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Impact of cryoballoon-guided pulmonary vein isolation on non-invasive autonomic tests in patients with paroxysmal atrial fibrillation

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ABSTRACT

Background: Pulmonary vein isolation (PVI) modulates the intrinsic cardiac autonomic nervous system (ANS). We evaluated the impact of PVI on 5 non-invasive autonomic tests.
Methods: Thirty patients (76% male, mean age 60.37 ± 9.02 years) with paroxysmal atrial fibrillation (AF) underwent cryoballoon-guided PVI. Five autonomic tests were performed 24hrs before and after PVI (N = 30) and repeated after 6months (N = 22). Parasympathetic function was measured by heart rate (HR) variability during deep breathing (E/I ratio, I-E difference), Valsalva maneuver (Valsalva-ratio) and head-up tilt test (30/15 ratio). Sympathetic function was measured by systolic BP response to sustained handgrip and 10' tilting and by diastolic BP response to cold water.
Results: 24hrs after PVI, baseline HR increased from 57.93 ± 9.06 bpm to 71.10 ± 12.75 bpm ($p < 0.001$). At 6 months, baseline HR was lower than immediately post-PVI (62.59 ± 7.89 vs 71.36 ± 13.58 bpm, $p = 0.032$) but still higher in comparison to pre-PVI (62.59 ± 7.89 vs 57.09 ± 8.80 bpm, $p < 0.001$). No differences were seen in baseline BP and parasympathetic tests acutely and at 6months. Besides an acute lowering in systolic BP increase during handgrip test, all sympathetic tests remained unchanged.
Conclusions: An acute HR increase attenuated at 6months and an acute lowered systolic BP response to sustained handgrip were the only changes after cryoballoon-guided PVI. Non-invasive autonomic tests seem therefore not appropriate to evaluate the autonomic modulatory effect of PVI, either due to a too limited sensitivity or a too localized effect of PVI to influence test results.

1. Introduction

The cardiac autonomic nervous system (ANS) can be divided into the extrinsic and intrinsic cardiac ANS [1]. The intrinsic cardiac ANS is composed of autonomic neurons and axons located in ganglionated plexi (GP). There are 7 major GP, including 4 located in the left atrium (LA) close to the LA–pulmonary vein (PV) junctions [1,2]. The role of the cardiac ANS in the development and maintenance of atrial fibrillation (AF) has been largely studied in animal and in vitro models [1,3]. Due to the proximity of the GP and the PVs, several studies have assessed the influence of pulmonary vein isolation (PVI) on the intrinsic cardiac ANS [4–8]. The consistent autonomic modulatory effect of PVI seems to be attenuation of parasympathetic activity (vagal denervation), as shown in previous studies, most of them using heart rate (HR) increase, heart rate variability (HRV) or vagal reactions (VR) during ablation as markers of intrinsic cardiac ANS modulation [4–6]. Current guidelines for the management of syncope [9] recommend the use of non-

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invasive autonomic function tests as Valsalva maneuver (class IIa) and deep breathing (class IIa) and to a lesser extent the 30:15 ratio, cold pressure and sustained hand grip test (all three IIb) in the identification of autonomic failure as the underlying cause of syncope.

By their non-invasive nature, these tests could be an attractive tool to study the autonomic influence of PVI, especially when post-procedural measurements are compared to pre-procedural baseline values. The aim is (1) to study the effect of PVI on the autonomic tests and, if possible, (2) delineate if modulation by PVI selectively involves the parasympathetic or also effect the sympathetic branch of the intrinsic cardiac ANS.

2. Methods

2.1. Patient population

Consecutive patients with symptomatic paroxysmal AF (PAF) scheduled for PVI by means of cryoballoon ablation, who were in sinus rhythm at the moment of inclusion were eligible for the study. The exclusion criteria were any contraindications for the procedure, including the presence of an intracavitary thrombus, uncontrolled heart failure, contraindications to general anesthesia and inability to provide informed consent. Patients who were considered not able to perform non-invasive autonomic function tests were also excluded from the study. Non-invasive autonomic function tests were performed the day before PVI and repeated the 24 h after PVI and again 6months post-PVI. All patients gave their written informed consent. The study protocol was approved by the hospital's institutional ethics board.

2.2. Ablation procedure

All procedures were performed under general anesthesia and using short acting neuromuscular blocking drugs for endotracheal intubation. Immediately prior to transseptal puncture an intravenous loading dose of 10.000 IU of heparin was administered. After puncture, a continuous infusion of heparin was started to maintain an activated clotting time >300 s throughout the procedure. A 14-F deflectable sheath (FlexCath, Medtronic, Minneapolis, MN, USA) was positioned in the left atrium. Transseptal sheath was continuously flushed with heparinized saline (flow rate of 10 mL/h) to avoid thrombus formation or air embolism. A 20 mm diameter Achieve inner lumen mapping catheter (ILMC) (Achieve, Medtronic, Minneapolis, MN, USA) was sequentially positioned in each PV ostium to obtain baseline electrical information. Then a 28 mm double walled cryoballoon (Arctic Front Advance) was inflated over the ILMC in the LA and positioned in the PV ostium of each vein. Optimal vessel occlusion was achieved when selective dye injection showed total contrast retention with no backflow to the atrium. Once occlusion was documented, cryothermal energy commenced. In the case of phrenic nerve injury, cryoenergy application was aborted and an immediate balloon deflation technique was performed in order to avoid further damage to the nerve. Recovery of diaphragmatic contraction was carefully monitored for 30 min. Further additional cryoenergy applications were not applied when the veins were already isolated.

2.3. Autonomic function tests

Autonomic function testing was performed following guidelines recommendations [10]. A standardized protocol was developed to make them comparable and to exclude as much confounding factors as possible. Patients were well instructed how to prepare for the tests: anti-arrhythmic drugs (AAD) were asked to be stopped two days before. Patients had to be sober and withhold alcohol for 8 h before testing. Caffeine and nicotine needed to be withheld for 3–4 h before testing. If possible, sympathomimetic drugs should be stopped for 24–48 h before testing, and anticholinergics for 48 h [10].

The patient was placed in supine position. Continuous beat-to-beat HR and blood pressure (BP) were registered by the Nexfin non-invasive Continuous Blood Pressure device using a finger cuff on the non-dominant arm [10]. Baseline HR and BP values were measure in supine position during 5 min, followed by 5 autonomic function tests in the following order: Valsalva maneuver, deep breathing test, cold pressor test, isometric (sustained) handgrip test and a 10' head-up tilt test [11,12]. Table 1 provides a scheme describing each test, including the measured parameter and the specific intrinsic cardiac ANS function evaluated.

Parasympathetic function was measured by HRV during Valsalva maneuver (Valsalva Ratio; highest HR at expiration/lowest HR after pressure release), deep breathing (E/I ratio; HR variability during respiration; highest HR during inspiration to the lowest HR during expiration, E-I difference; maximal inspiratory HR minus minimal expiratory HR), and head-up tilt test (30/15 ratio; highest HR 15seconds after tilting to the lowest HR 30seconds after tilting). Sympathetic function was measured by diastolic BP to cold pressor test, systolic BP response to isometric handgrip test and systolic BP response to 10' head-up tilt test.

2.4. Statistical analysis

Continuous variables are expressed as mean ± SD or median and interquartile range (IQR). Categorical data are expressed as number and percentages. The Shapiro-Wilk test and kolmoronov-smirnov test were used to analyze the distribution of our sample. A paired-samples *t*-test was used to compare the means of related variables before and after PVI. Wilcoxon signed rank test was used in case of variable abnormal distribution. All analyses were performed with SPSS Statistics for Windows (IBM SPSS Version 25.0. Armonk, NY: IBM Corp.).

3. Results

3.1. Baseline and procedural characteristics

Thirty patients were prospectively included in the study. The baseline characteristics are presented in Table 2. In brief, the mean age was 60.37 ± 9.02years (Female: 7 (23.3%)).

Six (20%) patients had a CHA_2DS_2-VASC score of ≥3 points. The mean LA diameter (LAD) was 40.80 ± 5.89 mm, mean BMI 26.63 ± 3.17 kg/m^2. Before ablation, 7 (23.3%) patients were receiving a Class Ic AAD plus a betablocker (BB), 9 (30%) were under BB therapy alone and 4 (13.3%) patients were receiving sotalol.

The mean procedural time was 65.37 ± 16.21 min and the mean fluoroscopy time was 11.30 ± 4.34 min. Complete PVI was obtained in all patients. Further information about procedural characteristics is described in Table 2. No vagal reactions were observed during ablation. Sudden HR increase occurred in all patients during cryoablation at the right superior PV.

3.2. Autonomic function tests 24 h after PVI

Results of the baseline HR, baseline BP, parasympathetic and sympathetic autonomic tests pre-PVI, 24 h and 6months post-PVI are summarized in Table 3.

Table 1
Resume of autonomic tests describing each test, including the measured parameter and the specific intrinsic cardiac ANS function evaluated.

Test	Technique	Measured parameter		Function tested
Valsalva maneuver	The patient was instructed to blow into a mouthpiece connected to a mercury manometer for 15 s at an expiratory pressure of 40 mmHg. The test was performed three times with a one-minute resting interval between tests in order to increase reproducibility.	Valsalva-ratio	Highest HR during the maneuver to the lowest HR after the maneuver	Parasympathetic
Deep breathing test	The patient was instructed to breathe deeply at the respiration rate of six breaths per minute for one minute under continuous HR and BP monitoring (five seconds of inspiration and five seconds of expiration per breath, timed with a chronometer).	E/I-ratio	HR variability during respiration: Ratio of the longest RR interval during expiration and the shortest RR interval during inspiration from 5 cycles.	Parasympathetic
		I-E difference	The difference between the average of the largest accelerations during inspiration and the average of the largest decelerations during expiration is calculated.	Parasympathetic
Cold pressor test	The patient's dominant hand was immersed in ice-cold water (0 °C - 10 °C) for one minute. He was instructed not to tough borders in order to maximize skin contact with the ice-cold water.	Diastolic BP response	Difference between diastolic BP before and after immersion	Sympathetic
Isometric (sustained) handgrip test	The patient was instructed to perform isometric pressing of a handgrip dynamometer at approximately thirty per cent of maximal strength during for three minutes.	Systolic BP rise during handgrip test	Difference between the highest systolic pressure during the examination and the average systolic pressure at rest	Sympathetic
Head-up tilt test	The patient was placed in supine position. Once baseline HR and BP values were achieved the	15/30-ratio	Highest HR 15 s after tilting to the lowest HR 30 s after tilting	Parasympathetic
	patient was tilted to a 70° angle during 10 min with continuous HR and BP monitoring as well as ECG-monitoring to detect cardiac arrhythmias.	Systolic BP increase during the 10-minute tilting	Difference between the highest systolic pressure after 10 tilting and the average systolic pressure at rest	Sympathetic

BP: Blood pressure; HR: Heart rate; E/I-ratio: Expiratory-inspiratory ratio; I-E difference: Inspiratory- Expiratory difference.

3.3. Baseline heart rate and blood pressure

At baseline the mean HR was 57.93 ± 9.06 bpm and significantly increased to 71.10 ± 12.75 bpm after PVI ($p < 0.001$). In 9 (30.0%) patients the HR increased less or equal to 10%, 7 (23.3%) patients presented an increase of HR between 10 and 20% and in the 14 (46.7%) remaining patients the HR increased equal or more than 20%. Baseline BP values did not significantly change after PVI (Fig. 1).

3.4. Parasympathetic function tests

The inspiratory (I)- Expiratory (E) heart rate difference (Pre-PVI: 15.70 ± 16.72 vs Post-PVI: 17.33 ± 26.61 bpm; $p = 0.794$), the E:I heart rate ratio (Pre-PVI: 1.30 ± 0.34 vs Post-PVI: 1.30 ± 0.52; $p = 0.989$), the 30/15 ratio during tilting (Pre-PVI: 1.16 ± 0.25 vs Post-PVI: 1.13 ± 0.29; $p = 0.589$) and the Valsalva ratio (Pre-PVI: 1.60 ± 0.46 vs Post-PVI: 1.43 ± 0.39; $p = 0.085$) were not significantly different (Fig. 2).

Only the Valsalva Ratio lowered acutely after PVI with a trend towards statistical significance. Also, when looking at subgroups divided by percentage of HR increase, no statistically significant difference in Valsalva Ratio was observed.

3.5. Sympathetic function tests

Diastolic BP response during the cold pressor test (24.63 ± 14.95 mmHg pre-PVI vs 21.17 ± 14.44 mmHg post-PVI; $p = 0.322$) as well as systolic BP response during 10' tilt-testing (2.50 (−9.75-11.50) pre-PVI vs −11.00 (−15.00-4.25), $p = 0.151$) remained unchanged. Systolic BP increase lowered significantly during sustained handgrip test (30.50 ± 25.49 mmHg pre-PVI vs 19.40 ± 22.40 mmHg post-PVI; $p = 0.041$).

3.6. Autonomic function tests 6months after PVI

At six months, in 22 out of 30 patients, autonomic tests were repeated. The baseline heart rate was significantly lower when compared with the immediate post-PVI values (62.59 ± 7.89 vs 71.36 ± 13.58 bpm, $p = 0.032$) but still significantly higher compared with pre-PVI values (62.59 ± 7.89 vs 57.09 ± 8.80 bpm, $p < 0.001$). There was no change in baseline BP values. Parasympathetic and sympathetic tests were not different in comparison to baseline values. Systolic BP increase during sustained handgrip test normalized at 6months (30.50 ± 25.49 mmHg pre-PVI vs 36.18 ± 29.46 mmHg 6months post-PVI; $p = 0.581$).

3.7. Clinical efficacy

At a mean follow-up of 6.43 ± 2.06 months, 3 out of 30 patients (10%) suffered a recurrence. There was no significant difference in AF freedom between patients with or without increase (≥20%) in HR (92.9% vs 87.5%, respectively; $p = 0.845$).

4. Discussion

The main findings of our study are: (1) After PVI by means of CB-A, HR at baseline significantly increased acutely, with unchanged baseline BP values (2) At 6 months, HR values were significantly lower than those registered acutely post-PVI but still higher than those found pre-PVI. (3) There were no significant differences in parasympathetic autonomic tests results after PVI by CB-A. (4) Besides a lowering in systolic BP increase during sustained handgrip acutely after PVI, no other differences in sympathetic autonomic test results were seen.

Table 2
Baseline and Procedural characteristics. Data are presented as the mean value ± SD or number (%) of patients. TIA, transient ischemic attack; TTE, trans-thoracic echocardiogram; PS-LAX, parasternal long axis view; SD, standard deviation.

Baseline Characteristics	
Age (Years)	60.37 ± 9.02
Female	7 (23,3%)
Hypertension	10 (33,3%)
Stroke/TIA	5 (16,7%)
Vascular disease	3 (10%)
Body mass index (kg/m^2)	26.63 ± 3.17
$CHADS_2VASC$	1.37 ± 1.88
$CHADS_2VASC > 2$ Points	6 (20%)
Left Atrial diameter (TTE, PS-LAX)	40.80 ± 5.89
Antiarrhythmic drugs	
• No medication	10 (33.3%)
• Class Ic + Betablocker	7 (23.3%)
• Only Betablocker	9 (30.0%)
• Sotalol	4 (13.3%)
Procedural Characteristics	
Procedure Time (Minutes)	65.37 ± 16.21
Fluoroscopy Time (Minutes)	11.30 ± 4.34
Minimal Temperature LSPV (°C)	−51.32 ± 4.05
Minimal Temperature RSPV (°C)	−53.00 ± 5.42
Minimal Temperature LIPV (°C)	−46.68 ± 3.67
Minimal Temperature RIPV (°C)	−50.93 ± 6.65
Number of freezes LSPV	1.43 ± 0.63
Number of freezes LIPV	1.30 ± 0.47
Number of freezes RSPV	1.27 ± 0.45
Number of freezes RIPV	1.17 ± 0.38
Temperature at 60 s. LSPV (°C)	−44.25 ± 3.95
Temperature at 60 s. RSPV (°C)	−45.11 ± 4.68
Temperature at 60 s. LIPV (°C)	−41.25 ± 3.35
Temperature at 60 s. RIPV (°C)	−43.22 ± 4.60
Isolation Temperature LSPV (°C)	−37.09 ± 8.53
Isolation Temperature RSPV (°C)	−29.10 ± 12.16
Isolation Temperature LIPV (°C)	−30.39 ± 9.48
Isolation Temperature RIPV (°C)	−30.53 ± 8.48
Isolation Time LSPV (Seconds)	43.39 ± 19.12
Isolation Time RSPV (Seconds)	33.38 ± 21.39
Isolation Time LIPV (Seconds)	37.28 ± 21.58
Isolation Time RIPV (Seconds)	32.73 ± 11.38

4.1. Heart rate increase after PVI

Heart rate increase after PVI has been widely described by several authors [8,13,14]. Pappone et al. [8] reported in a cohort of 297 PAF patients undergoing circumferential point-by-point PVI a significant increase in the mean and minimal HR 1 week, 1 month and 3 months after ablation. Interestingly, the maximal HR was not modified and after 6 months all values returned to pre-ablation levels. Nilsson et al. [13] also described a significant increase in the mean baseline HR at one month after ablation in 62 patients that underwent segmental or circumferential point-by-point PVI, but in contrast to Pappone's results, elevation of the mean HR persisted even up until 12 months after ablation. Subsequently, Ketels et al. [14] reported that acute HR changes invariably occur during radiofrequency (RF) energy delivery at the antero-superior junction between the LA and the right superior PV (RSPV). This finding leaded the authors to support the hypothesis that, during delivery of RF energy, parasympathetic fibers of the intrinsic cardiac ANS are ablated, resulting in vagal denervation of the LA, the sinoatrial node (SAN), and atrioventricular node (AVN). In a recent publication, Miyazaki et al. [5] reported that 66 out of 95 (69.5%) patients presented an increase of >20% in HR after PVI by means of CB-A. Interestingly, this increase was observed during the RSPV ablation in all 66 patients regardless of the order of the targeted PV. Furthermore, Qin et al. [15] studied a cohort of 62 patients that underwent anatomical GP ablation for the treatment of sinus bradycardia. The authors described a lack of obvious increase in the mean HR after ablation of the left superior (LS), left inferior (LI) and right inferior (RI) GP, only after ablation of the right anterior GP (RAGP) HR increased from 49.4 ± 5.5 beats/min to 55.6 ± 7.4 beats/min ($p < 0.05$). Moreover, ablation of the aorto-superior caval vein (AO-SCV) GP further increased the HR from 55.7 ± 7.1 beats/min to 63.6 ± 8.4 beats/min ($p < 0.05$). This finding suggests that solely ablating the RAGP (during ablation of the RSPV) is not enough to adequately denervate the SAN. In our cohort, 46.7% of our patients presented an increase ≥20% of the baseline HR, this percentage is lower than that reported by Miyazaki [5]. This difference might be explained by the smaller sample size of our cohort or by a more conservative approach during the ablation of the RSPV. The later seems plausible since, in order to avoid phrenic nerve palsy during RSPV ablation, we tend to place the balloon in a more antral position, avoiding deeper vein applications. In the study by Miyazaki, the authors do not describe the minimal temperature reached during RSPV ablation, but they describe 3 cases of persistent phrenic nerve palsy, which might suggest a more ostial approach. In fact, it is well described that phrenic nerve palsy is associated with faster temperature drop velocities [16,17]. Considering GP ablation also requires a transmural lesion similar to phrenic nerve damage, it might be possible that lower minimal temperatures and faster temperature drop velocities would create larger tissue destruction in the GP leading to more pronounced denervation.

Of note, at 6 months, we observed a partial normalization of HR

Table 3
Results of the baseline HR, baseline diastolic and systolic BP, sympathetic and parasympathetic autonomic tests pre-PVI, 24 h and 6months post-PVI. Data are presented as the mean value ± SD or Median; IQR. HR, Heart Rate; BP, Blood Pressure; I: Inspiratory, E: Expiratory. ^p values for the comparison between Pre and 24hrs Post-PVI, *p < 0.05 6months post-PVI compared with pre-PVI, & p < 0.05 6months Post-PVI compared with 24 h post-PVI.

		pre-PVI (N = 30)	24 h post-PVI (N = 30)	6 months post-PVI (N = 22)	p Value^
Baseline	Baseline HR	57.93 ± 9.06	71.10 ± 12.75	62.59 ± 7.89*&	0.000
	Baseline diastolic BP	72.13 ± 9.39	73.90 ± 7.58	76.91 ± 11.58	0.425
	Baseline systolic BP	129.63 ± 22.68	133.63 ± 15.03	137.41 ± 18.25	0.350
Sympathetic tests	Systolic BP increase during the 10-minutes tilting (Median; IQR)	2.50 (−9.75–11.50)	−11.00 (−15.00–4.25)	−8.00 (−20.00–1.00)	0.151
	Diastolic BP response to cold water	24.63 ± 14.95	21.17 ± 14.44	16.50 ± 20.63	0.322
	Systolic BP during hand grip	158.87 ± 28.13	153.03 ± 26.93	174.55 ± 38.77&	0.276
	Systolic BP increase during HG	30.50 ± 25.49	19.40 ± 22.40	36.18 ± 29.46	0.041
Parasympathetic tests	I-E heart rate difference	15.70 ± 16.72	17.33 ± 26.61	10.37 ± 12.61	0.794
	E: I heart rate ratio	1.30 ± 0.34	1.30 ± 0.52	1.19 ± 0.27	0.989
	15/30 heart rate Ratio during Tilt test	1.16 ± 0.25	1.13 ± 0.29	1.12 ± 0.27	0.589
	Heart rate ratio during Valsalva	1.60 ± 0.46	1.43 ± 0.39	1.61 ± 0.47	0.085

HR: Heart rate; BP: blood pressure; I: Inspiratory; E: Expiratory.
^ p values for the comparison between Pre and 24hrs. Post-PVI.
*p < 0.05 6months post-PVI compared with pre-PVI.
& p < 0.05 6months post-PVI compared with 24hrs post-PVI.

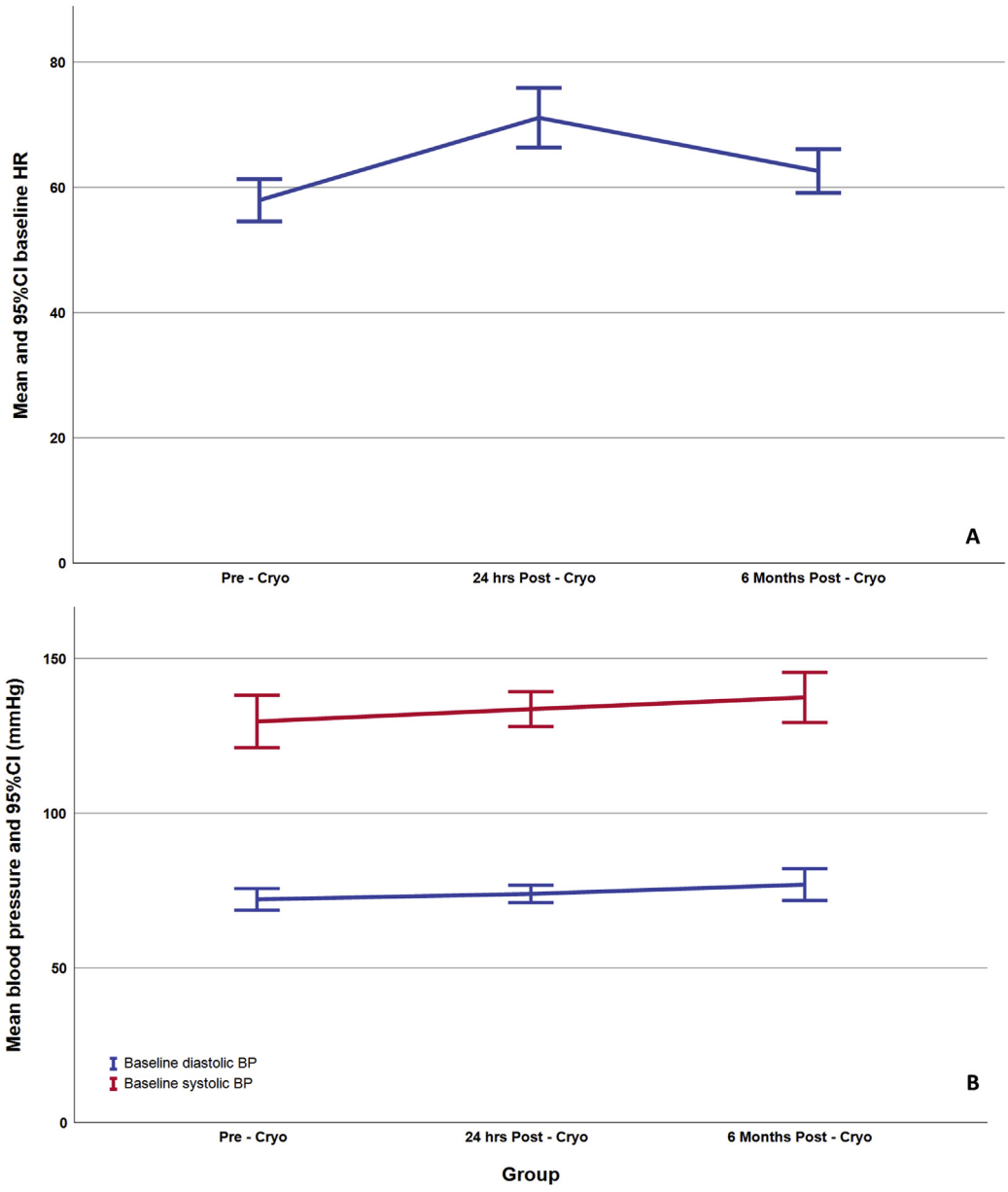

Fig. 1. Graphical representation of the mean baseline HR (upper panel) and of mean baseline systolic and diastolic BP pre-, 24hrs and 6months after cryoballoon-guided PVI. HR, Heart Rate; BP, blood pressure; CI, confidence interval.

to a level significantly lower than that registered 24 h after PVI but still higher than the pre-PVI values. Our results are in line with those of Pappone [8]. The recovery of the HR might be explained as a consequence of peripheral nerve injury in the GP resulting in Wallerian degeneration and subsequently regeneration via nerve sprouting similar to that occurring in myocardial infarction [18] or phrenic nerve injury [19].

In our cohort HR increase after PVI was not correlated with a more favorable clinical outcome. Data on the correlation of HR increase after PVI with clinical outcome are conflicting. Some report a positive correlation with clinical outcome [13] while no influence on outcome is seen in other studies [5,14].

4.2. Non-invasive autonomic tests

In this study, the value of 5 non-invasive autonomic tests proven to be of clinical use in syncope evaluation was evaluated. The reason to perform a 'battery' of tests is that due to the complexity of the intrinsic cardiac ANS no single test precisely reflects parasympathetic or sympathetic function [11]. Parasympathetic 'cardiovagal' function was measured by the analysis of HR response (Valsalva maneuver, deep breathing, 30/15 ratio) while sympathetic function was assessed by the BP response to physiological stimuli (cold pressor, sustained handgrip, Tilt testing).

Based on previous studies looking at autonomic modulation of PVI, the expectation was that PVI would result in cardiovagal denervation mainly affecting parasympathetic function and potentially could also influence sympathetic function tests. However acutely and 6months after PVI, no significant differences were seen in the parasympathetic function tests. For the sympathetic tests, systolic BP response during sustained handgrip test was lowered 24 h after PVI and normalized after 6months. The main difficulty of this test is standardizing muscle effort. Rather than a reduction in sympathetic outflow induced by PVI, most probable

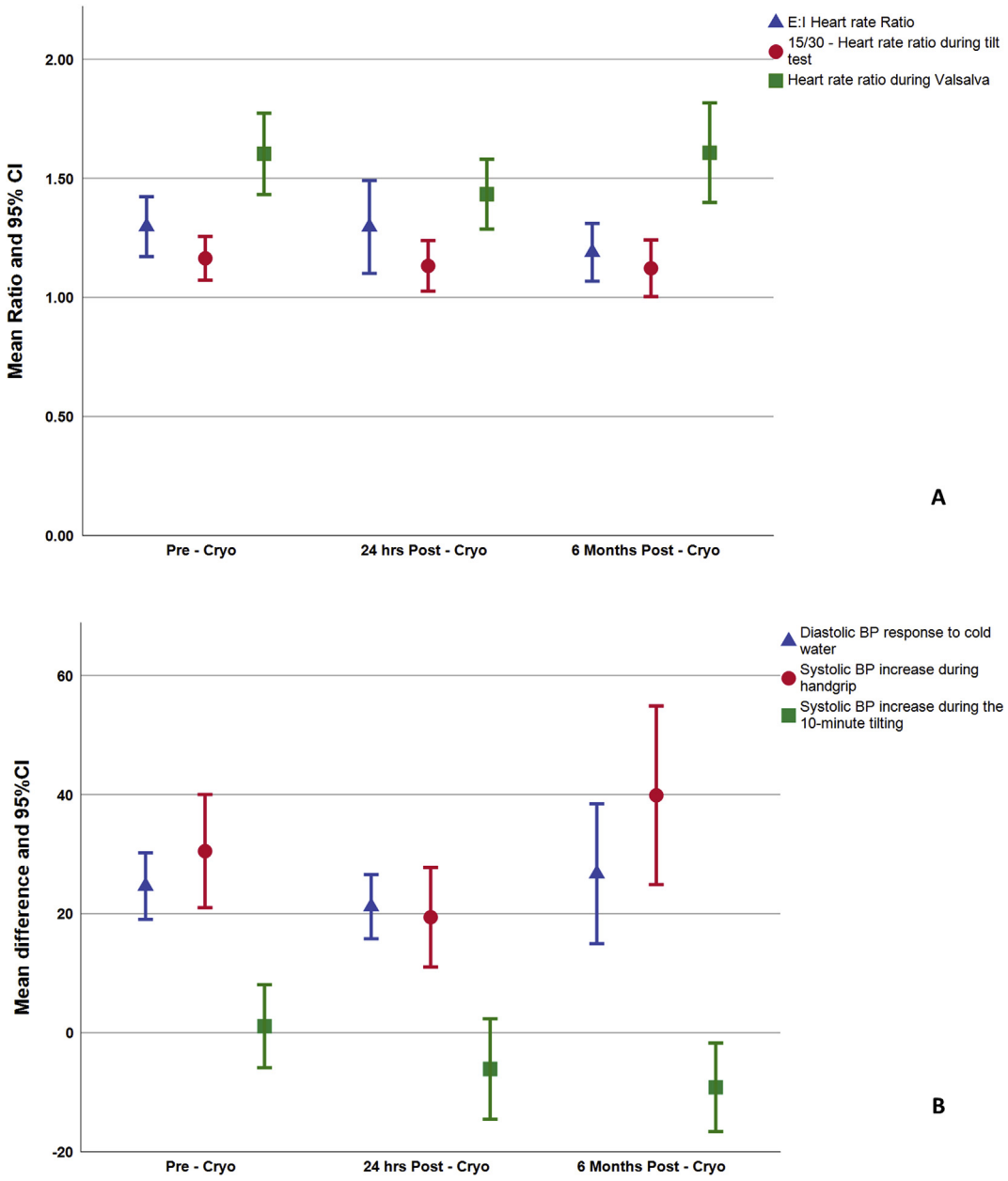

Fig. 2. Graphical representation of the parasympathetic test ratios (upper panel) and BP responses during sympathetic tests. See text for further explanation. E, expiratory; I, Inspiratory; BP, Blood Pressure.

explanation is a less firm execution of the test the day after PVI due to postoperative distress, recent anesthesia. The normal BP response to cold pressure and 10' tilting (both less dependent on patient compliance) and the normalization at 6months of the BP response to sustained hand grip further favors this explanation.

Our finding that PVI does not seem to affect autonomic test results might have 2 plausible explanations.

First, it is possible that the degree of denervation achieved during PVI by CB-A is not sufficient to affect the results of these tests. Even though CB-A is effective in modifying the 4 cardiac (PV related) GPs as shown in a recent study by Garabelli et al. [4], it largely affects the LSGP and RAGP with less effect on the RI and LIGP; additionally, during PVI, other GP (Ao-SCV, Marshall ligament and IVC) are not targeted. The importance of these non-PV related GPs may not be underestimated. As mentioned above, Qin et al. [15] reported that ablation of the Ao-SCV GP after ablation of all PV related GPs produced a significant further increase in HR. This suggests that PVI produces a significant but only partial denervation of the heart.

Second, clinical autonomic tests might not be sensible enough to detect subtle or specific changes in selective branches of the cardiac intrinsic ANS. They are now mainly used to provide objective physiological confirmation of a more generalized disease of the ANS as for example in neurogenic orthostatic hypotension in Parkinson's disease mostly in patients with clear-cut signs and symptoms. Previous studies looking at autonomic modulation of PVI used HRV as non-invasive marker of autonomic function and could demonstrate a significant decrease in HRV after PVI [4,5,8,13].

Although deep breathing test also measures HRV and is regarded as both a sensitive and specific measure of cardiovagal

function, no change in respiratory mediated HRV was observed after PVI. Most probably the PVI-induced HRV decrease is too weak or to localized to be picked up by the deep breathing test. Also a known pitfall of HRV evaluated by time domain methods is the length of the recording on which it is analyzed [20]. In consequence, guidelines [20] do not recommend using arbitrarily selected ECGs for the analysis since the standard deviation of the normal to normal intervals is not a well-defined statistical quantity because of its dependence on the length of the recording period. As explained in Table 1, clinical autonomic tests are based on a single point measurement in time of the HR during inspiration, expiration and Valsalva maneuver, which may lead to miss subtle variations that can occur after PVI.

4.3. Limitations

The present study is a single center study with a small sample size mainly due to the difficult study protocol. Autonomic tests are hampered by their indirect measurement, the need to perform several tests and combine testresults for interpretation, the intersubject variability in response, influence of age, lack of sensitivity/specificity (mainly for the sympathetic tests) and many confounding variables.

By comparing results after PVI with baseline values thereby making patients their own controls, influence of age and intersubject variability in response was overcome. Also, we used a well-defined standardized protocol excluding as many confounding factors as possible. Environmental noise for example was minimized as much as possible by using dimmed lights, keeping the doors locked and turning off the mobile phones. Also, every patient was seen during an outpatient consultation to maximize cooperation by explaining in detail the flow of the different tests. Despite this not all patients could be motivated to undergo the autonomic tests again after 6months.

5. Conclusions

Pulmonary vein isolation by the cryoballoon results in a significant acute increase in HR attenuated after 6months. Non-invasive autonomic tests are not appropriate to evaluate the autonomic modulatory effect of PVI, either due to a too limited sensitivity or a too localized effect of PVI to influence test results.

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