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Imbalance of autonomic nervous systems involved in ventricular arrhythmia after splenectomy in dogs

Nuttika PASTARAPATEE[1], Anusak KIJTAWORNRAT[1] and Chollada BURANAKARL[1]*

[1]Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University, Henri Dunant Rd., Pathumwan, Bangkok 10330, Thailand

ABSTRACT. The role of cardiac autonomic modulation on ventricular arrhythmia, known as ventricular premature complexes (VPC), after splenectomy was investigated. Twelve dogs undergoing splenectomy were divided into 2 groups: low VPC (<1,000/day, n=6) and high VPC groups (≥1,000/day, n=6). Electrocardiograph recording was performed prior to (D0), during the first three days (D1–3) and on day 9 (D9) after surgery. Arrhythmic indices, T_{peak}-T_{end}, corrected QT interval and short-term variability of QT interval as well as heart rate variability (HRV) were evaluated. Plasma concentrations of norepinephrine (NE) and epinephrine (E) were measured. In the high VPC group, the occurrences of VPC were significantly increased ($P<0.05$) after surgery, and reached the levels higher than those in the low VPC group. For the arrhythmic indices, only Tp-Te in the high VPC group increased significantly ($P<0.05$) after surgery. For HRV analysis, enhancement of both time and frequency domains were found postoperatively in both groups. On D2, however, the high VPC group showed significantly lower total power and high frequency with higher low to high frequency ratio ($P<0.05$) than the low VPC group. Plasma NE concentration significantly increased in the high VPC group after surgery. Dogs in the high VPC group had shorter survival time than those in the low VPC group. In conclusion, dogs with imbalance cardiac autonomic modulation accompanied with high circulating NE concentration after splenectomy are prone to ventricular arrhythmia, which leads to short survival time.

KEY WORDS: dog, heart rate variability, splenectomy, ventricular arrhythmia

Ventricular arrhythmia remains a major concern in dogs undergoing splenectomy since it can progress and lead to fatal outcome. Previous report showed that approximately 44% of splenectomized dogs had apparent ventricular arrhythmia [29]. A study of 10 dogs without preexisting cardiac disease found that ventricular premature complex (VPC) occurred from a few hour to 3 days postoperatively, and subsequently followed by ventricular fibrillation [24]. Numerous VPCs are detrimental factors that provoke a sudden cardiac death of which the mechanisms have been proposed [7].

Electrocardiographic markers for prediction of susceptibility to arrhythmia occurrence such as T_{peak}-T_{end} (Tp-Te), corrected QT interval (QTc) and short-term variability of QT interval (STV_{QT}) have been previously described [28, 32, 40]. Nevertheless, not all dogs undergoing splenectomy develop fatal arrhythmia. Therefore, the mechanism involving arrhythmia in these dogs should be identified along with changes in arrhythmia markers.

The autonomic nervous system is involved in the neurosecretory function and immune system of the spleen [10, 15] and may be one of the potential causes of arrhythmia after splenectomy. Herman and colleagues [18] found an increase in cardio-sympathetic activity via stimulation of splenic nerve, suggesting that the vulnerable situation from splenic nerve defect can ultimately lead to cardiovascular anomalies such as arrhythmia. The splenic afferents can alter cardiopulmonary and renal sympathetic efferent nerve activities which ultimately affect heart rate, contractility and blood pressure. Besides the cardiac sympathetic nerve activity, other factors may be involved in such as catecholamine-induced arrhythmia. There was a study which reported that enhanced intra-abdominal pressure either from mass or other abdominal pathologies positively correlated with hormone secretion including catecholamine from the adrenal gland [44].

The alterations in cardiac autonomic nervous system can be detected by a non-invasive, simple and practical procedure known as measurement of heart rate variability (HRV) [21, 23, 37]. Although a few data are available regarding HRV and abdominal surgery [13, 33], HRV in splenectomized dogs has not yet been identified.

*Correspondence to: Buranakarl, C.: bchollad@chula.ac.th

Therefore, this study was aimed to determine changes in electrocardiographic markers during arrhythmia. Additionally, the difference in responses of autonomic nervous system activity between dogs with low and high ventricular arrhythmia underwent splenectomy was demonstrated.

MATERIALS AND METHODS

Animals and grouping

Twelve dogs with non-specified gender, age, breed and body weight that were diagnosed with disease related to spleen and had plan to undergo splenectomy were recruited from the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University between July 2014 to July 2105. All dogs were subjected to history taking and physical examination; clinical signs of cardiac disease if present were not higher than stage B2 according to the guidelines of American College of Veterinary Internal Medicine (ACVIM) [6]. None of the dogs received any antiarrhythmic drugs or digoxin prior to the study and none had metastases to the heart or lungs. The dogs were subjected to blood collection, two views of thoracic radiographs and echocardiography.

The dogs were equally separated into 2 groups, low VPC and high VPC groups, according to the measurement of VPCs as described previously [30]. The low and high VPC groups were dogs in which the occurrence of VPCs measured postoperatively were less than 1,000 beats per 24 hr and equal to or more than 1,000 beats per 24 hr, respectively. Assessment of ventricular arrhythmia was performed by counting electrocardiographic waveforms that originated from the ventricle. Couplets and triplets of sustained VPC were counted by individual R waves within measurement periods.

Experimental protocol

The protocol followed the institutional guidelines for the care and use of animals and was approved by the Animal Care and Use Committee, Faculty of Veterinary Science, Chulalongkorn University (CU-ACUC) in protocol review number 1431110. In addition, informed written consent was obtained from all owners. The study was performed on preoperative day (D0), days 1 to 3 (D1, D2 and D3) and day 9 (D9) after splenectomy. Blood samples either from the cephalic or saphenous vein were collected on D0, D2 and D9 for measurement of concentrations of norepinephrine (NE) and epinephrine (E). Continuous electrocardiography (ECG), approximately 30 min, was recorded from a holter device on D0 and D9 during non-stressed condition while the continuous 24 hr ECG recording was performed on D1 to D3 in order to calculate HRV, Tp-Te, QTc and STV_{QT}. Blood pressure was measured using Doppler flow detection at approximately at 4:00 PM, 5 times each day and the average of each day (D0, D1, D2, D3 and D9) was calculated. Histopathology of the splenic tissue in each dog was determined by a pathologist from the Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University.

Experimental procedures

Blood collection for norepinephrine and epinephrine determinations: Three milliliters of venous blood was collected and simultaneously put into an ethylene glycol-bis (β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) tube which contained 90 mg/m*l* of ethylene glycol and 60 mg/m*l* of reduced glutathione was added for measurement of concentrations of NE and E.

Electrocardiographic parameters for arrhythmia prediction: ECG data obtained from the holter device and electrocardiographic parameters including Tp-Te, QTc and STV_{QT} were individually calculated. Tp-Te was calculated from the distance between the peak of T-wave and the termination of T-wave on ECG tracing. QTc was obtained from the onset of Q wave to the end point of T-wave according to Van de Water's equation (QTc=QT−0.087 [RR-1000]) [41]. Both Tp-Te and QTc parameters on D0 and D9 were calculated from 6 beats within 30-min duration while on D1-D3 they were calculated from 6 beats in every 6-hr duration throughout a 24-hr period and averaged. STV_{QT} was evaluated from 30 consecutive beats within 30-min duration on D0 and D9 and every 6-hr duration throughout a 24-hr period and averaged on D1-D3 according to the formula,

$$STV_{QT} = \sum |Dn+1-Dn|/30\sqrt{2}$$, where D refers to the duration of QT interval [40].

Holter monitoring and HRV analysis

The continuous ECG signals were recorded when the dogs were calm with minimal restraint. The standard 2-channel holter was used while 5-ECG electrodes were placed on the anterior thoracic wall of the dogs and connected to the monitor device (Fukuda Denshi Co., Ltd., Tokyo, Japan). ECG data were recorded in SD card and transferred and analyzed using SCM-510 software (Fukuda Denshi Co., Ltd.). For HRV, the ECG waveforms were carefully analyzed for VPC beats which were distinguished from normal beats automatically by a program and manually edited while data were used only when normal R waves were present more than 85%.

HRV was collected for 30 min on D0 and the same time frame as D0 was used to determine HRV in every single recording day (D1, D2, D3 and D9). In each 30-min duration, 3 consecutive 10-min intervals were used to analyze HRV and also to calculate average. For frequency domain analysis, parameters including very low frequency (VLF; 0.004–0.041 Hz), low frequency (LF; 0.041–0.15 Hz), high frequency (HF; 0.15–0.5 Hz), total power (TP; 0–0.5 Hz) and between low to high frequency ratio (LF/HF) were determined [38]. The Hamming window was used for filtering these signals and then data were converted into a spectrum term by Fast Fourier transformation algorithm. Parameters of time domain including SDANN, standard deviation (SD) of average value in division; SDNN index, average value of standard deviation in division; SDNN, SD of all normal RR intervals in the entire

recording; pNN50, percentage of the number of normal-to-normal intervals with differences >50 msec; RMSSD, the square root of the mean of the sum of the squares of differences between adjacent normal RR were determined [38].

Analytical procedures of plasma catecholamine

The methods for collection and preservation of blood for determination of plasma NE/E were described previously [3]. For the extraction steps, 500 μl of Extraction Buffer (#5011, Chromsystems Instruments & Chemicals GmbH, Gräfelfing, Germany) was added into a sample clean up cartridge (#5000, Chromsystems Instruments & Chemicals GmbH) with gentle shaking. Plasma sample of 1.5 ml was put together with 50 μl internal standard (3,4-Dihydroxy-benzyl-amine hydrobromide: DHBA 12 $pg/\mu l$ (#5004, Chromsystems Instruments & Chemicals GmbH), then mixed carefully by an inverting machine. The effluent was discarded by centrifugation at 2,000 rpm for a min. Afterward, 1 ml of Wash Buffer (#5005, Chromsystems Instruments & Chemicals GmbH) was added and triple centrifugations were then performed at 2,000 rpm for a min each except for the last time at 4,000 rpm for 2 min. A plastic tube was mounted to the input of the cartridge, 120 μl Elution Buffer (#5006, Chromsystems Instruments & Chemicals GmbH) was filled with soft shaking, followed by a 5-min pause, then vortex for 30 sec. The centrifugation at 2,000 rpm for a min was then performed in the attached plastic tube (#5007, Chromsystems Instruments & Chemicals GmbH) before elute solution was collected and injected into high performance liquid chromatography (HPLC) system.

Concentrations of epinephrine (E), norepinephrine (NE) and internal standard (DHBA) were delivered by HPLC pump (CLC 300, Chromsystems Instruments & Chemicals GmbH) and determined using an electrochemical detector (CLC 100, Chromsystems Instruments & Chemicals GmbH) linked to 15 cm HPLC column (#5100, Chromsystems Instruments & Chemicals GmbH). The eluent 40 μl containing vial was automatically injected into the column while the Mobile Phase Solution (#5001, Chromsystems Instruments & Chemicals GmbH) was run with a flow rate of 1 ml/min at 25°C. Data were graphically displayed in 20 min. The retention times of NE and E were at 5.5 and 7.0 min, respectively, by using DHBA as an internal standard. The standard curves were calibrated by plasma calibration standard (#5009, Chromsystems Instruments & Chemicals GmbH) with endocrine plasma control to verify normal range (#0010, Chromsystems Instruments & Chemicals GmbH) and pathological range (#0020, Chromsystems Instruments & Chemicals GmbH), respectively. The plasma calibration curves were also compared to the standard table for reliability of the quality control.

Statistical analysis

Data were expressed as mean ± SEM. One-way repeated measures analysis of variance (ANOVA) was performed followed by Dunnett's method for post-hoc analysis to compare data among periods while unpaired *t*-test was used to compare data between the low VPC and high VPC groups. Student's paired *t*-test was used to compare data of plasma NE and E concentrations between either D2 or D9 and D0. Relationship between either plasma NE or E and number of VPCs obtained from all dogs in all periods (D0, D2 and D9) was performed using Pearson's correlation. A survival curve was analyzed using Kaplan-Meier method and comparison between the two groups were performed by using log-rank test. The *P*-value <0.05 was considered as statistical significance.

RESULTS

General characteristics of dogs

The characteristics of dogs in both low VPC and high VPC groups are presented in Table 1. The age of the dogs was not different among the two groups. However, the high VPC group had higher body weight compared with the low VPC group due to the dogs being mostly Golden Retriever. The low VPC group, in contrast, consisted of a variety of breeds, half of which were small breeds. The dog sex in both groups included intact and castrated males and spayed females.

The histopathological diagnosis of spleen showed that hemangioma was presented in the low and high VPC groups with the incidence of 33.3% (2/6) and 50% (3/6), respectively (Table 1). Hemangiosarcoma was seen in one dog from each group. Other pathologic findings were splenic histiocytic sarcoma, adenocarcinoma, splenic lymphoma and splenic infarction.

Incidence of arrhythmia

The number of VPC was counted individually from single VPC and from either sustained or paroxysmal ventricular tachycardia. The dog in the low VPC group had no VPC at all before splenectomy while VPC was found in 5 from 6 dogs in the high VPC group during 30 min recording on D0 (Table 2). After splenectomy, VPCs were found, but rarely, in the low VPC group during D1-D3 while they were dramatically higher in the high VPC group with significant difference. The highest VPCs were found on D3. However, no VPC was found in the low VPC group while they were declined drastically in the high VPC when recorded at 30-min duration on D9.

Heart rate and systolic blood pressure

Heart rate (HR) in the low VPC and high VPC groups declined throughout 3 days postoperatively with significance on D3 in the low VPC group (*P*<0.05) compared with D0 (Table 2). The HR returned to the preoperative values on D9 in both groups. Systolic blood pressure (SBP) in both groups was unchanged and was not different among the periods and between the groups.

Table 1. General characteristics of all dogs that underwent splenectomy

Variables	Low VPC group (n=6)	High VPC group (n=6)	Histopathological diagnosis
Age (year)	10.5 ± 2.3	8.7 ± 0.8	
Body weight (kg)	14.6 ± 3.5	27.4 ± 5.2	
Breeds			
Labrador Retriever	1	-	Hemangioma
Golden Retriever	-	4	Adenocarcinoma
			Hemangioma with histiocytic splenitis
			Lymphoma
			Hemangiosarcoma
Siberian Husky	-	1	Hemangioma
Mixed	1	-	Splenic histiocytic sarcoma
Shetland Sheep dog	1	-	Splenic histiocytic sarcoma with cavernous hemangioma
Beagle	1	-	Hemangiosarcoma
Terrier	1	-	Splenic nodular hyperplasia with focal splenic infarction
Schnauzer	1	-	Splenic infarction with thrombus
Dachshund	-	1	Hemangioma
Gender			
M/Mc/Fs	3/2/1	3/1/2	

Data are presented as mean ± SEM. Abbreviations: M=male, Mc=castrated male, Fs=spayed female.

Table 2. Number of VPC, heart rate (HR) and systolic blood pressure (SBP) of dogs in low and high VPC groups

Parameter	Group	D0 beats/30 min	D1 beats/24 hr	D2 beats/24 hr	D3 beats/24 hr	D9 beats/30 min
VPCs	Low VPC group	0 ± 0	5 ± 2	2 ± 1	26 ± 21	0 ± 0
	High VPC group	5 ± 2[b]	2,552 ± 2,014[c]	1,801 ± 799[c]	6,385 ± 3,338[b]	2 ± 1[b]
HR	Low VPC group	108 ± 9	97 ± 15	79 ± 9	77 ± 6[a]	112 ± 15
(beats/min)	High VPC group	108 ± 12	91 ± 8	93 ± 14	90 ± 9	110 ± 7
SBP	Low VPC group	132 ± 15	132 ± 13	148 ± 15	143 ± 7	151 ± 9
(mmHg)	High VPC group	139 ± 14	143 ± 8	155 ± 16	131 ± 9	135 ± 8

Data are presented as mean ± SEM. a) indicates $P<0.05$ compared with preoperative period in the same group using one-way repeated measures ANOVA. b) indicates $P<0.05$, c) $P<0.01$ compared with low VPC group in each day using unpaired *t*-test.

Electrocardiographic parameters (Tp-Te, STV_{QT}, QTc)

All electrocardiographic parameters are shown in Fig. 1. The Tp-Te values in the low VPC group and the high VPC group increased with significances were found on D2 and D1 ($P<0.05$), respectively. On D9, they declined in both groups (Fig. 1a). The corrected QT intervals (QTc) in both groups were slightly lengthened on 1 to 3 days after surgery. On D9, QTc was still evident in the high VPC group and was significantly higher than that of the low VPC group ($P<0.05$) (Fig. 1b). STV_{QT} was not different between the groups and among the periods (Fig. 1c).

Heart rate variability

Time domain analysis: SDANN in the high VPC group was increased significantly on D3 after surgery compared with the preoperative period and showed a statistical change when compared with that of the low VPC group ($P<0.05$) (Table 3). SDNN index increased significantly in the low VPC group on D2 and D3 after surgery ($P<0.05$) and was higher than that in the high VPC group on D2 ($P<0.05$). SDNN was significantly higher ($P<0.05$) in both groups on D2 and D3 compared with D0, but without significant difference between groups. pNN50 was increased significantly ($P<0.05$) on D2 and D3 only in the low VPC group. RMSSD was elevated significantly ($P<0.05$) on D2 and D3 in the low VPC group and only on D3 in the high VPC group. All parameters of time domain in the high VPC group declined on D9 in comparison with D0.

Frequency domain analysis: VLF was increased in the low VPC group after splenectomy but not in the high VPC group (Table 4). LF tended to increase in both groups on D1-D3, but the increases were not significantly different compared with D0 and between the groups. The values on D9 declined compared with D3 in both groups. After surgery, the HF values in both groups, especially in the low VPC group, increased dramatically with statistical changes found on D3 compared with D0 in the low VPC group. When comparing among the groups, HF in the low VPC group was significantly higher on D2 and D3 compared with that in the high VPC group ($P<0.05$), resulting in significantly lower LF/HF on D2 ($P<0.05$). HF declined in both groups on D9. The same pattern was found for TP, which increased significantly on D2 and D3 compared with D0 only in the low VPC group and it was significantly higher than the high VPC group on D2 ($P<0.05$). The TP in both groups was also declined on D9.
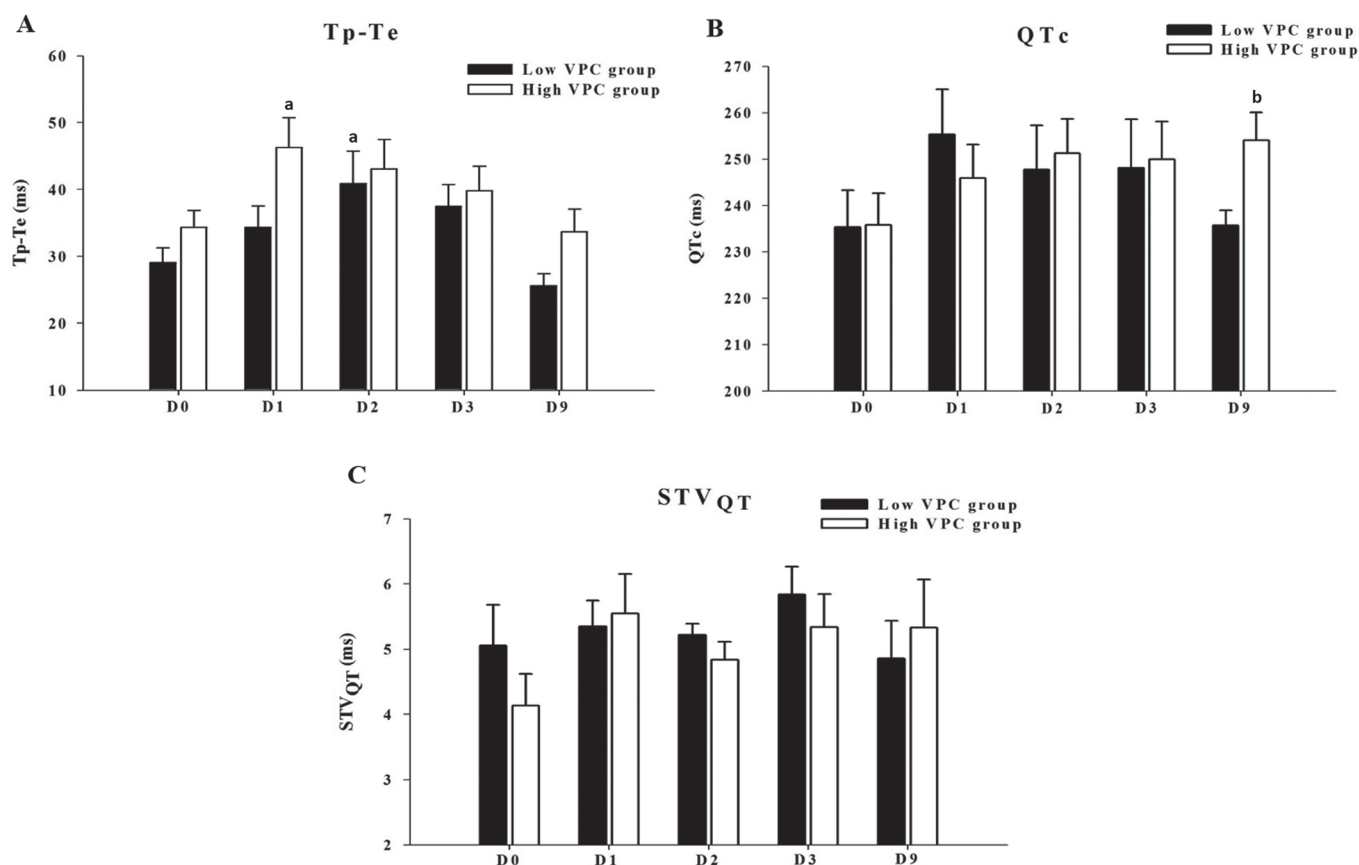
Fig. 1. Arrhythmic indices of dogs in low and high VPC groups. Data are presented as mean ± SEM. a) indicates $P<0.05$ compared with preoperative period in the same group using one-way repeated measures ANOVA. b)=$P<0.05$ compared with low VPC group in each day using unpaired *t*-test.

Concentrations of plasma catecholamine in splenectomized dogs

Plasma concentrations of NE and E on D0, D2 and D9 are shown in Table 5. In the high VPC group, enhanced NE ($P<0.05$) and E concentrations on D2 were found compared with D0. They were reduced on D9 compared with D2 but still higher than D0. NE concentration was unchanged in the low VPC group.

When investigating the relationships using all dogs in this study, correlations between the number of VPC and either NE concentration (n=36, r=0.372, $P<0.05$) or E concentration (n=36, r=0.421, $P<0.05$) were found.

Survival analysis between low VPC and high VPC groups in splenectomized dogs

The mean survival time in the low VPC group was 787 ± 137 days (range 517–1,056) with 75% of the dogs able to survive for 390 days (13 months) while the mean survival time in the high VPC group was 318 ± 69 days (range 182–453) with 75% of the dogs able to survive for 180 days (6 months) (Fig. 2). The difference between 2 group was not statistical significant (P=0.112).

DISCUSSION

The splenectomized dogs in the current findings were in middle to old age with male to female ratio of 9:3. The higher incidence in male was found in dogs subjected to splenectomy [14] and in dogs with hemangiosarcoma [17]. Golden Retriever was found the most in the present study corresponding to previous study which reported that splenic masses occurred in large dog breeds [14]. Among all splenectomy dogs, hemangioma was mostly found at 42% while the percentage of hemangiosarcoma was 17%. The incidence of hemangiosarcoma was much less than previous studies in which it was the most type of tumor found among splenic mass [14, 19]. Other lesions included splenic infarction and splenic hemorrhage. Arrhythmia was presented in all dogs prior to or after operation. Consequently, cardiac arrhythmia occurring in this study may not be related to the types of splenic disease.

VPC is commonly presented in dogs with splenic mass and subjected to splenectomy either prior to or after surgery [22, 24, 29]. Knapp *et al.* [24] and Marino *et al.* [29] demonstrated that the incidence of arrhythmia occurred within a few days postoperatively at 68 and 44%, respectively, which is similar to the present findings. One possible cause of VPC may be due to enhanced catecholamine secretion since inflammation can cause rapid increase in firing rate of sympathetic nerve innervating spleen and the release of noradrenaline within the spleen [8]. Another cause of VPC is myocardial hypoxia or ischemia or myocardial infarction

Table 3. Time domain analysis of HRV in low and high VPC groups

Parameter	Group	D0	D1	D2	D3	D9
SDANN (msec)	Low VPC group	60.77 ± 24.44	49.27 ± 14.27	62.23 ± 13.56	50.32 ± 14.76	57.6 ± 16.52
	High VPC group	51.67 ± 15.94	24.47 ± 5.67	97.71 ± 32.57	133.1 ± 28.2[a,b]	46.14 ± 11.64
SDNN index (msec)	Low VPC group	133.4 ± 44.1	182.9 ± 49.8	295.7 ± 44.8[a]	302.9 ± 47.0[a]	127.6 ± 36.8
	High VPC group	133.5 ± 38.8	187.1 ± 34.4	164.4 ± 25.3[b]	186.6 ± 22.4	127.4 ± 13.0
SDNN (msec)	Low VPC group	143.1 ± 47.8	230.8 ± 43.4	291.2 ± 45.1[a]	313.5 ± 45.9[a]	141.9 ± 36.9
	High VPC group	144.0 ± 38.5	219.6 ± 32.2	262.6 ± 43.9[a]	265.8 ± 32.3[a]	135.7 ± 12.3
pNN50 (%)	Low VPC group	39.59 ± 12.88	62.54 ± 11.7	77.98 ± 3.73[a]	75.27 ± 6.73[a]	37.3 ± 13.86
	High VPC group	37.24 ± 11.59	34.4 ± 23.33	56.54 ± 12.62	60.39 ± 9.81	43.0 ± 7.96
RMSSD (msec)	Low VPC group	165.1 ± 86.2	256.0 ± 62.2	335.7 ± 56.2[a]	364.8 ± 60.3[a]	163.4 ± 64.3
	High VPC group	142.4 ± 45.1	244.7 ± 41.3	221.1 ± 27.6	261.8 ± 36.8[a]	149.5 ± 21.9

Data are presented as mean ± SEM. a) indicates $P<0.05$ compared with D0 in the same group using one-way repeated measures ANOVA. b) indicates $P<0.05$ compared with low VPC group in each day using unpaired t-test. Abbreviations: SDANN, standard deviation (SD) of average value in division; SDNN index, average value of standard deviation in division; SDNN, SD of all normal RR intervals in the entire recording; pNN50, percentage of the number of normal-to-normal intervals with differences >50 msec; RMSSD, the square root of the mean of the sum of the squares of differences between adjacent normal RR.

Table 4. Frequency domain analysis of HRV in low and high VPC groups

Parameter (msec^2)	Group	D0	D1	D2	D3	D9
VLF	Low VPC group	2,512 ± 792	4,474 ± 1,856	11,050 ± 4,773	9,230 ± 2,613	2,533 ± 867
	High VPC group	8,073 ± 5,967	3,846 ± 1,218	3,738 ± 1,463	5,475 ± 1,772	5,578 ± 2,023
LF	Low VPC group	1,533 ± 641	3,436 ± 1,382	10,220 ± 5,125	10,712 ± 5,892	2,721 ± 1,163
	High VPC group	2,353 ± 1,115	4,218 ± 1,084	4,169 ± 1,302	11,770 ± 7,883	3,536 ± 850
HF	Low VPC group	19,838 ± 15,299	32,971 ± 16,971	62,055 ± 19,483	84,829 ± 25,689[a]	13,935 ± 7,000
	High VPC group	6,675 ± 3,595	22,040 ± 11,556	16,160 ± 3,129[b]	23,389 ± 9,294[b]	7,419 ± 2,205
TP	Low VPC group	26,863 ± 17,960	43,669 ± 19617	96,387 ± 28,708[a]	112,310 ± 29,713[a]	22,582 ± 9,920
	High VPC group	24,712 ± 13,671	37,774 ± 15,222	28,939 ± 5,947[b]	45,385 ± 16,123	20,928 ± 5,466
LF/HF	Low VPC group	0.78 ± 0.26	0.39 ± 0.18	0.16 ± 0.06	0.15 ± 0.06	0.89 ± 0.27
	High VPC group	1.17 ± 0.49	0.49 ± 0.16	0.35 ± 0.04[b]	0.46 ± 0.14	0.61 ± 0.08

Data are presented as mean ± SEM. a) indicates $P<0.05$ compared with preoperative period in the same group using one-way repeated measures ANOVA. b) indicates $P<0.05$ compared with low VPC group in each day using unpaired t-test. Abbreviations: VLF, very low frequency; LF, low frequency; HF, high frequency; TP, total power; msec^2, millisecond square.

(MI), which may occur during surgery or postoperative period. MI can cause heterogeneity among 3 cardiac layers, resulting in increased transmural dispersion of repolarization (TDR) and finally produced a malignant arrhythmia [45]. Moreover, a substance known as myocardial depressant factor, released during hypoxia condition, may cause an abnormality of the cardiac function [26]. Finally, an electrolyte imbalance such as hyponatremia may involve arrhythmia according to a previous study using Langendorff experiment [42]. There is no conclusion whether arrhythmia occurs in splenectomized dogs due to a specific cause. However, the positive relationship between NE and VPC suggests that sympathetic nervous system may be involved in the VPC occurrence in these dogs.

After splenectomy, the heart rate was reduced while the systolic blood pressure was unchanged, suggesting that cardiac parasympathetic nerve modulates heart rate while extra-cardiac factors are responsible for regulating and maintaining the systemic blood pressure. The heart rate was elevated to preoperative period levels on D9, which coincided with the return of all electrocardiographic parameters and HRV to preexisting levels.

The electrocardiographic parameters were investigated in splenectomized dogs. Tp-Te has been used to represent TDR, which is related to arrhythmia and has been studied previously [4, 5, 11]. The causes for enhanced Tp-Te may be due to sympathetic nerve stimulation [43]. Both QTc, and STV$_{QT}$ did not show any significant changes although previous studies demonstrated that STV$_{QT}$ could be used for prediction of drug-induced torsades de pointes [20, 40]. In this study, between the two study groups, the drug usage in either the anesthesia procedure or postoperative period was similar and uniform. Hence, the variations of electro-parameters between the groups unlikely originated from the anesthetic drugs.

For HRV parameters, their components and clinical significances were described previously [12, 36–39]. SDNN reflects a circadian rhythm while SDNN index and SDANN are involved in sympathetic and parasympathetic activities influenced by baroreceptor modulation. pNN50 and RMSSD stand for vagal modulation during respiratory change. Our findings showed that SDNN, SDNN index, pNN50 and RMSSD were more enhanced in the low VPC group than in the high VPC group, which implies that the modulation of baroreceptor or autonomic nervous system in the low VPC group was better than in the high VPC group. In the current study, LF, HF and TP were increased while LF/HF was decreased postoperatively in both groups, suggesting enhanced

Table 5. Plasma norepinephrine (NE) and epinephrine (E) in splenectomized dogs

Parameter		D0	D2	D9
NE (*p*g/m*l*)	Low VPC group	458.8 ± 133.5	472.4 ± 116.9	335.3 ± 68.9
	High VPC group	270.9 ± 25.6	687.0 ± 144.6[a]	440.3 ± 89.9
E (*p*g/m*l*)	Low VPC group	113.1 ± 43.0	160.9 ± 81.3	102.4 ± 39.3
	High VPC group	89.0 ± 49.6	207.5 ± 78.5	150.1 ± 64.4

Data are presented as mean ± SEM. a) indicates *P*<0.05 compared with preoperative period in the same group using paired *t*-test.
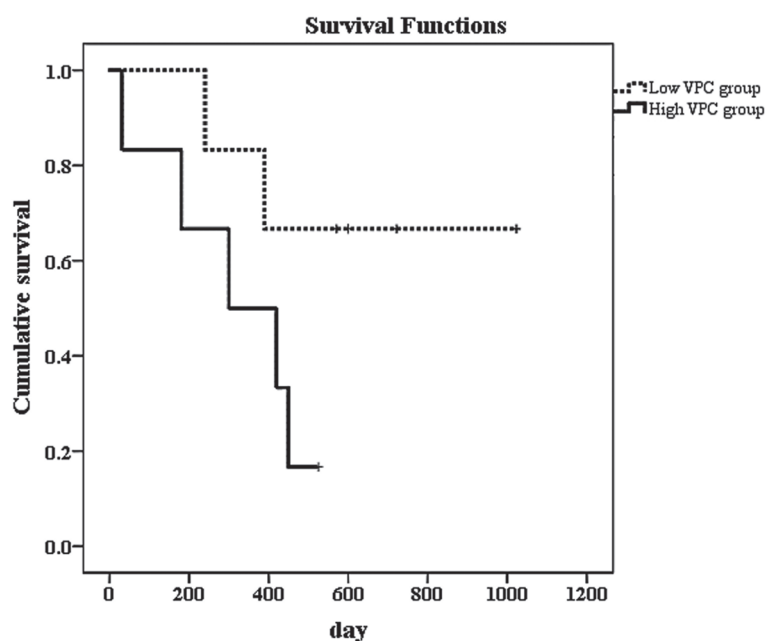
Fig. 2. Kaplan-Meier survival curves evaluated from day 1 after surgery in low VPC (dot line) and high VPC groups (solid line), respectively. +=censor which was estimated from D1 to either the death day or until October 10, 2016.

ANS activity, particularly parasympathetic pathway. The changes in HF were more pronounced in the low VPC group than in the high VPC group, causing lower LF/HF, which showed significant differences during 2 to 3 days after surgery. Thus, vagal activity was dominated and played a crucial role in controlling arrhythmia in the low VPC group. Our results, however, were contradicted by the study of Amar and co-workers [1], in which most parameters of HRV were decreased after a variety of major surgery in human. The reasons may be due to the species and variation of operation procedures or drug administered. Besides, the enhancement of sympathetic activity in this study may be due to surgical trauma or pain. However, after 9 days of surgery, all parameters returned to the preoperative levels. The data suggest that crucial cardiac death may occur around 1 week after operation.

The role of parasympathetic nervous system, especially vagus nerve, in modulating cardiac failure was reviewed [9, 35]. Mostly, the parasympathetic component improves the heart function and the treatments involved vagal nerve stimulation and baroreceptor modulation. In dogs with heart failure induced by high-rate pacing, chronic vagal nerve stimulation improved ventricular function [47]. The baroreflex sensitivity and heart rate variability improved with higher SDNN, RMSSD and HF component of HRV while LF was suppressed. Moreover, parasympathetic activation is also antiarrhythmic in many atrial and ventricular arrhythmias such as in ventricular tachyarrhythmia and in long QT syndrome as reviewed previously [25, 34]. The key modulation to control arrhythmia also focus on inhibition of sympathetic and stimulation of parasympathetic nerve activities. In dog model, with acute ischemia, low level carotid baroreceptor stimulation could prevent ventricular arrhythmias and increased HF component with decreased LF/HF ratio [27]. In dogs with acute cardiac ischemia by coronary occlusion and reperfusion, subthreshold vagal stimulation suppressed ventricular arrhythmia and decreased many inflammatory mediators; serum C-reactive protein, interleukin-6, tumor necrosis factor-α and high-mobility group box 1 (HMGB1) and noradrenaline during both ischemia and reperfusion periods [46]. In rat with ischemia-induced arrhythmia model, vagal nerve stimulation exerted anti-arrhythmogenic which was abolished by atropine, accompanied by prevention of the loss of phosphorylated connexin-43 effects during acute myocardial infarction [2]. Our results are in agreement with the previous findings and our study is the first to demonstrate the significant role of vagus in suppressing ventricular arrhythmia in dogs after splenectomy.

The elevation of both NE and E concentrations were found in both groups on D2, which may be due to postoperative stress/pain or hypoxia situation occurring either immediately or later on as seen in human after non-cardiothoracic surgery [1]. The hypoxia could induce catecholamine release as shown in cultured rat adrenal chromaffin cells [31]. Nevertheless, the high VPC group had higher responses to catecholamine release than the low VPC group. Higher catecholamine may be another factor responsible for higher VPC occurrence after surgery since the correlations were found between either NE or E and the number of VPC when considering all dogs in all periods (n=36, r=0.372, P<0.05 and n=36, r=0.421, P<0.05, respectively). Low response to NE release were found in dogs with induced ventricular arrhythmia using ischemic reperfusion model subjected to subthreshold vagal stimulation [46] and in dogs with heart failure induced by high-rate pacing after chronic cervical vagal nerve stimulation [47]. Thus, the dogs in the low VPC group of the present study had lower NE, suggesting that the high vagal activity was present in this group.

The survival times predicted from survival curve in the low VPC group was longer than in the high VPC groups (787 and 318 days, respectively) although records of 30% of dogs dying within a few months were demonstrated [16]. Thus, the length of survival in splenectomized dogs may in part depend upon the severity of cardiac arrhythmia. However, since most dogs in the high VPC group were Golden Retriever, the shorter survival curve might be affected in part by this large breed of dogs.

In conclusion, some splenectomized dogs had increased TDR along with high VPC occurrence after surgery corresponding to elevated catecholamine levels. Both enhanced sympathetic and parasympathetic nervous system activities were found in all dogs that underwent splenectomy, but impaired response of parasympathetic activation was demonstrated for the first time in dogs with high incidence of cardiac arrhythmia.

CONFLICT OF INTEREST. The authors had no conflict of interest.

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