeper lesion, minimize the RC gap appearance wich entail a lower arrhythmia recurrence rate, but involving a higher risk of damage extracardiac structures, such us PNP (9.2% CB2 vs 5.4% CB1). 8. Single CB technique is highly effective and safe for the definitive treatment of pts suffering from PAF with a 72.6% success rate, increasing up to 89.1% when this protocol is applied in a single procedure. After a second procedure performed in recurrences pts, the entire pt population group (100%), remain in sinus rhythm, free of arrhythmia, without AAD, in this very long term follow-up. 9. However, late recurrences, generates some concern about greater increased number of pts with recurrent arrhythmia over a longer course of time, especially for pts whom don't feel symptoms of the arrhythmia. 10. Patients with an estimated low ALARMEc score (≤ 1) have an excellent long term outcome. 11. Finally, to the best of our knowledge, this series includes the largest follow-up described to date, in pts treated for PAF with CB technique.

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