การเปลี่ยนแปลงของการกระจายตัวในระยะรีโพลาไรเซชันของหัวใจและสมดุลของระบบประสาทอัตโนวัติในสุนัขที่ตัดม้าม

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ALTERATIONS IN DISPERSION OF CARDIAC REPOLARIZATION AND AUTONOMIC BALANCE IN THE SPLENECTOMIZED DOG

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Animal Physiology
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Thesis Title	ALTERATIONS IN DISPERSION OF CARDIAC REPOLARIZATION AND AUTONOMIC BALANCE IN THE SPLENECTOMIZED DOG
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ณัฐฐิกา พัสตรเภที : การเปลี่ยนแปลงของการกระจายตัวในระยะรีโพลาไรเซชันของหัวใจและสมดุลของระบบประสาทอัตโนวัติในสุนัขที่ตัดม้าม (ALTERATIONS IN DISPERSION OF CARDIAC REPOLARIZATION AND AUTONOMIC BALANCE IN THE SPLENECTOMIZED DOG) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ. สพ.ญ. ดร. ชลลดา บูรณกาล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. น.สพ. ดร. อนุศักดิ์ กิจถาวรรัตน์, 88 หน้า.

ภาวะหัวใจเต้นผิดจังหวะชนิดมีจุดกำเนิดจากหัวใจห้องล่างหรือวีพีซีนั้น จัดเป็นข้อแทรกซ้อนสำคัญภายหลังการผ่าตัดม้ามในสุนัข เนื่องจากสามารถโน้มนำไปสู่การเกิดหัวใจเต้นผิดจังหวะชนิดรุนแรงได้ เช่น ภาวะหัวใจห้องล่างสั่นพริ้ว (ventricular fibrillation) ในการศึกษานี้ ผู้วิจัยทำการศึกษาในสุนัขจำนวน 12 ตัวที่เข้ารับการผ่าตัดม้าม โดยรายงานผลจากสุนัขทั้ง 12 ตัว และแบ่งเป็น 2 กลุ่มตามปริมาณของวีพีซีที่เกิดขึ้นใน 24 ชั่วโมง อันได้แก่ กลุ่มสุนัขที่มีปริมาณวีพีซีน้อยจำนวน 6 ตัว และกลุ่มสุนัขที่มีปริมาณวีพีซีมากจำนวน 6 ตัว ซึ่งสุนัขทุกตัวในงานวิจัยนี้ไม่ได้รับยาต้านการเต้นผิดจังหวะของหัวใจหรือยาไดจอกซินก่อนทำการศึกษา ทำการติดอุปกรณ์บันทึกคลื่นไฟฟ้าของหัวใจชนิดพกพาเป็นเวลา 30 นาที ก่อนผ่าตัดและวันที่ 9 หลังการผ่าตัด ระหว่างที่สุนัขพักฟื้นภายหลังการผ่าตัดนั้น ผู้วิจัยทำการติดอุปกรณ์บันทึกคลื่นไฟฟ้าของหัวใจชนิดพกพาอย่างต่อเนื่องเป็นเวลา 72 ชั่วโมง วัดความดันซิสโตลีทางอ้อมด้วยวิธีดอปเปลอร์ เก็บตัวอย่างเลือดไปวิเคราะห์ค่าทางโลหิตวิทยา ได้แก่ จำนวนเม็ดเลือดแดง ฮีมาโทคริต เกล็ดเลือดและจำนวนเม็ดเลือดขาว ค่าเคมีในเลือด ได้แก่ ระดับเอ็นไซม์ ALT ALP ค่ายูเรียไนโตรเจน ครีเอทินีน โปรตีนรวมในเลือด โซเดียมและโพแทสเซียม รวมถึงตรวจวัดระดับนอร์อิพิเนฟรินและอิพิเนฟรินในพลาสมาด้วย ผลการศึกษาพบว่า สุนัขมีปริมาณวีพีซีขึ้นสูงสุดในวันที่ 3 (p<0.05) และลดลงในวันที่ 9 หลังการผ่าตัด อัตราการเต้นของหัวใจในสุนัขทั้ง 12 ตัว และกลุ่มสุนัขที่มีปริมาณวีพีซีน้อย ลดลงภายหลังการผ่าตัดอย่างมีนัยสำคัญทางสถิติ (p<0.05) แต่ไม่พบการเปลี่ยนแปลงของความดันซิสโตลี ในส่วนการประเมินพารามิเตอร์ที่วิเคราะห์ได้จากคลื่นไฟฟ้าหัวใจนั้น ค่าความแปรปรวนของการเกิดรีโพลาไรเซชันในหัวใจห้องล่าง (transmural dispersion of repolarization: Tp-Te) เพิ่มขึ้นวันที่ 2 ในกลุ่มสุนัขที่มีปริมาณวีพีซีน้อย และวันที่ 1 ในกลุ่มสุนัขที่มีปริมาณวีพีซีมากอย่างมีนัยสำคัญทางสถิติ (p<0.05) ค่าความแปรปรวนในอัตราการเต้นของหัวใจแบบการวิเคราะห์ช่วงเวลา SDANN SDNN index SDNN pNN50 และ RMSSD มีแนวโน้มสูงขึ้นภายหลังการตัดม้ามเมื่อเทียบกับก่อนผ่าตัด ค่าความแปรปรวนในอัตราการเต้นของหัวใจแบบการวิเคราะห์ช่วงความถี่พบว่า ค่า HF และ TP ในสุนัขทั้ง 12 ตัว สูงขึ้นภายหลังการผ่าตัดเช่นกัน จึงส่งผลให้อัตราส่วนระหว่าง LF/HF มีค่าลดลงเมื่อเทียบกับก่อนผ่าตัด อย่างไรก็ตาม ในกลุ่มสุนัขที่มีปริมาณวีพีซีมากนั้น มีค่า HF ที่ต่ำกว่ากลุ่มสุนัขที่มีปริมาณวีพีซีน้อย ส่งผลให้มีอัตราส่วนของ LF/HF สูงกว่าอย่างมีนัยสำคัญทางสถิติในวันที่ 2 หลังการผ่าตัด (p<0.05) ค่านอร์อิพิเนฟรินและอิพิเนฟรินในสุนัขทั้ง 12 ตัว เพิ่มขึ้นในวันที่ 2 หลังการผ่าตัด พบค่านอร์อิพิเนฟรินสูงขึ้นอย่างมีนัยสำคัญทางสถิติในกลุ่มสุนัขที่มีปริมาณวีพีซีมาก (p<0.05) นอกจากนี้เมื่อนำข้อมูลมาวิเคราะห์ทางสถิติเพื่อศึกษาความสัมพันธ์ พบค่าสหสัมพันธ์เชิงบวกระหว่างปริมาณนอร์อิพิเนฟรินและอิพิเนฟรินกับปริมาณวีพีซีในสุนัขทั้ง 12 ตัวและกลุ่มสุนัขที่มีปริมาณวีพีซีมาก (p<0.05) จากการศึกษานี้สรุปได้ว่า สุนัขที่ตัดม้ามมักพบภาวะหัวใจเต้นผิดจังหวะชนิดวีพีซี ทั้งยังพบปริมาณนอร์อิพิเนฟริน อิพิเนฟรินและความแปรปรวนของการเกิดรีโพลาไรเซชันในหัวใจห้องล่างเพิ่มขึ้นภายหลังการผ่าตัดพร้อมกับการทำงานที่เพิ่มขึ้นของระบบประสาทอัตโนวัติที่หัวใจโดยเฉพาะระบบประสาทพาราซิมพาเทติก อย่างไรก็ตามการทำงานของระบบประสาทพาราซิมพาเทติกที่หัวใจในกลุ่มสุนัขที่มีปริมาณวีพีซีน้อยนั้นเด่นกว่ากลุ่มสุนัขที่มีปริมาณวีพีซีมาก

ภาควิชา สรีรวิทยา	ลายมือชื่อนิสิต .....
สาขาวิชา สรีรวิทยาการสัตว์	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
ปีการศึกษา 2559	ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5675305831 : MAJOR ANIMAL PHYSIOLOGY
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NUTTIKA PASTARAPATEE: ALTERATIONS IN DISPERSION OF CARDIAC REPOLARIZATION AND AUTONOMIC BALANCE IN THE SPLENECTOMIZED DOG. ADVISOR: PROF. CHOLLADA BURANAKARL, D.V.M., Ph.D., CO-ADVISOR: ASST. PROF. DR. ANUSAK KIJTAWORNRAT, Ph.D., 88 pp.

Ventricular premature contraction (VPC) in the splenectomized dogs remains a major concern during post-operative period since it may progress to fatal arrhythmia, ventricular fibrillation (VF). Twelve splenectomized dogs were recruited in the present study and further divided into 2 groups according to the number of VPC/24 hours, low VPC (n=6) and high VPC groups (n=6). None of the dogs received antiarrhythmic drug or digoxin prior to the study. Holter device was attached for 30 minutes in pre-operative period and 9 days after surgery while during the post-operative period, ECG signals were continuously recorded throughout 72 hours. Blood samples were collected at both pre-operative and post-operative (day 2 and 9) for complete blood count (RBC, Hct, platelet, WBC), blood chemical profiles (ALT, ALP, BUN, creatinine, total protein, Na^+, K^+) and concentrations of norepinephrine (NE) and epinephrine (E). The results revealed that the number of VPC was highest at day 3 (p<0.05) then declined at day 9. The HR in all dogs and low VPC group were decreased (p<0.05) compared with pre-operative period whereas SBP was unchanged. The Tp-Te was significantly increased on day 2 and day 1 (p<0.05) in low and high VPC groups, respectively. For time domain analysis of HRV, SDANN, SDNN index, SDNN, pNN50 and RMSSD tended to enhance after surgery compared with pre-operative period. In frequency domain analysis, HF and TP in all dogs increased having the decreased LF/HF after operation when comparing with pre-operative period. Nonetheless, lower HF and higher LF/HF were observed in high VPC group on day 2 (p<0.05) compared with low VPC group. NE and E increased significantly on day 2 after surgery in all dogs with the statistical significance of NE in high VPC group (p<0.05) compared with pre-operation. Additionally, concentrations of NE and E obtained from pre-operative, day 2 and day 9 after surgery were related to the number of VPC in all dogs and high VPC group (p<0.05). This study demonstrated that the splenectomized dogs had VPC after surgery. The catecholamine and Tp-Te were enhanced postoperatively along with enhanced cardiac ANS activity, particularly parasympathetic modulation. However, parasympathetic modulation in low VPC group was more dominant than high VPC group.

Department:	Veterinary Physiology	Student's Signature	.....
Field of Study:	Animal Physiology	Advisor's Signature	.....
Academic Year:	2016	Co-Advisor's Signature	.....

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CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	percent
#	number
β	beta
x g	times gravity
µl	microliter
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANS	autonomic nervous system
APD	action potential duration
BW	body weight
bpm	beat per minute
BUN	blood urea nitrogen
BVR	beat-to-beat variability of repolarization
°C	degree Celsius
CBC	complete blood count
cTn	cardiac troponin
CU-ACUC	Animal Care and Use Committee, Faculty of Veterinary Science, Chulalongkorn University
D_n	duration of QT interval of beat number n
D_{n+1}	duration of QT interval of beat number n+1
DCM	dilated cardiomyopathy
DIC	disseminated intravascular coagulopathy/coagulation
DHBA	3,4-dihydroxy-benzyl-amine hydrobromide

DMVD	degenerative mitral valve disease
E	epinephrine
e.g.	exempli gratia
EAD	early after depolarization
ECG	electrocardiogram
ERP	effective refractory period
etc.	et cetera
EDTA	ethylene diamine tetra-acetic acid
EGTA	ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid
F	female
F_s	spayed female
g/dl	gram per deciliter
GDV	gastric dilatation-volvulus
^{3}H	tritium
HF	high frequency
Hz	hertz
Hct	hematocrit
HRV	heart rate variability
HPLC	high performance liquid chromatography
HPLC-EC	high performance liquid chromatography-electrochemical detection
^{123}I	ioflupane
i.e.	id est

K^+	potassium ion
kg	kilogram
L2	the second lumbar vertebra
LF	low frequency
LF/HF	the ratio of LF to HF
M	male
M_c	castrated male
MI	myocardial infarction
ml	milliliter
MR	mitral regurgitation
ms	millisecond
ms^2	millisecond square
mV	millivolt
MAP	monophasic action potential
min	minute
MVD	mitral valve disease
mg/dl	milligram per deciliter
mmHg	millimeter of mercury
mmol/L	millimole per liter
n	number
Na^+	sodium ion
NE	norepinephrine
PET	positron emission tomography
PRV	pseudorabies virus

PSD	power spectrum density
pNN50	percentage of differences between adjacent normal RR intervals that >50 ms computed in the entire recording
pg/ml	picogram per milliliter
QTc	corrected QT interval
QT interval	time from the beginning of Q wave to the end of T wave on electrocardiogram
r	the correlation coefficient
RBC	red blood cell
rpm	revolutions per minute
RR interval	duration from peak of R wave to the next QRS complex R wave on electrocardiogram
RAAS	renin-angiotensin-aldosterone system
RMSSD	the square root of the mean squared differences between adjacent normal RR intervals
SAM	sympathetic-adrenal-medullary
SBP	systolic blood pressure
SCD	sudden cardiac death
SEM	standard error of mean
STV_{QT}	short-term variability of QT interval
SDNN	standard deviation of all normal RR intervals
SDANN	standard deviation of the average normal RR intervals calculated over short time periods (usually 5 minutes) for the entire recording period (usually 24 hours)

SDNN index	mean of the standard deviation of the normal RR intervals calculated over short period time (usually 5 minutes) for the entire recording period (usually 24 hours)
SF-MPQ	short form of the McGill pain questionnaire
T5	the fifth thoracic vertebra
TE	thromboembolism
TP	total power
TR	tricuspid regurgitation
Tp-Te	T_{peak} to T_{end}
TdP	torsade de pointes
TDR	transmural dispersion of repolarization
U/L	unit per liter
ULF	ultra-low frequency power
V	volt
VT	ventricular tachycardia
VLF	very low frequency
VPC	ventricular premature contraction
VVTI	vasovagal tonus index
WBC	white blood cell

CHAPTER I
INTRODUCTION

Splenectomy has been broadly performed for many decades with various indications, such as trauma, hematological disorder and neoplasia (Hosgood, 1986; Strickland and Lloyd, 2007). Several complications occurred after surgery, e.g., hemorrhage, infection, thrombosis and cardiac arrhythmia which was one of the most concerning complications (Hodam, 1970). The benign arrhythmia, ventricular premature contraction or VPC (wide and bizarre) is often developed during 3 hours to 3 days in post-operative period (Knapp et al., 1993). Moreover, VPC can degenerate into a fatal-arrhythmia, ventricular fibrillation. Those lethal-arrhythmia burdens are a detrimental factor to provoke a sudden cardiac death (SCD) which its mechanisms remain obscure (Bayes de Luna et al., 1989). Some investigators attempted to investigate the possible explanation of SCD. For instance, Cheriyath and colleagues (2011) reported that VPC could increase the SCD event by ratio 2:1 base upon left ventricular dysfunction, R on T phenomenon, overdrive of sympathetic tone, an abundance of VPC and the occurrence of ventricular tachycardia (VT) (Bruce et al., 2009).

In the study conducted by Knapp and colleagues (1993), ventricular arrhythmias especially VT and VPC were found in 10 dogs after splenectomy without preexisting cardiac disease. Similarly, Marino and colleagues (1997) demonstrated that approximately 44% of splenectomized dogs had the apparent ventricular arrhythmia.

In another study, splenectomy owing to splenic pedicle torsion resulted in one dog died from cardiac arrested because of ventricular arrhythmia (Stead et al., 1983).

Although the pathological mechanisms underlying arrhythmia after splenectomy are still unclear, autonomic nervous system (ANS) may be one of the potential causes (Felten et al., 1987; Buijs et al., 2008; Saladin, 2012). Even though a few data suggest the association between spleen and cardiovascular system due to the organ mediated by splenic nerve, Herman and colleagues (1982) found that while splenic nerve was stimulated, the cardio-sympathetic activity was simultaneously increased. These might be indicated that the vulnerable situation from splenic nerve defect can ultimately lead to the cardiovascular anomaly, i.e., arrhythmia. Besides the cardiac sympathetic nerve activity, other factors may be involved. Yu and colleagues (2013) reported that intra-abdominal pressure either from mass or other abdominal pathologies would be positively correlated to hormone secretion from adrenal gland.

Oscillation or dysfunction of cardiac autonomic nervous system can be early detected by non-invasive, simple, practical method known as the measurement of heart rate variability (HRV) (Sztajzel, 2004; Kleiger et al., 2005; Karim et al., 2011). Few data are available regarding HRV and abdominal surgery (Schubert et al., 1997; Chang et al., 2012; Rublenko et al., 2013). Nonetheless, the study of HRV in splenectomized dogs has not yet been identified.

Additionally, the interesting electrocardiographic markers used for prediction of susceptibility to arrhythmia occurrence, such as T_{peak}-T_{end}, short-term variability of QT interval or STV_{QT} and corrected QT interval (QTc) have been reported previously (Lubinski et al., 2000; Thomsen et al., 2004; Pham et al., 2015). However, the study

of the association between electrocardiographic parameter and splenectomy is extremely rare.

Nowadays, a number of evidences among HRV, electrocardiographic parameters and splenectomy have not been established. The causes of arrhythmia after surgery are still not well understood. Therefore, the present study was performed to investigate the alterations in dispersion of cardiac repolarization, cardiac autonomic balance and plasma levels of norepinephrine/epinephrine which may be a partial clarify of the arrhythmia complication in the splenectomized dogs.

Objectives of Study

1. To investigate the alteration in dispersion of cardiac repolarization in dogs after splenectomy
2. To investigate the alteration of cardiac autonomic nervous system activities in dogs after splenectomy assessed by heart rate variability and plasma norepinephrine concentration
3. To investigate associations between cardiac arrhythmia and dispersion of cardiac repolarization or autonomic balance in dogs after splenectomy

Keywords (Thai):

สมดุลของระบบประสาทอัตโนวัติ การกระจายตัวในระยะรีโพลาไรเซชันของหัวใจ สุนัข ความแปรปรวนของอัตราการเต้นของหัวใจ การตัดม้าม

Keywords (English):

autonomic balance, dispersion of cardiac repolarization, dog, heart rate variability, splenectomy

Research Questions

1. Is there enhancement of dispersion of cardiac repolarization in post-splenectomy situation?
2. Are there enhancements of autonomic nervous system activities and plasma norepinephrine in post-splenectomy situation?
3. Are there associations between cardiac arrhythmia and other parameters in the splenectomized dogs?

Research Hypotheses

1. Dispersion of cardiac repolarization is enhanced after splenectomy.
2. Cardiac autonomic nervous system activities (HRV) especially sympathetic are enhanced and plasma norepinephrine increase after splenectomy.
3. There are associations between cardiac arrhythmia and other parameters in the splenectomized dogs.

CHAPTER II
LITERATURE REVIEWS

2.1 Anatomy and Physiology of Spleen

In all vertebrates, spleen is a secondary lymphoid organ in which it consists of 2 compartments, the red and white pulps. In mammals, spleen serves as 3 essential functions. The first role is a phagocytic filter in which it removes pathogens from the bloodstream when infection occurs. Secondly, it is a dominant antibody-producing organ. Lastly, it acts as an "erythrocyte reservoir" in several animals (Steiniger et al., 2005). In canine, the spleen has been classified as a storage-spleen group (Tablin et al., 2002).

Celiac artery is the first major division of aorta and branches to supply the spleen known as splenic artery. The sympathetic nerve fibers (pre-ganglionic) leaving T5-L2 vertebra are splanchnic nerve routes which synapse to celiac ganglion and then travel along as splenic nerve (post-ganglionic fiber) to innervate the spleen.

The spleen is innervated by sympathetic nerves. Their terminals locate around white pulp region and release norepinephrine (NE) when being activated (Felten and Olschowka, 1987). Nevertheless, Brandon and Rand (1961) reported that acetylcholine was discharged along with splenic contraction after activating the splenic nerve (sympathomimetic performance) . In 2008, Buijs and colleagues performed the experiment by administration of Pseudorabies virus (PRV) into the splenic parenchyma using the retrograde technique. They found that Pseudorabies virus appeared in the

intermedio-lateral column of spinal cord in which sympathetic motor neurons were existed. Furthermore, some Pseudorabies positive neurons were presented in dorsal nucleus of vagus nerve. Hence, it can be implied from their experiment that spleen is actually innervated by both sympathetic and parasympathetic fibers. However, recent publications suggested that parasympathetic nervous system usually played a pivotal role in the immunological system (Huston et al., 2006; Vida et al., 2011).

2.2 Ventricular Arrhythmia and ANS

Autonomic nervous system (ANS), principally sympathetic section, is an essential key for expediting the arrhythmia. For instance, if the sympathetic nervous system is over-activated, it will markedly release the potent neurotransmitter into the bloodstream (Zipes, 2008). It also altered the substrate for arrhythmia in the cardiac. This was supported by the experiment in which the stimulation of sympathetic nerves with 8 volts electricity, 20 times per second resulted in ventricular tachycardia (VT) (Sosunov et al., 2001). The possible explanation was an upsurge of influx calcium currents mediated by epinephrine (E) and norepinephrine (NE) after stimulation (Vassalle et al., 1976). Accordingly, Szekeres and colleagues (1986) demonstrated that high concentration of plasma norepinephrine could activate the ventricular extra-systole by increasing the firing rates through ventricular muscle accompany with Purkinje fibers. Moreover, shortened refractory period (ERP) together with decreased diastolic threshold and elevated temporal dispersion can initiate the abnormal rhythm, i.e. ventricular ectopic beat. This ectopic beat may develop into other cardiac-harmed events in the future (Han et al., 1964; Stein et al., 1998).

2.3 Types of Splenic Mass and Survival Time

Splenic neoplasia has a high incidence in aged dogs. In this situation, surgery is the recommended choice for treatment (Slatter, 2002). In 2000-2011, the epidemiology of splenic mass revealed that hemangiosarcoma as well as nodular hyperplasia were the most prevalence in malignant and non-malignant subtypes by having incidences of 97% and 47%, respectively (Eberle et al., 2012). The metastases to other visceral organs**,** e.g. heart, lymph nodes, lung, peritoneum, omentum and liver should be concerned (Frey and Betts, 1977; Hosgood, 1986; Slatter, 2002).

The survival times after splenectomy are varied depending on multifactorial causes, such as an underlying disease prior to surgery or post-operative complications (Slatter, 2002). There were several reports about survival periods. For example, Frey and Betts (1977) reported that 1/3 of splenectomized dogs died within a couple months after surgery while a few patients lived for a year. Similarly, Slatter (2002) found that bearing-neoplasia dogs had a survival time less than 100 days. Johnson and colleagues (1989) demonstrated that the average survival time was 13 weeks and 36 weeks in splenic mass and non-splenic neoplasia dogs, respectively. Thus, in dogs with splenic neoplasia, the prognosis is poor.

2.4 Cardiovascular Complication after Splenectomy

Indications of splenectomy were widely divided into 3 major causes, trauma, hematological disorders (e.g. autoimmune hemolytic anemia, thrombocytopenia, blood

parasite) and mainly neoplasia (Strickland and Lloyd, 2007). The sarcoma was frequently found from Eberle and colleagues' study (2012).

Interestingly, the latent linkages among spleen, hemodynamic and cardiac function had been explored but the complete insights were not yet elucidated. In splenectomized dogs, they may encounter with the anemic situation, high blood viscosity, temporary thrombocytosis and augmentations of heart rate and left ventricular systolic pressure (Elenes et al., 1964; Hirsh and Dacie, 1966; Ffoulkes-Crabbe et al., 1976; Robertson et al., 1981). Besides, arrhythmia is a serious complication in splenectomized dogs (Slatter, 2002). According to the study of Knapp and colleagues (1993), they reported that ventricular arrhythmia especially VT and VPC were found in 10 dogs after splenectomy without primary cardiac disease. A year later, Marino and colleagues (1994) demonstrated the similar data in which splenectomized dogs had ventricular arrhythmia around 44%. Another study showed that one dog died from cardiac arrested owing to ventricular arrhythmia after splenectomy (Stead et al., 1983). Cardiac arrhythmia was developed not only in post-surgery conditions but also in other pathological stages before surgery, such as splenic infarction (Hardie et al., 1995). These findings suggested that spleen is likely associated with the cardiovascular system whether direct or indirect pathway.

Although the mechanism responsible for arrhythmia is still elusive, some researchers proposed that 1) myocardial ischemia from tumor pressure, 2) reduction in blood circulation from splenic rupture, tissue hypoxia, myocardial metastasis and 3) local/general catecholamine might be involved with the mechanism (Keyes et al., 1993).

2.5 Post-operative Arrhythmia in Non-cardiothoracic Surgery

Although arrhythmia is a problematic situation in post-cardiac surgery, it can also be presented in other operations (Goodman et al., 2007; Walsh et al., 2007). Various etiologies of post-operative arrhythmia were vastly assumed. Anesthetic drugs may be one of the causes of arrhythmias. Even though anesthetic regimes had been elucidated by several authors, their protocols in premedication, induction and maintenance of anesthesia were unlikely to produce arrhythmias unless patients with preexisting cardiovascular disorders (Buss et al., 1982; Buhl et al., 2005; Duerr et al., 2007).

2.6 General Evaluation Method of ANS activity

Sympathetic and parasympathetic components of ANS are the extrinsic control of the heart (Saladin, 2012). Acetylcholine is a mediate-neurotransmitter that reduces the heart rate and contractility when binds to the muscarinic receptor. On the other hand, norepinephrine increases the heart rate and contractility when binds to the $beta_1$-adrenergic receptor. Cléroux and colleagues (1984) found that norepinephrine level in circulation was augmented if sympathetic nerve fibers were stimulated. The half-life of plasma norepinephrine is approximately 1 to 3 minutes (Esler et al., 1984).

There are numerous techniques to examine autonomic nervous system function, such as HR measurement and HR recovery (HRR) (Lahiri et al., 2008). In addition, norepinephrine spillover contents can be estimated by calculating ^{3}H-norepinephrine

concentration (Keeton and Biediger, 1988). Another technique is cardiac sympathetic imaging using radiolabeled sympathomimetic amine, ^{123}I-metaiodobenzylguanidine. Nevertheless, this method needs a positron emission tomography (PET). In order to achieve the method for evaluating plasma norepinephrine/epinephrine with high specificity and sensitivity, the high performance liquid chromatography-electrochemical detection technique or HPLC-EC detector should be used (Freeman, 2006).

Recently, oscillation or dysfunction of autonomic nervous system can be early detected by a non-invasive method known as heart rate variability (HRV) (Sztajzel, 2004; Kleiger et al., 2005; Karim et al., 2011). This technique becomes one of the most favored procedures because of conveniences in device and software (Stein et al., 1994).

2.7 Heart Rate Variability (HRV)

Physiologically, variations of RR intervals in the cardiac cycle generally occur in resting phase (Bansal et al., 2009). These transient alterations of sequences during heart beats are called heart rate variability or HRV (Karim et al., 2011) (Figure 1). Periods of heart rate fluctuation can be used as an indicator for sympathetic/parasympathetic nervous system balance and for prognosis of cardiac pathological events (Kleiger et al., 2005). Thus, measurement of HRV by Holter recording is practical for clinical utility and research program (Stein et al., 1994).

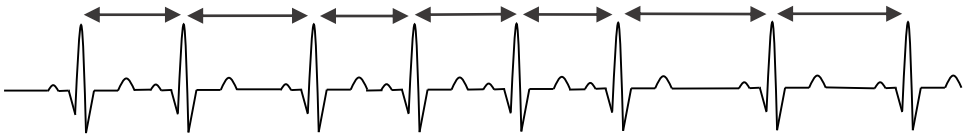


Figure 1. Electrocardiograms showing the variability of RR intervals (the length of arrows).

The HRV has been widely performed as the predictive tool (or detector) in many pathological conditions, such as cardiac arrhythmia, hypertension, congestive heart failure, myocardial infarction, coronary disease, diabetes neuropathy and renal failure (Dreifus et al., 1993; Huikuri et al., 1999; Stein et al., 1999; Reed et al., 2005; Rajendra Acharya et al., 2006). Reduction of HRV is a marker of various clinical situations. The Framingham Heart Study, assessed the correlation between HRV and hypertension, reported that low HRV was presented in the early stage of hypertensive patients (Singh et al., 1998). In 2012, Pirintr and co-workers found that cardiac autonomic nerve defect and decreased plasma norepinephrine concentration occurred in poorly-controlled diabetic dogs. The class IC anti-arrhythmic drugs were reported to significantly reduce HRV whereas the class III did not alter the HRV (Zuanetti et al., 1991). Nonetheless, there are numerous factors that may disturb HRV, such as temperature, endocrine, gender, age and some drugs (Stauss, 2003; Rajendra Acharya et al., 2006).

HRV can be assessed by using a 24-hour Holter monitoring device. The analytical constituents of HRV are classified into 2 compartments, statistical calculation of RR intervals (time domain) and spectral analysis of RR intervals (frequency domain) (Billman, 2011).

2.7.1 Time domain analysis

Time domain analysis quantified the short-term variability of RR intervals. It may concisely measure a segment of RR intervals of the interesting time window and subsequently calculated as time domain parameters shown in Table 1 (van Ravenswaaij-Arts et al.,1993; Billman, 2011).

SDNN is a mirror index of circadian rhythm (Kleiger et al., 2005). It is an average of variability in short period, usually 5 minutes. SDNN is represented as day and night alterations of HRV. The variations of autonomic tone especially vagal tone are reflected by RMSSD and pNN50 (Stein and Kleiger, 1999; Sztajzel, 2004).

Table 1. Time domain analysis parameters (Electrophysiology, 1996; Billman, 2011).

Variable	Unit	Definition
SDNN	ms	SD of all normal RR intervals
SDANN	ms	SD of the average normal RR intervals calculated over short time periods (usually 5 mins) for the entire recording period (usually 24 h)
RMSSD	ms	The square root of the mean squared differences between adjacent normal RR intervals
SDNN index	ms	Mean of the SD of the normal RR intervals calculated over short periods time (usually 5 mins) for the entire recording period (usually 24 h)
pNN50	%	Percentage of differences between adjacent normal RR intervals that >50 ms computed in the entire recording

2.7.2 Frequency domain analysis

Frequency domain analysis is a mathematic calculation of RR intervals for heart rate oscillations in frequency termed−power spectral density (PSD) (van Ravenswaaij-Arts et al., 1993; Sztajzel, 2004). The frequency analysis is usually presented as standard 3 bands (Figure 2).

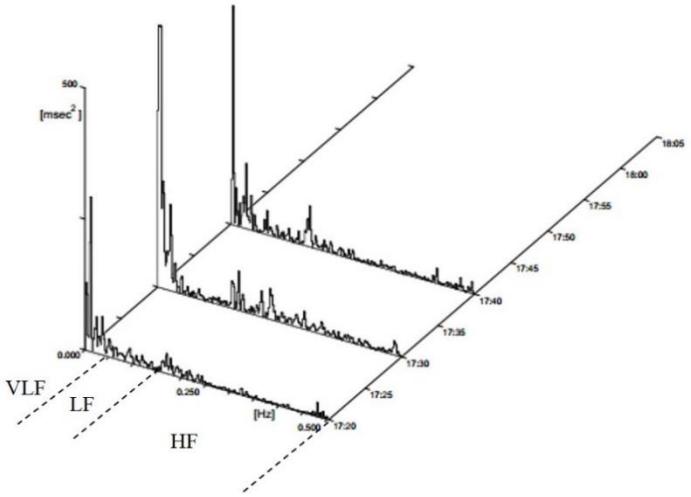


Figure 2. The frequency periods obtained from Holter recording.

Frequency domain parameters (Table 2), high frequency (HF), 0.15-0.5 Hz, is a marker of parasympathetic modulation. It is affected by respiratory oscillation (i.e. respiratory sinus arrhythmia). Low frequency (LF), 0.041-0.15 Hz, is influenced by both sympathetic and parasympathetic tones. Very low frequency (VLF) and ultra-low frequency (ULF) are assumed as the reflection of thermoregulatory, RAAS, vasomotor activity and neuroendocrine system. ULF (0-0.004 Hz) is recorded for a long period and closely associated with SDNN for indicating the circadian rhythm. The VLF (0.004-0.041 Hz) is determined by physiological actions and partial sympathetic nervous system activity. Total power (TP) is the sum of all frequencies and the ratio of LF to HF (LF/HF) is known as sympatho-vagal balance (Stein et al., 1994; Sztajzel, 2004; Kleiger et al., 2005).

Table 2. Frequency domain analysis parameters (Electrophysiology, 1996).

Variable	Unit	Definition
ULF	ms^2	Ultra-low frequency power (0-0.004 Hz)
VLF	ms^2	Very low frequency power (0.004-0.041 Hz)
LF	ms^2	Low frequency power (0.041-0.15 Hz)
HF	ms^2	High Frequency power (0.15-0.5 Hz)
TP	ms^2	Area under the entire power spectral curve (usually $\leq$0.40), variance of al normal RR intervals (0-0.5 Hz)
LF/HF		Ratio of the low-to high frequency power

2.8 Miscellaneous Factors that affect HRV

In human, there are some kind of factors that influence heart rate variability, e.g., age, gender, genetic, circadian rhythm, disease, body weight, smoking, alcohol, medication and noise (Sammito and Böckelmann, 2016). In dogs, which based on the study of Rasmussen et al. (2011), among these factors (i.e. breed, gender, age and body weight among Cavalier King Chales Spanial, Wire-haired Dachshund and Cairn Terrier) only breed is the considered factor that interfered with HRV (Rasmussen et al., 2011). Doxey and Boswood (2004) reported that vasovagal tonus index (VVTI) which representing parasympathetic activity was higher in brachycephalic than non-brachycephalic breeds. Additionally, HRV is implicated in abundant types of abdominal surgery. Both sympathetic and parasympathetic tones are concurrently triggered by organ manipulation (Hirata et al., 2012). Chang and colleagues (2012)

noticed that pain score assessment, SF-MPQ, in patients who underwent abdominal surgery was positively correlated to HRV.

2.9 Electrocardiographic Parameters

In most mammals, myocardium is composed of 3 layers: epicardial, M-cell and endocardial cells. Each layer possesses a unique electrophysiological property (i.e. action potential duration: APD) (Figure 3) (Antzelevitch, 2005). Arrhythmia may develop in the likelihood of lengthening of APD, QT interval prolongation, ectopic beat and enhancement of spatial repolarization dispersion. Dispersions are divided into 2 subtypes, spatial dispersion and temporal dispersion. The "spatial" (or transmural dispersion of repolarization, TDR) refers to the dissimilarity of repolarization across the ventricular wall which can be estimated from the duration of Tp-Te from ECG recording (Belardinelli et al., 2003). On the other hands, the temporal dispersion is variability of repolarization attributable to beat by beat in which it can be inferred from the QT interval variability, i.e., short-term variability of QT interval or STV_{QT} (Belardinelli et al., 2003; Thomsen et al., 2004; Schneider et al., 2005). In addition, there are many factors that influence the dispersion of depolarization (e.g. structural heart disease, myocardial infarction, neural stimulation and several drugs, such as cisapride and dofetilide) (Antzelevitch, 2008).

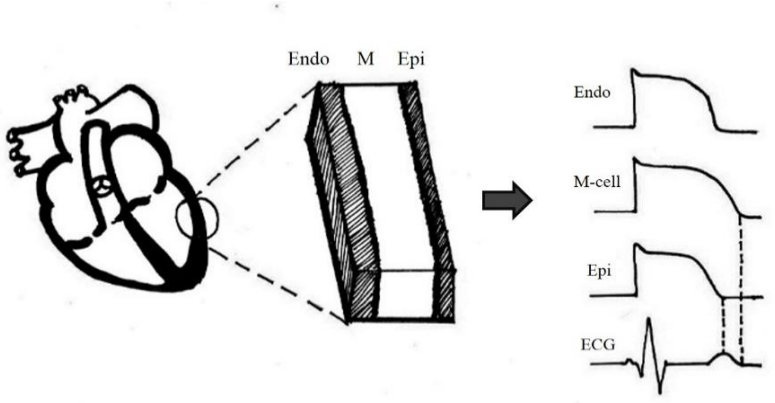


Figure 3. The transmembrane action potential of left ventricular wedge preparation (left) with their represented electrocardiogram. Notice that the end of action potential duration (APD) of M-cell equals to the end of T-wave on electrocardiogram (ECG) while the end of APD of epicardium equals to the peak of T-wave on ECG.

2.9.1 Tp-Te, TDR

The difference of action potential duration between M-cell and epicardial cell layers is simply reflected by the duration from the peak of T wave (Tp) to the end of T wave (Te) (Lubinski et al., 2000; Antzelevitch, 2001b) (Figure 4).

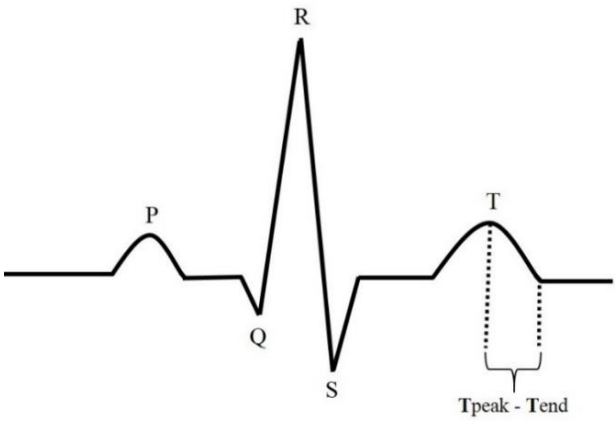


Figure 4. The duration of the peak of T-wave to the end of T-wave (Tp-Te) is shown by measurement of the distance of T_{peak} to T_{end}.

2.9.2 QTc (corrected QT interval)

The QT interval, the duration from the onset of Q wave to the end of T-wave on surface ECG, represents the ventricular depolarization and repolarization. The QT interval is influenced by heart rate leading to misinterpret of its duration. Hence, the corrected QT formula from Fridericia, Bazzette or Van de Water has been used for normalization of heart rate. The Van de Water formula in Equation 1 was generated from dogs with high correlation when compared to raw QT interval (Oliveira et al., 2014). The QTc is the QT interval corrected for heart rate. It has been known that when the heart has QTc interval prolongation, the ventricular arrhythmia may develop especially Torsade de pointes (TdP) (Pham et al., 2015).

2.9.3 Short-term variability of QT interval (STV_{QT})

The lengthening of the QT interval has been used for prediction of arrhythmic risk especially drug-induced TdP. It was used by the regulatory agencies to investigate the new chemical entities involving proarrhythmias. Poincaré plot from 30 consecutive beats of QT duration had been generated for quantification of the STV_{QT} as the following Equation 2 (Thomsen et al., 2004).

$$QTc\ (V) = QT - 0.087(RR-1000) \quad \text{.....1}$$

When QTc (V) = the corrected of QT interval from Van de water formula (ms)

QT = duration of QT (ms)

RR = duration of RR (ms)

$$STV_{QT} = \sum \frac{|Dn+1 - Dn|}{30\sqrt{2}} \quad \text{.....2}$$

When STV_{QT} = the short-term variability of QT interval (ms)

D_n = duration of QT from beat number n

D_{n+1} = duration of QT from beat number n+1

CHAPTER III
MATERIALS AND METHODS

3.1 Research Animals and Grouping

Twelve splenectomized dogs which non-specify of gender, age, breed and weight with lesion existing in the spleen under the veterinarian decision to perform splenectomy were recruited from Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University. The protocol was approved by the Animal Care and Use Committee, Faculty of Veterinary Science, Chulalongkorn University (CU-ACUC) and informed written consents were obtained from all owners before starting the procedures. All animals did not present the clinical signs of cardiac disease more than stage B2 following the guideline of American College of Veterinary Internal Medicine (ACVIM) and did not receive antiarrhythmic drug or digoxin prior to the study. In case of splenic tumor, dogs with metastases to the heart or lungs were excluded. Duration for collecting the animal data was between July 2014 to July 2015.

General history and patient signalments were documented while performing physical examinations. Dogs were subjected to blood collection. Two views of thoracic radiographs (right-lateral and ventro-dorsal views) and echocardiography were also performed.

Twelve splenectomized dogs in this study which were equally separated into 2 groups according to the study of Meurs and colleagues (2004) with low VPC (group I) and high VPC (group II) groups.

Low VPC group (group I) (n = 6) comprised of the dogs presenting with VPC less than 1000 beats per 24 hours throughout the experiment after surgery.

High VPC group (group II) (n = 6) comprised of the dogs presenting with VPC equal to or more than 1000 beats per 24 hours throughout the experiment after surgery.

3.2 Experimental Protocol

The study was performed on pre-operative, day 1 to 3 and the day of removing sutures or day 9 after surgery. On pre-operative day, an average blood pressure was measured using Doppler flow detection for 5 times. Two thoracic leads of Holter device were attached to the dog approximately 30 minutes continuously in non-stressed condition for calculation of heart rate variability (HRV), Tp-Te, QTc and STV_{QT}. On day 1 to 3 after surgery, the previous procedures were repeated except for the Holter monitoring which was attached continuously for 72 hours. On day 9, dogs were rechecked for blood pressure and 30 minutes ECG was recorded using Holter device. ECG data were transferred to the commercial program (SCM-510) and analyzed for 30 minutes on pre-operation, day 1 to 3 and day 9 after surgery. Four milliliters of blood were collected on pre-operative, day 2 and day 9 after surgery. Approximately 1 milliliter of blood was collected into either EDTA or heparinized tube for complete blood count (RBC, Hct, platelet, WBC) and blood chemistry (ALT, ALP, BUN, creatinine, total protein, Na^+, K^+), respectively. Three milliliters of blood were collected in EGTA tube (ethylene glycol 90 mg/ml + reduce glutathione 60 mg/ml) for plasma epinephrine/norepinephrine determinations. The biopsy results were obtained afterward from Department of Pathology, Faculty of Veterinary Science,

Chulalongkorn University. Diagram of timeline of experimental designs was performed as following.

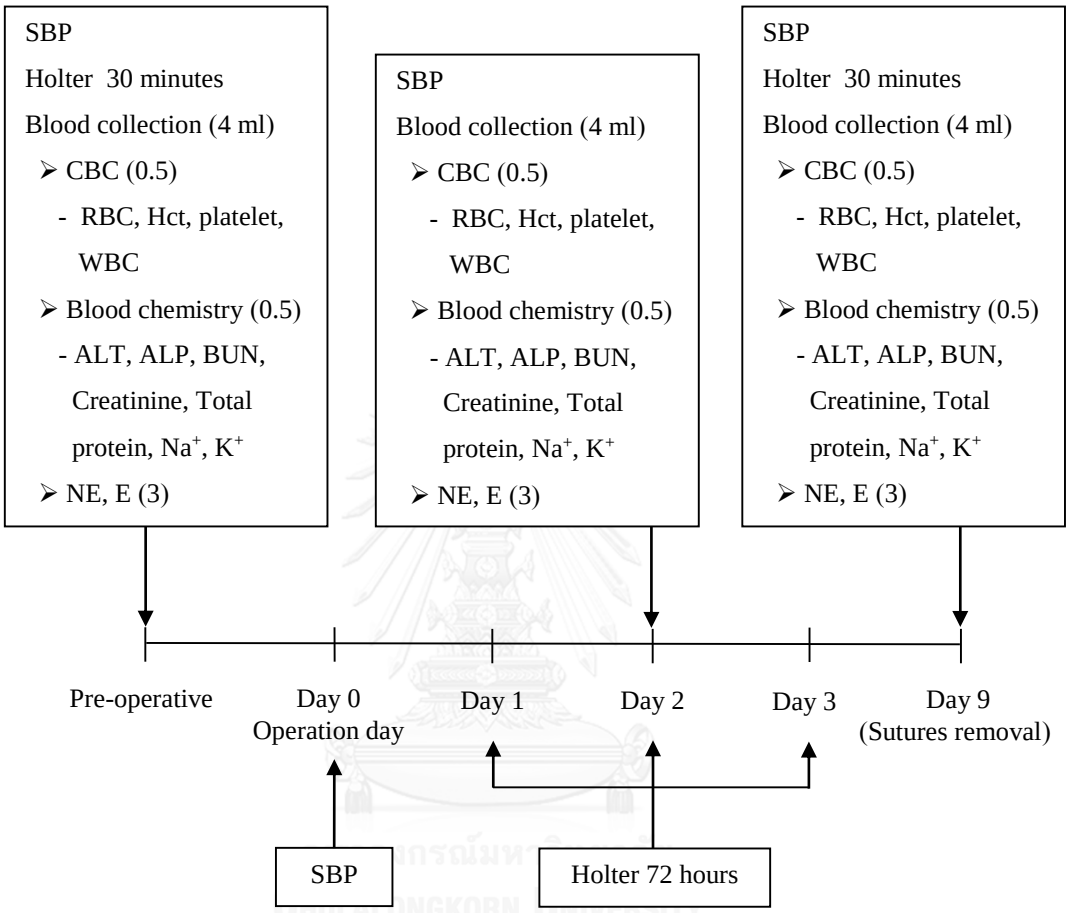


Figure 5. Diagram of timeline in pre-operative period, day 0 to day 3 and day 9 after surgery. Abbreviations: SBP, systolic blood pressure; CBC, complete blood count; RBC, red blood cell; Hct, hematocrit; WBC, white blood cell; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; Na^+, sodium; K^+, potassium; NE, norepinephrine; E, epinephrine.

3.3 Experimental Procedures

3.3.1 Blood collection and analytical process

Approximately 4 milliliters of venous blood were collected individually using a 21G needle. Animal's discomfort would be avoided during performing this routine. Portions of blood were divided by a half of milliliter of blood into EDTA (ethylene diamine tetra-acetic acid) tube for complete blood count determinations (RBC, Hct, platelet, WBC) using automated hematology analyzer (The CELL-DYN 3700, Abbott Laboratory, USA). A half milliliter of blood was collected into the heparinized tube for blood chemistry measurements (ALT, ALP, BUN, creatinine, total protein, Na^+, K^+) using automate chemistry analyzer (The IL ILab 650 Chemistry analyzer, Diamond diagnostic, MA, USA). A portion of 3 milliliters of blood was simultaneously put into EGTA (ethylene glycol 90 mg/ml + reduce glutathione 60 mg/ml) tube for further catecholamine measurement.

3.3.2 Electrocardiographic parameters

ECG data obtained from Holter device and electrocardiographic parameters were individually calculated according to descriptions of 3.3.2.1, 3.3.2.2 and 3.3.2.3 along with each algorithm.

3.3.2.1 Transmural dispersion of repolarization (TDR). It reflects the different of repolarization across ventricular layers and can simply be indicated by the components of T-wave (Lubinski et al., 2000; Antzelevitch, 2001b). The Tp-Te was measured from the 6 beats in every 6 hours during 24 hours period and averaged.

$$TDR = Tp\text{-}Te$$

When

TDR = transmural dispersion of repolarization (ms)

Tp-Te = distance between peak of T-wave to the termination of T-wave (ms)

3.3.2.2 The short-term variability of QT interval (STV_{QT}). The STV_{QT} was calculated according to modification of Thomsen et al. (2004). To examine the beat-to-beat variability, Poincaré plot was created by plotting the QT duration (QT_n : ms) versus the proceeding QT duration (QT_{n-1}: ms) for 30 consecutive beats. The distance from the diagonal line to averaged point of Poincaré plot perpendicularly refers to the short-term variability of QT interval. The STV_{QT} was measured from the 30 beats in every 6 hours during 24 hours period and averaged.

$$STV_{QT} = \sum \frac{|Dn+1 - Dn|}{30\sqrt{2}}$$

When

STV_{QT} = the short-term variability of QT interval (ms)

D_n = duration of QT from beat number n

D_{n+1} = duration of QT from beat number n+1

3.3.2.3 The corrected QT interval (QTc). The duration of QT (the onset of Q wave to the terminate point of T-wave) was influenced by heart rate. Hence, the exact QT interval needs to be corrected by using Van de Water equation (van de Water et al.,

1989). The QTc was measured from the 6 beats in every 6 hours during 24 hours period and averaged.

$$QTc\ (V) = QT - 0.087(RR-1000)$$

When

QTc (V) = the corrected of QT interval from Van de water formula (ms)

QT = duration of QT (ms)

RR = duration of RR (ms)

3.3.3 Holter monitoring and HRV analysis

According to Petrie (2005) with partial modifications, the Holter standard 2 channels type consisting of 2-ECG leads (FUKUDA DENSHI CO., LTD., JAPAN) were attached. The hair at 2 sides of chest area had been clipped before electrodes were placed. The uncovered area was cleaned up by alcohol then dried up with gauze. On the left-sided, 2 electrodes (Ambu® Blue Sensor P, ECG Electrodes) were attached at the intercostal space between the fifth and the sixth ribs (cardiac apex). On the right-sided, 2 electrodes were attached at the anterior site of thoracic wall and at the location of the last rib which representing as a ground (Figure 6). After that, Holter cables were connected to the interpretable device and put them between the scapula regions. Entire apparatuses were secured by conforming bandage and wrapped firmly by a nonwoven bandage. A new battery cell 1.5 V (AAA) was replaced in every single record.

A B

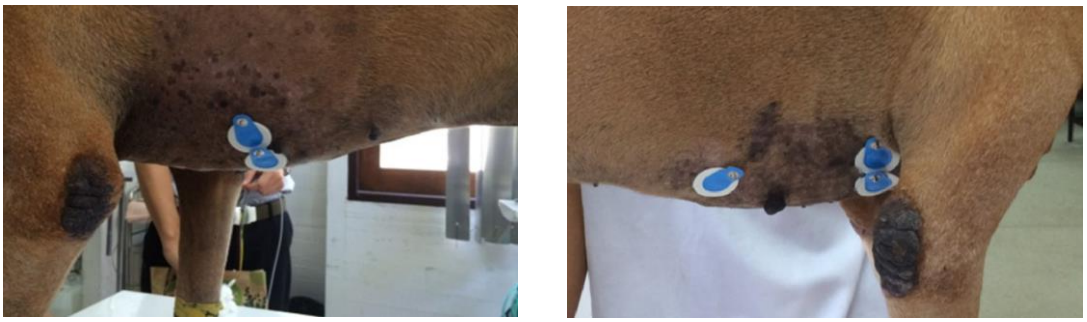


Figure 6. Locations of electrode placement on the left-sided (A) and right-sided (B).

Data analysis

Every 24 hours, ECG data recorded in SD card of Holter device were transferred to SCM-510 software (FUKUDA DENSHI CO., LTD., JAPAN). HRV was measured in 2 patterns; time domain as well as frequency domain. The acceptable ECG wave forms need to have normal R wave for at least 85%. VPC beats had been automatically distinguished both by program and by manual. To validate HRV, the same or similar time frame as the pre-operative day was selected. The analysis was performed for every 3 consecutive 10 minutes intervals. Frequency domain parameters included ultra-low frequency (ULF; 0-0.004 Hz), 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 the ratio between low frequency and high frequency (LF/HF). The Hamming window was used for filtering these signals and then the data were converted into a spectrum term by Fast Fourier transformation algorithm. Parameters of time domain composed of standard deviations of all normal RR intervals (SDNN), standard deviations of the average normal RR intervals calculated over short time periods (usually 5 mins) for the entire recording period (SDANN), mean of standard deviations of the average normal RR intervals calculated over short time periods (usually 5 mins) for the entire recording period (SDNN index), square root of the mean squared

differences between adjacent normal RR intervals (RMSSD) and percentage of differences between adjacent normal RR intervals that are > 50 ms computed in the entire recording (pNN50) (Billman, 2011).

3.3.4 Blood pressure evaluation process

Systolic blood pressure was indirectly measured by Doppler flow detection while an animal was calm and relax with minimal restraint. A cuff was applied on an appendage either at antebrachium between the carpal pad and elbow or upon the hock joint of the hind limb. The selected cuff size width is 40% of limb circumference and the cuff was placed securely around the appendage. The hair over the metacarpal (palmar metacarpal artery) or metatarsal (ventral metatarsal artery) area was clipped for locating the pulse using Doppler transducer probe. Ultrasound transmission gel was applied as generous amount on a probe for clear sound conduction then put the probe on shaved area, wrapped tape around the paw with moderately firm pressure. Thereafter, attached a manometer to a single hose cuff and inflated the pressure up until all signals were disappeared and then gradually released the pressure for listening the signal-returned sound which was determined as a systolic blood pressure. Mean values were assessed from the average of 5 consecutive measurements.

3.3.5 Measurement of plasma catecholamine concentration

Three milliliters of blood sample containing EGTA (ethylene glycol bis (beta-aminoethyl ether)-N,N,N',N'-tetraacetic acid) 90 mg/ml as well as reduce glutathione (anti-oxidant) 60 mg/ml were used for preserving catecholamine. The blood sample was centrifuged at 1500-x g, 4 °C for 5 minutes and the supernatant was stored at -80

°C for further analysis which was performed at Central Laboratory, Faculty of Medicine, Chulalongkorn University. In the extraction steps, 500 µl of Extraction Buffer (#5011 MassTox® TDM Series A, Germany) were added to a sample clean up cartridge (#5000 Chromsystems, Germany) with gentle shaking. Plasma sample 1.5 ml were applied together with 50 µl internal standard (3,4-Dihydroxy-benzyl-amine hydrobromide : DHBA 12 pg/µl) (#500 4 Chromsystems, Germany), then mixed carefully by inverting machine. The discard effluent was removed by centrifugation at 2000 rpm for a minute. Thereafter, in wash steps, 1 ml Wash Buffer (#5005 MassTox® TDM Series A, Germany) was added and triple centrifugations were then performed at 2000 rpm for a minute with the last time at 4000 rpm for 2 minutes. The plastic tube was mounted to the outlet of the cartridge, 120 µl Elution Buffer (#5006 MassTox® TDM Series A, Germany) were filled with soft shaking and waited for 5 minutes followed by vortex for 30 seconds. The centrifugation at 2000 rpm for a minute in attached plastic tube (#5007 Chromsystems, Germany) had been performed before elute solution was collected for injecting into HPLC system.

Concentrations of epinephrine (E), norepinephrine (NE) and DHBA were determined by HPLC pump (Chromsystems, CLC 300, Germany) and electrochemical-detector (HPLC-EC, Chromsystems, CLC 100, Germany) linked to 15 cm HPLC column (#5100 Chromsystems, Germany) for catecholamine in plasma. The eluent 40 µl containing vial were automatically injected and Mobile Phase Solution (#5001 MassTox® TDM Series A, Germany) was also run. The system values were set including flow rate 1 ml/min at temperature 25 °C. Data were graphically displayed in 20 minutes via Easy-Line software.

The standard curves were calibrated by Plasma Calibration Standard (#5009 Chromsystems, Germany) with Endocrine Plasma Control in normal range (#0010 Chromsystems, Germany) and pathological range (#0020 Chromsystems, Germany). The plasma calibration curves were compared to the standard table for reliability of the quality control.

3.4 Statistical Analysis

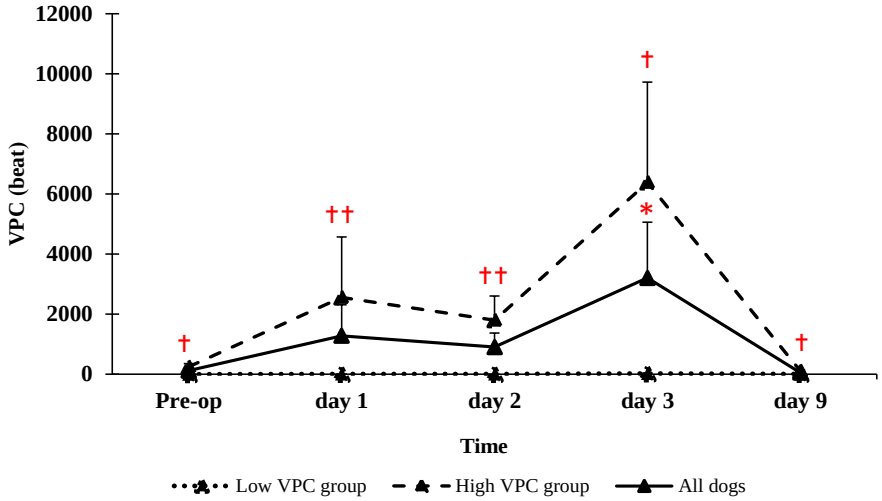
Data were expressed as mean ± SEM. The unpaired t-test or Mann-Whitney U test was used to compare data between low VPC and high VPC groups. To compare data among periods, the one-way repeated measures ANOVA or repeated measures ANOVA on ranks was performed. The Dunnett's method was used for post-hoc analysis. Comparisons between plasma catecholamine levels in day 2 or day 9 and pre-operative period were individually compared by Student paired t-test. Pearson's correlation was performed to examine the relationships between parameters. The survival curve analysis was measured by Kaplan–Meier method and comparisons between 2 groups were performed by using log-rank test. The p-value < 0.05 is considered as statistical significance.

CHAPTER IV
RESULTS

4.1 Prevalence of Arrhythmia Event

Even though VPC mostly occurred, other arrhythmia types, such as atrial premature beat and supraventricular premature beat were trivially found as 8% and 0.006% of total beats, respectively. The number of VPC in all dogs and 2 groups is shown in Figure 7. According to our criteria categorized for groups, the number of 24 hours VPC in high VPC group was significantly higher by 510 (p<0.01), 901 (p<0.01) and 246 (p<0.05) times compared with low VPC group on day 1, day 2 and day 3 after surgery, respectively. The number of VPC in high VPC group declined when recording at 30 minutes duration at day 9 after surgery but still higher than low VPC group (p<0.05). It was demonstrated that the number of VPC was highest at day 3 in all dogs (p<0.05) compared with pre-operative period. By determining the VPC in both groups, the VPC were the highest on day 3 after surgery. The number of VPC was counted individually from single VPC and from either sustained or paroxysmal ventricular tachycardia.

The examples of ECG tracing obtained from Holter device in low VPC and high VPC groups are shown in Figure 8 and 9, respectively.



Period	Low VPC group (beat)	High VPC group (beat)	All dogs (beat)
Pre-op	0 ± 0	248 ± 106†	124 ± 63
day 1	5 ± 2	2552 ± 2014††	1278 ± 1034
day 2	2 ± 1	1801 ± 799††	901 ± 468
day 3	26 ± 21	6385 ± 3338†	3205 ± 1858*
day 9	8 ± 8	104 ± 31†	56 ± 21

Figure 7. The number of VPC in all dogs (—▲—), low VPC group (···▲···) and high VPC group (-·▲·-) was plotted during pre-op, 1, 2, 3 and 9 days after surgery. Data are presented as mean + SEM (upper)/mean ± SEM (lower) and values from pre-op and day 9 after surgery were normalized for 24 hours.

* indicates $p<0.05$ compared with pre-operative period in the same group using one-way repeated measures ANOVA.

† $p<0.05$; †† , $p<0.01$ compared with low VPC group in each day using unpaired t-test.

Abbreviation: VPC, ventricular premature contraction.

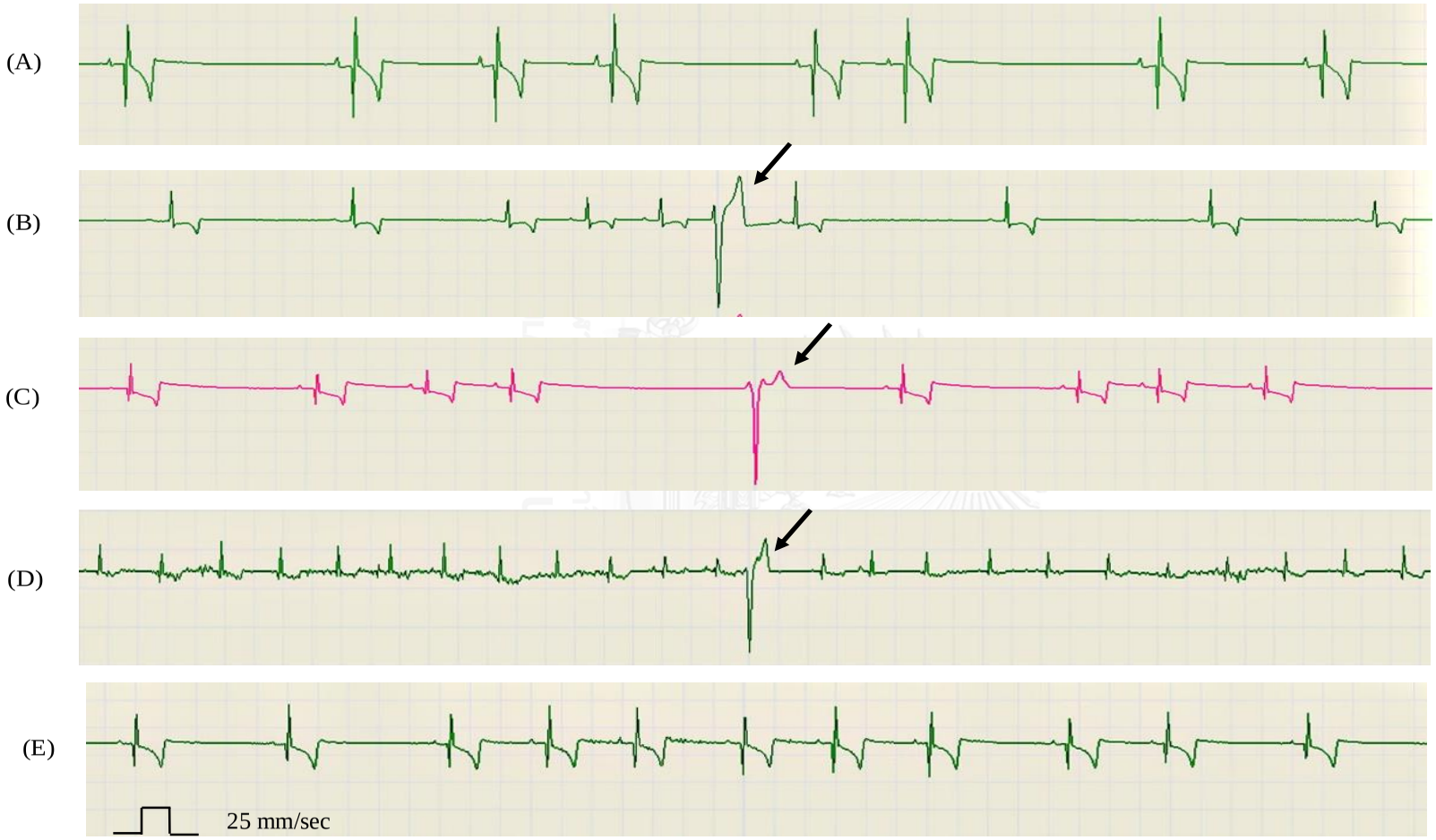


Figure 8. Examples of VPC (arrows) obtained from a dog in low VPC group. Top panel to the bottom panel were recorded from pre-operative period (A), day 1 (B), day 2 (C), day 3 (D) and day 9 (E) after surgery, respectively.

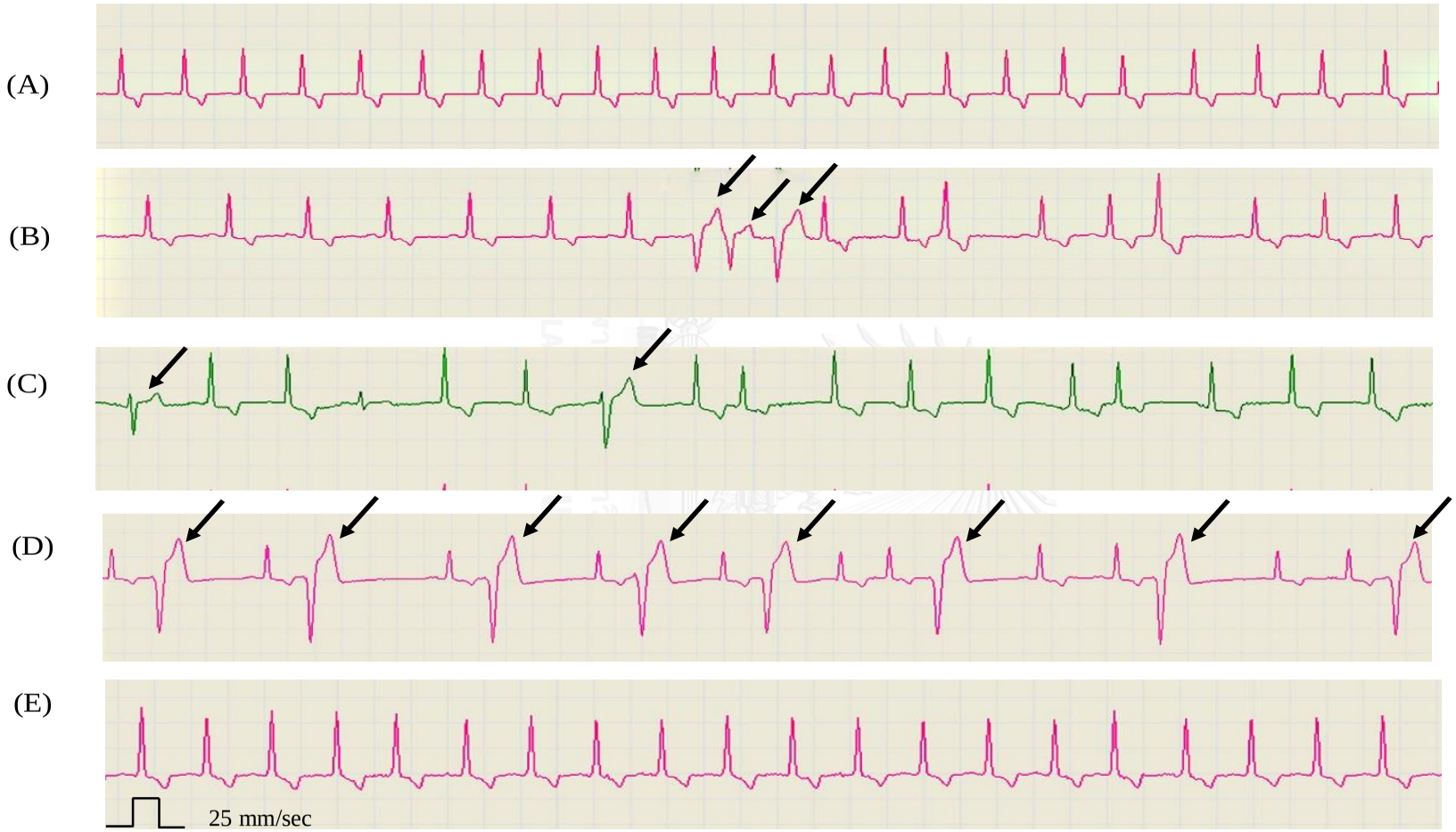


Figure 9. Examples of VPC (arrows) obtained from a dog in high VPC group. Top panel to the bottom panel were recorded from pre-operative period (A), day 1 (B), day 2 (C), day 3 (D) and day 9 (E) after surgery, respectively.

4.2 Characteristics of Dogs and Histopathology of Spleen

4.2.1 General profile

This study investigated 12 splenectomized dogs under the veterinary judgment at the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University. Despite the fact that various breeds were indicated, Golden Retriever was frequently found in current research (Table 3). The mean ages of all dogs, low VPC and high VPC groups were 9.6 ± 1.2 (range 1-16), 10.5 ± 2.3 (range 1-16) years and 8.7 ± 0.8 (range 7-12) years, respectively. The gender ratio of male to female was 9:3 in all dogs. There were 3 castrated, 6 intacted male and 3 spayed female dogs. In low VPC group, the sex ratio of male to female was 5:1 consisted of 2 castrated, 3 intacted male and one spayed female dogs. In high VPC group, the sex ratio of male to female was 4:2 which composed of one castrated, 3 intacted male and 2 spayed female dogs. The mean values of body weight were 21.0 ± 3.6 kg, 14.6 ± 3.5 kg and 27.4 ± 5.2 kg in all dogs, low VPC and high VPC groups, respectively.

The results of radiology revealed that all splenectomized dogs had normal heart and lungs appearances. According to echocardiographic data, some dogs had cardiac abnormalities. In low VPC group, 2 dogs had non-remarkable findings, 2 dogs had mild MR and TR, 2 dogs had moderate to severe DMVD with tricuspid insufficiency. For high VPC group, 2 dogs had unremarkable changes of cardiac dimension and function, one dog had diastolic dysfunction, one dog should be aware for DCM due to increasing chamber size with poor fractional shortening, one dog had mild DMVD and the last one had both MR and TR with diastolic dysfunction.

Table 3. General information of 12 splenectomized dogs.

Group	Breed	Sex	Age (year)	BW (kg)	X-ray	Echocardiography
Low VPC group						
Dog 1	Labrador Retriever	M	1	29.6	Normal thorax	Non-remarkable
Dog 2	Mixed	M	8	18.0	Normal thorax	Non-remarkable
Dog 3	Shetland Sheep dog	M_c	11	15.0	Normal thorax	Mild MR and TR
Dog 4	Beagle	M_c	16	8.9	Normal thorax	Mild MR and TR
Dog 5	Terrier	M	16	10.0	Normal thorax	Moderate to severe DMVD and mild TR
Dog 6	Schnauzer	F_s	11	6.0	Normal thorax	Moderate to severe DMVD and normal systolic function with tricuspid insufficiency
High VPC group						
Dog 7	Golden Retriever	F_s	12	26.6	Normal thorax	Primary diastolic dysfunction
Dog 8	Golden Retriever	M	7	43.0	Normal thorax	Non-remarkable
Dog 9	Golden Retriever	M	8	36.7	Normal thorax	Normal cusp valves, DCM should be aware
Dog 10	Golden Retriever	M	8	32.0	Normal thorax	Non-remarkable
Dog 11	Siberian Husky	F_s	10	18.1	Normal thorax	Severe MR with moderate TR and diastolic dysfunction
Dog 12	Dachshund	M_c	7	8.1	Normal thorax	Mild DMVD

Abbreviations: VPC, ventricular premature contraction; BW, body weight; kg, kilogram; M, male dog; F, female dog; M_c, castrated dog; F_s, spayed dog; DCM, dilated cardiomyopathy; MR, mitral regurgitation; TR, tricuspid regurgitation; DMVD, degenerative mitral valve disease.

4.2.2 Blood and electrolyte profiles

The complete blood count (CBC) and blood chemistry are shown in Table 4 from 3 periods, pre-operative, day 2 and day 9, postoperatively. Red blood cell and hematocrit were not different after splenectomy and between groups. Platelet was significantly higher at day 9 compared with pre-operative period with no difference between groups. White blood cell count was elevated similarly on day 2 after surgery in both groups but presented significant alteration ($p<0.05$) only in all dogs. ALT and ALP were not different among groups. The BUN, creatinine and total protein in all dogs were decreased significantly ($p<0.05$) at day 2 compared with pre-operative period. However, no obvious differences were shown between groups among these parameters. Na^+ and K^+ although had differences in some periods, they stayed within normal reference range.

Table 4. The blood and electrolyte profiles of 12 dogs undergoing splenectomy in all dogs, low VPC and high VPC groups.

Parameter	Group	Pre-op	Day 2	Day 9
R.B.C. ($x10^6$) (per µl) (5.2-8.06)	All dogs	5.39 ± 0.30	5.00 ± 0.31	5.30 ± 0.34
	Low VPC group	5.34 ± 0.36	5.03 ± 0.51	5.33 ± 0.33
	High VPC group	5.43 ± 0.50	4.96 ± 0.40	5.27 ± 0.64
Hematocrit (%) (29.8-57.5)	All dogs	36.50 ± 2.31	33.25 ± 2.09	34.50 ± 2.12
	Low VPC group	36.83 ± 3.20	35.17 ± 3.74	36.00 ± 2.76
	High VPC group	36.17 ± 3.63	31.33 ± 1.93	33.00 ± 3.37
Platelet ($x10^3$) (per µl) (160-525)	All dogs	200 ± 37	284 ± 38	595 ± 71*
	Low VPC group	203 ± 65	327 ± 60	705 ± 87*
	High VPC group	197 ± 41	241 ± 44	485 ± 99*
W.B.C. ($x10^3$) (per µl) (5.4-15.3)	All dogs	19306 ± 7095	24928 ± 3058*	15800 ± 2271
	Low VPC group	10813 ± 2066	21440 ± 4130	16983 ± 3489
	High VPC group	27798 ± 13724	28417 ± 4384	14617 ± 3157
ALT (U/L) (4-91)	All dogs	52 ± 12	55 ± 14	54 ± 15
	Low VPC group	50 ± 17	65 ± 24	71 ± 28
	High VPC group	55 ± 20	44 ± 14	37 ± 10
ALP (U/L) (3-60)	All dogs	227 ± 82	223 ± 68	236 ± 93
	Low VPC group	266 ± 133	312 ± 124	386 ± 169
	High VPC group	188 ± 104	133 ± 43	85 ± 22
BUN (mg/dl) (7-26)	All dogs	15.51 ± 3.20	9.13 ± 2.00*	16.76 ± 4.50
	Low VPC group	15.02 ± 6.23	10.67 ± 4.00	21.85 ± 8.74
	High VPC group	16.00 ± 2.46	7.60 ± 0.86*	11.67 ± 1.54
Creatinine (mg/dl) (0.6-1.4)	All dogs	0.91 ± 0.09	0.74 ± 0.06*	0.85 ± 0.10
	Low VPC group	0.88 ± 0.15	0.73 ± 0.09	0.90 ± 0.17
	High VPC group	0.93 ± 0.12	0.75 ± 0.10*	0.80 ± 0.13*
Total protein (g/dl) (5.8-7.9)	All dogs	6.47 ± 0.38	5.69 ± 0.34*	5.87 ± 0.48
	Low VPC group	6.83 ± 0.64	6.12 ± 0.58	6.57 ± 0.46
	High VPC group	6.10 ± 0.40	5.27 ± 0.32	5.17 ± 0.78
Na^+ (mmol/L) (146-156)	All dogs	148.13 ± 2.05	146.76 ± 2.18	145.12 ± 2.23
	Low VPC group	150.18 ± 2.47	148.20 ± 3.62	149.48 ± 3.25
	High VPC group	146.07 ± 3.28	145.32 ± 2.63	140.75 ± 1.92†
K^+ (mmol/L) (3.8-5.1)	All dogs	4.25 ± 0.17	4.00 ± 0.24	3.97 ± 0.23
	Low VPC group	4.58 ± 0.27	4.21 ± 0.34	3.92 ± 0.39
	High VPC group	3.93 ± 0.11	3.79 ± 0.34	4.02 ± 0.27

Data are shown as mean ± SEM.

*indicates $p<0.05$ compared with pre-operative period in the same group using one-way repeated measures ANOVA. † indicates $p<0.05$ compared with low VPC group using unpaired t-test.

Abbreviations: RBC, red blood cell; WBC, white blood cell; ALT, alanine aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; Na^+, sodium; K^+, potassium.

4.2.3 Histopathological diagnosis

Histopathological results were achieved after surgery by the Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University as shown in Table 5. Hemangioma was presented in all dogs, low VPC and high VPC groups with the incidences of 41.7% (5/12), 33.3% (2/6) and 50% (3/6), respectively. Hemangiosarcoma was shown with incidences of 16.7% (2/12, 1/6 and 1/6) in each group. The remaining (5/12) was histiocytoma, infarction, adenocarcinoma and lymphoma.

Table 5. The histopathological diagnosis of spleen of 12 splenectomized dogs.

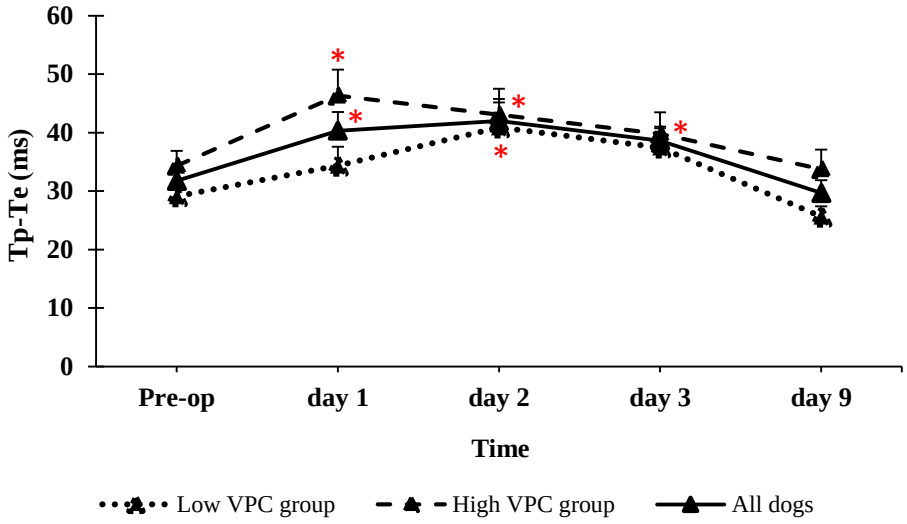
Group	Histopathological diagnosis
Low VPC group	
Dog 1	Splenic hemangioma
Dog 2	Splenic histiocytoma with inflammation
Dog 3	Malignant splenic fibrous histiocytoma with cavernous hemangioma
Dog 4	Splenic hemangiosarcoma
Dog 5	Regenerative nodule with focal splenic infarction
Dog 6	Splenic infarction with thrombus
High VPC group	
Dog 7	Splenic adenocarcinoma
Dog 8	Splenic hemangioma with histiocytic splenitis
Dog 9	Splenic lymphoma
Dog 10	Splenic hemangiosarcoma
Dog 11	Splenic hemangioma
Dog 12	Splenic hemangioma with lymphoid depletion

Abbreviation: VPC, ventricular premature contraction.

4.3 Electrocardiographic Parameters

4.3.1 Tp-Te

According to ECG of Holter device, the Tp-Te from all 12 splenectomized dogs was increased significantly during day 1 to day 3 ($p<0.05$) by 27.1%, 32.5% and 21.8%, respectively compared with pre-operative period (Figure 10). On day 9 after surgery, Tp-Te was declined similar to pre-operative value. The Tp-Te values in low VPC group were increased on day 1, 2 and 3 by 17.9%, 40.5% ($p<0.05$) and 28.9% when compared with pre-operative period. On day 9 after surgery, the Tp-Te in low VPC group was decreased by 12%. In high VPC group, Tp-Te increased significantly by 34.6% ($p<0.05$) on day 1 as compared with pre-operative period. The 25.3% and 15.7% increases in Tp-Te were found on day 2 and 3, respectively while it declined on day 9 after surgery. When comparing between groups, Tp-Te was not significantly different ($p = 0.06$ at day 1).



Period	Low VPC group	High VPC group	All dogs
Pre-op	29.1 ± 2.2	34.4 ± 2.5	31.7 ± 1.8
day 1	34.3 ± 3.2	46.3 ± 4.5*	40.3 ± 3.2*
day 2	40.9 ± 4.8*	43.1 ± 4.5	42.0 ± 3.2*
day 3	37.5 ± 3.3	39.8 ± 3.7	38.6 ± 2.4*
day 9	25.6 ± 1.8	33.7 ± 3.4	29.7 ± 2.2

Figure 10. Values and plots of Tp-Te in all dogs (▲), low VPC group (●) and high VPC group (■). Data are presented as mean + SEM (upper) and mean ± SEM (lower). *indicates $p<0.05$ compared with pre-operative period in the same group using one-way repeated measures ANOVA.
Abbreviations: ms, millisecond; VPC, ventricular premature contraction.

4.3.2 STV_{QT}

The short-term variability of QT interval in all dogs, low VPC and high VPC groups of splenectomized dogs before and after surgery are shown in Table 6. The STV_{QT} obtained from all groups were slightly increased on day 1, 2 and 3 after surgery without significance and was not different between groups.

Table 6. The STV_{QT} measured from continuous ECG in all dogs, low VPC and high VPC groups of splenectomized dogs before and after surgery.

Period	Low VPC group	High VPC group	All dogs
Pre-op	5.1 ± 0.6	4.1 ± 0.5	4.6 ± 0.4
day 1	5.4 ± 0.4	5.6 ± 0.6	5.5 ± 0.4
day 2	5.2 ± 0.2	4.8 ± 0.3	5.0 ± 0.2
day 3	5.8 ± 0.4	5.3 ± 0.5	5.6 ± 0.3
day 9	4.9 ± 0.6	5.3 ± 0.7	5.1 ± 0.5

Data are shown as mean ± SEM.

The example of Poincaré plot in one splenectomized dog which generated from durations of QT; QT_{n-1} against QTn is shown in Figure 11. The cluster pattern of consecutive QT intervals in a dog from high VPC group obtained from pre-operative period was superimposed with that obtained from day 1 after surgery.

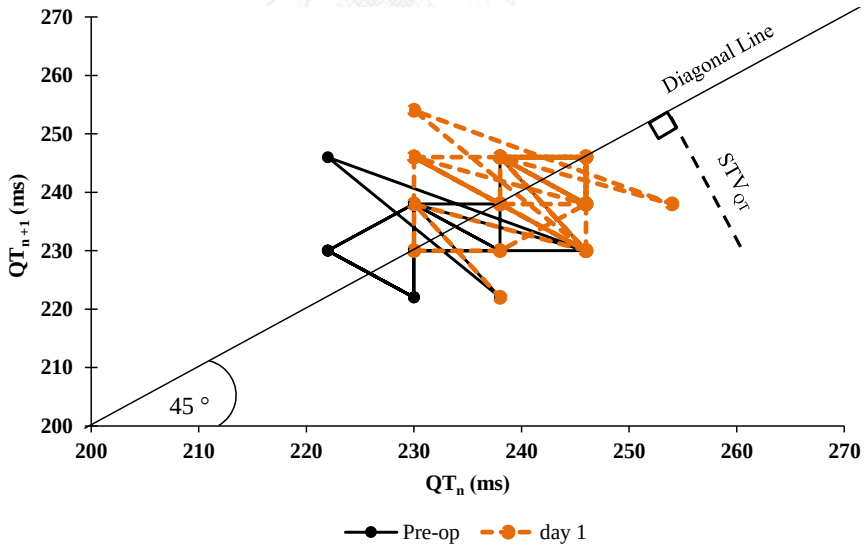
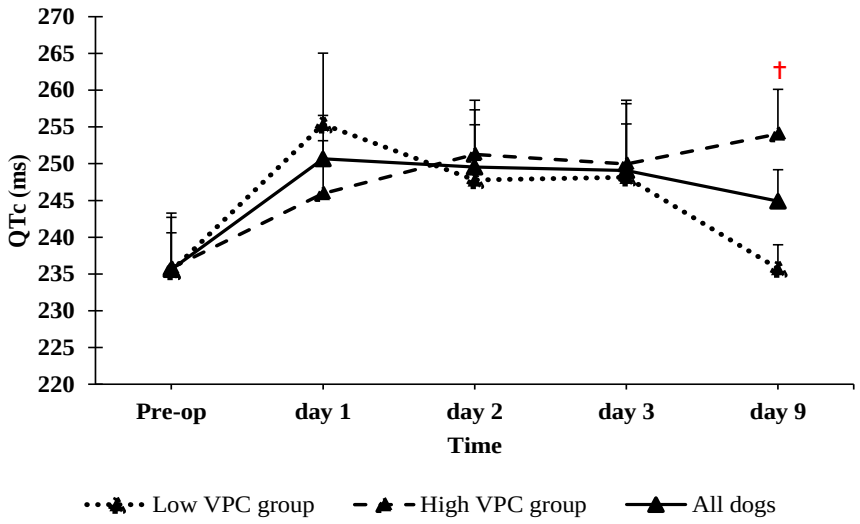


Figure 11. The STV_{QT} parameter by Poincaré plot from QT durations (QT_n : ms) versus the proceeding QT durations (QT_{n-1} : ms). STV_{QT} is the perpendicular distance from the diagonal line to averaged points of Poincaré plot. Each circular data came from 30 consecutive QT intervals of one dog in high VPC group from 2 periods.
Abbreviations: ms, millisecond; STV_{QT}, short-term variability of QT interval.

4.3.3 QTc

Since QT interval is influenced by heart rate; therefore, corrected QT interval (QTc) following Van de water equation was performed. Their values, in all dogs and 2 groups, were slightly lengthen at 1 to 3 days after surgery with significant difference (p<0.05) between groups at day 9 (Figure 12).



Period	Low VPC group	High VPC group	All dogs
Pre-op	235 ± 8	236 ± 7	236 ± 5
day 1	255 ± 10	246 ± 7	251 ± 6
day 2	248 ± 10	251 ± 7	250 ± 6
day 3	248 ± 10	250 ± 8	249 ± 6
day 9	236 ± 3	254 ± 6†	245 ± 4

Figure 12. The QTc (ms) calculated from Van de water equation in all dogs (-▲-), low VPC group (···▲···) and high VPC group (- -▲- -) during pre-op, 1, 2, 3 and 9 days after surgery. Data are presented as mean + SEM (upper) and mean ± SEM (lower).

† p<0.05 compared with low VPC group in each day using unpaired t-test.

Abbreviations: QTc, corrected QT interval; ms, millisecond; VPC, ventricular premature contraction.

4.4 Heart Rate Variability

4.4.1 Time domain analysis

The SDANN in high VPC group was increased significantly at day 3 after surgery compared with pre-operative period and showed the statistical alteration when comparing with low VPC group ($p<0.05$) (Table 7). The SDNN index increased significantly in all dogs and low VPC group 2 to 3 days after surgery. The percent increase was almost twice of that obtained during pre-operative period. This tendency was similar to high VPC group; although, it was not significant. The SDNN index in high VPC group at day 2 and 3 were lower than low VPC group with $p<0.05$ and $p = 0.05$, respectively. The SDNN was higher significantly ($p<0.05$) in all dogs from day 1 to day 3 after surgery while, in both groups, it was increased significantly during day 2 and 3 compared with pre-operative period. No difference was found when comparing between groups. The pNN50 was increased significantly during day 2 and 3 after splenectomy in low VPC group ($p<0.05$) but it was slightly enhanced without significant difference in high VPC group. No difference was found when considering between groups. The RMSSD was elevated significantly from 1 to 3 days after surgery in all dogs. In low VPC group, the RMSSD was increased during day 2 and 3 ($p<0.05$) while, in high VPC group, it was significant ($p<0.05$) only at day 3 compared with pre-operative period. No statistical change was observed when comparing between groups. All parameters of time domain were fallen to pre-operative values when measuring at 9 days after surgery.

Table 7. Time domain parameters of HRV of splenectomized dog in all dogs, low and high VPC groups before and after surgery.

Parameter	Group	Pre-op	Day 1	Day 2	Day 3	Day 9
SDANN	All dogs	56.22 ± 13.98	36.87 ± 8.22	79.97 ± 17.65	91.70 ± 19.63	51.87 ± 9.79
	Low VPC group	60.77 ± 24.44	49.27 ± 14.27	62.23 ± 13.56	50.32 ± 14.76	57.60 ± 16.52
	High VPC group	51.67 ± 15.94	24.47 ± 5.67	97.71 ± 32.57	133.1 ± 28.2*,†	46.14 ± 11.64
SDNN index	All dogs	133.4 ± 28.0	185.0 ± 28.9	230.1 ± 31.5*	244.8 ± 30.4*	127.5 ± 18.6
	Low VPC group	133.4 ± 44.1	182.9 ± 49.8	295.7 ± 44.8*	302.9 ± 47.0*	127.6 ± 36.8
	High VPC group	133.5 ± 38.8	187.1 ± 34.4	164.4 ± 25.3†	186.6 ± 22.4	127.4 ± 13.0
SDNN	All dogs	143.6 ± 29.3	225.2 ± 25.8*	276.9 ± 30.4*	289.7 ± 27.7*	138.8 ± 18.6
	Low VPC group	143.1 ± 47.8	230.8 ± 43.4	291.2 ± 45.1*	313.5 ± 45.9*	141.9 ± 36.9
	High VPC group	144.0 ± 38.5	219.6 ± 32.2	262.6 ± 43.9*	265.8 ± 32.3*	135.7 ± 12.3
pNN50	All dogs	38.42 ± 8.27	48.47 ± 13.15	67.26 ± 7.06	67.83 ± 6.10	40.15 ± 7.67
	Low VPC group	39.59 ± 12.88	62.54 ± 11.70	77.98 ± 3.73*	75.27 ± 6.73*	37.30 ± 13.86
	High VPC group	37.24 ± 11.59	34.40 ± 23.33	56.54 ± 12.62	60.39 ± 9.81	43.00 ± 7.96
RMSSD	All dogs	153.8 ± 46.5	250.4 ± 35.6*	278.4 ± 34.5*	313.3 ± 37.1*	156.4 ± 32.5
	Low VPC group	165.1 ± 86.2	256.0 ± 62.2	335.7 ± 56.2*	364.8 ± 60.3*	163.4 ± 64.3
	High VPC group	142.4 ± 45.1	244.7 ± 41.3	221.1 ± 27.6	261.8 ± 36.8*	149.5 ± 21.9

Data are presented as mean ± SEM. * indicates $p<0.05$ compared with pre-operative period in the same group using one-way repeated measures ANOVA, † indicates $p<0.05$ compared with low VPC group in each day using unpaired t-test.

Abbreviations: SDANN, standard deviations of the average normal RR intervals calculated over short time periods (usually 5 mins) for the entire recording period; SDNN index, mean of standard deviations of the average normal RR intervals calculated over short time periods (usually 5 mins) for the entire recording period; SDNN, standard deviations of all normal RR intervals; pNN50, percentage of differences between adjacent normal RR intervals that are > 50 ms computed in the entire recording; RMSSD, square root of the mean squared differences between adjacent normal RR; VPC, ventricular premature contraction.

4.4.2 Frequency domain analysis

The ULF, VLF, LF, HF, TP and LF/HF ratio are exhibited in Table 8. The ULF was significantly enhanced at day 2 after surgery compared with pre-operative period ($p<0.05$) while it did not show statistical changes in all dogs and high VPC group. A significant difference was found at day 2 by lower value in high VPC group compared with low VPC group ($p<0.05$). The VLF in all dogs and low VPC group tended to increase while it decreased in high VPC group without significant difference compared with pre-operative period or between groups. The LF tended to increase in all dogs and both groups postoperatively compared with pre-operation. No statistical alterations were observed when comparing with pre-operative or between low and high VPC groups. After surgery, the HF values in all dogs and low VPC group tended to increase by 2, 3 and 4 times compared with pre-operative period on day 1, 2 and 3, respectively. Statistical significance was shown during 2 and 3 days after surgery in all dogs while it was found at day 3 in low VPC group compared with pre-operative period. Lower HF values were found in high VPC group although it had tendency to increase compared with pre-operation. When considering between groups, HF values in high VPC group were lower than low VPC group in 2 and 3 days after surgery ($p<0.05$). The TP increased significantly at day 3 in all dogs whereas it increased significantly during day 2 and 3 after splenectomy in low VPC group compared with pre-operative period ($p<0.05$). The TP values in high VPC group tended to increase but still lower which resulting in lower HF when comparing with low VPC group. A significant difference of TP was found at day 2 compared with low VPC group ($p<0.05$). The LF/HF ratio was decreased in all groups after surgery due to enhanced HF. However,

slightly lower LF/HF was found in low VPC group. At day 2, higher ratio in high VPC group was presented significantly when comparing with low VPC group.

Table 8. Frequency domain parameters of HRV of splenectomized dog in all dogs, low and high VPC groups before and after surgery.

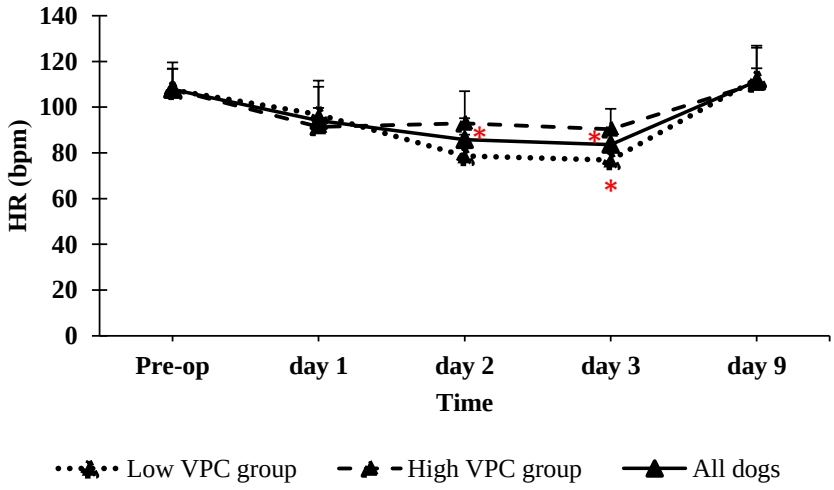
Parameter	Group	Pre-op	Day 1	Day 2	Day 3	Day 9
ULF	All dogs	5295 ± 1974	5230 ± 1549	8967 ± 1963	6145 ± 1138	4519 ± 1141
	Low VPC group	2979 ± 1338	2788 ± 939	13061 ± 2882*	7539 ± 2080	3393 ± 1497
	High VPC group	7611 ± 3635	7671 ± 2701	4873 ± 1398†	4751 ± 770	5645 ± 1726
VLF	All dogs	5293 ± 2990	4160 ± 1063	7394 ± 2623	7353 ± 1608	4055 ± 1145
	Low VPC group	2512 ± 792	4474 ± 1856	11050 ± 4773	9230 ± 2613	2533 ± 867
	High VPC group	8073 ± 5967	3846 ± 1218	3738 ± 1463	5475 ± 1772	5578 ± 2023
LF	All dogs	1943 ± 625	3827 ± 846	7194 ± 2681	11241 ± 4695	3128 ± 698
	Low VPC group	1533 ± 641	3436 ± 1382	10220 ± 5125	10712 ± 5892	2721 ± 1163
	High VPC group	2353 ± 1115	4218 ± 1084	4169 ± 1302	11770 ± 7883	3536 ± 850
HF	All dogs	13256 ± 7750	27505 ± 9926	39107 ± 11678*	54109 ± 15981*	10677 ± 3634
	Low VPC group	19838 ± 15299	32971 ± 16971	62055 ± 19483	84829 ± 25689*	13935 ± 7000
	High VPC group	6675 ± 3595	22040 ± 11556	16160 ± 3129†	23389 ± 9294†	7419 ± 2205
TP	All dogs	25787 ± 10765	40722 ± 11871	62663 ± 17284	78847 ± 19014*	21755 ± 5405
	Low VPC group	26863 ± 17960	43669 ± 19617	96387 ± 28708*	112310 ± 29713*	22582 ± 9920
	High VPC group	24712 ± 13671	37774 ± 15222	28939 ± 5947†	45385 ± 16123	20928 ± 5466
LF/HF	All dogs	0.97 ± 0.27	0.44 ± 0.11	0.25 ± 0.04	0.31 ± 0.08	0.75 ± 0.14
	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†	0.46 ± 0.14	0.61 ± 0.08

Data are present as mean ± SEM. * indicates $p<0.05$ compared with pre-operative period in the same group using one-way repeated measures ANOVA, † indicates $p<0.05$ compared with low VPC group in each day using unpaired t-test.

Abbreviations: ULF, ultralow frequency; VLF, very low frequency; LF, low frequency; HF, high frequency; TP, total power; ms^2, millisecond square; VPC, ventricular premature contraction.

4.4.3 Heart rate

Heart rate (HR) in all dogs, low VPC and high VPC groups declined in the same direction throughout 3 days, postoperatively. Significant changes were found at day 3 in low VPC group (p<0.05) and at both day 2 and 3 when considering from all dogs compared with pre-operative period. At the 9 days after surgery, HR remained the same as those during pre-operative period in both groups (Figure 13).



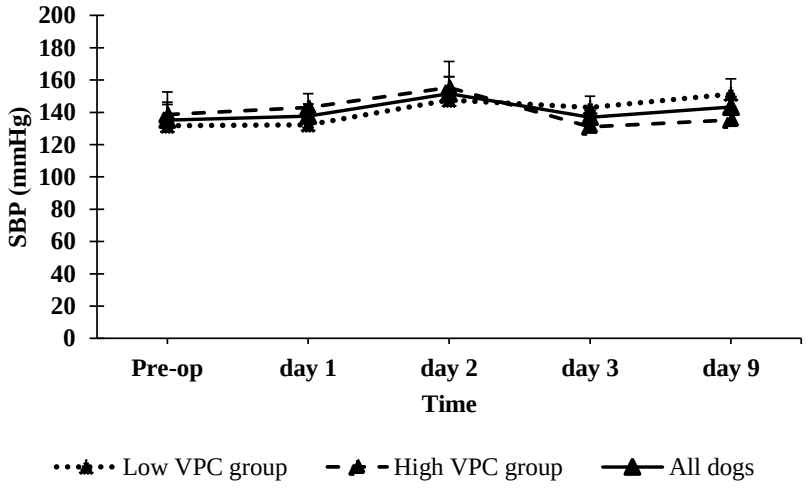
Period	Low VPC group	High VPC group	All dogs
Pre-op	108 ± 9	108 ± 12	108 ± 7
day 1	97 ± 15	91 ± 8	94 ± 8
day 2	79 ± 9	93 ± 14	86 ± 8*
day 3	77 ± 6*	90 ± 9	84 ± 5*
day 9	112 ± 15	110 ± 7	111 ± 8

Figure 13. The heart rate from all dogs (▲), low VPC group (···▲···) and high VPC group (-▲-) during pre-op, 1, 2, 3 and 9 days after surgery. Data are presented as mean + SEM (upper) and mean ± SEM (lower). *indicates p<0.05 compared with pre-operative period in the same group using one-way repeated measures ANOVA.

Abbreviations: HR, heart rate; bpm, beat per minute; VPC, ventricular premature contraction.

4.4.4 Systolic blood pressure

Systolic blood pressure (SBP) in all groups was unchanged; although, it slightly increased 1 to 2 days after surgery (Figure 14).



Period	Low VPC group	High VPC group	All dogs
Pre-op	132 ± 15	139 ± 14	135 ± 10
day 1	132 ± 13	143 ± 8	138 ± 7
day 2	148 ± 15	155 ± 16	152 ± 10
day 3	143 ± 7	131 ± 9	137 ± 6
day 9	151 ± 9	135 ± 8	143 ± 6

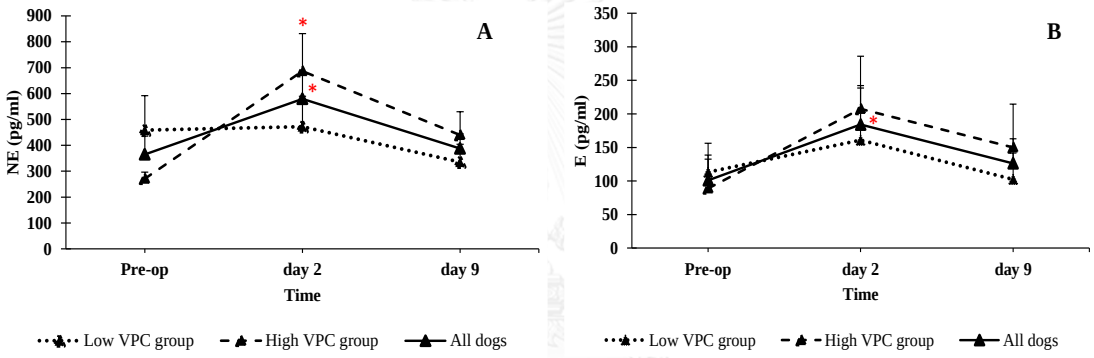
Figure 14. The systolic blood pressure in all groups. Data are presented as mean + SEM (upper) and mean ± SEM (lower).

Abbreviations: SBP, systolic blood pressure; mmHg, millimeter of mercury; VPC, ventricular premature contraction.

4.5 Catecholamine Analysis

Norepinephrine (NE) and epinephrine (E) at pre-operative period, day 2 and day 9 using HPLC-EC detector are shown in Figure 15. The results revealed that NE at day 2 was enhanced in all dogs and high VPC group ($p<0.05$) by 2 and 3 times after

surgery, respectively and reduced on day 9 while it was unchanged in low VPC group. The E increased in both low VPC and high VPC groups by 42.3% and 133.1%, respectively compared with pre-operative period. The E was declined on day 9 after surgery in both groups; although, the value in high VPC group was slightly higher than pre-operation. When comparing the E in all 12 dogs, it increased significantly 2 days after surgery (p<0.05) and declined on day 9 after operation. The examples of the peaks of NE, E and DHBA (internal standard) are shown in Figure 16.

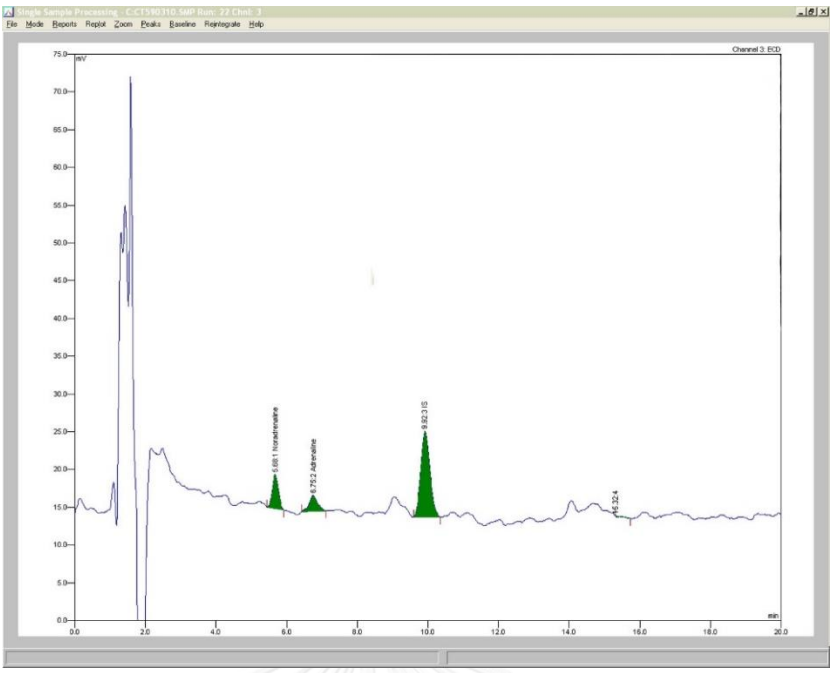


Parameter		Period		
		Pre-op	day 2	day 9
NE (pg/ml)	All dogs	364.9 ± 70.7	579.7 ± 94.4*	387.8 ± 56.3
	Low VPC group	458.8 ± 133.5	472.4 ± 116.9	335.3 ± 68.9
	High VPC group	270.9 ± 25.6	687.0 ± 144.6*	440.3 ± 89.9
E (pg/ml)	All dogs	101.1 ± 31.5	184.2 ± 54.3*	126.2 ± 36.7
	Low VPC group	113.1 ± 43.0	160.9 ± 81.3	102.4 ± 39.3
	High VPC group	89.0 ± 49.6	207.5 ± 78.5	150.1 ± 64.4

Figure 15. The values of NE (A) and E (B) in all dogs (—▲—), low VPC group (···▲···) and high VPC group (- -▲- -) throughout the experiment. Data are presented as mean + SEM (upper) and mean ± SEM (lower). * indicates p<0.05 compared with pre-operative period in the same group using paired t-test.

Abbreviations: NE, norepinephrine; E, epinephrine; pg/ml, picogram per milliliter; VPC, ventricular premature contraction.

(A)



(B)

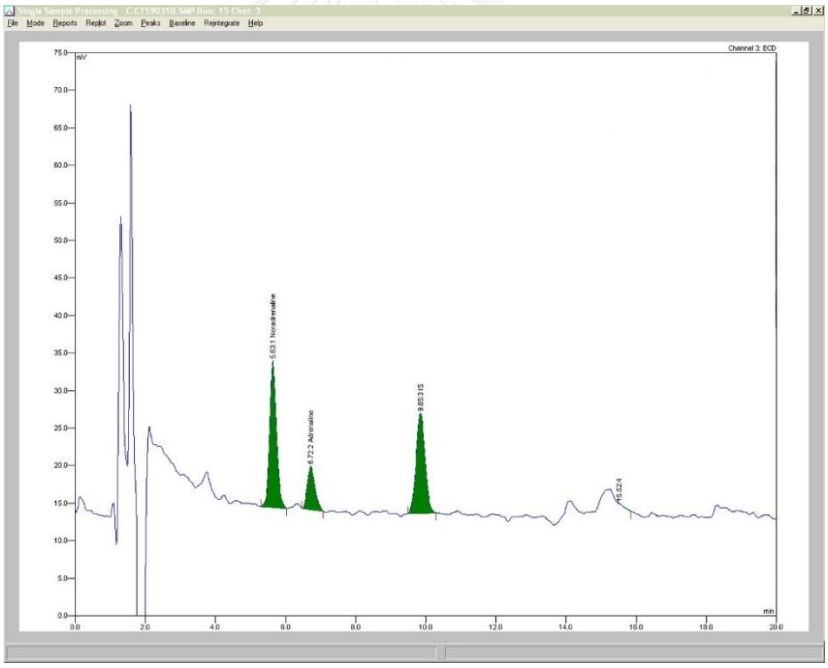


Figure 16. The results from HPLC running in two dogs at day 2 after surgery. One in low VPC group (A) and another one in high VPC group (B). Three apparent shaded peaks are NE, E and DHBA with mean retention time of 5.5, 7 and 11 minutes, respectively. Abbreviation: mV, millivolt.

4.6 Correlations between Parameters

The correlations between NE/E and the number of VPC obtained from pre-operative period, day 2 and day 9 in both all 12 dogs and high VPC group were performed. Together with other parameters in all dogs obtained from day 1, 2 and 3 after surgery including heart rate, systolic blood pressure, time domain and frequency domain of heart rate variability, the results are presented in Table 9. However, no correlations were apparent between HRV or electrocardiographic parameters and the number of VPC.

Table 9. The correlations of NE, E, HR, SBP and HRV of all dogs.

Parameter	n	r	*p*-value
NE and VPC	36[b]	0.372	<0.05
	18[a]	0.500	<0.05
E and VPC	36[b]	0.421	<0.05
	18[a]	0.555	<0.05
HR and SBP	36[c]	0.464	<0.01
HR and SDNN index	36[c]	-0.772	<0.001
SDNN	36[c]	-0.692	<0.001
pNN50	36[c]	-0.508	<0.01
RMSSD	36[c]	-0.649	<0.001
ULF	36[c]	-0.502	<0.01
VLF	36[c]	-0.583	<0.001
LF	36[c]	-0.399	<0.05
HF	36[c]	-0.551	<0.001
TP	36[c]	-0.620	<0.001
LF/HF	36[c]	0.348	<0.05
SBP and SDNN index	36[c]	-0.394	<0.05
SDNN	36[c]	-0.380	<0.05
RMSSD	36[c]	-0.442	<0.01
LF	36[c]	-0.329	<0.05

[a] values in pre-operative period, day 2 and day 9 after surgery were grouped when considering in 6 dogs of high VPC group.

[b] values in pre-operative period, day 2 and day 9 after surgery were grouped when considering in all 12 dogs.

[c] values in day 1, day 2 and day 3 after surgery were grouped when considering in all 12 dogs.

Abbreviations: NE, norepinephrine; E, epinephrine; VPC, ventricular premature contraction; HR, heart rate; SBP, systolic blood pressure; SDNN index, mean of standard deviations of the average normal RR intervals calculated over short time periods (usually 5 min) for the entire recording period; SDNN, standard deviations of all normal RR intervals; pNN50, percentage of differences between adjacent normal RR intervals that are > 50 ms computed in the entire recording; RMSSD, square root of the mean squared differences between adjacent normal RR; ULF, ultralow frequency; VLF, very low frequency; LF, low frequency; HF, high frequency; TP, total power.

4.7 Survival Analysis

The survival time was counted from day 1 after surgery to the day of death. In case of surviving, the survival time was counted from day 1 after surgery to the 10th October 2016 which represented as a censored observation.

In all dogs (n = 12), mean survival time was 594 ± 109 days (range 380-808) and survival curve showed that 75% of the dogs survived for 240 days (8 months) (Figure 17). The mean survival time in low VPC group was 787 ± 137 days (range 517-1056) with 75% of the dogs survived for 390 days (13 months) while the mean survival time in high VPC group was 318 ± 69 days (range 182-453) with 75% of the dogs survived for 180 days (6 months) (Figure 18).

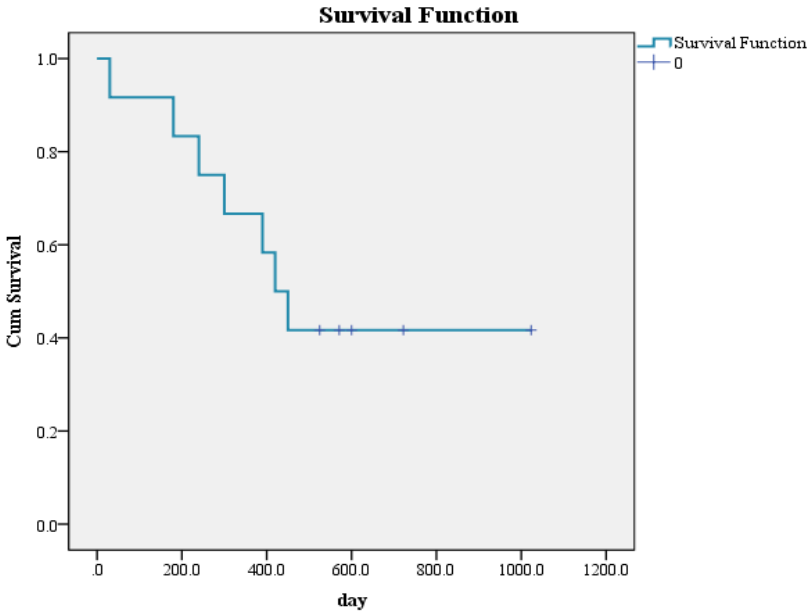


Figure 17. Kaplan-Meier survival curve evaluated in days, from day 1 after surgery as the beginning for all 12 splenectomized dogs.

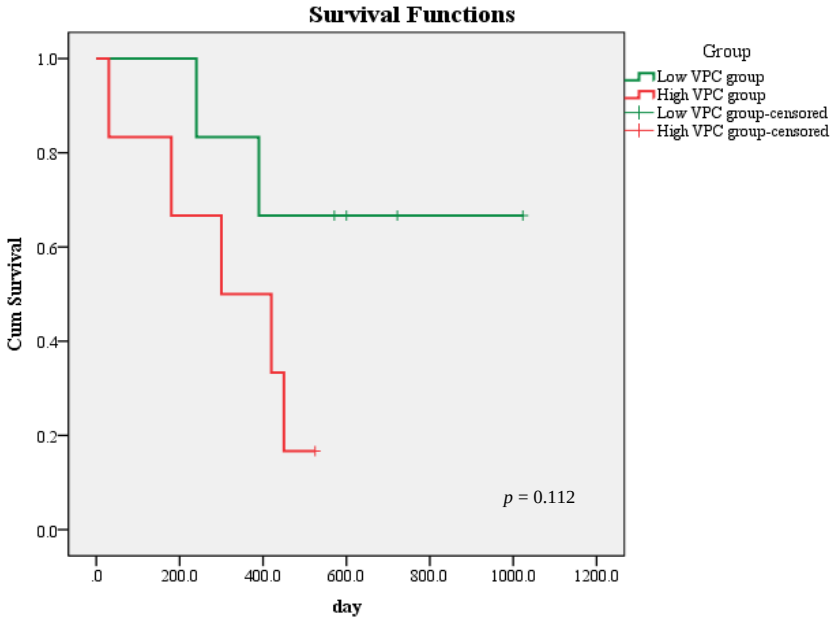


Figure 18. Kaplan-Meier survival curves evaluated in days, from day 1 after surgery as the beginning for each 6 splenectomized dogs in low VPC (green line) and high VPC groups (red line), respectively. Comparison between 2 groups by log-rank test.

4.8 Limitation

According to the Veterinary Professional Ethics, the animals were treated with analgesic and antibiotic drugs postoperatively as needed. Blood transfusions were performed for 2 dogs; one in low VPC group during the surgical procedures and another one in high VPC group on day 3 after surgery.

CHAPTER V
DISCUSSION

5.1 Incidence of Arrhythmia and Possible Etiology

The VPC were commonly presented in canine splenectomy either prior to or after operation (Keyes et al., 1993; Knapp et al., 1993; Marino et al., 1994; Neath et al., 1997). In current study, the VPC in one dog although were undetected prior to surgery but it was found more than 1000 beats/24 hours after surgery and was categorized into the high VPC group.

Importantly, Knapp et al. (1993) and Marino et al. (1994) recommended that incidences of arrhythmia occurred postoperatively by 68% and 44%, respectively which similar to the present findings. Rather than splenectomy, the arrhythmia complications were frequently found in canine GDV, hepatic resection and colorectal surgery (Muir and Lipowitz, 1978; Yanaga et al., 1996; Batra et al., 2001; Walsh et al., 2006; Buranakarl et al., 2007; Hekman et al., 2014; Lopes-Junior et al., 2014; Sharp and Rozanski, 2014). Study of Knapp and co-workers (1993) reported that VPC after spleen removal occurred within 3 hours and persisted for 3 days. The origin of VPC remained unknown but there were several observations suggested that the complication was developed from multifactorial factors. Four possible explanations in current study were: **(i)** catecholamine secretion which was a result of surgical trauma or pain response via sympathetic-adrenal-medullary (SAM) axis and activation of general sympathetic fibers (Kook et al., 2007; Hekman et al., 2014). Anatomically, at least 25 branches of

vessels were found prior to supply the spleen in dog (Slatter, 2002). Therefore, cutting of vessels during surgery may induce more pain than operation on other internal organs. Moreover, spleen is abundantly innervated by sympathetic nerves; hence, splanchnic and splenic nerves disturbing via abdominal manipulation could induce catecholamine releasing into the circulation due to contraction of spleen (Barcroft et al., 1932; Davies et al., 1973; Straub, 2004; Padro et al., 2013). Another source may occur via stimulation of splenic nerve linking to renal sympathetic known as splenorenal reflex which may involve the release of catecholamine from adrenal gland (Herman et al., 1982; Deng and Kaufman, 2001). This catecholamine may be the cause of arrhythmia genesis through triggered activity (Gaztanaga et al., 2012). In addition, when sympathetic activity was stimulated due to pain, angiotensin II following RAAS may be increased. The angiotensin II was related to arrhythmia based on Gαq/11 protein via angiotensin II type 1 receptor and a signaling was down-regulated to increase calcium influx and further triggered activity (Goette and Lendeckel, 2008; Iravanian and Dudley, 2008). Angiotensin II can elevate transmural dispersion of repolarization (TDR) and local catecholamine release (Goette and Lendeckel, 2008). **(ii)** Myocardial hypoxia, ischemia or myocardial infarction (MI) which may occur during surgery and post-operative period. In case of myocardial ischemia, an acidosis was accumulated with decreased I_{Na}, I_{Ca} but enhanced I_K currents in epicardium as well as endocardium resulting in shortening APD (Cuntai et al., 2002). On the contrary, M-cell had I_K less than epi- and endocardium caused a prolongation of APD and increment of the transmural dispersion of repolarization (TDR) (Cuntai et al., 2002). The TDR along with EAD-induced extrasystole produced a malignant arrhythmia (Belardinelli et al., 2003). Furthermore, anesthetic procedures, surgical methods and pain stimulating

sympathetic tone were the major causes to increase myocardial oxygen demand (Priebe, 2004; Puelacher et al., 2015). Several studies revealed that arrhythmia including ventricular premature beat, non-sustained VT and accelerated idioventricular rhythm occurred due to MI had a delayed onset (6 to 72 hours) (Campbell et al., 1981; Northover, 1982) which was similar to our complication. The surviving Purkinje cells from subendocardium which sensitive to catecholamine may generate an abnormal automaticity and may be a reason of delayed arrhythmia (Sugi et al., 1985; Janse and Wit, 1989). However, proof of MI requires the further evidence, such as measurement of plasma cardiac troponin (cTn) (Lucreziotti et al., 2006). **(iii)** A myocardial depressant factor which was a substance releasing from the hypoxic organ, i.e., spleen, and may cause an abnormality of cardiac function (Lefer, 1982). **(iv)** An electrolyte imbalance, such as hyponatremia in high VPC group (Table. 4, page 36). The Langendorf study demonstrated that hyponatremia may delay conduction velocity while, during ischemia, it induced arrhythmia at epicardial layer (Wolk et al., 1998).

In human, the data of body weight linking to the occurrence of arrhythmia were widely recorded based on several mechanisms, such as obesity-induced electrophysiological remodeling, myocardial infarction and sympathetic hyperinnervation (Mozos, 2014). Nevertheless, those data had not been found in dog. In the present experiment, only 3 dogs were overweight according to Breed Weight Chart.

5.2 Characteristics of Dogs

5.2.1 General profile

Twelve splenectomized dogs in current findings were middle to old age. The mean age in all dogs was 9.6 ± 1.2 years old while in low VPC as well as high VPC groups were 10.5 ± 2.3 and 8.7 ± 0.8 years old, respectively. These data are in agreement with prior studies (Johnson et al., 1989; Spangler and Kass, 1997; Eberle et al., 2012; Goritz et al., 2013). Although underlying causes of splenic lesion are not precisely clarified, some investigators considered the splenic mass in immune-mediated, genetic factor, infections or environmental factors (Mortier et al., 2012). Thus, tumor was developed in an elderly dog. Major splenectomized dogs were male similar to several studies (Johnson et al., 1989; Spangler and Kass, 1997). Whereas the ratio between spayed and non-spayed dogs was different by 54:51 and 19:29 according to previous reports (Eberle et al., 2012; Goritz et al., 2013). At this point, the variations may be due to the scale of sample size in this research.

Despite the fact that numerous breeds of splenectomized dogs including Labrador Retriever, Siberian Husky, Shetland Sheep dog, Beagle, Terrier, Schnauzer, Dachshund and mixed were presented, Golden Retriever was found the most in present study. Previous studies reported that splenic tumor was found in large breed dog same as our report except for German Shepherd, Boxer and Molossoid breeds (Eberle et al., 2012; Goritz et al., 2013). The reasons may depend on popularity of breeds in each country and the number of dogs in each experiment. Mean body weight was around 21.0 ± 3.6 kg which similar to the study of Eberle and assistants (2012) which reported that the splenectomized dogs had an average of body weight of 30 kg. In our study,

low VPC group had mean body weight lower than high VPC group because the large breed dogs were mainly presented in high VPC group.

Since the VPC may be related to systemic disease or directly due to organic heart disease; therefore, the radiology and echocardiography were further examined in all dogs. The thoracic radiograph did not show an abnormal appearance which was in accordance with previous reports (Marino et al., 1994; Mortier et al., 2012). Although echocardiography was barely recorded. The mitral regurgitation (MR) was currently shown in both groups with various degrees similar to the earlier study (Marino et al., 1994). MR dogs may present some types of arrhythmia. Crosara and co-workers (2010) reported that 57% of pre-clinical group (asymptomatic) and 86% of symptomatic group represented with ventricular arrhythmia. The tricuspid regurgitation (TR) mostly occurred together with chronic mitral valve disease (MVD), pulmonary hypertension and right ventricular enlargement. Electrocardiogram alteration in TR may be seen as atrial fibrillation with less common in supraventricular and ventricular premature beats (Bruce and Connolly, 2009; Olsen et al., 2010). Hence, these data suggest that arrhythmia in either MR or TR was unreliable and non-specific. Therefore, occurring of arrhythmia due to MR or TR depending on the severity of clinical signs, progression of disease and may not be involved in the present study.

Dilated cardiomyopathy or DCM is one important source of arrhythmias, such as atrial fibrillation and ventricular tachycardia (Brachmann et al., 1997; Meurs, 2010). The etiology of arrhythmia in DCM has not yet clearly elucidated but there were some supporting hypotheses. For instance, myocardial fibrosis which easily triggered became the arrhythmogenic substrate and catecholamine elevation resulting in myocardial fibers stretching due to enhancement of left ventricular end-diastolic

volume (Brachmann et al., 1997; Towbin and Lorts, 2011). Additionally, a recent study demonstrated that DCM-associated arrhythmia may be developed through gene mutation, electrical dysfunction, mechanical instability and myocardial dysfunction (Towbin and Lorts, 2011). In this research, although many dogs were large breeds, the DCM was not diagnosed from echocardiography.

Consequently, it was assumed that preexisting cardiac diseases prior to surgery may not be the cause of arrhythmia in these splenectomized dogs.

5.2.2 Blood and electrolyte profiles

Red blood cell and hematocrit in both groups slightly reduced in day 2 after operation compared with pre-operative period which similar to the earlier studies (Waldmann et al., 1960; Richardson and Brown, 1996). The platelet counts of low and high VPC groups were significantly increased at day 9 after surgery. The high platelet occurring in this situation known as post-splenectomy thrombocytosis which was defined according to platelet count > 500-600 x 10^3/µl (Hammer, 1991; Neel et al., 2012). This occurrence was in agreement with findings of Waldmann et al. (1960) and Dellenback et al. (1969) who reported that platelet enhancement or thrombocytosis was developed in the splenectomized dogs within 2 to 3 weeks after surgery. Furthermore, thrombocytosis in human which occurred by 75-82% in 48 hours to at least day 5 postoperatively was predisposing to thromboembolism (TE). Nevertheless, there was a low incidence of TE occurring in dogs (Boxer et al., 1978; Khan et al., 2009; Neel et al., 2012; Lee et al., 2015; Pommerening et al., 2015). The etiology or pathophysiology of post-splenectomy thrombocytosis has remained obscure. The spleen is recognized as a primary organ to regulate the platelet count by destroying the senescent or damaged

platelet from the circulation; therefore, removal of spleen can impair these abilities (Boxer et al., 1978; Bullen and Losowsky, 1979; Ahmed and Isaac, 2009; Khan et al., 2009). High platelets responsible for hemostasis during surgery or from catecholamine, infection, tumor and stress (Ahmed and Isaac, 2009). The platelet destruction may be impaired due to splenectomy.

The post-splenectomy leukocytosis has been widely documented in both human and dog (Maxie et al., 1970; Richardson and Brown, 1996; Chaudhry et al., 1997; Wernick et al., 2016). The present study revealed that leukocytosis occurred particularly at day 2 after surgery in all dogs. The mechanism may be due to the absence of factor to promote cell apoptosis or lacking of the organ to destruct WBC after removal of spleen (Spencer and Pearson, 1975; Djaldetti et al., 2003). Furthermore, NE and E elevations at day 2 may potentiate the number of WBC which was released from other organs, such as lung, blood vessel and bone marrow as called the catecholamine-induced leukocytosis phenomenon (Benschop et al., 1996).

The levels of ALT and ALP, neither in low nor high VPC group had a significant difference when compared with pre-operative period. These results showed the consistent information with the previous report in 15 healthy mongrel dogs performed splenectomy (Stedile et al., 2009).

BUN and creatinine were maintained within the normal reference range but they were slightly decreased 2 days after surgery. This may be a consequence of fluid supplement during post-operative period. The total protein slightly decreased in agreement with the earlier reports (Dellenback et al., 1969; Richardson and Brown, 1996; Chaudhry et al., 1997). Potassium (K^+) remained in normal range while hyponatremia in high VPC group was found in day 9 after surgery.

5.2.3 Histopathology of splenic defect

The causes of splenic lesions that required spleen removal in this study were malignant-, benign- or non-neoplastic types which similar to previous reports (Johnson et al., 1989; Spangler and Kass, 1997). Hemangioma, surrogate of a benign tumor, was mostly found by 42%. The percentage was found for hemangiosarcoma, a malignant tumor, by 17% which lower than previous studies due to the number of dogs in current findings (Frey and Betts, 1977; Hosgood, 1986; Johnson et al., 1989; Spangler and Kass, 1997; Eberle et al., 2012). Other splenic lesions including splenic infarction and splenic hemorrhage were presented in this report. Importantly, we found arrhythmia in all 12 dogs after surgery although there were various numbers of VPC. Our results differed from the study of Marino and colleagues (1994) which reported that only arrhythmia according to hemangiosarcoma, hematoma and splenic torsion were found. Consequently, cardiac arrhythmia occurring in this study may not be related to types of splenic disease.

5.3 Electrocardiographic Parameters

This is the first investigation of electrocardiographic parameters in canine splenectomy. The estimation of VPC tendency may be validated using many parameters from electrocardiographic components, such as Tp-Te, STV_{QT} and QTc (Lubinski et al., 2000; Thomsen et al., 2004; Pham et al., 2015). In present findings, Tp-Te values increased significantly when compared with pre-operative period. The Tp-Te represents the TDR between epicardial layer and M-cell (Antzelevitch, 2001a; Fish et al., 2004; Antzelevitch et al., 2007; Xia and Yuan, 2007). TDR enhancement

may induce arrhythmia by providing a substrate for re-entry (Burton and Cobbe, 2001; Belardinelli et al., 2003). The causes for enhancement of Tp-Te in both groups may be due to many reasons. **(i)** Sympathetic activity; Yagishita and colleagues (2015) performing the experiment in Yorkshire pigs showed that stimulation of sympathetic nerve markedly increased Tp-Te interval. Similar to another study by Mantravadi and co-workers (2007) in rabbit Langendorf model suggested that the mechanism according to TDR enhancement may be related to the difference of potassium channel density in each cardiac layer as described previously. However, vagus nerve stimulation did not show the alteration of TDR (Mantravadi et al., 2007). **(ii)** Circulating catecholamine; epinephrine increased significantly in all dogs during post-surgery. Fish et al. (2004) reported that epinephrine infusion increased TDR via enhancement of outward K^+ currents like sympathetic stimulation. Although epinephrine prolonged phase 2 (plateau) of the action potential, K^+ outward currents were still predominated (Fuenmayor et al., 2016).

Temporal heterogeneity of repolarization or beat-to-beat variability of repolarization (BVR) was evaluated by STV_{QT} (Belardinelli et al., 2003). In the present findings, STV_{QT} did not show any significant changes in either 12 splenectomized dogs or among 2 groups which may be due to the experimental procedures in which monophasic action potential (MAP) catheter was used before (Thomsen et al., 2004). Another possible explanation may be the fact that STV_{QT} was mainly performed in the pharmacological entity to evaluate the pro-arrhythymic property of drug especially drug-induced TdP (Schneider et al., 2005; Jacobson et al., 2011). In current observations, none of this drug-induced TdP was administered in either pre- or post-

operative period. Accordingly, there was an inadequate potential to elevate the oscillation of STV_{QT}.

The QTc interval did not show the significant alteration in post-operative period which was in agreement with the earlier study in human performing in non-cardiac surgery (Nagele et al., 2012). However, in this study, QTc at day 9 in high VPC group was higher than another group. Some investigators suggested that transient increase of QTc may be due to electrolyte imbalance or impairment of drug clearance (Smithburger et al., 2010; Joyce et al., 2015). In the present study, none of those reasons was found. The prolonged QTc value in high VPC group on day 9 was corresponding to the existing VPC found on that day.

Since influences of anesthetic drugs on overall electro-parameters have not been widely explored. Only some drugs were examined, e.g., midazolam, propofol and isoflurane (Whyte et al., 2005; Owczuk et al., 2009; Owczuk et al., 2012). In this study, drug usages in either anesthesia procedure or post-operative period were similar and uniform between 2 groups. Hence, variations of electro-parameters between groups were not originated from the anesthetic drugs.

5.4 Heart Rate Variability (HRV)

5.4.1 Time domain

The SDNN reflects a circadian rhythm (Electrophysiology, 1996; Stein and Kleiger, 1999; Sztajzel, 2004). The SDNN index and SDANN are involved with sympathetic and parasympathetic activities influenced from baroreceptor modulation (Electrophysiology, 1996; Stein and Kleiger, 1999). The pNN50 and RMSSD stand

for vagal modulation during respiratory change (Stein and Kleiger, 1999; Sztajzel, 2004). Enhancements of SDNN, SDNN index, pNN50 and RMSSD in low VPC group more than high VPC group were found after surgery implied that baroreceptor or autonomic nervous system modulation was better in low VPC group than high VPC group.

5.4.2 Frequency domain

The frequency domain was enhanced in all dogs and both groups after surgery. Interestingly, the higher changes were found in low VPC group compared with high VPC group. The components of ULF remain unclear but neuroendocrine and physical activity may be involved (Electrophysiology, 1996; Stein and Kleiger, 1999). Although components of VLF are partially questionable, mainly parasympathetic outflow, moderately renin-angiotensin-aldosterone system (RAAS) and thermal regulation may be a contributor (Taylor et al., 1998). Reduction of VLF indicated the enhancement of RAAS fluctuation, withdrawal of parasympathetic activity and impaired the ability of thermal regulation (Taylor et al., 1998). In our study, VLF in high VPC group tended to decrease while it increased in low VPC group. These results implied that the dogs in low VPC group had better improvement of parasympathetic modulation, thermal adjustment but attenuated the RAAS coupling. Although temperature of splenectomized dogs was not completely documented, postoperative hypothermia commonly occurred after major surgery following the earlier studies (Abelha et al., 2005; Belayneh et al., 2005). The LF comprises dual interplays of sympathetic and parasympathetic activities which derived from baroreceptor while HF is predominantly parasympathetic. The TP is the sum of all cardiac autonomic nervous system activities

and LF/HF refers to cardiac sympatho-vagal balance (Electrophysiology, 1996; Calvert, 1998; Stein and Kleiger, 1999). In this study, LF, HF and TP were increased while LF/HF was decreased during post-operation in all dogs and both groups suggesting that enhanced ANS activity with higher parasympathetic pathway. Nevertheless, the changes were more pronounced in low VPC group than high VPC group especially HF causing lower LF/HF. Thus, vagal activity was dominated along with lower VPC occurrence. Our findings were contradicted by the study of Amar and co-workers (1998) in which the entire parameters of HRV were decreased after a variety of major surgery in human (only one patient had splenectomy). No record of duration that performing the HRV (day or night) and patients were administered morphine which already examined to reduce the HRV via control-pump for 72-96 hours (Michaloudis et al., 1998). Enhancement of sympathetic activation in this study may be due to surgical trauma or pain (Nicholson, 2005). Chang et al. (2012) reported that LF/HF positively related to the degree of pain after abdominal surgery.

Effects of anesthetic and analgesic drugs on heart rate variability were established. The reduced heart rate variability (propofol, midazolam) and reduced LF and LF/HF (fentanyl, morphine) were reported (Vettorello et al., 2008; Mazzeo et al., 2011). However, in present study, usages of anesthetic and analgesic drugs were similar between 2 groups.

5.4.3 Heart rate and systolic blood pressure

After operation, heart rate was reduced while systolic blood pressure was unchanged suggesting that improvement of parasympathetic modulation in low VPC group could be reflected on lower heart rate compared with high VPC group

5.5 Relationships between Parameters

5.5.1 NE and E levels

The dogs after operation had high NE and E levels which were more pronounced in high VPC group. Change in E was not affected by external administration because it was not given during surgery. Augmentation of epinephrine after operation was similar to the previous study in human non-cardiothoracic surgery (Amar et al., 1998). The reasons for enhancements of E and NE after surgery may be due to post-operative stress/pain. Although a discrepancy occurred following study of Ledowski et al. (2012) which demonstrated that post-operative pain levels were not correlated to plasma catecholamine. Another possible cause for catecholamine elevation was post-operative hypoxia occurring in either immediately or later on (Powell et al., 1996; Filho et al., 2001). The hypoxia could induce catecholamine releasing via Chromaffin cells which consisting of epinephrine 60-70% (Mochizuki-Oda et al., 1997).

5.5.2 Correlations between catecholamine and VPC

The relationships were found between either NE or E and the number of VPC in all dogs ($n = 36$, $r = 0.372$, $p<0.05$ and $n = 36$, $r = 0.421$, $p<0.05$, respectively). When considering in 2 groups, the relationship was found only in high VPC group. Thus, catecholamine may responsible for VPC occurrence. Higher correlation of E suggested that it may be predominated than NE.

5.5.3 Other correlations

After surgery, heart rate was correlated to systolic blood pressure due to pain. The heart rate was negatively correlated to time domain and frequency domain of HRV suggesting that ANS modulation was enhanced. On the contrary, a positive correlation was found between heart rate and LF/HF due to parasympathetic stimulation resulting in lower heart rate in low VPC group.

Systolic blood pressure was also negatively correlated to heart rate, SDNN index, SDNN, RMSSD and LF suggesting that blood pressure was regulated via cardiac ANS activity.

5.6 Survival Time

In case of splenic tumor, surgery is the recommended choice for treatment; otherwise, the tumor may rupture fatally (Slatter, 2002). There were various reports for survival durations after splenectomy ranging from 2-3 months to 36 weeks (Frey and Betts, 1977; Johnson et al., 1989; Slatter, 2002). Nonetheless, in present study, survival times in all dogs, low VPC and high VPC groups were longer than previous studies (594, 787 and 318 days, respectively) and dogs without surgery.

CHAPTER VI
CONCLUSION

In conclusion, the splenectomized dogs may be suffered from VPC especially after surgery. Some dogs had high VPC depending upon various factors, such as catecholamine secretion or impairment of autonomic response after surgery etc. The electrocardiographic parameter which increased along with high VPC occurrence after splenectomy was transmural dispersion of repolarization or Tp-Te. The catecholamine was enhanced postoperatively resulting from post-operative pain, splenic contraction and possibly adrenal gland via splenorenal reflex. These hormones may responsible for cardiac arrhythmia supported by correlations between the number of VPC and catecholamine in all dogs especially in high VPC group.

The elevations of cardiac ANS activities both sympathetic and parasympathetic pathways were demonstrated in splenectomized dogs. However, parasympathetic activity was dominated in low VPC group compared with high VPC group which may be responsible for lower heart rate and less ventricular arrhythmia.

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APPENDIX

VITA

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