CIRCARDIAN VARIABILITY PATTERNS PREDICT AND GUIDE PREMATURE VENTRICULAR CONTRACTION ABLATION PROCEDURAL INDUCIBILITY AND OUTCOMES

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Running title: Procedural impact of PVC circadian variability

Manuscript word count: 5000

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ABSTRACT

Background: Infrequent intra-procedural premature ventricular complexes (PVCs) may impede radiofrequency catheter ablation (RFA) outcome and pharmacological induction is unpredictable.

Objective: To determine whether PVC circadian variation could help predict drug response.

Methods: Consecutive patients referred for RFA with detailed Holter monitoring and frequent monomorphic PVCs were included. Patients were divided into 3 groups based on hourly PVC count relationship to corresponding mean HR during each of the 24 hours on Holter: fast-HR-dependent PVC (F-HR-PVC) type for a positive correlation (Pearson, $P<0.05$), slow-HR-dependent PVC (S-HR-PVC) type for a negative one and independent-HR-PVC (I-HR-PVC) when no correlation was found.

Results: Fifty one of the 101 pts (50.5%) had F-HR-PVC, 39.6% I-HR-PVC and 9.9% S-HR-PVC. 30.7% of pts had infrequent intra-procedural PVC requiring drug infusion. The best predictor of infrequent PVC was number of hours with a PVC count<120/h on Holter (AUC=0.80, Se=83.9%, Spe=74.3%, for ≥2h). Only F-HR-PVC pts responded to isoproterenol. Isoproterenol washout or phenylephrine infusion were successful for the 3 S-HR-PVC pts; and no drug could increase PVC frequency in the 12 I-HR-PVC pts. Long term RFA success rate in pts with frequent PVCs at baseline (82.9%) was similar to those with infrequent PVC who responded to a drug (77.8%, $P=0.732$), but significantly higher than for those who did not respond to any drug (15.4%, $P<0.0001$).

Conclusion: A simple analysis of Holter PVC circadian variability provides incremental value to guide pharmacologic induction of PVCs during RFA and predict outcome. Patients with infrequent I-HR-PVC had the least successful outcomes from RF ablation.

Key words: premature ventricular complexes, radiofrequency ablation, circadian, isoproterenol, autonomic nervous system.

INTRODUCTION

Premature ventricular contractions (PVCs) are frequently encountered in clinical practice and may cause symptoms, lead to cardiomyopathy,[1, 2] or even cause sudden cardiac death.[3, 4] Radiofrequency ablation (RFA) of PVCs is routinely performed, aiming to prevent these risks and manage drug-refractory symptoms.[5]

However, infrequent intra-procedural PVCs makes activation mapping difficult, impeding the ablation procedure and resulting in reduced short and long- term ablation success rates.[6] Pace-mapping is of limited value as an alternative approach. Isoproterenol, sometimes in combination with pacing maneuvers, is used in such instances to increase intra-procedural PVC frequency. Successful induction of PVCs with isoproterenol has been reported in less than 50% of cases,[6] and no specific criteria have been developed to predict the likelihood of success. In addition to its unreliability in enhancing PVC frequency, isoproterenol is also a very expensive drug in the United States (~$1200/dose); and may in rare instances lead to serious complications.[7, 8]

Idiopathic PVCs are thought to be mainly sympathetically-mediated, making the utilization of isoproterenol to increase intra-procedure PVC burden a rational strategy. However, the circadian distribution of PVCs is highly variable between patients suggesting a more complex autonomic neural control. A subset of patients seems to have a preferentially higher burden during daytime and as heart rate (HR) accelerates (e.g., exercise, stress), while PVCs are suppressed at night or rest. However, some patients have the opposite distribution pattern, where PVCs are mainly present at night/rest, but infrequent during the day/activity. A third group of patients do not seem to have PVC frequency linked to any discernable circadian distribution or HR changes.

We hypothesized that PVC circadian variation could help predict: 1) which patients may have infrequent intra-procedural PVCs, and 2) the likelihood of drug (i.e. isoproterenol) response when used to increase PVC frequency to allow activation mapping. In addition, we sought to describe patients' clinical characteristics associated with each PVC profile, to assess possible mechanistic and clinical implications.

METHODS

Patient Characteristics

Retrospective data collection was approved by the local institutional review boards at five international centers. Between December 2013 and May 2016, 101 consecutive patients with detailed 24-hour Holter monitoring and frequent idiopathic monomorphic PVCs referred for RFA were included in this study. Patients with cardiomyopathy were excluded if the PVCs were thought to be secondary to the underlying cardiomyopathy (i.e. scar-related). Cases of cardiomyopathy due to a high burden of PVCs were included as long as alternative etiologies of cardiomyopathy such as severe obstructive coronary artery or significant valvular disease were ruled out.

Patient classification based on Holter PVC circadian distribution

Patients were divided into 3 groups based on their hourly PVC relationship to the corresponding mean HR during the pre-procedure Holter monitoring (Figure 1). When their hourly PVC number had a positive correlation with the mean HR (Pearson, $p<0.05$), they were defined as fast HR-dependent PVC (F-HR-PVC) type. Those with a negative correlation were defined as slow HR dependent PVC (S-HR-PVC) type. Finally, when there was no statistical link between PVC number and mean HR, they were defined as independent HR PVC (I-HR-PVC).

Ablation procedure

Antiarrhythmic drugs other than amiodarone were discontinued at least five half-lives prior to ablation while amiodarone was usually stopped approximately 6 weeks prior as per institutional protocols. Intravenous sedation was minimized as tolerated to avoid anesthesia-related PVC suppression. Isoproterenol infusion was used as needed to induce PVCs when judged by the operator to be too infrequent for activation mapping, often after attempting burst pacing induction as well as decrease in intravenous sedation as tolerated. Mapping of the PVC origin was performed targeting the earliest site of activation compared with the onset of the surface PVC QRS complex, after which RFA was attempted using standard or irrigated radiofrequency energy after excluding proximity to a major coronary artery or the conduction system. In most cases, ablation was facilitated by use of a 3-dimentional electroanatomic mapping systems, with CARTO (Biosense-Webster, Diamond Bar, CA) or NavX (St. Jude Medical, Minneapolis, MN). Acute procedural success was defined as the absence of spontaneous or inducible clinical PVCs after an at least 30-minute waiting period following the successful RF application, either at baseline or after isoproterenol infusion if required to induce PVCs prior to ablation.

Baseline evaluation and follow-up

The most recent detailed baseline Holter monitoring including hourly PVC count and mean HR was analyzed. Transthoracic echocardiography was also performed prior to ablation to assess left ventricular ejection fraction (LVEF), quantified by using the Simpson's biplane formula in sinus rhythm after at least 3 consecutive sinus beats.

At least 1 post-ablation Holter monitor was obtained at each institution to document intermediate-long-term success; and echocardiography was commonly repeated once or twice a year for patients with initial impaired LVEF, until normalization or stabilization. Long-term ablation success was defined by a >80% decrease in Holter PVC burden associated with a

symptom improvement, at 3 months or later when available.[9] As previously described, a PVC-induced cardiomyopathy (PVC-CMP) was defined as an LVEF<50% that normalized after successful RFA.[10]

Data collection

Surface electrocardiograms (ECGs) from the diagnostic electrophysiological study were analyzed using electronic calipers at a 100 mm/s sweep speed. Only the clinical PVC was studied. At baseline, PVC count per minute was averaged over 5 minutes prior to catheter insertion. Other measurements included the PVC coupling (averaged 6 consecutive PVCs when possible) the coupling variability (max-min) and the sinus beat RR and QT intervals.

Infrequent intra-procedural PVCs was defined as a burden preventing adequate activation mapping (usually ≤1 or 2 PVC/min),[6] requiring isoproterenol infusion. When isoproterenol was used, the same parameters were collected at the peak response. If isoproterenol could not increase PVC frequency, we again analyzed the same parameters during the washout period, which was defined as the time period when HR was decreasing, between isoproterenol cessation until return to baseline conditions. Finally, in one center (UCLA), when PVCs were not sustainably present at baseline or during isoproterenol infusion/washout, phenylephrine (repeated boluses) was used in order to obtain a 25% increase in systolic blood pressure (~30 mmHg) triggering a vagal response decreasing HR (~10%). Again, PVC frequency, coupling, RR and QT intervals were measured during maximal drug effect. A significant response to drug was defined an increase in PVC frequency allowing comfortable mapping (≥5 PVCs/min).

Statistical analysis

Normally distributed variables were expressed as mean ± SD and compared, using Student's *t*-test or Mann-Whitney's U-test, as appropriate. Categorical variables were expressed as counts

and percentages and were compared using the Chi-square test or Fisher's exact test as appropriate. Receiver operating characteristic curves (ROC) were constructed to determine the cut-off value, sensitivity, and specificity of variables associated with infrequent intra-procedural PVCs. The area under the curve (AUC) was measured to discriminate the power of each parameter. To test whether there was a linear correlation between the 24-hour-holter hourly mean HR and their corresponding PVC frequencies, the Pearson correlation test was used to assess the strength of this relationship and classify patients into the F-HR-PVC, the S-HR-PVC or the I-HR-PVC groups given a positive ($p<0.05$), a negative ($p<0.05$) or no correlation (P=NS) was found, respectively. PVC count/min at baseline versus during drug infusions were compared using the paired *t*-test or Wilcoxon test as appropriate. ANOVA model was used to compare patient's characteristics between the 3 subgroups. A $P<0.05$ was considered statistically significant. Analyses were performed on SPSSv16.0 (SPSS Inc., Chicago, IL).

RESULTS

One hundred and one consecutive patients were included in this study. Clinical characteristics are summarized in table 1. Most patients referred for ablation were symptomatic (87.1%), with decreased LVEF (<50%) in 21.8% of cases. According to baseline Holter monitoring, the most common circadian PVC profile was a F-HR-PVC type, found in half (50.5%) of our patients (r=0.68±0.17), followed by I-HR-PVC (39.6%, r=0.09±0.21) and S-HR-PVC (9.9%, r=-0.56±0.15) profiles. Pharmacologic therapy had been prescribed prior to the index ablation in 75.2%, mainly beta-blockers (66.3%) and 28.7% had had at least 1 previous ablation attempt before the index procedure, and this subgroup had similar baseline characteristics. Right (43.6%) and Left (17.8%) outflow tracts were the most common PVC origins (Table 1). We could not identify a significant relationship between the different circadian PVC profiles and the PVC origin.

Predictors of infrequent intra-procedural PVCs

Almost a third (30.7%) of our patient population had infrequent intra-procedural clinical PVC requiring drug infusion for activation mapping. These patients had a dramatically lower PVC count at the beginning of EPS compared to those who did not require drug infusion (1.1±1.9 PVC/min vs 16.4±9.7 PVC/min; P<0.0001). It is noteworthy that they also had a significantly lower baseline HR during EPS (68.4±12.9 vs 77.7±13.1, P=0.001), despite the fact that the mean HR was similar between the two groups on their Holter recordings (74.3±11.3 vs 74.8±11.3, P=0.953) and they had similar anesthesia protocols utilized.

Approximately one third of patients with infrequent intra-procedural PVCs were present in each PVC profile group (31.4% of F-HR-PVC, 30% of I-HR-PVC and 30% of S-HR-PVC types; P=0.989). The best predictor of infrequent intra-procedural PVC was the number of hours with a PVC count<120/h (AUC=0.80, P<0.0001, sensitivity [Se]=83.9%, specificity [Spe]=74.3% for a cutoff ≥2 hours) on Holter monitoring. The overall PVC burden (%) was the second most powerful, but the most specific predictor (AUC=0.77, P<0.0001, Se=67.7% and Spe=75.7% for a cutoff ≤11.7%; Table 2).

Impact of PVC circadian profile on intra-procedural drug response

Among patient with infrequent intra-procedural PVC (n=31), 58.1% had a favorable response to at least 1 drug (Table 3). Patients responding to isoproterenol (48.4%, Figure 2) all had a F-HR-PVC profile (r range [0.395 to 0.845], p<0.05) and isoproterenol was successful in all but 1 patient, belonging to this group, who had polymorphic non-clinical PVCs induced. It is noteworthy that the magnitude of the PVC frequency response on isoproterenol in this group was not associated to the strength of the correlation coefficient (r) (p=0.489), nor it was to the intensity of the HR increase (p=0.545). Isoproterenol washout or phenylephrine was successful for the 3 patients with a S-HR-PVC type (r[-0.350 to -0.480], p<0.05); no drug could increase

PVC frequency in the 12 patients with an I-HR-PVC profile (r[-0.315 to 0.359], p=NS). It is noteworthy that the minimum cycle length at the peak response of isoproterenol was similar in patients responding and those who did not (545±112 versus 486±66 milliseconds; p=0.153). Examples of successful drug responses to isoproterenol and phenylephrine are shown in Figure 3.

PVC ablation outcome and impact of PVC frequency during EP study

After a mean follow-up of 9±7 months, ablation success was achieved in 73.3% patients, the mean PVC burden in this group was 0.4±1.4% and none of them remained symptomatic. Intensification of medical therapy after failed ablation resulted in a significant PVC suppression in 4 additional patients; therefore, overall clinical success in our patient cohort was 77.2%. The highest ablation success rate (82.9%) was obtained for patients with frequent PVCs at baseline (Figure 4). Importantly, ablation success was not significantly different in the group of patients with infrequent PVC at baseline who responded to a drug (77.8%, P=0.732), but dramatically lower in those who did not respond to any drug (15.4%, P<0.0001). Therefore, PVC frequency during EPS (spontaneous or on drug) was an important determinant of ablation success (AUC=0.82, P<0.0001, Se=83.8%, Spe=74.1% for a cutoff of >7 PVC/min). Lastly, PVC origin was also associated with procedural outcome, with mitral annulus, para-hisian, papillary muscles, and epicardial foci displaying the poorest outcome. A PVC-CMP could be diagnosed with confidence (successful ablation with mean LVEF recovering from 35±9% to 53±8%) in 13 (12.9%) patients; 6 (11.8%) from the F-HR-PVC, 6 (15%) from the I-HR-PVC and 1 (10%) from the S-HR-PVC group (P=0.868).

DISCUSSION

Main findings

This is the first study assessing the impact of PVC circadian variation on procedural outcome, including drug responses. Our main findings are:

1) There are 3 distinct PVC profiles based on the correlation of hourly PVC frequency distribution to HR changes and a significant positive correlation (F-HR-PVC) characterized 50% of cases.
2) Infrequent intra-procedural PVCs affected about one third of patients and may reduce ablation success. PVC burden (≤11.7%) as well as at least 2 hours with less than 120 PVCs on a 24-hour ECG-Holter, best predicted patients where ablation was likely to be unsuccessful.
3) Infrequent intra-procedural F-HR-PVC types likely responded to isoproterenol, S-HR-PVC types to washout or phenylephrine, while no drug could increase PVCs in the I-HR-PVC type.
4) Finally, patients with drug response had the similar intermediate-term ablation success rates to patients with frequent PVC at baseline, while patients in whom drugs failed to increase PVCs (i.e. infrequent I-HR-PVC type) displayed a poor outcome.

PVC ablation has been used to manage drug-refractory symptoms and has become the preferred approach to treat and prevent PVC-induced cardiomyopathy.[5] Long-term PVC suppression is essential for total and durable recovery because a delayed recurrence of frequent PVCs has been reported to result in recurrence of PVC-induced cardiomyopathy.[11] A classically described cause of RFA failure is the inability to reach the site of origin for technical or anatomical reasons. However, another important cause, independent of the PVC location, is when there is insufficient intra-procedural PVCs for mapping.[6] When there are insufficient PVCs to map this leads to the inability to precisely identify the site of origin. Some operators then utilize pace mapping as surrogate, which has poor spatial resolution, and lacks a reliable procedural endpoint.

Baser and colleagues reported that patients with a PVC count <32 during the first 30 min of procedure had lower success rates at 3 months. Similarly, they found that approximately one third of their patients were affected by low burden at baseline and that most of those patients (59%) did not respond to isoproterenol.[6] The current study provides data that will help predict which patients are at risk of infrequent intra-procedural PVCs, and more importantly, in which cases an increase in PVC frequency can be expected in response respond to drug (and to which drugs). In our study, patients who had a pharmacological response, compared to those who did not, had a better clinical outcome (Figure 4).

Autonomic involvement in arrhythmogenesis is well established.[12] We report in this study distinct circadian PVC profiles. The I-HR-PVC suggests a pathogenesis likely independent from autonomic variation, while the F-HR-PVC type is evoked by adrenergic triggers and the S-HR-PVC evoked by vagal triggers. Increased sympathetic and parasympathetic activity estimated through heart rate variability parameters has been specifically described during the onset of PVCs displaying these respective profiles.[13] The fact that patients with F-HR-PVC responded with increased PVC frequency to isoproterenol, a beta2-receptor agonist, which has been used for decades to reproduce sympathetically-mediated arrhythmias, further supports this concept.

Phenylephrine, an α-adrenergic agonist, induces a vagal baroreflex activation, mediated through acute rise in blood pressure following vasoconstriction.[14] Attempts to induce arrhythmias with this drug in Brugada syndrome patients has been made in the past, because these patients are at risk of sudden cardiac death specifically at rest.[15] Because only 3 patients in the S-HR-PVC type needed drug infusion and phenylephrine was used in only one center, we report in this study only one attempt. Nonetheless we believe it is a promising patient-customized approach to increase PVC frequency during mapping in this subgroup that should

be evaluated further. This specific patient had a mild (6/min) and temporary (≤1 min) increase of PVC during isoproterenol washout that was more intense and more sustained with phenylephrine infusion (12/min until RF delivery). Isoproterenol washout corresponds to a more complex situation mimicking exercise recovery during which, while sympathetic drive is decreasing, parasympathetic drive is progressively increasing in healthy subjects.[16] Therefore, the suitable autonomic balance targeted to increase PVC frequency in the S-HR-PVC group may be achieved only for a brief period. Nonetheless, we could successfully use this technique for the 2 other patients requiring drug challenge is this subgroup.

There are several possible mechanistic explanations for how increased vagal tone could increase PVC frequency. In the setting of a normally automatic focus surrounded by a region of depressed excitability (i.e. allowing entrance block but not always exit block), a slower sinus rate may result in appearance of PVC without any change in the underlying substrate (modulated parasystole). Furthermore, increased vagal activity has been shown to prolong ventricular action potential duration [17] and also transiently elicit inexcitable zones near an ectopic pacemaker in atrial tissue. Thus, it may protect it from conducted sinus impulses and, in the setting of slow heart rate, it may result in spontaneous discharges likely manifest as late coupled PVCs.[18]

It this study, no differences in the mean CI, the CI variability or the PVC location between subgroups was found that could have helped decipher the plausible pathophysiology linked to each subtype (Table 4). It is also noteworthy that although recent studies shed light on some of the pathophysiologic aspects and consequences of the coupling interval and its variability, it remains a complex interaction, not yet fully elucidated.[3, 19] Nonetheless, similar to vagally mediated atrial fibrillation, patients in the S-HR-PVC subgroup were significantly younger; therefore, increased normal automaticity (of a focus), is a likely plausible mechanism (unlike reentry or afterdepolarizations) in this subgroup of “healthier” patients.

Clinical Implications

This study, based on a detailed analysis of the PVC circadian variation on Holter monitoring allowing a classification in 3 distinct subgroups, provides a simple clinical tool that may guide ablation management in patients with frequent PVCs. Further, it may help inform patients with a more realistic and customized outcome estimation.

Mapping in F-HR-PVC ($r \geq 0.4$) patients with a high likelihood of infrequent intra-procedural PVCs should be managed with isoproterenol use. Likewise, for slow-HR-PVC ($r \leq -0.35$), phenylephrine may be useful, but further data are needed. However, I-HR-PVC patients with infrequent intraprocedural PVCs have a low likelihood of success, as our data demonstrates that no commonly used drug can induce PVCs in these cases. Therefore, when patients are known to have I-HR-PVCs, we believe PVC frequency should be monitored before entering the EP lab and the procedure potentially rescheduled when PVC burden is not sufficient at the time. In addition, we believe that this data can be made easily accessible to clinicians by having Holter software automatically generate the PVC/HR correlation coefficient and display it on reports.

An additional point can me made regarding procedural cost. In many centers after ablation of the targeted PVC, isoproterenol is used to test whether PVCs can be re-induced regardless of whether the drug was needed to allow for mapping. Knowing isoproterenol is effective in less than 50% of cases and, only in the F-HR-PVC subgroup, it should not be used post-ablation, except in these specific cases. Knowing when isoproterenol can be useful ahead of time has the potential to save both time and procedural cost.

Limitations

This is a retrospective study. Since isoproterenol always increased PVC frequency in the setting of infrequent F-HR-PVC, phenylephrine was not tested in the subgroup. Different drug administration approaches (bolus vs continuous infusions, maximal dose, etc.) were used in the different centers in this study; nonetheless infusion protocol did not seem to interfere with drug responses and this is likely to increase the applicability of our results to centers using different ablation protocols to manage PVCs. Predictors of infrequent intra-procedural PVCs as well as our circadian variation PVC classification were based a single Holter monitor prior to the ablation procedure; yet inter-Holter PVC burden variability has been described in patients with repeated Holters. More specifically, whether the correlation between PVC frequency and HR could vary between different Holter monitoring sessions and whether a patient could have several PVCs displaying different circadian profiles remains unclear and should be the focus of further research. Our classification that has been developed based on Pearson correlation to identify a linear correlation between the hourly mean HR and PVC frequency may not identify unusual association such as PVC frequency concentrated during a very narrow range of HR or specific moments of the day, and therefore classify them as I-HR-PVC. It is noteworthy that PVCs were not excluded from the mean HR count that is automatically generated by Holter software. Nonetheless, interpolated PVCs were not common and therefore PVC presence did not affect the mean HR calculation.

Conclusion: A simple analysis of Holter PVC circadian variability may provide incremental value to predict infrequent intra-procedural PVCs, guide pharmacologic induction of PVCs during RFA, and predict outcome. Patients with infrequent Fast or Slow HR-PVCs have a high likelihood of successful ablation when PVC frequency can be pharmacologically enhanced, while those with infrequent I-HR-PVC have a low ablation success rate regardless of intervention when infrequent baseline PVCs are present.

Funding: This work was made possible by support from NIH grants HL084261 & OT2OD023848 to KS and from the Federation Francaise de Cardiologie to DH.

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4 **Table 1 Baseline characteristics**

	All (n=101)
Age, yrs	49.9±16.9
Male	57(56.4)
LVEF, %	55.5±12.1
Symptoms	88(87.1)
Palpitations	76(75.2)
Dyspnea	23(22.8)
Sustained ventricular tachycardia	1(1.0)
Syncope	3(2.9)
Non-sustained ventricular tachycardia	8(7.9)
PVC burden, %	19.7±12.3
SDNN, ms	145.2±51.6
SDANN	113.2±38.4
Fast-HR-PVC	51(50.5)
Independent-HR-PVC	40(39.6)
Slow-HR-PVC	10(9.9)
Drug prior ablation	76(75.2)
Beta-blocker	67(66.3)
CCB	12(11.9
Class I AAD	17(16.8)
Amiodarone	5(4.9)
Other	5(4.9)
PVC origin:	
RVOT	44(43.6)
Para-Hisian/intra-septal	9(8.9)
Tricuspid annulus	2(2.0)
Other RV Endo	4(4.0)
LVOT	18(17.8)
Mitral annular	7(6.9)
Papillary muscle	2(2.0)

Other LV endo	**6(5.9)**
Epicardial	**7(6.9)**
Unknown	**2(2.0)**

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Table 2: Predictors of infrequent intra-procedural PVC during EP study requiring drug trial

	Patients with Infrequent Intra-procedural PVC (n=31)	Patients Without Infrequent intra-procedural PVC (n=70)	P	AUC	Cutoff	Se	Spe
Holter-PVC burden (%)	12.2±10.1	23.0±11.8	**<0.001**	0.77	≤11.7	67.7	**75.7**
Holter-lowest hourly PVC count (n/h)	120±342	362±462	**<0.001**	0.74	≤88	82.8	65.2
Holter-hours (n) with PVC <120/h	7.4±7.1	1.8±3.3	**<0.001**	**0.80**	≥2	**83.9**	74.3
EPS baseline mean heart-rate	68.4±12.9	77.7±13.1	**0.001**	0.69	≤78	86.7	49.3

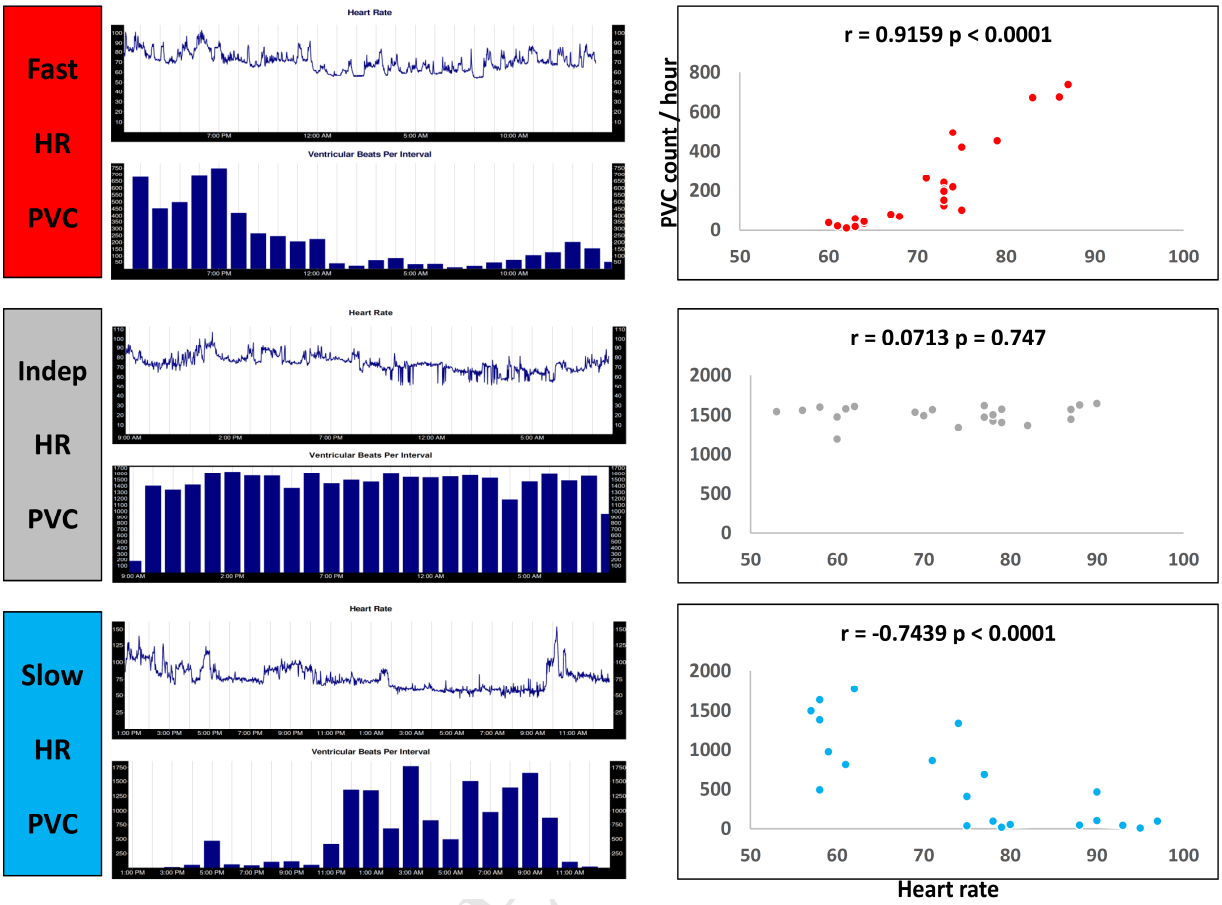
Table 3 Response to different drugs during EPS in patients with infrequent intra-procedural PVC at baseline.

Patients needing Drug infusion	Isoproterenol infusion	Isoproterenol washout	Phenylephrine infusion	Epinephrine infusion
All n (% success)	15/31(48.4)	3/16(18.7)	1/9(11.1)	0/6(0)
Fast-HR-PVC	15/16(93.7)	0/1(0)	0/1(0)	Ø
Indep-HR-PVC	0/12(0)	0/12(0)	0/7	0/6(0)
Slow-HR-PVC	0/3(0)	3/3(100)	1/1(100%)	Ø

Table 4: Patients/PVC characteristics in the 3 subgroups

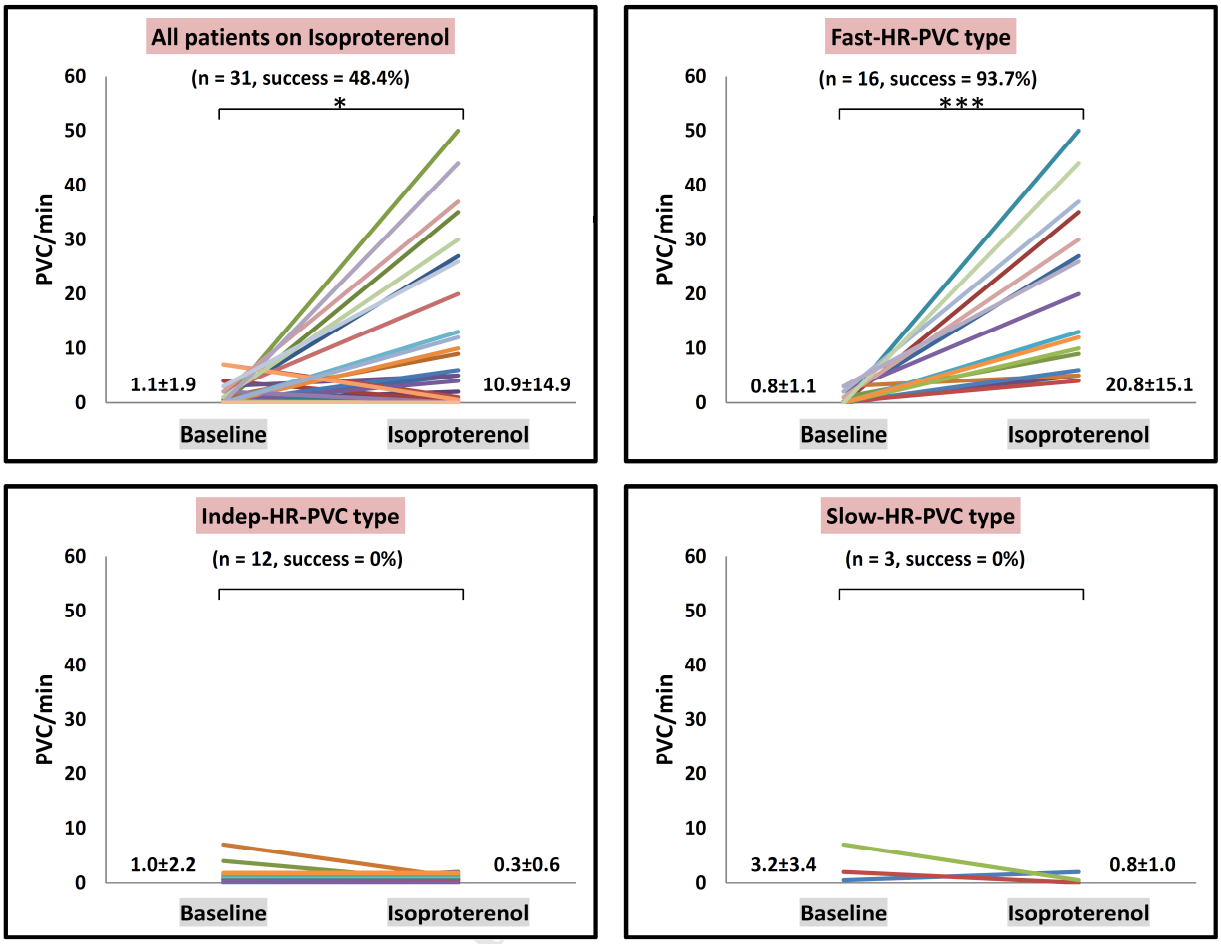
	Fast-HR PVC	Indep-HR PVC	Slow-HR PVC	p
N(%)	**51(50.5)**	**40(39.6)**	**10(9.9)**	
Age	52.7±17.4	50.1±14.9	34.3±12.8	**0.006**
Male	28(54.9)	24(60)	5(50)	0.848
PVC burden, %	20.4±13.2	19.3±11.6	18.0±11.0	0.820
LVEF, %	55.2±12.3	55.7±12.2	56.5±7.3	0.946
PVC/min at baseline	10.5±10.8	12.1±10.5	13.5±12.5	0.484
Mean Coupling	485.3±69.3	478.4±70.7	463.4±83.8	0.669
Coupling variability (max-min)	63.0±70.9	63.3±43.8	36.4±42.0	0.451
PVC localization				NS
RVOT	21(41.2)	17(42.5)	6(60)	
Para-Hisian/intra-septal	4(7.8)	4(10.0)	0(0)	
Tricuspid annulus	1(2.0)	1(2.5)	0(0)	
Other RV Endo	3(5.9)	1(2.5)	1(10)	
LVOT	9(17.6)	8(20.0)	1(10)	
Mitral annular	2(3.9)	1(2.5)	0(0)	
Papillary muscle	1(2.0)	1(2.5)	0(0)	
Other LV endo	6(11.8)	2(5.0)	2(20.0)	
Epicardial	4(7.8)	3(7.5)	0(0)	
Unknown	0(0)	2(5.0)	0(0)	

Figure 1: Holter example of the 3 PVC circadian profiles



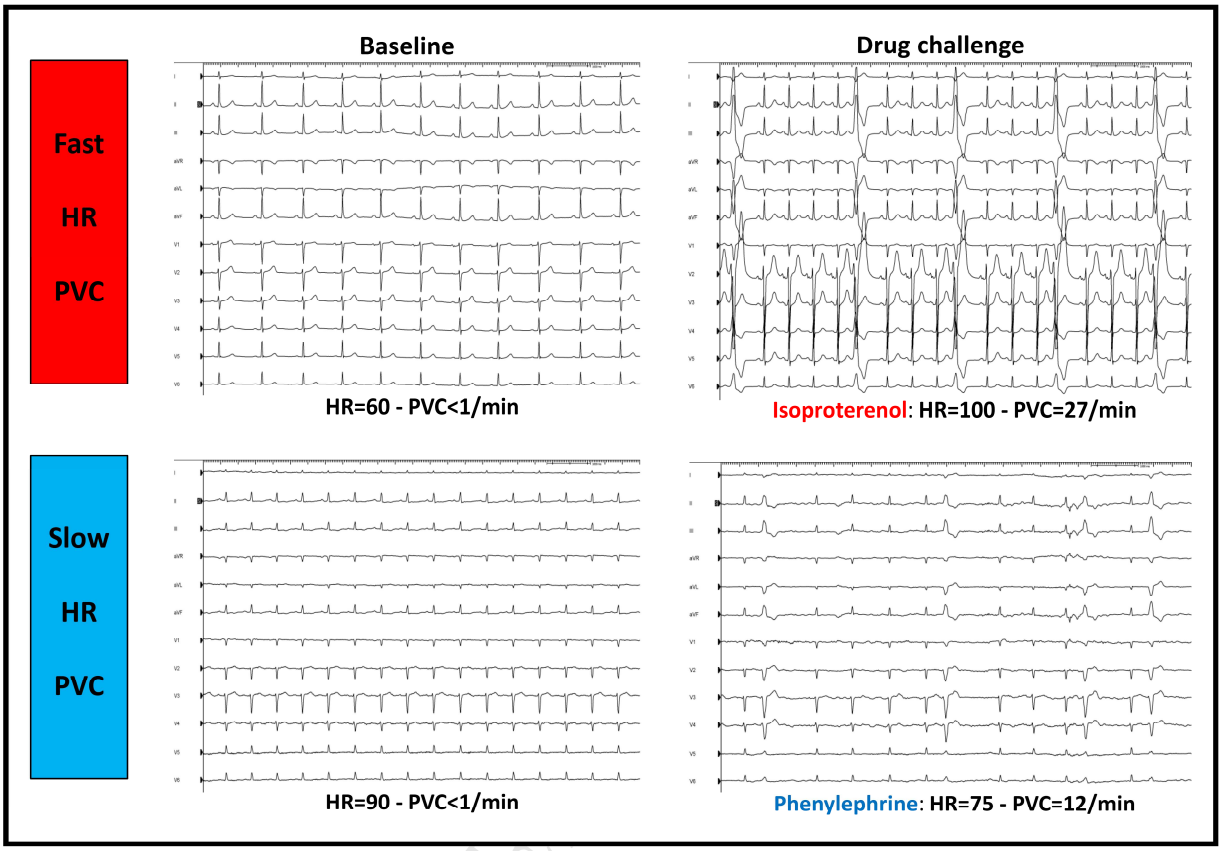
The left panels, generated by holter software, represent mean heart rate (top) and PVC count/hour (bottom) over the 24 hours. The right panels represent the PVC count (y) over the mean heart rate (x) distribution (correlation) derived from holter hourly summary raw data.

Figure 2: Isoproterenol response in patients with infrequent intraprocedural PVC



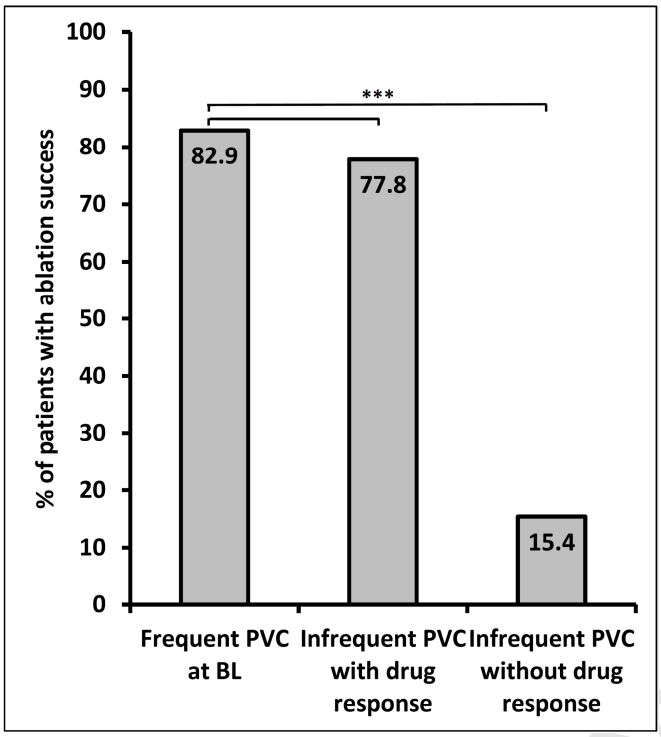
Representation of the PVC frequency (PVC/min) during the electrophysiological study at baseline and during isoproterenol infusion in all and subgroups of patients with infrequent intra-procedural PVC. Each line represents a different patient.

Figure 3: Example of drug response to isoproterenol for F-HR-PVC and to phenylephrine for S-HR-PVC



The first patient (F-HR-PVC) had no PVC spontaneously present at baseline while the PVC frequency dramatically increased during isoproterenol infusion allowing mapping. Likewise, the second patient (S-HR-PVC) had very infrequent PVCs at baseline but also during isoproterenol infusion and phenylephrine could successfully increase its frequency. Therefore, the 2 patients had successful ablations.

Figure 4: Impact of PVC frequency during EP study on ablation outcome



*** p<0.0001