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CRYOABLATION OF PARA-HISIAN AND MID-SEPTAL ACCESSORY PATHWAYS:

LONG-TERM OUTCOME OF A SPECIFIC CRYOABLATION PROTOCOL

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1. INTRODUCTION

1.1 SUPRAVENTRICULAR TACHYCARDIAS

Supraventricular tachycardias (SVTs) denote all tachyarrhythmias that originate from supraventricular tissue or require it to be a part of the reentrant circuit. This term is used to describe any heart rhythm disturbances generated by cardiac tissue above the ventricles with ventricular rate exceeding 100 beats/min with a narrow QRS complex (QRS width, <120 ms) or, less commonly, wide QRS complex (QRS width, >120 ms) tachycardias. The increased QRS width is due to concomitant aberrancy of the normal conduction system, such as that associated with bundle branch block [1].

SVTs include a large spectrum of tachyarrhythmias (Table 1) characterized by different pathophysiologic mechanisms. SVTs are relatively common, often repetitive, occasionally persistent, and rarely life-threatening. The precipitants of SVTs vary with age, gender, and associated comorbidity.

SVTs are frequent cause of emergency room and primary care physician visits, and have an impact on quality of life, which varies according to the frequency and duration of episodes. Patients might be also completely asymptomatic, or feel isolated palpitations, fatigue, light-headedness, chest discomfort, dyspnea, or even present with presyncope and syncope if tachycardia is severe [2-6].

Direct risks due to SVTs are unusual, but in specific situations (e.g. in patients with Wolff-Parkinson-White syndrome and concomitant atrial fibrillation) may lead to sudden cardiac death [7].

The incidence of SVT is approximately 35 per 100000 person-years with a prevalence of 2.25 per 1000 persons in the general population. The presence of a regular and rapid tachycardia with abrupt onset and termination define a distinct clinical subtype defined paroxysmal supraventricular tachycardia (PSVT) [8].

When adjusted by age and sex, the incidence of PSVT is estimated to be 36 per 100000 persons per year [8]. There are approximately 89000 new cases per year and 570000 persons with PSVT [8]. Compared with

patients with cardiovascular disease, those with PSVT without any cardiovascular disease are younger and have faster PSVT. Women have twice the risk of men of developing PSVT [8,9].

Individuals >65 years of age have five times the risk of younger persons of developing PSVT [9,10].

Atrioventricular node reentry tachycardia (AVNRT) is the most frequently type of SVT after atrial fibrillation (AF), followed by atrial flutter and atrioventricular reentry tachycardia (AVRT).

Table 1. Conventional classification of supraventricular tachycardias. 2019 ESC Guidelines for the management of patients with supraventricular tachycardia. European Heart Journal (2019)

Atrial tachycardias
Sinus tachycardia
• Physiological sinus tachycardia
• Inappropriate sinus tachycardia
• Sinus nodal re-entrant tachycardia
Focal AT
Multifocal AT
MRAT
• Cavotricuspid isthmus-dependent MRAT - Typical atrial flutter, counter-clockwise (common) or clockwise (reverse) - Other cavotricuspid isthmus-dependent MRAT • Non-cavotricuspid isthmus-dependent MRAT - RA MRAT - LA MRAT
AF
AV junctional tachycardias
Atrioventricular nodal re-entrant tachycardia (AVNRT)
• Typical
• Atypical
Non-re-entrant junctional tachycardia
• JET (junctional ectopic or focal junctional tachycardia)
• Other non-re-entrant variants
Atrioventricular re-entrant tachycardia (AVRT)
• Orthodromic (including PJRT)
• Antidromic (with retrograde conduction through the AVN or, rarely, over another pathway)

AT, atrial tachycardia; MRAT, macro-re-entrant atrial tachycardia; RA, right atrial; LA, left atrial; AF, atrial fibrillation; AV, atrioventricular; PJRT, permanent junctional reciprocating tachycardia; AVN, atrioventricular node;

1.2 MECHANISMS OF SUPRAVENTRICULAR TACHYCARDIAS

The two principal mechanisms of the genesis of cardiac dysrhythmias, included SVTs, are automaticity and reentry [11,12].

1.2.1 Automaticity

Automaticity is a normal property of some specialized cardiac cells; it is responsible for sinus node function and for the appearance of subsidiary ("escape") pacemaker activity seen in atrial, atrioventricular (AV) nodal, and His-Purkinje cells. Common automatic dysrhythmias include sinus tachycardia and multifocal atrial tachycardia [11,12].

1.2.2 Reentry

Excluding sinus tachycardia, the vast majority of sustained SVTs are due to reentry [13].

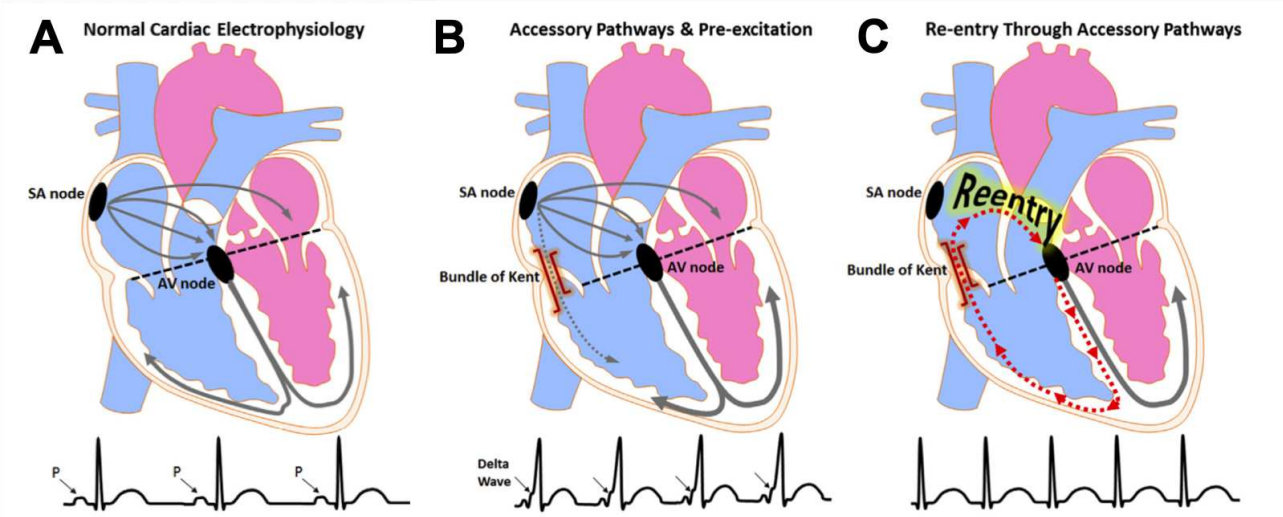
In the normal heart, conduction of electrical impulses proceeds rapidly and uniformly, from the sinus node, through the AV node, to the specialized conducting tissues of the ventricles (Figure 1-A). Normal conduction depends upon electrical homogeneity: adjacent conducting pathways have similar refractory periods and conduction velocities. In contrast, in most patients who are prone to develop paroxysmal supraventricular reentrant tachycardia, two or more anatomically or functionally separate conducting pathways exist, which differ in refractoriness and conduction speed. During sinus rhythm, impulses conduct over both pathways but reach the His bundle or the ventricle via the rapid pathway.

In this condition the electrocardiogram demonstrates only fast pathway conduction (short PR interval and delta wave) (Figure 1-B) [14].

In the presence of reentry, SVT is initiated when a critically timed atrial premature contraction occurs and finds one pathway refractory. The impulse is then conducted over the nonrefractory pathway. If the first pathway is capable of retrograde conduction and is excitable, the impulse can travel back to its point of origin, thus completing the reentry circuits [11,13,14]. It is the presence of at least two distinct pathways, with unidirectional block in one of them, that creates the potential for a reentrant (or "circus") tachycardia (Figure 1-C) [12,15]. Conduction velocity in the anterograde limb must be slower than the refractory period of the return limb; the leading edge of the wave front must always meet excitable tissue, or the tachycardia cannot be sustained [13]. The two pathways that participate in a reentrant tachycardia may be anatomically distinct. One example is the accessory Kent bundle of the Wolff-Parkinson-White (WPW) syndrome [16]. Sometimes, the two pathways cannot be distinguished anatomically, and in most patients with SVTs, the two pathways both lie within or near the AV node.

As shown in table 1, SVT includes several distinct tachycardias that are classified according to the anatomic location of the subsidiary pacemaker activity or of the reentry.

Figure 1. Schematic representation of cardiac conduction system and reentry mechanism.
Image adapted from Salah S. Al-Zaiti et al. Paroxysmal Supraventricular Tachycardia. Pathophysiology, Diagnosis, and Management. Crit Care Nurs Clin North Am. 2016.



In normal sinus rhythm, electrical impulses (gray arrows) travel through the AV node using homogenous adjacent fibers. The result is narrow QRS complexes preceded by P waves, with regular P-R intervals (A). Sinus rhythm when an accessory pathway exists (eg, bundle of Kent, bars), the electrical impulse bypasses the AV node and results in premature excitation of the ventricles; the result is short P-R intervals with a delta wave that widens the QRS complex (B). The accessory pathways typically have a longer refractory period, so a critically timed premature beat can be conducted through the AV node but not the accessory pathway. Upon the ventricular excitation, the accessory pathway is ready for excitation and is used for retrograde conduction and circuit formation (dotted line) (C).

2. ATRIOVENTRICULAR REENTRY TACHYCARDIA

Atrioventricular reentry tachycardias are SVTs that use an anatomically defined re-entrant circuit that consists of two limbs: first, the AV node – His Purkinje system (HPS), and second, an accessory pathway (AP) also called Kent bundle. The two limbs are characterized by differences in refractoriness and conduction times, with critically timed premature atrial or ventricular beats initiating re-entrant tachycardia (Figure 1-C). On rare occasions, the circuit consists of two APs.

3. ACCESSORY PATHWAY

Atrial septation during cardiac development is an extremely complex process. The concept of converting a single myocardial tube into four cardiac chambers involves multiple mechanisms, including looping, the formation of extra cardiac mesenchyme, the segmental transformation of endocardial endothelium into valvulo-septal mesenchyme, and finally the remodeling of the myocardial epithelium into muscular partitions [17]. It is important to note that the septation process is reflected in the position of the primitive conduction system. At the level of the atrium, the sinus venosus and sinoatrial transition to form the sinoatrial node [18]. During this period of development, multiple APs run through the areas that are to become the right and left cardinal veins, pulmonary veins, coronary sinus, and right atrioventricular ring [19]. However, in the mature adult heart, this primitive conduction tissue is not recognizable anymore, and usually undergoes electrical insulation via formation of the annulus fibrosus. When insulation does not occur appropriately, the result is the persistence of APs [19,20]. Most APs are found within the parietal atrioventricular junctional areas, including the para-septal region [19]. These APs consist mostly of myocardium and are rarely specialized cells. Since they are derived from the same precursor, many of their characteristics are similar to normal cardiac

myocytes [19]. However, refractory periods during cardiac repolarization differ in length and provide the ability for faster conduction or faster preexcitation.

APs present characteristic electrophysiological features that differ from AV nodal conduction properties. They typically exhibit fast conduction, dependent on a sodium current similar to that of myocardial cells.

At the end of this process, APs result in single or multiple strands of myocardial cells that bypass the physiological conduction system, and directly connect atrial and ventricular myocardium [19].

APs are considered “manifest” if they conduct in the anterograde direction, demonstrating ventricular pre-excitation (VPE) with a delta wave on surface 12-lead electrocardiogram (ECG) (Figure 1-B). Manifest pathways occur in 0.1% to 0.3% of the population and may conduct in both the anterograde and retrograde directions or, less commonly, only in the anterograde direction [19]. Conversely, “concealed” APs conduct only in the retrograde direction and therefore do not cause VPE on the 12-lead ECG.

APs are commonly located along the tricuspid or mitral annulus or within the subepicardial pyramidal space in the septal region forming connections between atrial and ventricular tissue in addition to that of the normal conduction system and AV node.

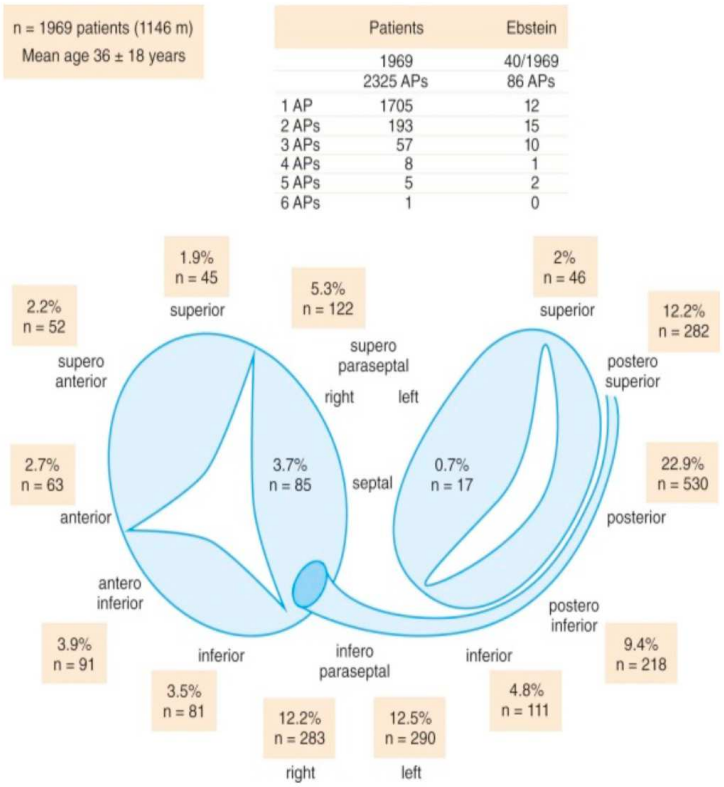
The most common are those that connect the atrium and the ventricle along the mitral or tricuspid annulus. Approximately 60% are located along the mitral valve and are referred to as left free wall APs, 25% insert along the septal aspect of the mitral or tricuspid annulus, close proximity to the His bundle and AV node, and a low percentage insert along the right free wall [21-24, 25].

According with the nomenclature introduced by NAPSE and ESC in 1999 [26], accessory pathways are also defined basing on the anatomical distribution in three main groups and relative subgroups as shown in Table 2 and schematically represented in Figure 2.

Table 2. nomenclature and terminology of accessory pathways based on anatomical positions.
Living anatomy of the atrioventricular junctions. A guide to electrophysiological mapping. European Heart Journal (1999)

Right	Superior
	Supero-anterior
	Anterior
	Infero-Anterior
	Inferior
Left	Superior
	Supero-posterior
	Posterior
	Infero-posterior
	Inferior
Septal/paraseptal	Superoparaseptal
	Inferoparaseptal
	Septal

Figure 2. Distribution of accessory pathway location. Schematic of tricuspid and mitral atrioventricular (AV) rings, in a left anterior oblique projection, showing the locations of accessory pathways from 1969 consecutive patients. The table shows the number of accessory pathways per patient in those with and without Ebstein anomaly. From Ernst S, Ouyang F, Antz M, et al: Catheter ablation of atrioventricular reentry. In Zipes DP, Jalife J, editors: Cardiac Electrophysiology from Cell to Bedside, ed 4, Philadelphia, 2004, WB Saunders, pp 1078–1086.



4. WOLFF PARKINSON WHITE SYNDROME

In 1921, a phenomenon of "intraventricular block and a PR interval of 0.08 ms" in a 19 years-old patient with paroxysms of tachycardia was described by Wedd [27]. In 1930, Louis Wolff, John Parkinson, and Paul D. White, described a set of eleven healthy young patients with Bundle-branch block with short P-R interval prone to paroxysmal tachycardias [28].

"WPW syndrome" refers to the presence of ventricular pre-excitation on surface ECG plus the presence of symptoms suggestive of arrhythmia related to the pre-excitation, such as palpitations, episodic lightheadedness, pre-syncope, syncope, or cardiac arrest.

In the general population, the prevalence of VPE pattern on surface ECG ranges from 0.15-0.25% [29], increasing to 0.55% among first-degree relatives of affected patients [30]. However, not all these patients develop AVRT and consequently WPW syndrome. Compared with the remaining population, the pre-excitation population is generally younger, predominantly male, and has less comorbidity [19,31].

In most cases, APs giving rise to the VPE pattern are seen in structurally normal hearts.

The most common tachycardia associated with the presence of AP is the AVRT.

Two mechanisms of re-entry are possible according to the antegrade or retrograde conduction over the AV node-AP and are classified as orthodromic and antidromic AVRT.

Orthodromic AVRT recognize a circuit where the re-entrant impulse conducts from the atrium to the ventricle through the AV node - HPS, which is the anterograde limb of the re-entrant circuit, whereas the AP conducts from the ventricle to the atrium and serves as retrograde limb of the re-entrant circuit.

Orthodromic AVRT accounts for approximately 90% to 95% of AVRT episodes in patients with accessory pathway, and for 20-30% of all sustained SVTs.

Antidromic AVRT occurs in 3-8% of patients with WPW syndrome. The impulse travel from the atrium to the ventricle through the AP with anterograde conduction, meanwhile retrograde conduction occurs via the AV node.

AVRT in WPW syndrome is not per se a life-threatening condition, but non-decremental property of the AP holds the key to the potential for sudden death in patients with the WPW syndrome.

Paroxysmal AF has been found in 50% of patients with WPW syndrome. In these patients, AF may result in extremely rapid conduction to the ventricle, very frequent ventricular depolarization and high rates could lead to degeneration into ventricular fibrillation (VF) and sudden cardiac death (SCD). This dramatic event can result in patients with a manifest accessory pathway, with a 10-year risk ranging from 0.15% to 0.24% [32,33]. Unfortunately, SCD may be the first presentation of patients with undiagnosed WPW syndrome.

Increased risk of SCD is associated with a history of symptomatic tachycardia, multiple accessory pathways, and a shortest pre-excited R-R interval of <250 ms during AF.

The risk of SCD associated with WPW syndrome appears highest in the first 2 decades of life [32-37].

In 1998, M. Arruda et al. developed an algorithm for accessory pathway localization correlating the surface 12-lead ECG pattern with the successful ablation site in 135 patients with manifest anterogradely conducting pathways. The authors demonstrate that the vector of the initial portion of the surface delta wave in leads I, II, aVF, and V1, as well as the R-to-S ratio in lead III and V1 accurately predicted 1 of 10 sites around the atrioventricular annuli or subepicardial region with a sensitivity of 90% and a specificity of 99% [38].

Since then, several authors developed further algorithms to predict the anatomic localization of accessory pathways basing on surface 12-lead ECG [39, 40].

5. CATHETER ABLATION OF ACCESSORY PATHWAY

In Wolff-Parkinson-White syndrome, catheter ablation is the treatment of choice for patients with symptomatic and recurrent AVRT, or pre-excited AF [41].

In patients with asymptomatic pre-excitation, invasive screening with an electrophysiology study (EPS) should be performed in selected cases, as well as in competitive athletes or subjects with “high-risk” occupations.

Identification of an abrupt and complete normalization of the PR interval with loss of delta wave during exercise stress test (EST), or following procainamide or propafenone administration, has been considered a valid marker of low risk [42, 43-45].

In patients undergoes screening with an EPS and is found to have an AP with ‘high-risk’ characteristics, catheter ablation should be performed.

Clinical and electrophysiological features associated with an increased risk of sudden cardiac death include younger age, [46,47,48] inducibility of AV-reciprocating tachycardia during EPS [48-52], multiple APs [48,49,53,54], and demonstration of a capability of the AP to allow rapid conduction to the ventricles [46,48,49,51-54].

These variables include the shortest pre-excited RR interval during AF (SPERRI) of ≤ 250 ms at baseline or a short antegrade effective refractory period (ERP) of the AP (≤ 250 ms) [46,48-50-56].

5.1 RADIOFREQUENCY CATHETER ABLATION

Since its introduction in the 1980s, radiofrequency (RF) catheter ablation of APs is regarded as a highly safe and effective procedure in both adults and children [10]. Several large series support the use of RF catheter ablation of the accessory pathway as first-line therapy in patients who have had AF, AVRT or "high-risk" APs. These series report a success rate of approximately 93% to 95% and a 3% risk of major complications when patients are followed up for 6 months to 8 years [41].

Major complications include cardiac tamponade (0.13 - 1.1%) and complete AV block (0.17 - 2.7%) in patients in whom ablation of septal APs is attempted. Nevertheless, the high success rate and the low risk of complications reported in these studies are related to APs located anywhere within the AV junction surrounding the orifices of the mitral and tricuspid valves, but a different analysis has to be done regarding septally located APs.

RF ablation of APs that run in right septum (near the His bundle), is related with a higher risk of physiological AV node conduction injure, with complete AV block requiring pace-maker implant. In fact, a lower efficacy and safety profile is reported for RF ablation of septally located APs, in particular para-Hisian (P-H) and mid-septal (M-S) APs, in which the risk of AV block seems to be as high as 12.9% [57].

Subgroups data analysis limited to ablation procedures targeted to septal accessory pathways (P-H and M-S APs) revealed a higher rate of complications on AV node-HPS physiological conduction.

H. Kalkis et al. [58] in a cohort of 250 patients underwent radiofrequency ablation of accessory pathways, reported 1.2% incidence of complete heart block (three patients) in the overall cohort. Two patients which developed complete AV nodal block had a septal AP. Data analysis of this subgroup of patients with septal APs, revealed a 13% relative incidence of AV block related to direct-current energy, and 86% success rate.

Similarly, R. Kobza et al. [59] in a cohort of 323 patients reported 0.6% AV node injury during ablation (overall complication rate less than 2%). The two patients that experienced AV node block had both P-H AP

and required pace-maker implant. Even in this case, the sub-analysis of patients with septal APs revealed an 8% incidence of AV node injury related to RF, with a 23% reintervention rate, that was significant higher respect to other subgroups with different anatomical APs location.

Even if not life threatening, adverse events as AV block requiring pacemaker implant, result in a dramatic consequence, especially when happen in young healthy individuals and possibly athletes.

In addition to the risk of AV node conduction damage, RF catheter ablation of septal APs (especially postero-septal APs) can lead to others rare but serious consequences as coronary artery injury. This event may present acutely or several weeks after the ablation. Stenosis of the coronary arteries immediately and during long-term follow-up after ablation has been described in animal models [60-62]. In one large retrospective study, clinically evident coronary injury incidence is estimated to be 0.06% - 0.1% in adults [63, 64]. This ~~low~~ risk is surprising given the close proximity of the coronary arteries to common sites of ablation and may be due to under-recognition and under-reporting [65].

5.2 CRYOENERGY CATHETER ABLATION

Cryoablation (CA), or the use of freezing temperatures to elicit a specific tissue response, has been regarded as a safer alternative in ablation procedures on septal APs. CA results in discrete homogenous lesions that are sharply demarcated, with preserved ultrastructural tissue integrity and low propensity for thrombosis [66–68]. The major mechanisms of cold-induced cellular and tissue injury result from a combination of (1) direct cellular damage attributable to the deleterious effects of ice crystal formation during hypothermia and (2) ischemic cell death attributable to microcirculatory failure and subsequent vascular stasis during thawing [69]. The complex mechanisms underlying cryo-thermal lesion formation can be divided into sequential stages: freeze, thaw, hemorrhage and inflammation, and replacement fibrosis [70].

In general, the early effects are transient, provided that the duration of nonfreezing cooling does not exceed a few minutes. Thereafter, progressive cooling is associated with the formation of ice crystals.

Progressive cooling to below – 40°C results in the formation of intracellular ice crystals.

Whereas ice crystals are associated with mechanical cellular disruption, the predominant mechanism of cellular injury is biochemical [71-73]. The formation of ice crystals in the extracellular space results in it becoming relative hypertonic. Furthermore, the newly established osmotic gradient precipitates a diffusion gradient between extracellular and intracellular spaces, resulting in the net movement of H^+ ions out of the cell, and the migration of solute ions into the cell. On completion of the freezing phase, the tissue passively returns to body temperature (thawing effect).

This second phase induces cellular damage through a combination of two mechanisms. First, recrystallization and coalescence of intra- and extracellular ice crystals increase the osmotic damage and generate shear forces, which further disrupt tissue architecture. Second, restoration of microcirculatory function is associated with a hyperemic vascular response characterized by hemorrhage and inflammation [71].

The final phase of cryoinjury begins concurrent to thawing and is characterized by reactive inflammation, followed by tissue repair and replacement fibrosis.

During the subsequent weeks, these processes culminate in the generation of a mature lesion, which has a distinct, well-circumscribed central region of dense fibrosis surrounded by a narrow border zone of variable cellular death (attributable to microvascular injury and apoptosis) [74].

When compared with RF energy, cryo-thermal ablation offers several advantages related to its two specific properties: cryomapping and cryoadhesion [75].

Cryomapping is the ability to assess the safety and efficacy of a potential ablation lesion site dynamically and prospectively, because a period of reversible tissue inhibition obligatorily precedes irreversible tissue destruction. Whereas extreme freezing (i.e., tissue temperatures colder than −50°C) results in near instantaneous permanent tissue injury, the degree of permanent cellular damage with relatively warmer tissue temperatures (−10°C to −25°C) is directly related to duration of freezing. Thus, the use of milder freezing temperatures and shorter ablation times facilitates the assessment of clinical effect at the target lesion site, and confirmation that a target site does not result in adverse clinical outcomes.

Cryoadhesion is the freeze-mediated catheter adhesion to the target tissue. In contrast to RF catheter ablation, where the catheter remains free-floating during ablation, cryoadhesion provides several advantages. First, freeze-mediated catheter stability facilitates the efficacious ablation of technically challenging regions, such as sites where contact is difficult to maintain. Second, cryoadhesion eliminates the brushing effect, whereby RF energy is applied to the target and surrounding non-target tissue because of cardiac and respiratory motion. Consequently, the major advantage of cryo-thermal energy is the ability to assess the safety and efficacy of a potential ablation lesion site dynamically and prospectively, because a period of reversible tissue inhibition obligatorily precedes irreversible tissue destruction. Furthermore, the ablation lesion can be safely delivered

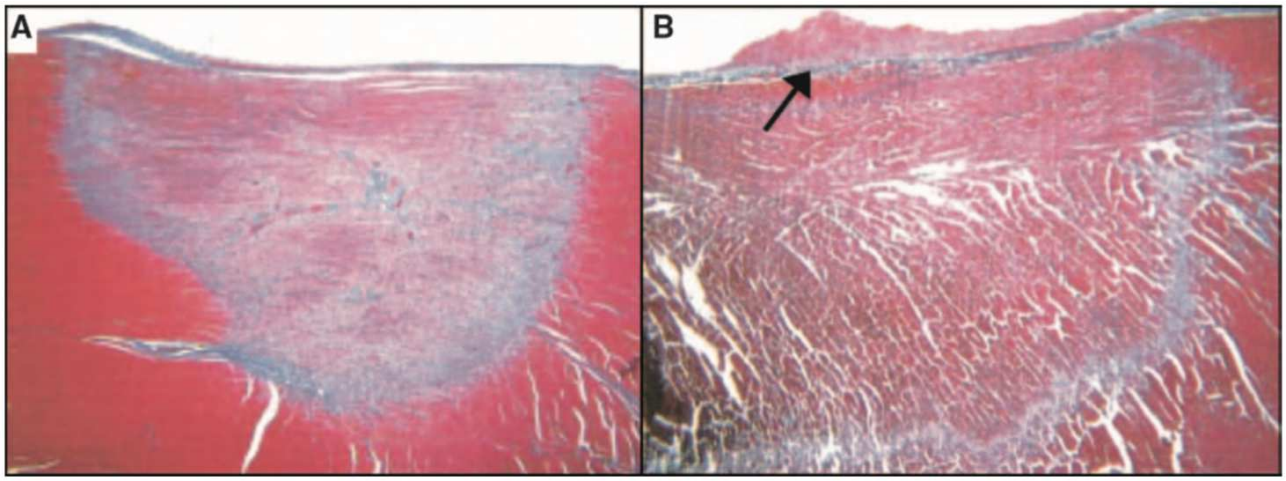
during arrhythmia, or programmed electric stimulation, as there is no concern for catheter dislodgement on arrhythmia termination.

In comparison with RF, lesions associated with focal cryo-thermal ablation have a smaller surface area attributable, in part, to cryoadhesion-induced loss of the brushing effect, with no difference in lesion depth (Figure 3) [74]. While cryo-thermal ablation results in a histologically dense, homogenous fibrosis, clearly demarcated from normal myocardium (Figure 3-A) [69,76], the hyperthermic injury induced by RF results in diffuse cellular destruction characterized by intralesional hemorrhage and ragged edges less clearly demarcated from underlying normal myocardium (Figure 3-B) [74].

In addition, although devitalized, lesions produced by cryo-thermal ablation are associated with preservation of ultrastructural integrity. Preservation of tissue ultrastructural integrity should theoretically result in a lower risk of myocardial perforation, aneurysmal dilation, and are associated with a lower incidence of venous or arterial stenosis, (given the minimal tissue contraction observed with lesion healing) [71,72,75], even if there are not data concerning coronary artery injury in long term.

For all these reasons, when targeting septal APs, CA reach a higher safety profile if compared to RF ablation, quantified in 0% vs. 5.4%, respectively, of persistent AV block even if recurrence of previously blocked pathways has been reported to be significantly higher in CA respect to RF ablation (21.1% vs. 7.1%, respectively) [77].

Figure 3. Histology of cryoenergy and radiofrequency lesions. Typical histological characteristics 1 week after cryo-thermal (A) and RF (B) ablation when stained with Masson's trichrome and magnified 16-fold. Note the more homogeneous nature of cryolesion, with a smoother, sharper demarcation from intact myocardium (A). In contrast, RF lesion is less well circumscribed, with serrated edges (B). Arrow, Endocardial thrombus formation at ablation site.



Khairy P et al. Lower incidence of thrombus formation with cryo-energy versus radiofrequency catheter ablation. Circulation. 2003.

6. RATIONALE OF THE STUDY

Cryoenergy offers a safe therapeutic option for arrhythmia substrates deemed to be associated with an unacceptably high risk of AV block using other energy modalities. Despite the high safety profile of CA, the efficacy is still lower than RF ablation [77], furthermore data derived from follow-up of previous studies are unsatisfactory in terms of time and method.

The aim of the present study was to assess efficacy and safety during acute procedure and long-term follow-up of CA of P-H and M-S APs in a cohort of consecutive patients using a specific protocol for cryo-mapping and CA.

7. METHODS

7.1 PATIENT POPULATION

A cohort of 24 consecutive patients undergoing EPS and CA of P-H or M-S APs from 2004 to 2014, were considered. All patients were enrolled in 2018 and prospectively considered during the same year or 2019, receiving a follow-up.

EP study findings and CA procedure were considered as the *acute procedure*.

Absence of conduction over the AP after CA was defined as *successful acute procedure*, otherwise persistence of conduction over the AP after CA or the resumption of preexcitation during the first week after procedure were both considered as *failed acute procedure*.

Follow-up included cardiac examination, EST, and in some cases 24-hour Holter monitoring. Furthermore, data regarding symptoms and anti-arrhythmic drugs (AADs) therapy were collected. In patients on AAD therapy, medication was continued during the FU.

Resumption of VPE at 12-lead ECG or evidence of AVRT or pre-excited AF at EST or 24-hour Holter monitoring were considered as *failed procedure at follow-up.*

This study conforms to the Declaration of Helsinki on human research and was approved by the Ethical Committee at our institution. All patients or their legal guardian signed informed consent.

7.2 ACUTE PROCEDURE

7.2.1 Electrophysiologic study

The procedure was performed by two experienced operators in the fasting non-sedated state after withdrawal of any antiarrhythmic drug for at least five half-lives. Multipolar catheters were introduced by the right femoral vein and the left or right antecubital vein and advanced to the high right atrium, coronary sinus, His bundle area, and right ventricular apex. Antegrade and retrograde conduction properties of the AP were assessed and induction of AVRT was tested at baseline and during continuous isoproterenol up to 4 mcg/min, if required. Burst pacing with progressive shortening of the pacing cycle length until the lack of atrial capture was used to induce atrial fibrillation and test the shortest pre-excited R-R interval.

Location of the AP was defined by using both bipolar and unipolar recordings during sinus rhythm and/or AVRT. Only in case of P-H location, if the positioning and stability of the CA catheter were unsatisfactory using the inferior vena cava (IVC) approach, then a superior vena cava (SVC) approach was used by advancing the catheter through an antecubital vein (Figure 4, a–d). An AP had a P-H location if a distinct His-bundle potential was recorded during sinus rhythm in the site where the earliest ventricular bipolar electrogram preceding the delta-wave and coincident with a fast and negative intrinsicoid ventricular deflection in the unipolar recording was observed. If VPE hampered His-bundle recording or the AP had concealed conduction, then its location relative to the His-bundle was defined during orthodromic AVRT based on the earliest retrograde atrial activation in bipolar recordings and negative atrial intrinsicoid deflection in the unipolar

recording (Figure 5, panel a). An AP had a M-S location if, based on the above electrophysiologic criteria, it was localized in an area bounded superiorly by the proximal His-bundle potential and inferiorly by the roof of the coronary sinus as assessed by the 30° left anterior oblique fluoroscopic projection view. During the ESP procedure, catheters were manipulated carefully to avoid traumatic lesions and consequent conduction modification of the AV node, right bundle branch, and AP.

Figure 4. Panel **a–d**: Fluoroscopic image in 30° left (**a, c**) and right (**b, d**) anterior oblique view of the cryoablation catheter positioned via inferior (**a, b**) and superior (**c, d**) vena cava access.
CSos, coronary sinus ostium; HBA, His-bundle area

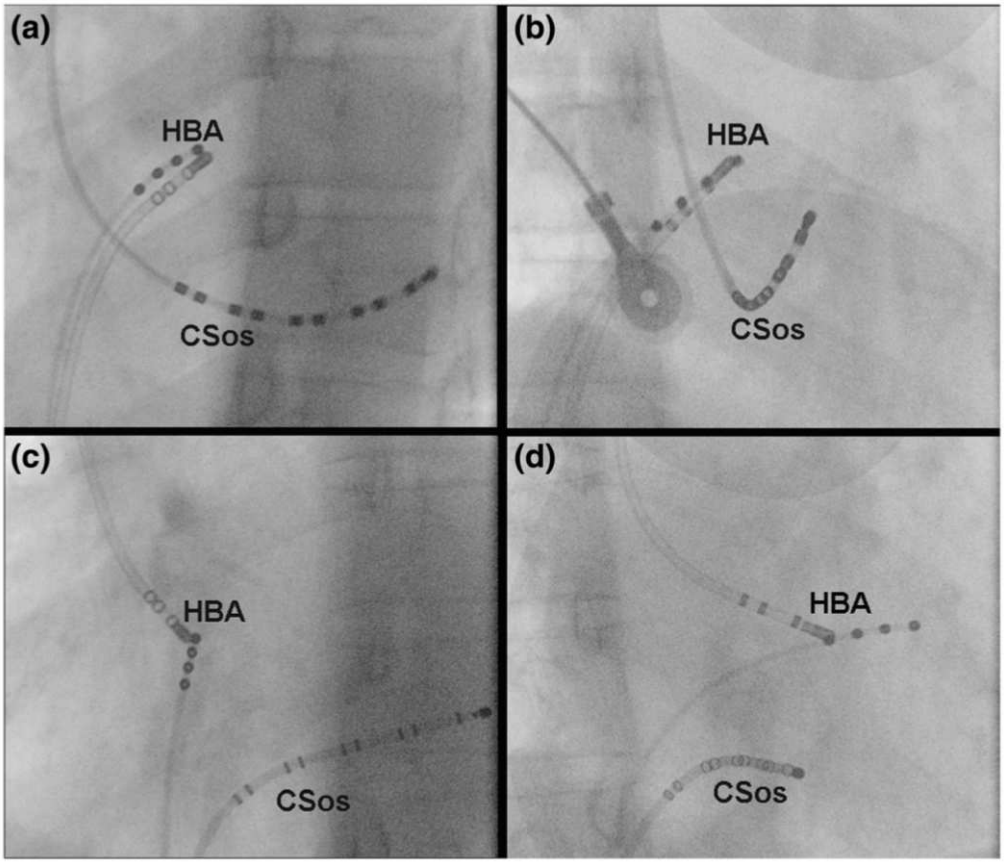
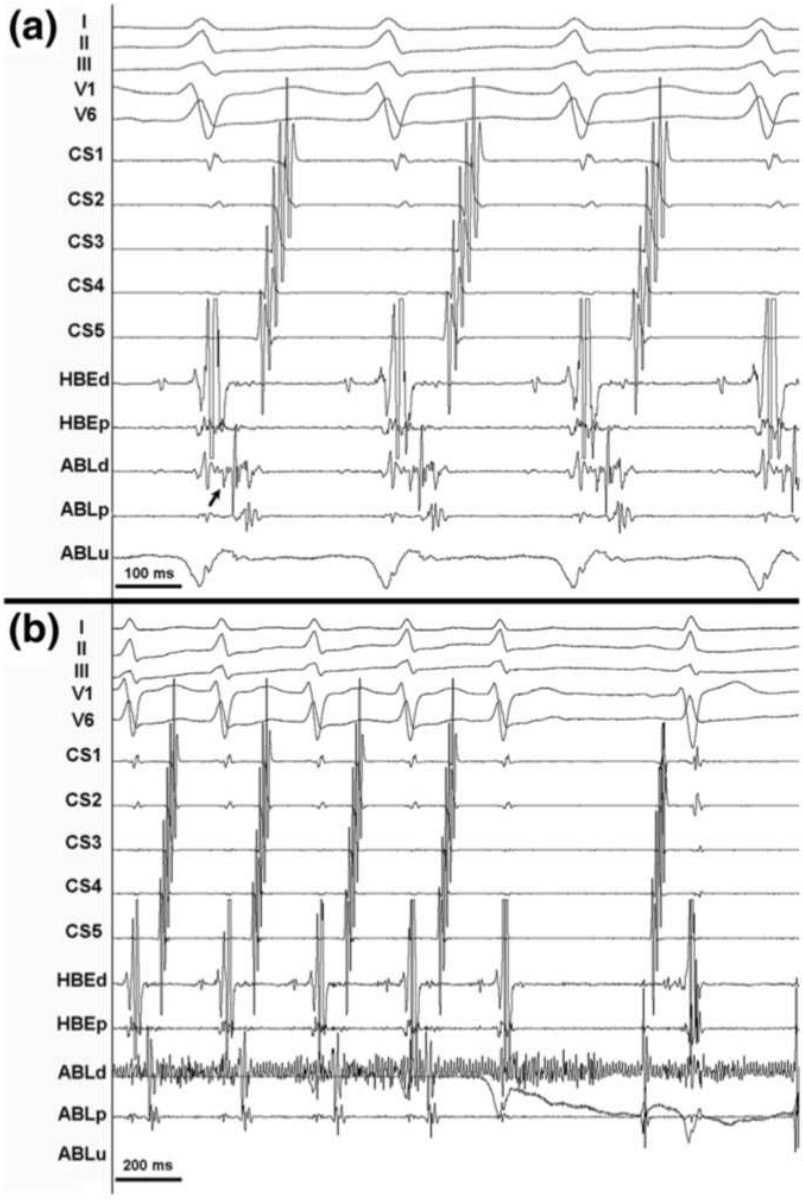


Figure 5. Panel **a – b**: Mapping and cryoablation during orthodromic atrio-ventricular re-entry tachycardia in a case of para-Hisian accessory pathway. In this and in the following figures, tracings are as follows from top to bottom: surface ECG, coronary sinus from distal (CS1) to proximal (CS5), distal (HBEd), and proximal (HBEp) His-bundle electrograms, distal (ABLd) and proximal (ABLp) bipolar recordings form the cryoablation catheter and unipolar (ABLu) recording from the distal electrode of the same catheter. In panel **a**, the earliest retrograde atrial activation is observed in ABLd, where the shortest V-A interval with a putative Kent bundle potential is observed between the ventricular and atrial deflection (arrow); in ABLd, a far-field His-bundle potential is also observed. Of note, bipolar signals at the target site are stable over time, indicating good catheter/tissue contact. In panel **b**, during test application at − 40 °C, conduction interruption over the accessory pathway is obtained with tachycardia interruption



7.2.2 Cryoablation protocol

CA was performed in the same procedure with a dedicated console (Cryoconsole, Medtronic CryoCath, Kirkland Quebec, Canada) and a 7F 4-mm-tip catheter (Freezor, Medtronic CryoCath, Kirkland Quebec, Canada) was initially used; in case of failure, a 7F 6-mm-tip catheter was used (Freezor Xtra, Medtronic CryoCath, Kirkland Quebec, Canada). Whenever a left component of the AP was suspected, trans-septal (TSP) catheterization was performed and the left antero-septal region beneath the aortic valve mapped. In the most suitable area, the ablation catheter was positioned so that the A/V ratio in the distal electrode pair was >1 to prevent permanent lesion to the right bundle branch. In this site, cryomapping was applied using the specific stepwise protocol of our electrophysiology laboratory [75,78]. Briefly, an application of 30 sec. at −30 °C was delivered in the most suitable site. If it interrupted conduction over the AP with no modification of normal conduction, then direct transition to CA at –75 °C up to 480 s was performed. If cryomapping was unsuccessful, after defrost, further 30-s applications were tested step-by-step, decreasing for each test the temperature by 10 °C to a minimum of - 70 °C. If cryomapping was ineffective at –70°, then a 6-mm-tip catheter was used. Throughout the cryomapping protocol steps, the cryocatheter was firmly kept in the most suitable position, assessed by stability of bipolar recordings (Figure 5, panel a). Electroanatomic mapping system compatible with the cryocatheter was not used to assess catheter stability throughout the procedure because of the significant catheter deformation visualized by the system that based on previous experience was due to the abrupt impedance change during ice formation at the catheter tip. Figures 5 panel b, and figure 6 panel a show interruption of AP conduction during cryomapping. Throughout CA, normal AV conduction and suppression of arrhythmia inducibility were tested using programmed electrical stimulation, while the catheter/tissue contact was maintained by cryoadherence (Figure 6 panel b, and figure 7 panels a-b). If during CA, prolongation of the A-H interval or appearance of right bundle branch block was observed, cryoenergy delivery was promptly interrupted and resumption of the baseline conduction properties was assessed.

Similarly, if any increase of the degree of the VPE during sinus rhythm or if interruption of the AVRT over the antegrade re-entry limb was observed, cryomapping or CA was immediately stopped to avoid inadvertent damage to the normal AV conduction system. After successful ablation, the absence of conduction resumption over the AP was assessed for 60 min. If AP conduction resumed, then further CA was performed.

Patients were discharged after 24 h from the procedure with no antiarrhythmic drugs.

AP conduction resumption within 7 days after CA ablation, in patients underwent already two CA procedures, was considered as acute failed procedure.

Figure 6. Panel **a–b** Cryomappingat−30°C and ablation during sinus rhythm in a patient with a manifest para-Hisian accessory pathway. In panel **a**: early after application initiation, pre-excitation disappears on surface ECG in the third last beat with no modification of the A-H and H-V interval in HBEd. In panel **b**, during defrost, after cryoablation at − 75 °C for 480 s, the artifact due to ice formation disappears in ABLd and a His-bundle poten- tial is seen in the same electrogram (arrow). Notably, A-H and H-V intervals in HBEd are normal

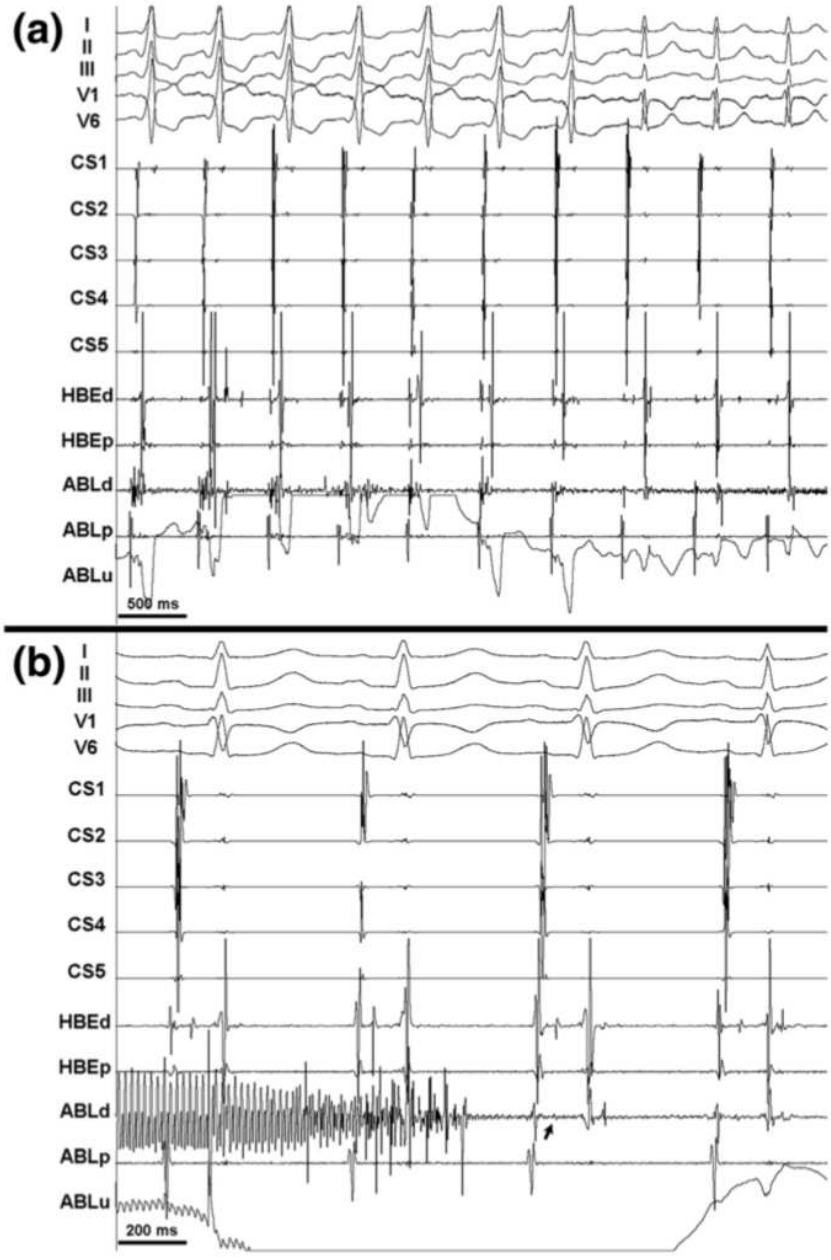
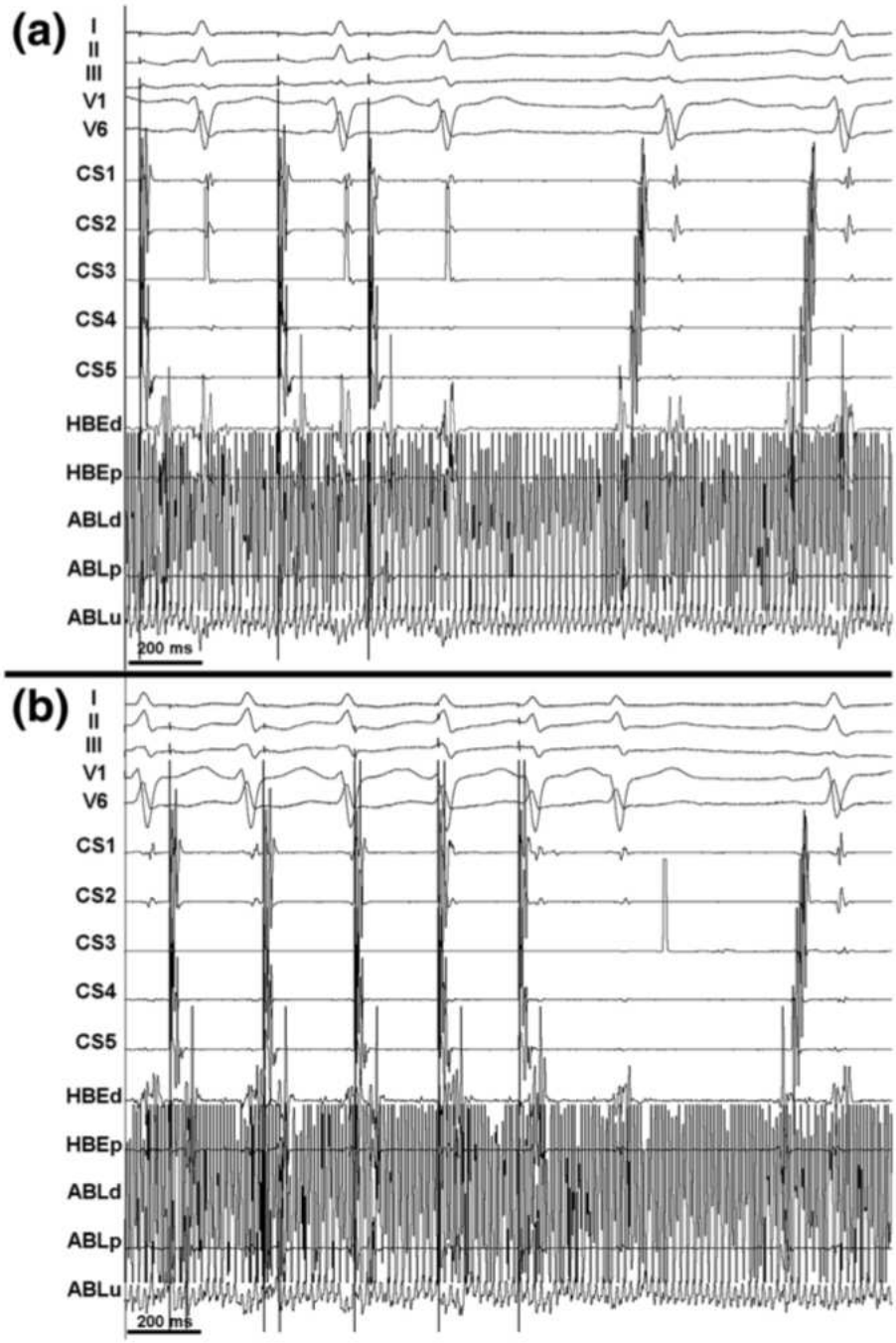


Figure 7. Panel **a–b**: same patient as in **Figure 4**. In panel **a**, after tachycardia interruption during cryomapping programmed atrial stimulation from coronary sinus (p = 400, 220 ms) fails to induce re-entry during cryoablation at − 75°C, responsible for massive artifact on the ablation catheter recordings. In panel **b**, incremental atrial pacing up to 200 ms shows preserved conduction over the normal AV conduction pathway during a 480-s cryoablation



7.3 LONG TERM FOLLOW-UP

All patients underwent CA received a medical FU. Data concerning cardiac symptoms (chest pain, palpitations, syncope, breathlessness), and cardiac risk factors were collected, furthermore cardiac examination, blood pressure measurement and surface 12-lead ECG were evaluated.

All patients underwent EST. During the EST, ST-segment disturbances, or other findings suggestive of myocardial ischemic alterations, were evaluated.

In patients with manifest VPE, EST was aimed to identify subjects with low risk for SCD with loss of delta wave during the increase of HR. The EST was also performed to detect AVRT or other arrhythmias related to AP, considering the exercise a predisposing factor to initiate tachyarrhythmias in WPW syndrome [41].

AV node conduction disturbances during EST were also carefully evaluated. AV pathological conduction delay at rest or during HR increase might have been a consequence of previous CA.

Subjects with previous *failed acute procedure* or reported symptoms, and patients with VPE recurrence at 12-lead ECG, underwent 24-hour Holter monitoring.

7.4 STATISTICS

Continuous variables are expressed as mean ± standard deviation (SD), if normally distributed according to D'Agostino– Pearson test for normality. Student's t test for independent samples with a confidence interval of 95% was used for metric variables; otherwise, either logarithmic transformation of variables or nonparametric tests (Mann–Whitney) were used. A chi-squared test was performed to examine whether there was an association between categorical variables; when the sample size was too small for the chi-squared test to be valid, a Fisher exact test was used. All data were analyzed using commercially available software (SPSS Statistics, IBM). P values < 0.05 were considered significant.

8. RESULTS

8.1 ACUTE PROCEDURE DATA

Table 3. Clinical characteristics of considered population at acute procedure time

	n. (%)
Patients	24
Patients referred from other centers, n. (%)	21 (88)
Patients with previous EPS, n. (%)	15 (63)
Patients with previous RF ablation on AP, n. (%)	5 (21)
Patients with AV node iatrogenic injury related to RF, n. (%)	3 (13)
Male gender, n. (%)	15 (63)
Age (mean ± SD)	24 ± 12 (range 12 -52) years
Cryoablation procedure per patients (mean ± SD)	1.13 ± 0.34
Acute success, n. (%)	20 (83)
Manifest VPE, n. (%)	15 (63)
Symptomatic patients, n. (%)	23 (96)
Prior use of AADs, n. (%)	15 (63)
Para-Hisian AP, n. (%)	21 (87)
Mid-Septal AP, n. (%)	3 (13)
IVC access, n. (%)	24 (100)
SVC access, n. (%)	11 (46)
TSP access, n. (%)	2 (8)
Cryoablation time, s (mean ± SD)	496 ± 320
Procedure time, min (mean ± SD)	181 ± 45
6 mm-tip-catheter, n. (%)	6 (25)

EPS electrophysiology study; RF, radiofrequency; AP, accessory pathway; AV, atrioventricular; SD, standard deviation; VPE, ventricular pre-excitation; AADs, anti-arrhythmic drugs; IVC inferior vena cava, SVC, superior vena cava; TSP, trans-septal

As shown in Table 3, twenty-four patients underwent 27 CA procedures. 21/24 patients (88%) referred from another center, of these 15/24 (63%) had undergone a previous EP study procedure and 5/24 (21%) received an unsuccessful RF ablation. 3/24 (13%) experienced iatrogenic AV conduction lesion related to the RF ablation attempted, in 1 case (4%) with 12-lead ECG evidence of first-degree AV block.

Twenty-one out of 24 patients (87%) had a P-H AP while 3/24 (13%) had a M-S AP.

Fifteen patients (63%) had a manifest AP while the remaining had a concealed one.

The SVC approach was required in 11/24 patients (46%) while trans-septal (TSP) catheterization was performed in 2/24 patients (8%). The mean procedural and CA times were 181 ± 45 min. and 496 ± 320 sec., respectively.

The majority of patients 23/24 (96%), referred palpitations symptoms despite AAD therapy in 15/24 patients (63%).

Table 4. Comparison of clinical and procedural parameters between patients with and without cryoablation acute success

	Acute success	Acute failure	p value
Patients, n	20 (83)	4 (17)	
Cryoablation procedures per patients, (mean ± SD)	1	1.75 ± 0.5	*P <0.05*
Age, years (mean ± SD)	25 ± 13	20 ± 5	*ns*
Male gender, n (%)	12 (60)	3 (75)	*ns*
Manifest VPE, n (%)	12 (60)	3 (75)	*ns*
Prior use of AADs, n (%)	12 (60)	3 (75)	*ns*
P-H AP, n (%)	17 (85)	4 (100)	*ns*
M-S AP, n (%)	3 (15)	0	*ns*
SVC access, n. (%)	7 (35)	4 (100)	*P < 0.05*
TSP sccess, n (%)	0 (0)	2 (50)	*p < 0.05*
6-mm-tip catheter, n (%)	3 (15)	3 (75)	*P < 0.05*
Cryoablation time, s (mean ± SD)	486 ± 325	548 ± 334	*ns*
Procedure time, min (mean ± SD)	183 ± 48	172 ± 26	*ns*
Patients with previous RF ablation on AP, n (%)	3 (15)	2 (50)	*ns*

SD, standard deviation; VPE, ventricular pre-excitation; AADs, anti-arrhythmic drugs; AP, accessory pathway; M-S, mid-septal; P-H, para-Hisian; SVC, superior vena cava; TSP, trans-septal; RF, radiofrequency; ns, non-significant.

As shown in table 4, among all the clinical and procedural variables investigated, a statistically significant difference between the two groups was only found for the SVC approach, the 6-mm-tip catheter, and the TSP catheterization, more frequently used in the group of patients with acute failure, associated with a greater number of procedures per patient.

8.2 LONG TERM FOLLOW-UP DATA

Follow-up was performed after a mean of 120 ± 37 months since the acute procedure, data are reported in the table below.

Table 5. Follow-up data of considered population

Follw-up	n. (%)
Patients, n	24
Age, years (mean ± SD)	34 ± 13
FU time, months (mean ± SD); range	120 ± 37; 60-180
Manifest VPE, n (%)	4 (17)
VPE disappearance at EST, n (%)	1 (4)
Persistent VPE during Holter monitoring, n (%)	4 (17)
Symptomatic patients, n (%)	3 (13)
Use of AADs, n (%)	3 (13)

SD, standard deviation; FU, follow-up; VPE, ventricular pre-excitation, EST, exercise test; AADs, anti-arrhythmic drugs

Four out of twenty-four patients (17%) showed VPE at 12-lead ECG; three belonged to the group of *failed acute procedure* with manifest P-H AP, and one to the group of *successful acute procedure* with manifest M-S AP. In the three patients with *failed acute procedure*, EST showed constant PE during the test, while in the patient with *successful acute procedure* with new resumption of VPE, the EST showed loss of the delta wave during the test.

Three out of the twenty-four patients (13%) reported palpitations. Two belonged to the group of *failed acute procedure* with concealed P-H AP and manifest P-H AP respectively, while the third belonged to group of *successful acute procedure* with concealed P-H AP. In the patient with *failed acute procedure* with concealed PH-AP, EST showed a prolonged time to restore the HR to the pre-exercise level, the patient had identified this event as the clinical symptom reported and took AAD therapy discontinuously.

The subject with *failed acute procedure* with manifest PH-AP, took AAD therapy and reported sporadic palpitation episodes (about 1 time a month for 1-2 minutes). In this case EST and Holter monitoring did not show arrhythmias sustained by AP, even if the patient remained asymptomatic during the tests.

In the third subject reporting symptoms that belonged to group of *successful acute procedure* with concealed P-H AP, EST disclosed bigeminy ventricular ectopic beats during the recovery phase. This event was recognized as the reported clinical symptoms, and the patient did not take AAD therapy chronically.

Three out of twenty-four patients (13%) took AAD therapy, two subjects were already described above in the analysis of patients reporting symptoms, while the third belonged to the group of *failed acute procedure* with manifest P-H AP, in this case the subject did not reported symptoms, and EST and Holter monitoring did not detect arrhythmias.

In overall population, EST did not show ischemic alterations, PR pathological prolongation during the increase of HR, or induction of tachyarrhythmias related to AP. The only subject with first-degree AV block showed reduction of PR interval during the EST.

Holter monitoring did not show arrhythmias, nevertheless the presence of persistent VPE during the whole test was confirmed in all the 4 subjects with manifest PE.

Table 6 shows clinical characteristics of each patient with VPE and symptoms reported at follow-up.

In this table the acute procedural success/failure was reported for each patient.

Only 1/20 (5%) of the patients discharged with *successful acute procedure* can be considered a *failed procedure at follow-up*, this single case was the only one with resumption of pre-excitation at 12-lead ECG.

Table 6. Patients with ventricular pre-excitation and/or reported symptoms at follow-up.

	Group	AAD therapy before CA	Symptoms before CA	AAD therapy at FU	Symptoms at FU	EST findings	Holter monitoring findings	Failed procedure at FU time
Patient 1	Failed CA, Manifest PH AP	Target dose	Continuous (Incessant AVRT)	NO	NO	Persistent VPE, NO arrhythmias	Persistent VPE, No arrhythmias	NO
Patient 2	Failed CA, Manifest P-H AP	Target dose	Frequent	Target dose	Sporadic	Persistent VPE, NO arrhythmias	Persistent VPE, No arrhythmias	NO
Patient 3	Failed CA, Manifest P-H AP	Target dose	Frequent	Target dose	NO	Persistent VPE, NO arrhythmias	Persistent VPE, No arrhythmias	NO
Patient 4	Failed CA, Concealed P-H AP	Target dose	Frequent	Discontinuous	Sporadic	Prolonged time to restore the basal HR*	No arrhythmias	NO
Patient 5	Successful CA, Manifest P-H AP	NO	NO	NO	NO	VPE disappearance during EST	Persistent VPE, No arrhythmias	**YES**
Patient 6	Successful CA, Concealed P-H AP	Target dose	Frequent	NO	Sporadic	Bigeminy ventricular ectopic beats during recovery phase*.	No arrhythmias	NO

CA, Cryoablation; FU, follow-up; AADs, anti-arrhythmic drugs; EST, exercise stress test; P-H, para-Hisian; M-S, mid-septal; AP, accessory pathway; HR, heart rate; VPE, ventricular preexcitation. ** event related to reported symptoms*

The table below compares the different clinical parameters at the time of acute procedure and at follow-up.

Table 7. Comparison between clinical parameters during acute procedure and at follow-up.

	Acute procedure	Follow-up	p
Patients	24	24	
Age, years (mean ± SD)	24 ± 12	34 ± 13	
Failed procedures, n (%)	4/24 (17) †	1/20 (5) #	
AAD therapy	15/24 (63) *	3/24 (13)	$p < 0.05$
Symptoms	23/24 (96) *	3/24 (8)	$p < 0.05$
First-degree AVB	1/24 (4) *	1/24 (4)	ns

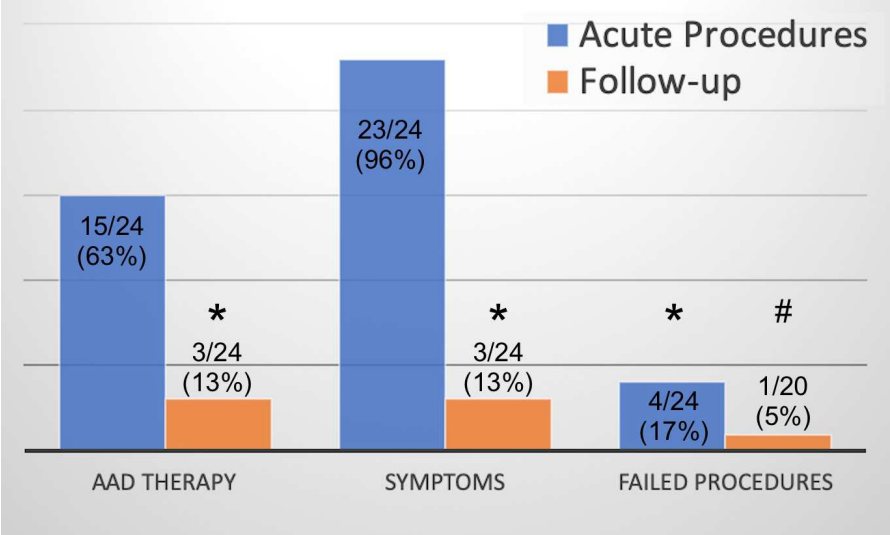
AAD, anti-arrhythmic drug, AVB, atrioventricular block
† $p < 0.05$ vs overall patients
 # $p < 0.05$ vs successful acute procedures (20 patients)
** data refers to "prior acute procedure"*

A statistically significant reduction in the AAD therapy use with a drastic reduction of symptoms can be observed 10 years after acute procedure, associated with a low rate of VPE recurrence (Figure 8).

No adverse events, (new onset of AV block, neither ischemic alterations) were observed at follow-up.

Of note, as already explained, the three patients that reported palpitation symptoms did not show arrhythmias related to AP at EST neither at Holter monitoring; in particular in two of them the symptoms occurred during the EST were not related to the presence of AP.

Figure 8. Comparison of anti-arrhythmic drug therapy, symptoms and VPE recurrence between acute procedure time and follow-up.



AAD, Anti-arrhythmic drug.

AAD therapy and Symptoms of acute procedure group refer to time prior CA.

** p < 0.05 vs acute procedure group*

 # p < 0.05 vs successful acute procedure group (20 pts)

8.2.1 Analysis of failed acute procedures at follow-up

Data concerning the follow-up of each patient with *failed acute procedure* are reported in table 6 (Patients 1-4).

These patients (3 with manifest P-H AP and 1 with concealed P-H PA), were deeply symptomatic before CA because of AVRTs, despite use of AAD therapy on target dose.

At follow-up, the three patients with manifest AP confirmed VPE pattern. Only two patients took AAD therapy on target dose, and symptoms were reported in two cases even if deeply reduced in frequency and duration respect to time prior the acute procedure.

9. DISCUSSION

As shown in Table 8, CA of P-H and M-S APs in previously published papers [79-93], each including more than 10 APs, confirm a good acute success with only minor complications of CA with, however, a non-negligible rate of recurrences. Different technical variables, such as a longer time to effect during cryomapping [87] or a longer time to success [85], avoidance of bonus application [79], and the size of freezing electrode [88,89,92], may play a role in higher recurrence rate in patients treated with CA. However, none of these variables have shown an overwhelming importance in determining recurrences, with the only potential exception of an abrupt vs. a gradual disappearance of conduction over the AP during cryothermal energy delivery [88].

These studies generally report a less aggressive cryomapping and CA protocol, compared to the one used in the present study.

Our data confirms that CA of P-H and M-S APs with the specific protocol used achieves very good results in term of acute success and safety, with 86% of acute success rate and no major complications.

This has to be considered especially in the light that the patients in our series were consecutive but represented a selected group of cases referred from other centers after an invasive EP procedure. In fact, in 2/4 patients who received an unsuccessful CA, a previous RF ablation had been unsuccessful as well.

In our patient series, acute failure was associated with more frequent use of the 6-mm-tip catheter, SVC, and TSP approach. This shows that in these cases, the AP was resistant to a more aggressive approach, suggesting a more complex or deeper anatomical substrate not suitable for CA.

However, the clinical conditions of these patients did not justify a more aggressive approach at risk of major complication.

The longer follow-up time reported in previous similar studies has been 51.1 ± 25.9 months (range 6-69) [92] (Table 8) and was performed without an accurate method and often not in all patients.

For the first time we obtained a relevant follow-up time (120 ± 37 months, range 60 – 180) that confirmed the positive procedural success rate with a recurrence of only 5%, that has resulted lower if compared with other similar studies [79-93], with a solid safety during the time. In fact, concerning *successful acute procedure* group, only 1/20 patient (5%) can be considered a *failed procedure at follow-up* because of pre-excitation resumption. This subject did not referred symptoms and did not assumed AAD therapy at follow-up time as well as before the CA, but at follow-up, EST identified a low risk profile of the resumed AP.

The CA procedure in this patient was performed through inferior vena cava approach, delivering 240 sec of cryo-energy. Therefore, the failure at follow-up seems not related to any procedural or clinical feature.

As attended, at follow-up the use of AAD therapy and presence of symptoms resulted significantly lower when compared to data derived before the acute procedure, underlining the optimal clinical outcome of CA during the time.

A further reflection has to be done regarding the patients who failed the acute procedure. In this group, only 2/4 patients referred symptoms at follow-up. In one case, EST and Holter monitoring excluded the link between symptoms and arrhythmias related to AP, while in the second case, the presence of arrhythmias sustained by AP could not be excluded/confirmed definitely because the lack of reported symptoms during the tests, that resulted both negative anyway. Nevertheless, the lower frequency and duration of symptoms reported, underlined the better clinical outcome also in this subgroup of patients.

Furthermore, the reduction of symptoms in patients with previous failed acute procedure is more interesting in the light of concurrent AAD reduction.

Considering these data, CA showed a remarkable improvement in clinical outcome also in patients with failed procedures, during the long time period. This finding could be probably related to a potential cryothermal

lesion modification over the time, able to modulate the AP conduction capability, mitigating the presence of sustained AVRTs.

Table 8. Overview of the previously published papers with cryoablation. Adapted from Marazzato J et al. Efficacy and safety of cryoablation of para-Hisian and mid-septal accessory pathways using a specific protocol: single-center experience in consecutive patients. J Interv Card Electrophysiol 2019.

First author	n. APs (P-H/M-S)	Cryomapping Min T (°C) and time (s)	CA time (s)	Acute success rate	Persistent complications	Follow-up time	Follow-up recurrences	Success after recurrence treatment
Gaita [79]	20 (11/9)	− 30 (60)	240	20/20 (100%)	0	15 ± 6 months	4/20 (20%)	20/20 (100%)
Atienza [80]	22 (10/12)	− 30 (45)	240	20/22 (91%)	2 RBBB	Median 286 days IR 123-421	3/20 (15%)	19/20 (95%)
Kirsh [81]	16 (11/5)	− 30 (60)	240	12/16 (75%)	0	3 monts (in 28 Pts)	0/12 (0%)	12/12 (100%)
Drago [82]	11 (7/4)	− 30 (60)	240-480	9/11 (82%)	0	Range: 1 to 22 months	3/9 (33%)	6/9 (67%)
Bar-Cohen [83]	12 (0/12)	− 30 (10–20)	240 + 480	9/12 (75%)	1 RBBB	Median 207 days (range 2-695 days	7/9 (77%)	2/9 (22%)
Gaita [84]	39 (24/15)	− 30 (NA)	240	37/39 (95%)	0	mean 27 ± 12.9 months	8/37 (20%) 1	37/37 (100%)
Kaltmann [85]	14 (9/4)	− 80 (25)[a]	240	13/14 (93%)	0	Mean 7.6 ± 3.8 months	1/13 (8%)	13/13 (100%)
Bastani [86]	27 (27/0)	− 30 (20)	240	26/27 (96%)	0	996 ± 511 days	7/26 (27%)	24/26 (92%)
Drago [87]	52 (35/17)	≤ −30(60)[b]	240-480	50/52 (96%)	0	18.6 ± 6.6 months; range 3-111	12/50 (24%)	NA
Ergul [88]	24 (24/0)	− 30 (30–45)	NA	23/24 (96%)	0	14.2 ± 7.7 months	2/23 (9%)	23/23 (100%)
Yildirim [89]	25 (25/0)	− 30 (60)	240-300	23/25 (92%)	2 RBBB	17.5 months (range 6–34 months)	1/23 (4%)	22/23 (96%)
Insulander [90]	100 (NA)	− 30 (20)	240	81/100 (81%)	0	24 months, (range 6-96 months)	11/81 (14%)	70/81 (86%)
Karadeniz [91]	28 (20/8)	− 30 (45)	240-360	28/28 (100%)	0	Mean 8.8 ± 4.8 months	2/28 (7%)	26/28 (93%)
Swissa [92]	50 (50/0)	− 30 (30)	240	47/50 (94%)	0	59.7 months (range 6-102 months	7/47 (15%)	47/47 (100%)
Jang [93]	26 (16/10)	− 40 (10)	240	23/26 (88%)	1 1° AVB, 2RBBB	18 monts range 10-24	3/23 (13%)	20/23 (87%)
Total	466	−30 to −80 (10–60)	240 - 480	421/466 (90%)	8 (1.8%)	NA	71/421 (17%)	341/371 (92%)

APs, accessory pathways; AVB, atrio-ventricular block; CA, cryoablation; Min T, minimum temperature reached; M-S, mid-septal; P-H, para-Hisian; RBBB, right bundle branch block. NA, not available

a Initially tested during cryoablation

b Two different protocols used: (1) − 30° for 60 s (2) from − 30 to − 70 with a 10° decremental step each 10 s

10. CONCLUSIONS

Our study confirms that the cryoablation protocol used is associated with a lower recurrence rate, and a high safety profile in acute procedure. Successful CA can be obtained at first procedure even in patient with unsuccessful prior RF ablation.

Acute failure is possibly related to anatomic features of the AP, which is resistant to an even more aggressive CA approach in repeated procedures.

Long term follow-up, over 10 years since the acute procedure, confirms the efficacy and safety of our method.

CA of P-H and M-S septal APs is associated, during the time, to an improvement in clinical outcome in terms of discontinuation of medical therapy and reduction of symptoms, also in patients that received a failed acute procedure.

The CA protocol described is an acceptable procedure in terms of clinical outcome and safety in short term as well as during long time.

On the other hand, different more aggressive cryomapping and CA protocols could be taken into account, with the aim to get a prompt interruption of conduction over the AP and to avoid tissue edema related to repetitive applications, which may prevent success. However, our protocol could be better compared to other possibly more aggressive strategies. In fact, an initial more aggressive application may produce inadvertent modification of normal AV conduction forcing early interruption of the application and potentially leading to unsuccessful ablation.

11. LIMITATIONS

The major limitation of this single-center study is the relatively small cohort of patients.

Moreover, during the follow-up, we evaluated data derived from first line screening tests as EST and Holter monitoring that, nevertheless, acquire more relevance because they are associated with a deep clinical investigation over a long time period.

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