Original Article

Vagal response in cryoballoon ablation of atrial fibrillation and autonomic nervous system: Utility of epicardial adipose tissue location

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ABSTRACT

Background: Mechanism and effects of vagal response (VR) during cryoballoon ablation procedure on the cardiac autonomic nervous system (ANS) are unclear. The present study aimed to evaluate the relationship between VR during cryoballoon catheter ablation for atrial fibrillation and ANS modulation by evaluating epicardial adipose tissue (EAT) locations and heart rate variability (HRV) analysis.
Methods: Forty-one patients with paroxysmal atrial fibrillation (11 with VR during the procedure and 30 without VR) who underwent second-generation cryoballoon ablation were included. EAT locations and changes in HRV parameters were compared between the VR and non-VR groups, using Holter monitoring before ablation, immediately after ablation and one month after ablation.
Results: The total EAT volume surrounding the left atrium (LA) in the VR and non-VR groups was 29.0 ± 18.4 cm^3 vs 27.7 ± 19.7 cm^3, respectively ($p=0.847$). The VR group exhibited greater EAT volume overlaying the LA-left superior pulmonary vein (PV) junction (6.1 ± 3.6 cm^3 vs 3.6 ± 3.3 cm^3, $p=0.039$) than the non-VR group. HRV parameters similarly changed following ablation in both the groups. EAT volume overlaying LA-right superior PV junction was significantly correlated with the relative changes in root-mean-square successive differences ($r=-0.317$, $p=0.043$) and high frequency ($r=-0.331$, $p=0.034$), immediately after the ablation.
Conclusions: Changes in HRV parameters following ablation were similarly observed in both the groups. EAT volume on the LA-PV junction is helpful for interpretation of VR occurrence and ANS modulation.
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1. Introduction

Cryoballoon-based catheter ablation is a recently developed therapeutic device for treating atrial fibrillation (AF), and has demonstrated efficacy and prognosis similar to that of radio-frequency catheter ablation for paroxysmal AF patients [1,2]. With a unique combination of balloon occlusion of the pulmonary vein (PV) and cooling of the attached surface, PV triggers can be eliminated, and substrate modification of the left antrum (LA) obtained. During cryoballoon catheter ablation of AF, vagal responses (VRs) such as bradycardia, asystole, and atrioventricular block, occasionally occur [3–6]. Recent reports have shown that these responses may be associated with intrinsic cardiac autonomic nervous system (ANS)

expression. However, the underlying mechanism and association between VR during cryoballoon ablation procedure and the cardiac ANS remain unclear.

In discussing the cardiac ANS for catheter ablation, the ganglionated plexi (GP) located between the LA and PV junction, which are part of the ANS, may have an important role in the initiation and maintenance of AF [7,8]. The GP are located on the epicardial surface of the atria, and are involved in the epicardial adipose tissue (EAT) surrounding the LA. As the GP are unable to attach directly to the LA surface, the surrounding EAT may play an accessory role in receiving neural activity associated with the GP, and in producing pro-inflammatory adipokines [9]. Several studies have demonstrated the precise location of EAT through imaging modalities, and have suggested the presence of potential overlap between the EAT and GP areas during radiofrequency catheter ablation [10,11]. Therefore, EAT volume on the LA-PV junction area could represent an area of interconnected cardiac ANS tissue.

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This study investigated the relationship between the VR during cryoballoon ablation and the effects of the cardiac ANS, using both, visual imaging modalities and autonomic nerve function assessment. We compared the EAT volume surrounding the LA and changes in pre- and post-procedural heart rate variability (HRV) parameters between the VR and non-VR groups.

2. Material and methods

2.1. Study population

Subjects were recruited retrospectively from a catheter ablation database at Nagoya University Hospital in Japan. This ablation database was approved by our institutional ethics committee. Total 148 patients who underwent second-generation cryoballoon catheter ablation for paroxysmal AF for the first time between July 2014 and December 2015 were firstly evaluated. We excluded patients who were lost to follow-up within three months after the ablation, and those who received intravenous atropine administration prior to the procedure. For the HRV assessment, we additionally excluded patients who were administered beta-blocker, digoxin, amiodarone, and/or bepridil agents during the procedure; those who presented with structural heart diseases, diabetes mellitus, stroke and/or unusual pacing rhythms; and those who had an inadequate HRV assessment. Finally, 41 patients (11 in whom VRs, such as sinus bradycardia, asystole, and atrioventricular block occurred during the procedure, and 30 patients in whom VR did not occur) were included in the present study. The indications for catheter ablation of AF complied with the latest guidelines [12]. Before the procedure, informed consent was obtained from all patients, according to our hospital guidelines.

2.2. Examination course

Patients scheduled for catheter ablation treatment were admitted the day before the procedure. At admission, baseline blood testing, echocardiography, electrocardiography and Holter examination were performed. Antiarrhythmic agents were discontinued five half-lives before ablation. Transesophageal echocardiography was performed in all patients to confirm the absence of atrial thrombus. All patients underwent three-dimensional (3D) computed tomography (Aquilion ONE™, TSX-301C, Toshiba Medical Systems, Japan) using contrast medium (OMINIPAQUE™ 350 mgL/mL) for the visualizing LA and PV. Anticoagulant drugs, including direct oral anticoagulants, were continued during the procedure [13].

2.3. Ablation procedure

For the cryoballoon ablation procedure, the entire technique was performed with the patient conscious, using a minimal sedation strategy. Boluses of hydroxyzine pamoate 25 mg and buprenorphine 0.1 mg were administered intravenously before vascular puncture. After administration of an 80–100 IU/kg bolus of heparin, a transseptal puncture was performed using a radiofrequency needle (Baylis Medical, Inc., Montreal, QC, Canada) and an 8-French (Fr) sheath under intracardiac echocardiography monitoring. A 15-Fr steerable sheath (Flexcath Advance, Medtronic, Minneapolis, MN, USA) was introduced into the LA. Following all PV venography during rapid ventricular pacing, a second-generation 28-mm cryoballoon system (Arctic Front Advance, Medtronic, Minneapolis, MN, USA) was advanced and placed on the ostium of each PV using an inner circular mapping catheter (Achieve, Medtronic, Minneapolis, MN, USA). Following confirmation of PV ostium occlusion with the cryoballoon using contrast medium, a 180-second cycle freeze ablation was repeated until electrical isolation of the PV was achieved using the mapping catheter. During the freeze ablation of the right PV, pacing phrenic nerve stimulation was performed with compound motor action potential monitoring to avoid phrenic nerve injury [14]. During the procedure, we systematically began with isolation of the left superior PV (LSPV), followed by the left inferior PV (LIPV), right inferior PV (RIPV) and right superior PV (RSPV), respectively. An 8-mm tip cryocatheter (Freezor MAX, Medtronic, Minneapolis, MN, USA) was also available for additional touch-up freeze applications. All procedures were performed using a 3D electroanatomical mapping system (EnSite™, NavX™, St. Jude Medical, Inc., St Paul, MN, USA). After confirmation of PV isolation, a bipolar voltage amplitude map of the LA was generated with the aforementioned EnSite™ NavX system. Using a post-ablation voltage map, the level of ablation scar was quantitatively assessed by measuring post-ablation low-voltage areas (< 0.5 mV). The ablation area was defined as a low-voltage area surrounded by the voltage borderline of the left atrial side and ipsilateral PV ostia [15].

VR was defined as sinus bradycardia (< 40 bpm), asystole and/or atrioventricular block at any time during the procedure, from the time of balloon occlusion to deflation following the thawing period. Temporal ventricular pacing and intravenous atropine administration were applied in cases in which VR occurred.
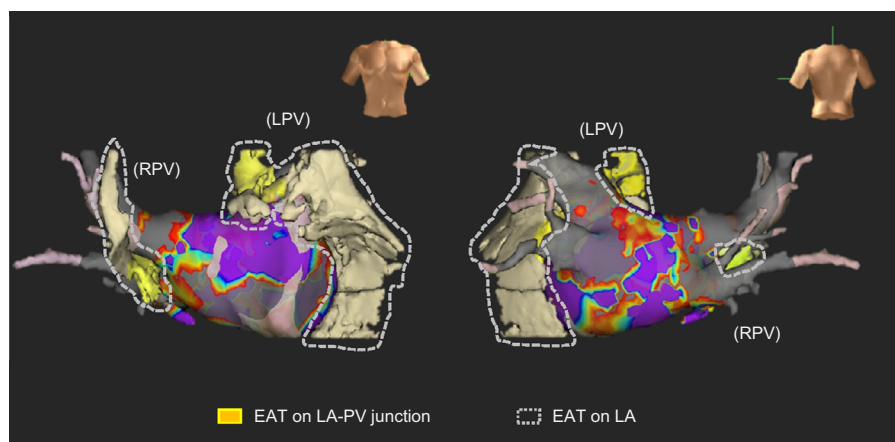

Fig. 1. Representative case showing the distribution of EAT on the LA. Fusion between the EAT image and voltage mapping in sinus rhythm after cryoballoon ablation was performed. In the voltage map, areas under 0.05 V were mapped to gray, between 0.05 and 0.5 V to colors between red and purple, and above 0.5 V to purple.

2.4. Follow-up

Patients remained hospitalized under continuous rhythm monitoring for three days after the procedure. Holter monitoring was performed in all patients immediately after ablation. Following discharge, patients were followed-up through the outpatient clinic in our hospital and by a nearby practitioner, at a minimum of once a month following ablation. At one month after the procedure, Holter examination was also performed. At the time of each follow-up visit, patients underwent 12-lead electrocardiography, and were questioned about any symptoms related to arrhythmia. If patients had an AF episode, any antiarrhythmic drugs that were discontinued before the procedure were re-administered. AF recurrence was defined as any episode of AF or atrial tachycardia of > 30 s duration. However, instances of AF or atrial tachycardia occurring within three months (blanking period) after ablation were not considered recurrent.

2.5. EAT measurement

EAT volume was evaluated from contrast CT imaging using the NavX™ system imaging software (EnSite™ Verismo™, St. Jude Medical Inc., St Paul, MN, USA). This software, which accompanied the NavX™ system, is commercially available and has already been validated for EAT assessment in several reports [10,11]. First, 0.5–1.0 mm slices of axial CT images were transferred to the NavX™ imaging software. We selected the range of imaging from the level of the pulmonary artery bifurcation to the diaphragm vertically, and from the level of aortic cusp to the vertebral body anteroposteriorly. Surface reconstruction of the LA and PV volume was semi-automatically segmented from each chamber of interest. Thereafter, the EAT surrounding the LA was identified and reconstructed by assigning Hounsfield units from −200 to −50. EAT located on the ventricular site of the mitral annulus and the lower side of the coronary sinus was excluded. As components of the total EAT volume, EAT values surrounding the LA at each PV junction were determined by the area from the border zone between healthy voltage area and the scar zone from cryoablation, as determined in voltage mapping on EnSite™ imaging system, to 15 mm inside each PV from the PV ostium. EAT assessment was performed by two independent blinded experts. Fig. 1 includes a representative fusion image between EAT locations and voltage mapping in the LA.

2.6. HRV analysis

We analyzed HRV using Holter monitoring at baseline, immediately after ablation and at one month following ablation in the hospital. Time-domain and frequency-domain HRV analysis was performed using commercially-available software (MemCalc/CHIRAM3, GMS, Tokyo, Japan). All instances of premature atrial and/or ventricular beats, AF, atrial flutter and/or electrical noise were excluded from the analysis. Following automatic exclusion, the recording file was carefully reviewed by an expert technician, with only artifact-free episodes ultimately included. Records with abnormal beats that constituted > 5% of all available beats were also excluded. In the present study, we used a period of time overnight between 2300 and 0600 the next day for HRV analysis, as a means of minimizing potential biases due to circadian variation and patient activity during the day [16]. In the time-domain HRV analysis, root-mean-square successive differences in the adjacent NN intervals (rMSSD) and the standard deviation of the NN intervals (SDNN) were evaluated. The frequency-domain analysis was performed via maximum entropy method evaluation of the NN intervals for each five-min segment of data. The low frequency (LF; range 0.04–0.15 Hz), high frequency (HF;

Table 1
Comparison of demographic and baseline characteristics between the vagal response and non-vagal response groups.

	Vagal response (n=11)	Non-vagal response (n=30)	p-value
Age, years	65.6 ± 10.8	61.9 ± 11.9	0.377
Male sex	9 (82%)	20 (67%)	0.457
Body mass index, kg/m^2	23.4 ± 2.7	25.2 ± 3.6	0.139
Duration of AF, years	3.3 ± 2.8	2.0 ± 2.4	0.247
Symptoms	10 (91%)	26 (87%)	0.999
Comorbidities			
Hypertension	5 (46%)	13 (43%)	0.999
Dyslipidemia	3 (27%)	6 (20%)	0.680
Echocardiographic data			
LA diameter, mm	38.4 ± 5.3	36.5 ± 5.4	0.320
Left ventricular ejection fraction, %	65.1 ± 4.4	65.2 ± 5.0	0.939
CHADS2 score	0.7 ± 0.8	0.7 ± 0.8	0.831
CHA2DS2-VASc score	1.8 ± 1.5	1.6 ± 1.3	0.703
Laboratory data			
eGFR, mL/min/1.73m^2	74.5 ± 14.5	72.7 ± 15.9	0.742
Total cholesterol, mg/dL	181.6 ± 23.2	185.0 ± 26.5	0.711
Ablation procedures			
Pulmonary vein isolation	11 (100%)	30 (100%)	N/A
Number of cryo-ablations	9.2 ± 3.3	8.6 ± 2.4	0.557
LSPV	3.1 ± 2.5	2.3 ± 1.0	0.146
LIPV	1.9 ± 0.5	2.3 ± 1.1	0.315
RSPV	2.6 ± 1.7	1.9 ± 0.8	0.112
RIPV	1.6 ± 0.8	2.2 ± 1.2	0.146
Cavotricuspid isthmus	0 (0%)	6 (20%)	0.167
Additional RA or LA ablation	2 (18%)	2 (7%)	0.288
Total session times, min	115.9 ± 98.2	105.6 ± 75.9	0.722
Total cryoablation time, sec	1549 ± 674	1479 ± 545	0.734
Mean voltage in the LA posterior wall, mV	2.6 ± 1.2	2.3 ± 1.2	0.583
The ablation area, cm^2	19.9 ± 5.2	21.7 ± 6.7	0.428

Data are presented as number (%), mean ± standard deviation. AF, atrial fibrillation; eGFR, estimated glomerular filtration rate; LA, left atrium; LI, left inferior; LS, left superior; PV, pulmonary vein; RA, right atrium; RI, right inferior; RS, right superior.

range 0.15–0.40 Hz) and LF/HF ratio were calculated. In general, rMSSD and HF indicates parasympathetic nervous activity, whereas LF/HF reflects sympathetic nervous activity. All values of the frequency-domain HRV were logarithmically transformed as a means of lessening the substantial range of distribution. We defined the outcome as changes in HRV parameters between baseline and post-ablation procedures [17,18].

2.7. Other examination

For echocardiography parameters, the left ventricular ejection fraction was calculated using Simpson's method and the LA diameter was assessed using M-mode methods. This retrospective study was performed in accordance with the Declaration of Helsinki. Baseline patient characteristics, comorbidities and relevant therapeutic details were obtained from hospital medical records.

2.8. Statistical analysis

Differences in baseline characteristics were analyzed using Student's t-test. Categorical variables were compared using the chi-square test or Fisher's exact test. Differences in outcome parameters between baseline and follow-up evaluations were compared using the paired t-test. Correlations between two samples were made using Spearman's rank correlation coefficient. A p-value < 0.05 was considered statistically significant.

Table 2
Changes in HRV parameters between the vagal response and non-vagal response groups.

Parameter	Baseline	Post ablation	1 month after ablation[a]	Relative change from baseline to post ablation (%)	Relative change from baseline to 1 month (%)[a]
Mean heart rate, bpm					
Vagal response	58.1 ± 7.2	71.7 ± 5.9	60.7 ± 9.1	24.6 ± 15.6	12.2 ± 15.7
P (vs baseline)		< 0.001	0.225		
Non-vagal response	57.9 ± 6.3	74.7 ± 11.8	66.2 ± 8.0	29.1 ± 14.6	16.6 ± 11.7
P (vs baseline)		< 0.001	< 0.001		
P (vs vagal response)	0.912	0.424	0.233	0.402	0.524
SDNN, ms					
Vagal response	60.2 ± 20.1	32.3 ± 23.2	37.5 ± 6.4	-42.5 ± 38.5	-50.3 ± 18.0
P (vs baseline)		0.009	0.040		
Non-vagal response	64.1 ± 24.4	25.8 ± 12.6	32.9 ± 11.9	-56.8 ± 20.0	-44.8 ± 23.9
P (vs baseline)		< 0.001	< 0.001		
P (vs vagal response)	0.653	0.257	0.461	0.128	0.665
rMSSD, ms					
Vagal response	26.5 ± 7.5	16.1 ± 5.3	23.5 ± 9.5	-32.5 ± 35.2	-12.0 ± 64.4
P (vs baseline)		0.008	0.429		
Non-vagal response	30.8 ± 15.4	16.4 ± 5.9	20.9 ± 8.8	-37.6 ± 30.3	-19.2 ± 48.1
P (vs baseline)		< 0.001	0.018		
P (vs vagal response)	0.387	0.900	0.603	0.648	0.797
Ln LF, ms²					
Vagal response	5.90 ± 0.87	4.05 ± 1.12	5.32 ± 0.59	-30.1 ± 22.2	-10.9 ± 9.6
P (vs baseline)		0.001	0.089		
Non-vagal response	5.95 ± 0.83	3.78 ± 1.22	4.64 ± 1.21	-35.2 ± 22.2	-22.3 ± 19.0
P (vs baseline)		< 0.001	< 0.001		
P (vs vagal response)	0.851	0.531	0.291	0.511	0.263
Ln HF, ms²					
Vagal response	4.89 ± 0.55	3.63 ± 0.81	4.56 ± 1.02	-24.8 ± 20.2	-10.9 ± 9.6
P (vs baseline)		0.002	0.438		
Non-vagal response	4.94 ± 0.86	3.56 ± 0.80	4.13 ± 0.94	-26.3 ± 18.3	-12.8 ± 21.9
P (vs baseline)		< 0.001	0.009		
P (vs vagal response)	0.863	0.797	0.415	0.824	0.766
LF/HF ratio					
Vagal response	1.20 ± 0.12	1.14 ± 0.30	1.20 ± 0.25	-5.8 ± 22.0	1.5 ± 17.3
P (vs baseline)		0.414	0.832		
Non-vagal response	1.22 ± 0.15	1.06 ± 0.30	1.14 ± 0.28	-13.1 ± 21.7	-8.1 ± 21.4
P (vs baseline)		0.002	0.102		
P (vs vagal response)	0.745	0.488	0.651	0.349	0.409

[a] HRV analysis 1 month after the procedure was conducted with 4 and 20 patients in the vagal response and non-vagal response groups, respectively. HRV, heart rate variability; rMSSD, root-mean-square successive differences; SDNN, standard deviation of the NN intervals; LF, low frequency; HF, high frequency.

3. Results

The baseline characteristics, examination results and ablation procedures in the VR and non-VR groups are shown in Table 1. There were no significant differences found in either baseline characteristics or ablation procedures between the two groups.

All cryoballoon ablation procedures were successfully performed, but one patient in each of the groups exhibited a transient phrenic nerve injury. However, these injuries exhibited complete recovery during the follow-up period. All patients had successful electrical isolation in all PVs. Touch-up application for the purpose of PV isolation was applied in five non-VR patients. One patient in the VR group had atrioventricular reentry tachycardia during the procedure, and additional ablation for the accessary pathway was applied.

All VRs occurred during the thawing period following the first LSPV cryoballoon ablation, except for in the case of a second LSPV ablation in one patient. Additionally another VR occurred 60 seconds after the first LIPV ablation in one patient. VRs included asystole in three patients, bradycardia in four and atrioventricular block in four. Atrioventricular block in one patient occurred during AF rhythm, whereas atrioventricular block in the other three patients occurred during atrial stimulus pacing from the coronary sinus electrode catheter; all other VRs occurred in sinus rhythm. The median time to the VR occurrence after the end of cryo-

application was 50 [25.5–69.3] seconds. On the coronary angiography, performed before the ablation procedure, three patients in the VR group exhibited a well-developed sinus node artery branching from the left circumflex artery and directed to the sinus node.

Changes in HRV parameters from baseline to post-ablation among the groups are shown in Table 2. In both groups, mean heart rate, SDNN, rMSSD, ln LF and ln HF were significantly different after ablation. Moreover, in the non-VR group, the LF/HF ratio was significantly decreased after ablation. At one month after the ablation, an HRV analysis of the data from the Holter recording was performed in four and 20 patients in the VR and non-VR groups, respectively. The relative changes in HRV parameters from baseline to post-ablation and at one month after ablation were not significantly different between the two groups. Fig. 2 demonstrates that changes in HRV parameters were seen equivalently in both the groups.

The total EAT volume surrounding the LA in the VR and non-VR groups was 29.0 ± 18.4 cm³ vs 27.7 ± 19.7 cm³, respectively ($p=0.847$). The VR group had a significantly larger EAT volume on LA-LSPV junction than did the non-VR group (6.1 ± 3.6 cm³ vs 3.6 ± 3.3 cm³, $p=0.039$; Table 3).

Correlations between EAT volume surrounding the LA and changes in HRV parameters are shown in Table 4. EAT volume on the RSPV was significantly correlated with relative changes in
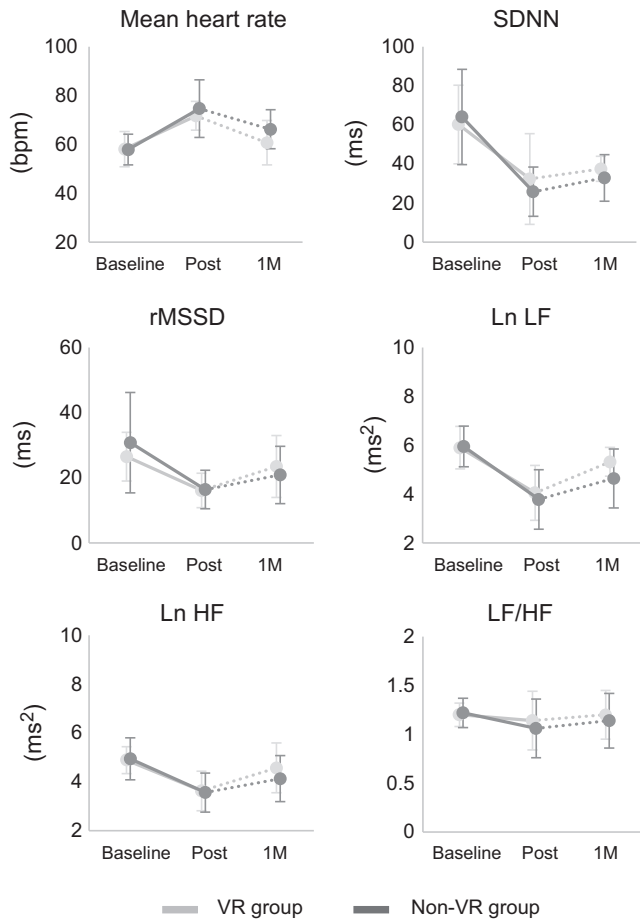
Fig. 2. Changes in HRV parameters from baseline to post-ablation and one-month after ablation among the VR and non-VR groups. HRV analysis one month after the procedure was conducted with four and 20 patients in the VR and non-VR groups, respectively. Data are presented as mean values. Error bars indicate standard deviation.

Table 3
Distribution of EAT surrounding the LA between the vagal response and non-vagal response groups.

	Vagal response ($n=11$)	Non-vagal response ($n=30$)	p-value
Total LA volume, mL	95.2 ± 17.5	94.1 ± 36.0	0.918
Total EAT volume, cm^3	29.0 ± 18.4	27.7 ± 19.7	0.847
Total EAT/BSA, cm^3/m^2	16.8 ± 9.8	15.6 ± 11.1	0.768
EAT on LSPV, cm^3	6.1 ± 3.6	3.6 ± 3.3	0.039
EAT on LIPV, cm^3	1.8 ± 1.8	1.4 ± 1.6	0.489
EAT on RSPV, cm^3	4.2 ± 3.2	3.9 ± 3.0	0.803
EAT on RIPV, cm^3	1.5 ± 1.2	2.0 ± 1.8	0.371
Total EAT/LA volume ratio	0.30 ± 0.17	0.28 ± 0.17	0.768

BSA, body surface area; EAT, epicardial adipose tissue; LA, left atrium; LIPV, left inferior pulmonary vein; LSPV, left superior pulmonary vein; RIPV, right inferior pulmonary vein; RSPV, right superior pulmonary vein.

rMSSD ($r=-0.317$, $p=0.043$) and ln HF ($r=-0.331$, $p=0.034$) post-ablation. In contrast, the EAT volume on the LSPV was not significantly correlated with any changes in HRV parameters post-ablation. Distribution of EAT on the RSPV with relative changes in rMSSD and ln HF is demonstrated in Fig. 3. In addition, no significant association was found between the EAT volume and the changes in HRV parameters at one month after ablation.

Moreover, the time course of the mean cycle length changes in the sinus rhythm during the cryoballoon ablation procedure is demonstrated in Fig. 4. The mean cycle length did not change after

the LPV ablation procedure relative to the baseline (from 886.4 ± 149.6 ms to 895.4 ± 149.3 ms, $p=0.667$), but did significant shorten following the RPV ablation procedure relative to the cycle length after the LPV ablation (from 895.4 ± 149.3 ms to 736.1 ± 114.7 ms, $p < 0.001$). Similar observations were made in both the groups.

After a follow-up of 10.1 months, recurrence occurred in one (9%) and five patient(s) (17%) in the VR and non-VR groups, respectively ($p=0.999$). Anti-arrhythmic agents were present at the follow-up in four and four patients in the VR and non-VR groups, respectively ($p=0.178$).

4. Discussion

The present study demonstrated the association between VR during cryoballoon ablation and cardiac ANS modulation by evaluating EAT locations and performing HRV analysis. The VR group had a greater EAT volume overlaying the LA-LSPV junction area than did the non-VR group. Changes in HRV parameters after ablation were also similarly observed in both the groups, while the larger EAT volume on the RSPV was associated with greater relative reduction of rMSSD and ln HF immediately after the ablation.

VR during cryoballoon catheter ablation has been described in several studies. From 36% to 40.7% of patients exhibit VR during cryoballoon ablation, and the prevalence of VR is reasonably high [3–6]. Particularly, in the case of the second-generation cryoballoon procedure, the act of pushing an inflated 28-mm balloon to the PV ostia with an enlarging LA-PV junction and performing an ablation for the wide-ranged LA antrum area all at once with cryo-application could have a larger effect on the pericardial ANS network, with higher VR prevalence. To evaluate the extent of ANS modulation, HRV parameters calculated from Holter recordings served as a surrogate for intrinsic cardiac ANS modulation. In radiofrequency catheter ablation, an association between the VR and HRV parameters has been evaluated over decades ago. In 1999, Hsieh and colleagues used HRV to evaluate autonomic function in 30 patients (six VR and 24 non-VR patients) with paroxysmal AF who underwent radiofrequency ablation. They found that both the groups had similar acute decrease in HRV time- and frequency-domains post-procedure [19]. As for the cryoballoon ablation, Oswald et al. reported a significant decrease in HRV parameters with 14 paroxysmal AF patients was present one month after performance of a first-generation cryoballoon ablation procedure, and noted that five patients showed bradycardia during the procedure [3]. However, they did not assess the individual effects or perform a functional analysis of the ANS according to the presence or absence of VR. Our findings of the similar change in HRV parameters, regardless of VR presence or absence during the cryoballoon ablation, were consistent with those of the previous studies [18,19]. This may support the suggestion that VR presence does not necessarily indicate a larger cardiac ANS modulation, even during a cryoballoon ablation procedure.

Besides the HRV parameters, a change in heart rate during the ablation procedure is also the key to accurately assessing cardiac ANS modulation. Ketels et al. described acute heart rate acceleration in 36 patients who underwent radiofrequency ablation occurring during ablation of the anterio-superior element of the LA-RSPV junction [18]. In contrast, all marked vagal reflex was observed during the left PV (LPV) ablation procedure, and the vagal reflex did not seem to cause any subsequent heart rate acceleration. Recently, Miyazaki et al. found that VR was detected in 14 of 39 patients who underwent primary cryoballoon ablation of the LSPV [6]. However, heart rate was significantly increased following RSPV ablation, regardless of status of VR occurrence during the LSPV ablation.

Table 4
Correlations between EAT volume surrounding the LA with changes in HRV parameters.

		Total EAT volume, cm³	EAT on LSPV, cm³	EAT on LIPV, cm³	EAT on RSPV, cm³	EAT on RIPV, cm³
Post-ablation (n=41)	%Mean heart rate, bpm	0.134 (0.404)	0.070 (0.661)	0.283 (0.073)	0.176 (0.270)	0.123 (0.444)
	%SDNN, ms	0.019 (0.907)	0.046 (0.777)	−0.045 (0.778)	−0.067 (0.679)	0.020 (0.903)
	%rMSSD, ms	−0.215 (0.177)	−0.177 (0.269)	−0.249 (0.117)	**−0.317 (0.043)***	−0.152 (0.343)
	%Ln LF, ms²	−0.197 (0.217)	−0.150 (0.348)	−0.243 (0.126)	−0.189 (0.236)	−0.212 (0.184)
	%Ln HF, ms²	−0.193 (0.227)	−0.215 (0.177)	−0.228 (0.151)	**−0.331 (0.034)***	−0.210 (0.188)
	%LF/HF ratio	−0.114 (0.477)	−0.035 (0.830)	−0.065 (0.687)	0.044 (0.785)	−0.045 (0.780)
1 month after ablation (n=24)	%Mean heart rate, bpm	0.071 (0.743)	0.115 (0.593)	0.063 (0.769)	−0.055 (0.798)	0.243 (0.252)
	%SDNN, ms	0.342 (0.101)	0.035 (0.869)	0.363 (0.081)	0.374 (0.072)	0.204 (0.340)
	%rMSSD, ms	−0.151 (0.480)	−0.123 (0.568)	0.052 (0.810)	−0.140 (0.514)	−0.030 (0.890)
	%Ln LF, ms²	−0.146 (0.495)	−0.096 (0.656)	0.131 (0.541)	0.059 (0.783)	−0.243 (0.253)
	%Ln HF, ms²	−0.230 (0.280)	−0.172 (0.422)	0.063 (0.769)	−0.185 (0.387)	−0.173 (0.418)
	%LF/HF ratio	0.074 (0.731)	0.064 (0.767)	0.044 (0.838)	0.243 (0.254)	−0.107 (0.619)

Values are given as correlation, r (p-value). %=relative change in heart rate variability (HRV) parameter (absolute change/baseline). EAT, epicardial adipose tissue; LA, left atrium; LPV, left pulmonary vein; RPV, right pulmonary vein.
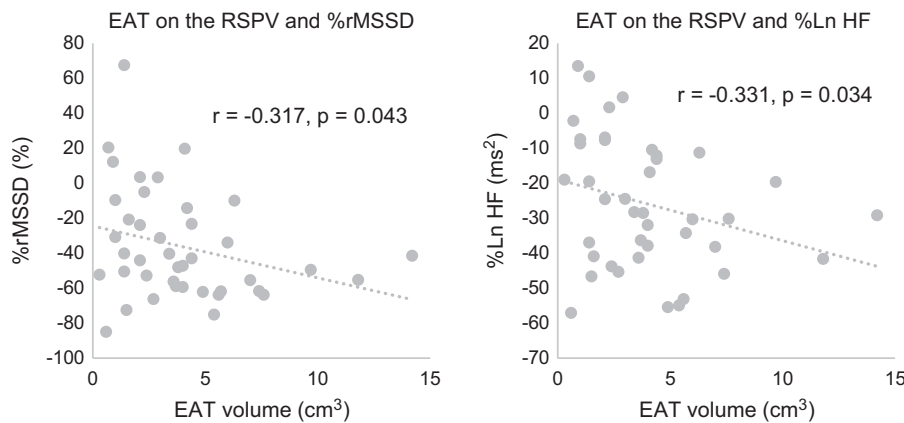
* $p < 0.05$.

Fig. 3. Distributions of EAT volume on the RSPV, with relative changes in rMSSD and ln HF from baseline to post-ablation.

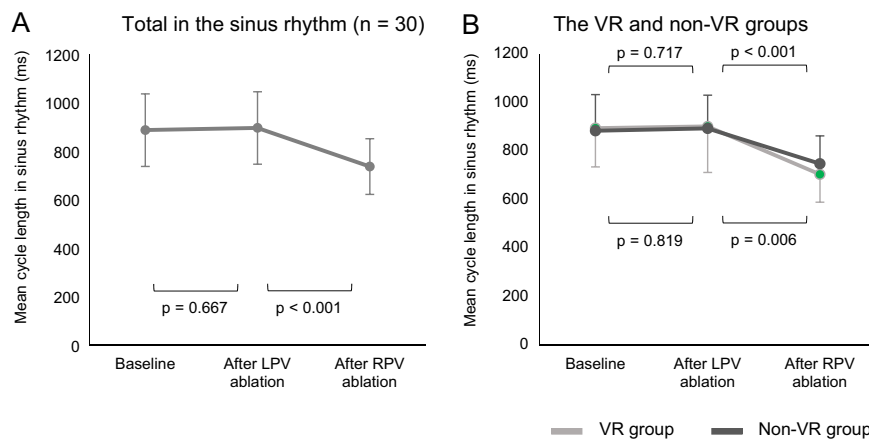**Fig. 4.** Time course of the cycle length in the sinus rhythm during the ablation procedure (n=30) (A), and between the VR (n=8) and non-VR (n=22) groups (B). Patients with atrial fibrillation rhythms or a change of rhythms during the ablation procedure were excluded. Data is presented as a mean value. Error bars indicate standard deviation.

Similar with the results of former studies, mean cycle length did not change following the LPV ablation, but significantly decreased after the RPV ablation procedure in the present study. More interestingly, the non-VR group had a significant cycle length reduction after the RPV ablation, which was comparable to that of the VR group. Based on these findings, VR occurrence and changes in heart rate may be distinct entities. Changes in heart rate, as with ANS modulation,

could primarily be a product of ablation of the anterior-right GP (ARGP), which lies close to the LA–right PV (RPV) junction. Our findings regarding the significant correlation between a decrease in rMMSD and ln HF immediately after ablation and larger EAT volume on the LA-RSPV junction could also support the previous hypothesis that suggested a dominant role for the ARGP in cardiac ANS modulation [18]. The presence of considerable cardiac ANS network

growth in the ARGP area, as represented by an increase in the amount of overlapping EAT, could be affected by RPV ablation. By contrast, we did not find any association between the EAT volume and the changes in HRV at one month after the ablation in this study with a limited sample size. Further study with a larger sample and longer-term HRV analysis would be required for such an evaluation.

In addition, we found that EAT volume on the LA-LSPV junction area was significantly greater in the VR group than in the non-VR group. This result could indicate the potential development of a cardiac ANS connection network in the SLGP area. During cryoballoon ablation of the LSPV, the neural pathway (SLGP–ARGP–sinoatrial node) could be affected, causing sinoatrial node stimulation and subsequent VR [6,20]. The larger EAT volume surrounding the LA-LSPV junction, as a growth of ANS in the SLGP area connecting to the ARGP, might play an important role in the integration and occurrence of VR. However, a larger EAT volume on the LA-LSPV junction might not indicate a direct ANS modulation, because we did not find a significant relationship between EAT on the LSPV and changes in HRV parameters. Two cases in the present study had VR occurrences from the second instance of cryoballoon ablation of the LPV. Another previous report showed that VR was reproducible during the LPV cryo-application any number of times, without any heart rate or HRV parameter changes [6]. Therefore, VR during cryoballoon ablation may be considered to be an intrinsic cardiac ANS "temporal stimulation" marker rather than a cardiac ANS modulation/denervation" marker [21]. A previous report has also demonstrated a possible mechanism of VR occurrence as a sympathovagal reflex (i.e., the so-called Bezold-Jarisch reflex) via the central neurons [22]. EAT surrounding the LSPV might thus be a collection of surrogate neurons transmitting to the central neurons, causing a vagal reflex. Another possible mechanism of the VR is the transient ischemic injury of the sinus node artery during the cryoballoon ablation procedure. Previous reports showed that a sinus node artery dominantly originating from left circumflex artery courses over the anterior LA, and that the proximity of the ablation application site to the endocardium of LA could disrupt the artery flow, resulting in bradycardia [23,24]. The present study showed that some patients, but not all of the VR patients, exhibited a well-developed sinus node artery branching from the left circumflex artery. Miyazaki et al. also showed that the VR did not occur following the initial RSPV cryoballoon ablation. Further examination and case accumulation would be needed to validate this association.

The predominant parasympathetic attenuation after the ablation was similar to the result of the previous studies on radio-frequency catheter ablation [18,19,25]. A potential reason for this phenomenon is that the higher concentrated proportion of ganglion cells in the PV junction was cholinergic [26]. Furthermore, cholinergic nerve locations were limited, as compared with the adrenergic nerves locations that were more widely distributed in the LA [27]. Thus, PV junction catheter ablation may eliminate a greater portion of the parasympathetic nerve innervation related to the sympathetic nerve innervation.

The clinical implication of the present study and its direct links to the therapeutic approach of catheter ablation for AF may be limited. However, the study findings could approach an insight mechanism of VR during cryoballoon ablation for AF by evaluating HRV analysis and EAT distribution. We could hypothesize that an ablation for the right septal LA-PV junction associates with a larger modulation of the cardiac ANS, not the VR occurrence during the LPV ablation. Moreover, a simple imaging assessment of EAT location surrounding the LA might provide helpful information in predicting VR occurrence during the ablation and the extent of the autonomic nerve modulation.

4.1. Study limitations

The main limitation of our study was the definition of intrinsic cardiac ANS development according to the EAT volume on the LA-PV junction area in the imaging analysis. We did not perform precise assessment of GP locations in the LA-PV junction area using high-frequency stimulation from the endocardium, or electrophysiological function testing. Although a previous study has confirmed that EAT can be found overlaying more than 90% of the five major anatomical GP areas in the LA, indicating a strong relationship between EAT and GP [11], all EAT does not include GPs. However, we think that this non-invasive method of EAT evaluation closely linking to GP locations in an imaging modality can be an alternative to GP stimulation that would offer less procedural stress to avoid provoking AF occurrences and to save more time. Second, the present study consisted of a small sample taken from a single institution. Compared to the remaining included population, more than half of the total population was excluded. In addition, the number of patients who could obtain a HRV analysis at one month following ablation was limited because of frequent arrhythmia occurrence and administration of anti-arrhythmic drugs at the time of Holter recording. To obtain pure autonomic nervous function parameters, we had to exclude all potential factors that could influence HRV analysis in this study. Third, touch-up application was needed for PV isolation only in the non-VR patients, probably because of the low contact force of the cryoballoon in these patients. The low contact force of the cryoballoon might have influenced GP modulation. Fourth, concerning the assessment of autonomic nerve balance, only using HRV analysis may not have sufficient reliability. Moreover, post-ablation HRV analysis was performed on the day after ablation. Thus, there may be several confounding variables that could influence ANS immediately after the ablation procedure. Finally, the correlation test results for the HRV parameters with EAT volume were individually analyzed without performance of multiple testing. Thus, a significant association between HRV parameters and EAT volume could be validated in a larger sample study.

5. Conclusions

Changes in HRV parameters after ablation were similarly observed in both groups. The VR group had a greater EAT volume on the LA-LSPV area than the non-VR group. In contrast, a greater EAT volume on the LA-RSPV might be associated with cardiac ANS modulation immediately following ablation. Knowing the location of EAT on the LA-PV junction area is helpful for the interpretation of VR occurrence during cryoballoon ablation and ANS modulation.

Disclosures

All authors declare no conflict of interest related to this study.

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