THE LEFT VENTRICULAR FUNCTIONS AND HEART RATE VARIABILITY IN DOGS WITH PULMONIC VALVULAR STENOSIS

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การทำงานของหัวใจห้องล่างซ้ายและความแปรปรวนของอัตราการเต้นของหัวใจในสุนัขลิ้นหัวใจพัลโมนิคตีบ

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โรคลิ้นหัวใจพัลโมนิคตีบ (pulmonic valvular stenosis; PS) เป็นหนึ่งในโรคหัวใจพิการแต่กำเนิดที่พบได้บ่อยในสุนัขซึ่งสามารถนำไปสู่การเปลี่ยนแปลงของหัวใจที่ไม่พึงประสงค์ นอกจากนี้โรค PS ยังมีผลต่อระบบประสาทอัตโนวัติที่มีผลต่อหัวใจ อย่างไรก็ตามข้อมูลทางคลินิกเกี่ยวกับการบีบตัวของหัวใจและการเปลี่ยนแปลงของระบบประสาทอัตโนวัติที่มีผลต่อหัวใจในสุนัขที่มีโรค PS ยังมีข้อมูลที่ไม่แน่ชัด วัตถุประสงค์ของการศึกษานี้เพื่อประเมินคุณสมบัติการทำงานด้านไฟฟ้าของหัวใจ การทำงานของหัวใจห้องล่างซ้าย และประเมินระบบประสาทอัตโนวัติที่มีต่อผลหัวใจด้วยการวิเคราะห์ความแปรปรวนของอัตราการเต้นของหัวใจ (heart rate variability) ในสุนัขที่มี PS เปรียบเทียบกับสุนัขสุขภาพดี สุนัขในการศึกษานี้แบ่งออกเป็นสองกลุ่มคือ สุนัขที่มีโรค PS จำนวน 13 ตัว และสุนัขควบคุมที่มีสุขภาพดีจำนวน 12 ตัว ทำการตรวจร่างกาย วัดความดันโลหิตด้วยวิธีออสซิลโลเมตริก ตรวจการทำงานของหัวใจด้วยการวัดคลื่นไฟฟ้าหัวใจ บันทึกภาพหัวใจด้วยคลื่นเสียงสะท้อนความถี่สูง บันทึกคลื่นไฟฟ้าหัวใจแบบต่อเนื่องเป็นเวลา 30 นาที และเก็บตัวอย่างเลือดไปวิเคราะห์ค่าทางโลหิตวิทยา ผลการศึกษาพบว่า สุนัขที่มีโรค PS ทุกตัวแสดงอาการไม่ทนต่อการออกกำลังกายและมี 6 ตัวแสดงอาการหมดสติชั่วคราว การตรวจคลื่นไฟฟ้าหัวใจด้วย lead II พบว่าสุนัขที่มีโรค PS มีความสูงของ P, และ T สูงขึ้น (P<0.01) และ S ลึกขึ้น (P<0.01) มีอัตราส่วนความสูงของ R:S ต่ำลง (P<0.001) และช่วงเวลาของ QRS ยาวกว่า (P<0.001) สุนัขกลุ่มควบคุม การศึกษาโครงสร้างและการทำงานของหัวใจพบว่า ความเร็วของการไหลของเลือดจากลิ้นหัวใจพัลโมนิค (pulmonic flow velocity; PV) และความแตกต่างของความดันระหว่างหัวใจห้องล่างขวาและหลอดเลือดแดงพัลโมนารี (pressure gradient; PG) ของสุนัขที่มีโรค PS มากกว่าสุนัขกลุ่มควบคุม (P<0.001) สุนัขที่มีโรค PS มีความหนาเพิ่มขึ้นของผนังหัวใจล่างขวา (P<0.001) และผนังกั้นระหว่างหัวใจห้องซ้ายและขวา (P<0.01) มีการเพิ่มขนาดหัวใจห้องบนขวา (P<0.001) และมีขนาดหัวใจห้องล่างซ้ายที่เล็กลงเมื่อเปรียบเทียบกับสุนัขกลุ่มควบคุม (P<0.001) การหดตัวของหัวใจห้องล่างขวาในสุนัขที่มีโรค PS มีค่า pulmonic valve velocity time integral (PVVTI) (P<0.001) และ pulmonic valve ejection time (PVET) (P<0.01) ที่เพิ่มขึ้น อย่างไรก็ตามไม่พบความแตกต่างของความแปรปรวนของอัตราการเต้นหัวใจระหว่างสุนัขสองกลุ่ม PG มีความสัมพันธ์เชิงบวกกับความสูงของ P (r=0.597, P<0.01) S (r=0.569, P<0.01) และ T (r=0.423, P<0.05) และมีความสัมพันธ์เชิงลบต่อความสูง R (r=-0.599, P<0.01), อัตราส่วนความสูง R:S (r=-0.677, P<0.001) และช่วงเวลาของ QRS (r=-0.423, P<0.05) นอกจากนี้ PG มีความสัมพันธ์เชิงบวกต่อพารามิเตอร์ของคลื่นเสียงสะท้อนความถี่สูง ได้แก่ IVSdN (r=0.560, P<0.01) IVSsN (r=0.538, P<0.01) PVVTI (r=0.812, P<0.001) PVET (r=0.408, P<0.05) อัตราส่วน RVFW:LVPW (r=0.688, P<0.001) และอัตราส่วน RA:LA (r=0.802, P<0.001) จากการศึกษานี้สรุปได้ว่า การที่หัวใจห้องล่างขวาหนาตัวจาก PS อาจนำไปสู่การลดลงของปริมาตรเลือดที่ไหลกลับสู่หัวใจและการทำงานของหัวใจห้องล่างซ้าย การเพิ่ม PVET ในสุนัขที่มีโรค PS อาจเป็นหนึ่งในการปรับตัวของหัวใจเพื่อรักษาปริมาณการไหลของเลือดสู่ปอด อย่างไรก็ตามการเปลี่ยนแปลงของระบบประสาทอัตโนวัติที่มีผลต่อหัวใจที่ไม่เด่นชัดอาจไม่เป็นตัวควบคุมหลักของการเปลี่ยนแปลงอย่างเรื้อรังทางพยาธิสรีรวิทยาของการไหลเวียนโลหิต

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Ploypanut Trikhun : THE LEFT VENTRICULAR FUNCTIONS AND HEART RATE VARIABILITY IN DOGS WITH PULMONIC VALVULAR STENOSIS . Advisor: Prof. CHOLLADA BURANAKARL, D.V.M., Ph.D. Co-advisor: Assoc. Prof. SIRILAK SURACHETPONG, D.V.M., Ph.D,Dr. SAIKAEW SUTAYATRAM, D.V.M., Ph.D

Pulmonic valvular stenosis (PS) is one of the most common congenital heart diseases in dogs that can lead to the cardiac maladaptation. Also, PS can affect the cardiac autonomic nervous system (ANS). However, clinical information regarding to systolic function and cardiac ANS alteration in PS dogs had not been fully elucidated. The objectives of this study were to evaluate cardiac electrical property, left ventricular (LV) function, and to assess cardiac ANS from heart rate variability (HRV) analysis in PS dogs compared with healthy dogs. The dogs in this study were divided into 2 groups, PS dogs (n=13) and healthy control dogs (CONT) (n=12). Physical examination, oscillometric blood pressure measurement and assessments of cardiac function including electrocardiography (ECG), echocardiography and 30-min heart rate variability (HRV) were performed. The blood sample were collected for determination of complete blood count and blood chemistries. The results showed that all PS dogs had exercise intolerance while 6 developed syncope. ECG findings indicated that PS dogs had higher amplitudes of P, and T waves (P<0.01), deeper S wave, lower R:S ratio (P<0.001) with longer QRS duration than CONT dogs (P<0.001). For cardiac structural and functional studies, the pulmonic flow velocity (PV) and pressure gradient (PG) between the right ventricle (RV) and the pulmonary artery (PA) of PS dogs were significantly higher than CONT dogs (P<0.001). PS dogs had thicker RV free wall (P<0.001) and interventricular septum (IVS) (P<0.01), bigger right atrium (RA) (P<0.001) with smaller LV chamber during diastole and systole compared with CONT dogs (P<0.001). The RV systolic function in PS dogs showed higher pulmonic valve velocity time integral (PVVTI) value (P<0.001) and longer pulmonic valve ejection time (PVET) (P<0.01). However, there was no significantly difference in HRV parameters between groups. PG had positive correlations with amplitudes of P (r=0.597, P<0.01), S (r=0.569, P<0.01) and T waves (r=0.423, P<0.05) and negative correlations with amplitude of R wave (r=-0.599, P<0.01), R:S ratio (r=-0.677, P<0.001) and QRS duration (r=-0.423, P<0.05). Also, PG had positive correlations with IVS during diastole normalized by body weight (IVSdN) (r=0.560, P<0.01), IVS during systole normalized by body weight (IVSsN) (r=0.538, P<0.01), PVVTI (r=0.812, P<0.001), PVET (r=0.408, P<0.05), right ventricular free wall thickness to left ventricular posterior wall thickness (RVFW:LVPW) ratio (r=0.688, P<0.001) and right atrium to left atrium diameter (RA:LA) ratio (r=0.802, P<0.001). In conclusion, RV hypertrophy induced by PS may lead to reduction of LV preload and function. The increased PVET in PS dogs may be one of the cardiac compensations to maintain pulmonary blood flow. However, the unremarkable cardiac ANS changes in PS dogs may not play a crucial role in chronic pathophysiological alteration of the hemodynamics.

Field of Study:	Animal Physiology	Student's Signature .....
Academic Year:	2019	Advisor's Signature .....
		Co-advisor's Signature .....
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Ploypanut Trikhun

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LIST OF ABBREVIATIONS

%	percent
µl	microliter
ACEi	angiotensin converting enzyme inhibitor
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANS	autonomic nervous system
AO	aorta
AON	aorta diameter normalized by body weight
AO:PA	aorta to pulmonary annulus ratio
ASD	atrial septal defect
AVET	aortic valve ejection time
AVPEP	aortic valve pre-ejection period
AVPEP:AVET	aortic valve velocity time integral to aortic valve pre-ejection period ratio
AVVTI	aortic valve velocity time integral
BP	blood parasite
bpm	beats per min

BUN	blood urea nitrogen
BV	balloon valvuloplasty
CA	coronary artery
CCHD	cyanotic congenital heart diseases
CF	color flow
CHD	congenital heart disease
CHF	congestive heart failure
CO	cardiac output
CONT	control
Cr	creatinine
CSA	cross sectional area
CW	continuous wave
DMVD	degenerative mitral valve disease
ECG	electrocardiogram
EDTA	ethylene diamine tetra-acetic acid
EF	ejection fraction
EPO	erythropoietin
ET	ejection time
F	female
FS	fractional shortening

g%	gram percent
g/dL	gram per deciliter
Hb	hemoglobin
HRV	heart rate variability
IACUC	Institutional Animal Care and Use Committees
IU/L	unit per liter
IVS	interventricular septum
IVSdN	interventricular septum thickness during diastole normalized by body weight
IVSsN	interventricular septum thickness during systole normalized by body weight
LA	left atrium
LA:AO	left atrium to aortic diameter ratio
LAN	left atrium diameter normalized by body weight
LF	low frequency
LF:HF ratio	low frequency to high frequency ratio
LV	left ventricular
LVET	left ventricular ejection time
LVFS	left ventricular fractional shortening
LVIDd	left ventricular internal diameter during diastole

LVIDdN	left ventricular internal diameter during diastole normalized by body weight
LVIDs	left ventricular internal diameter during systole
LVIDsN	left ventricular internal diameter during systole normalized by body weight
LVOT	left ventricular out flow tract
LVPWd	left ventricular posterior wall thickness during diastole
LVPWs	left ventricular posterior wall thickness during systole
LVPW	left ventricular posterior wall
LVSTI	left ventricular systolic time interval
LVPWdN	left ventricular posterior wall thickness during diastole normalized by body weight
LVPWsN	left ventricular posterior wall thickness during systole normalized by body weight
M	male
m/s	meter per second
mg%	milligram percent
ml	millimeters
mmHg	millimeter mercury
ms	millisecond

mV	millivolt
NE	norepinephrine
NN50	number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording
NSR	normal sinus rhythm
NT-proBNP	plasma N-terminal proBNP
PA	pulmonary artery
PCV	packed cell volume
PE	physical examination
PEP	pre-ejection period
PEP:ET	pre-ejection period to ejection time
PG	pressure gradient
PLAX	parasternal long axis
PSAX	parasternal short axis
PLT	platelet
pNN50	percentage of number of normal-to-normal intervals with differences >50 ms divided by the total number of all NN intervals
PNS	parasympathetic nervous system
PO_2	partial pressure of oxygen

proBNP	pro B-type natriuretic peptide
PS	pulmonic valvular stenosis
PV	pulmonic flow velocity
PVET	pulmonic valve ejection time
PVPEP	pulmonic valve pre-ejection period
PVPEP:PVET	pulmonic valve pre-ejection period to pulmonic valve ejection time
PVVTI	pulmonic valve velocity time integral
PVVTI:AVVTI	pulmonic valve velocity time integral to aortic valve velocity time integral
PW	pulse wave
QTc	corrected QT interval
R:S ratio	R wave to S wave ratio
RA	right atrial
RA:LA	right atrium to left atrium diameter ratio
RBC	red blood cell
RCHF	right sided heart failure
rMSSD	the square root of the mean of the sum of the squares of differences between adjacent NN intervals
RSA	respiratory sinus arrhythmia

RV	right ventricle
RVFW	right ventricular free wall
RVFW:LVPW	right ventricular free wall thickness to left ventricular posterior wall thickness ratio
RVOT	right ventricular outflow tract
SBP	systolic blood pressure
SDANN	standard deviation of the averages of NN intervals in all five-min segments of the entire recording
SDNN	standard deviation of the all NN interval
SDNN index	mean of the standard deviation of NN intervals
SNS	sympathetic nervous systems
SEM	standard error of the mean
ST	sinus tachycardia
STI	systolic time interval
SV	stroke volume
TdP	torsade de pointes
TP	total power
TPr	total protein
TR	tricuspid regurgitation
ULF	ultra low frequency

VLF	very low frequency
VSD	ventricular septal defect
VTI	velocity time integral
WBC	white blood cell

CHAPTER I

INTRODUCTION

Importance and rationale

Pulmonic valvular stenosis (PS) is a congenital narrowing of the pulmonic valve located between the right ventricle (RV) and the pulmonary artery (PA). Types of PS are classified according to the stenotic levels as subvalvular (below the pulmonic valve), valvular (at the valve) and supravalvular (above the valve) types. Also, PS can be divided into type A and type B depending on thickening and hypoplasia of the valve (Patterson et al., 1981; Bussadori et al., 2000). The severity of PS is graded by the pressure gradient (PG) across RV and the right ventricular outflow tract (RVOT) measured by echocardiography (Bussadori et al., 2000). Dogs with severe PS will gradually develop right-sided heart failure due to pressure overload (Loyer, 2000). The clinical signs that could be found in PS dogs are exercise intolerance, syncope and sudden death depending on the severity of PS (Loyer, 2000). Upon physical examination, left heart base systolic murmur and the signs of right-sided heart failure such as ascites or pleural effusion are commonly found. The cardiac electrical abnormalities and cardiac structural alterations especially RV hypertrophy can be found in PS dogs (Bussadori et al., 2000; Loyer, 2000). Nevertheless, systolic function

and relationship between the right side and the left side of the heart have not yet fully elucidated in PS dogs.

Supportive medications in PS dogs depend on clinical signs. However, the medical therapy cannot correct the stenosis. As a result, balloon valvuloplasty (BV) procedure that can correct RVOT obstruction has been recommended in moderate to severe cases because it can improve clinical outcome in terms of clinical signs, survival time and incidence of sudden death in dogs (Johnson et al., 2004; Locatelli et al., 2011). Moreover, BV has high success rate (Bussadori et al., 2001a) and short recovery times due to its minimal invasiveness (Glenn, 1987). Both cardiac electrical and mechanical, together with hemodynamic parameters are routinely assessed and monitored in cardiology clinic. Cardiac electrical alterations associated with PS mostly related with structural cardiac abnormalities and enhanced sympathetic activities. This cardiac electrical property can be monitored by electrocardiograph (ECG) and holter device while cardiac mechanical property can be evaluated using the conventional echocardiography.

Heart rate variability (HRV) is a variation in heart rate which can be modulated by the autonomic nervous system (ANS). In dogs, the alterations cardiac autonomic nervous activity was demonstrated in diabetes mellitus (DM) (Pirintr et al., 2012) and degenerative mitral valve disease (DMVD) that received medications (Chompoosan et al., 2014; Pirintr et al., 2017). The alterations in HRV were demonstrate in PS human

patients (Alyan et al., 2008) and dogs (Trikhun et al., 2019) before and after balloon valvuloplasty. However, no information is obtained in PS dogs compared with control healthy dogs.

Therefore, the objectives of this study are first, to investigate the alterations of cardiac functions including electrical activity and left ventricular (LV) function in dogs with PS compared with normal healthy dogs. Secondly, to evaluate the cardiac ANS activity using HRV analysis in both groups.

Objectives of the study

1. To compare cardiac electrical properties of dogs with PS using ECG.
2. To measure LV function of dogs with PS using conventional echocardiography.
3. To assess cardiac ANS activity of dogs with PS using holter device.

Keywords (Thai)

สุนัข คลื่นไฟฟ้าหัวใจ ความแปรปรวนของอัตราการเต้นของหัวใจ การทำงานของหัวใจด้านซ้าย ลิ้นหัวใจพัลโมนิกตีบ

Keywords (English)

Dogs, Electrocardiography, Heart rate variability, Left ventricular function, Pulmonic valvular stenosis

Research questions

1. Do electrical activities performed by ECG markedly change in dogs with PS compared with control healthy dogs?
2. Do LV echocardiographic indices impair in dogs with PS compared with control healthy dogs?
3. Do the cardiac ANS activities change in dogs with PS compared with control healthy dogs?

Research hypothesis

1. ECG in dogs with PS is different from that of control healthy dogs.
2. Left ventricular function assessed by echocardiography especially systolic function is impaired in dogs with PS compared with control healthy dogs.
3. Dogs with PS have alteration in cardiac autonomic nervous system activity compared with control healthy dogs.

CHAPTER II

LITERATURE REVIEWS

2.1 Pulmonic valvular stenosis (PS)

PS, characterized by a narrowing of the pulmonic valvular valve, located between the right ventricle (RV) and the pulmonary artery (PA) and PS is the most common congenital heart disease (CHD) in dogs (Oliveira et al., 2011; Schrope, 2015). Breeds that commonly found are Fox Terrier, Chihuahua, Boxer, Beagle and brachycephalic breeds including English Bulldog and French Bulldog (Patterson, 1968; Patterson et al., 1981; Bussadori et al., 2001b; Oliveira et al., 2011). Common clinical signs in PS cases included exercise intolerence, lethargy, evidence of right-sided heart failure (RCHF) signs (i.e. ascites), syncope and sudden death (Guthery and Schumann, 1998; Loyer, 2000). PS can be characterized using abnormality of the anatomy and the morphology of the pulmonic valve. In terms of the anatomy, PS can be classified by the location of the stenotic part: valvular, subvalvular and supravalvular regions. However, the valvular type is the most common form in dogs (Loyer, 2000; Johnson et al., 2004) while supravalvular stenosis is rare (Oliveira et al., 2011). In subvalvular stenosis, the coronary artery (CA) anomaly (right coronary aberrance that encircles the pulmonary trunk at below the valve) can be a common cause (Bussadori et al., 2000). Bulldog is a breed predisposition to CA anomalies as 75% of Bulldogs with PS had CA

abnormality (Buchanan, 1990; Bussadori et al., 2000). This CA abnormality is considered as a major limitation of BV procedure as it can increase risk of death during BV procedure (Bussadori et al., 2000). However, success of PS correction in Bulldog by BV was reported (Buranakarl et al., 2017). By using morphological criteria, PS is categorized into two types which are type A and type B using the degree of valvular leaflet and pulmonary hypoplasia measured from the aorta to pulmonary annulus ratio (AO:PA ratio) (Bussadori et al., 2000). Dogs with type A PS have mild to moderate pulmonic valvular leaflet thickening with AO:PA ratio less than 1.2 without pulmonary annulus hypoplasia. While, dogs with type B PS have severe pulmonic valvular leaflet thickening with AO:PA ratio more than 1.2 with or without the presence of pulmonary annulus hypoplasia (Bussadori et al., 2000). In dogs, the prevalence rate in type A is higher than type B (Oliveira et al., 2011). However, type B is more problematic since RV hypertrophy and the narrowing of the RVOT are usually more severe (Bussadori et al., 2001a).

This cardiac structural maladaptation induced by PS can lead to several alterations in both cardiac electrical and mechanical properties, which have been reported in both humans and animals. Regardless to type of PS, the narrowing of the RVOT can lead to pressure overload resulting in increased myocardial wall stress, right ventricular enlargement with right axis deviation (i.e., deep S wave) and prolonged ventricular conduction (i.e., widened QRS complex and right bundle branch block (RBBB) (Loyer, 2000; Alyan et al., 2008; Urashima et al., 2008; Buranakarl et al., 2016).

All of the above alterations can result in mechanical changes including dilatation of the tricuspid valve annulus leading to functional tricuspid regurgitation (TR), leftward of interventricular septum (IVS) shift, RCHF, ventricular function impairment and decreased survival time (Urashima et al., 2008).

Pressure overload at the RVOT is one of the major risk factors of RCHF and cardiac death in humans and animals. Francis and co-workers (2011) found that the independent predictor of cardiac death in PS dogs was the PG over 60 millimeter mercury (mmHg) with 86% sensitivity and 71% specificity. Moreover, dogs with symptomatic PS that resulted in TR had a 16-fold increase in a risk of death compared with dogs with asymptomatic PS (Johnson et al., 2004). Therefore, proper treatment is very important in all symptomatic cases.

The medical treatment aims to relieve symptoms includes diuretics, vasodilators, β-blockers and positive inotropes (Loyer, 2000). Diuretics have been known to alleviate congestive heart failure symptom while β-blockers have been used to control over sympathetic activation. In addition, vasodilators and inotropic drugs are prescribed to regulate hemodynamic parameters including LV filling pressure, right atrial (RA) pressure and cardiac output (CO) (Guthery and Schumann, 1998). However, these medications are only supportive treatment, but the primary cause of pressure overload has not yet been corrected.

BV is categorized as an intervention that can dilate pulmonary outflow tract using a balloon tip catheter. It is recommended in moderate to severe PS cases (Johnson et al., 2004). BV procedure does not require either thoracotomy or cardiotomy, thus, it could reduce hospitalization time and recovery period (Glenn, 1987; Sisson and MacCoy, 1988). After BV, an immediate reduction of systolic PG and right ventricular systolic pressure (RVSP) with increased CO had been reported (Sisson and MacCoy, 1988). The RV free wall thickness and the size of the RV papillary muscle measured from echocardiography were also reduced in dogs after BV (Sisson and MacCoy, 1988). Moreover, BV can alleviate clinical signs and prolong survival time in dogs with moderate to severe PS (Johnson et al., 2004).

The success of BV, determined by reduction in PG after BV procedure, was 100% and 66% in PS dogs with type A and type B, respectively (Bussadori et al., 2001a). The median PG in the success cases should be reduced by 50% from pre-BV value in dogs (Belanger et al., 2018). BV can reduce PG up to 60% in type A and 48% in type B compared with the PG before BV (Bussadori et al., 2001a). However, in another study of Belanger and collogues (2018), PG reduction was not affected by valvular morphology (i.e., type A and B) or severity of the RV hypertrophy, but depended on pulmonary valve annulus size. Other factors related to BV successful rates and clinical outcomes included balloon to valve hinge diameter, concurrent cardiac defects and systemic diseases (McCrindle, 1994; Bussadori et al., 2000; Loyer, 2000). Nevertheless,

clinical complications can be found after BV. Recurrent stenosis in PS patients after BV intervention had been periodically reported, especially in the case that balloon:PV annulus ratio was less than 1.2 or PG after BV was over 30 mmHg (Rao et al., 1988). In dog clinical report, 6 from 22 dogs developed restenosis at average of 5.5 months (range 1.5-68.2 months) after BV (Sunahara et al., 2014). Other severe complications such as sudden death due to cardiac arrest, rupture of the coronary artery, coronary ischemia, severe hypotension, bleeding, arrhythmias and RBBB had been reported (Johnson et al., 2004; Sunahara et al., 2014).

2.2 Electrocardiogram (ECG) in pulmonic valvular stenosis

Long-term pressure overload in PS can cause cardiac electrical and functional maladaptations, typically found in RV concentric hypertrophy (Loyer, 2000; Alyan et al., 2008; Urashima et al., 2008). The electrical alterations in both of the amplitude and duration of ECG had been reported in moderate to severe PS patients. In terms of the amplitude of ECG, PS patients showed the RV hypertrophy pattern including right axis deviation, increased amplitude of R and T waves, with high R:S ratio in lead V1, as well as, deep S wave in lead V6 (Lasser and Genkins, 1957; McCrindle and Kan, 1991; Alyan et al., 2008). For the duration of ECG, widening of QRS complex and prolongation of corrected QT interval (QTc) were found in PS patients (Alyan et al., 2008). The RV enlargement pattern and right axis deviation were also reported in PS dogs including tall R and T waves, deep S wave and widening of QRS complex (Loyer, 2000; Ristic et

al., 2001; Trikhun et al., 2019). Moreover, an experimentally induced severe PS mice model presented widened QRS complex with incomplete RBBB and ST-T elevation (Urashima et al., 2008). These ECG adaptations can be altered by the improvement of cardiac structural and function, as well as, disease progression (Alyan et al., 2008; Urashima et al., 2008). In PS patients, BV can improve both of the ECG alteration and cardiac function (McCrindle and Kan, 1991). The reductions of ECG amplitudes of R and S waves, R:S ratio, duration of QRS complex and QT interval were reported in PS patients at 1 month after BV (Alyan et al., 2008). The duration of QT interval depends on the duration of ventricular action potential (Nachimuthu et al., 2012). Therefore, beside cardiac maladaptation, other several factors can affect QT interval included mutation of genes that encode the potassium and sodium channels of myocytes, gender, heart rate, cardiac conduction defects and some drugs such as antiarrhythmic drugs, diuretics and antihistamines (Nachimuthu et al., 2012; Vandael et al., 2017). Among these factors, heart rate is considered as a major influence of the QT interval, thus the QTc, QT duration normalized by HR, is recommended (Beardow, 2000). Several QTc formulas had been proposed for humans and animals. However, the QTc from the Van de water's formula is a recommended in dogs (Spence et al., 1998; Oliveira et al., 2014). Furthermore, the prolongation of QTc had been used to predict ventricular arrhythmia such as Torsade de Pointes (TdP) and sudden cardiac death in humans (Straus et al., 2006; Vandael et al., 2017). However, the study of the QTc in PS dog has not been fully elucidated.

2.3 Conventional echocardiography techniques in PS

Echocardiography is a non-invasive and inexpensive tool for cardiovascular assessment (Hozumi et al., 1998). Conventional echocardiography consists of several modalities. Conventional 2D, M-mode and Doppler echocardiographic examinations are common tools for general evaluation of cardiac structure and function, severity assessment, as well as treatment monitoring in dogs (Boon, 2011; Park et al., 2014).

Evaluation of myocardial systolic function can be indirectly assessed by 2D, M-mode and TDI echocardiographic measurements. The LV functions can be assessed from changes in the LV dimensions and volumes during diastole and systole. The common parameters of LV systolic function include fractional shortening (FS) using modified Simpson's method (Boon, 2011). FS can be assessed from the LV diameter from 2D and M-mode echocardiographic measurements on the parasternal long axis (PLAX) view at the tip of mitral valve leaflets during diastole and systole or in the parasternal short axis (PSAX) view at the papillary muscle level. The FS can be calculated using the formula as figure 1. However, there are some limitations of FS as this parameter is calculated from the consumption that the LV shape is a cylinder. Thus, when LV shape is altered, these parameters may be under or over-estimated. Therefore, velocity time integral (VTI) and systolic time interval (STI) has also been used for indirect estimation of systolic function.

VTI is an area under the velocity curve at the valvular area that is generated from Doppler technique. VTI can assess blood flow distance or stroke distance across the valve during ejection phase of cardiac cycle (Nishimura et al., 2018). The concepts of VTI measuring is presented in Figure 2. Stroke volume (SV) can be calculated from VTI multiply by cross sectional area (CSA); SV = VTI x CSA (Nishimura et al., 2018). In clinical setting, VTI has been used for cardiac monitoring, evaluation and prediction of clinical outcomes in patients with acute myocardial infarction, congestive heart failure (CHF) and pulmonary hypertension (Trent and Rawles, 1999; Koestenberger et al., 2016; Tan et al., 2017). In patients with CHF, low VTI at the left ventricular out flow tract (LVOT) correlated with predictive adverse outcomes (Tan et al., 2017). It was suggested that, low VTI at LVOT was an accurate marker of low CO and may be able to identify the stage of CHF (Tan et al., 2017).

STI is a method to measure ventricular performance related to electromechanical delay property using Doppler technique equipped with ECG (Hamada et al., 1990). STI consists of pre-ejection period (PEP) and ejection time (ET) (Figure 3). PEP is a duration of electromechanical delay during isovolumic contraction. Therefore, it starts from Q wave of ECG to the beginning of valvular opening to ejection phase of cardiac cycle. While, ET represents the ejection time interval of interested valve that is measured from the beginning of ejection phase to the complete valvular closure (Boon, 2011). The details on PEP and ET measuring are presented in Figure 4.

As STI depends on electromechanical property, STI can be affected by several factors including heart rate, preload, afterload, inotropic and dromotropic stages. In human, the PEP can be altered by cardiac conduction property, load condition, inotropic agent and ventricular failure condition (Harris et al., 1967; Lewis et al., 1976). However, PEP was reported to be minimally affected by changes in heart rate (Cokkinos et al., 1976). Whereas, ET can be significantly interfered by heart rate, load condition, inotropic agent and heart failure situation (Cokkinos et al., 1976; Lewis et al., 1976). From these factors, the pre-ejection period to ejection time (PEP:ET) is more accuracy for ventricular performance assessment because PEP:ET value is normalized by heart rate. PEP:ET had more sensitivity in ventricular function assessment in healthy subject compared with PEP or ET alone (Cokkinos et al., 1976). In human medicine, STI can evaluate chronic myocardial disease, mitral valve disease and clinical medication response (i.e. inotropic agents) (Lewis et al., 1976; Lewis et al., 1977). Furthermore, the shortened LVET with prolonged LVPEP (high PEP:ET) was a characteristic pattern found in CHF patients with reduced SV and CO (Lewis et al., 1976). However, STI characteristic in patients with originally right-side heart problems including PS has not been fully elucidated in either humans or dogs.

$$FS\ (\%) = \frac{LVIDd - LVIDs}{LVIDd} \times 100$$

Figure 1. The systolic function formula calculated from echocardiographic parameters**.** Abbreviations: FS: fractional shortening, LVIDd: left ventricular internal diameter during diastole and LVIDs: left ventricular internal diameter during systole

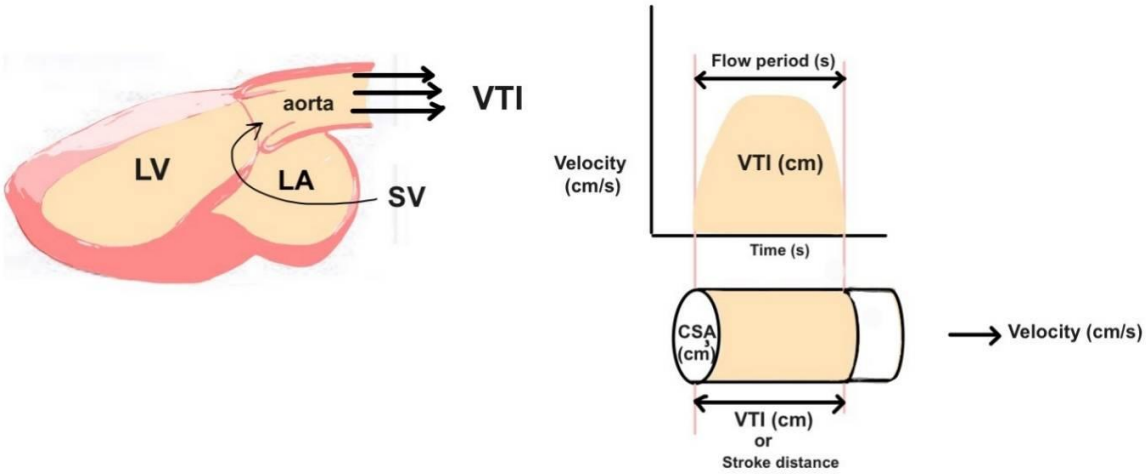

Figure 2. Diagrams showed the velocity time integral (VTI) calculation and association with stroke volume (SV) (modified from Nguyen and Squara, 2017). A: The Left ventricular (LV) stroke volume (SV) (cm3) is equal to multiplication of the distance of ejected blood (i.e. VTI) (cm) in one ejection phase with cross sectional area (CSA) of the aorta (cm^2).

B: The drawing figure showed VTI calculation from area under the curve of blood flow velocity and duration of blood flow across the valve. Abbreviations: LV: left ventricle, LA: left atrium, SV: stroke volume, VTI: velocity time integral and CSA: cross sectional area

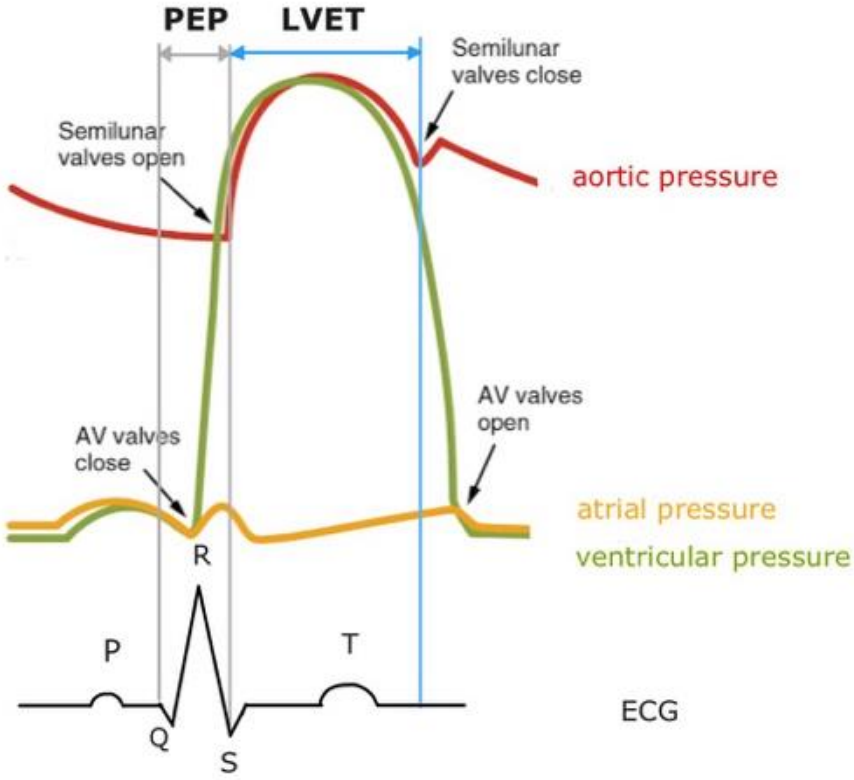

Figure 3. Electromechanical activity in each cardiac cycle phase. In left ventricular systolic time integral (LVSTI), pre-ejection period (PEP) represents electromechanical delay and isovolumetric contraction of the LV, while, LV ejection time (LVET) equals to the duration that blood is ejected into the aorta (modified from Afonso et al., 2009). Abbreviations: PEP: pre-ejection period, LVET: left ventricular ejection time, AV: atrioventricular valve and ECG: electrocardiogram

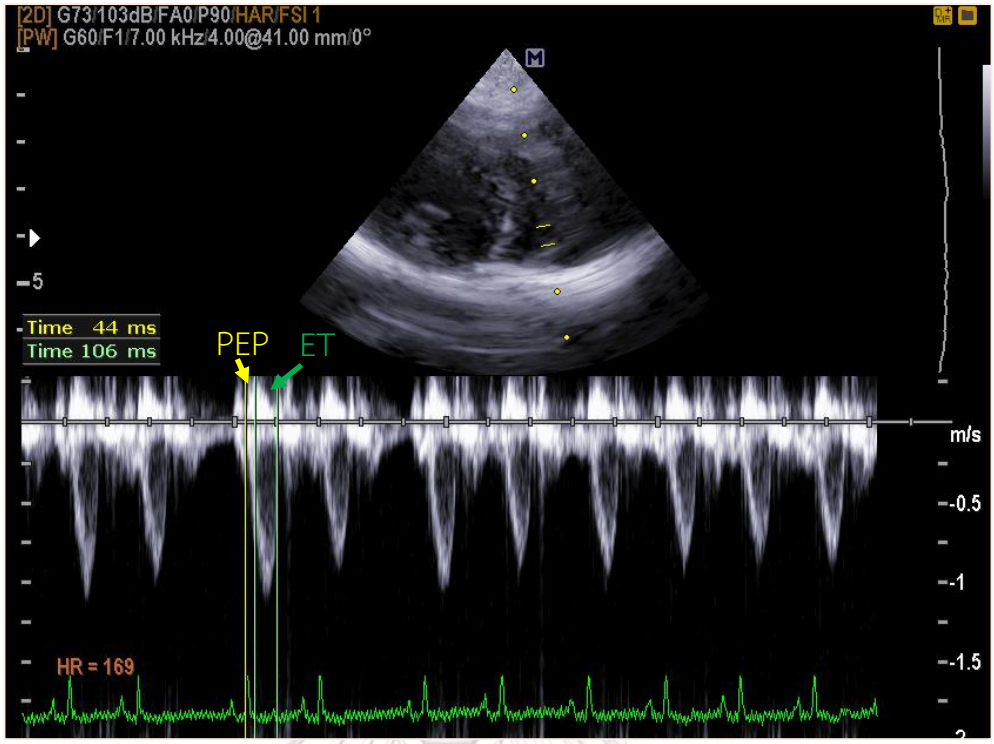

Figure 4. Measuring of the systolic time interval (STI) of the right ventricle (RV) using pulse wave (PW) Doppler. The right parasternal short axis (PSAX) view at pulmonic valve level echocardiographic picture of the RV. Pre-ejection period (PEP) is measured from the beginning of Q wave of ECG to the pulmonic valve opening. Ejection time (ET) is measured from the duration of the pulmonic valve opening with blood ejection. Abbreviations: PEP: pre-ejection period, ET: ejection time and HR: heart rate

The definitive diagnosis and severity determination of PS require all conventional echocardiography including 2D, M-mode, color flow (CF), pulse wave (PW) and continuous wave (CW) Dopplers to elucidate the alterations in both of the cardiac structures and functions especially PG across the pulmonic valve. The common view used for PS evaluation is the right PSAX view in terms of the pulmonic valve malformations and pulmonic valve annulus during systole. Another view is the right

PLAX view which is used to assess pulmonary flow velocity and peak PG across the stenotic location. In this view, the RV wall thickness and AO:PA ratio can also be assessed during diastole (Bussadori et al., 2000).

The 2D echocardiography can diferentiate the locations of stenosis into valvular, subvaluvular and supravalvular types. However, multiple stenotic levels were also reported in a dog (Buranakarl et al., 2016). The 2D echocardiography can also classify PS into type A and B (Bussadori et al., 2001a). Dogs with type A PS have mild to moderate thickening of the pulmonic leaflets with a commissural fusion, doming shape of the valve during systole, AO:PA ratio less than 1.2 and presence of post-stenotic dilatation of the pulmonary trunk. In PS dog with type B, the pulmonic valve is severely thickening with or without annulus hypoplasia (i.e., AO:PA ratio diameter more than 1.2). The post-stenotic dilatation is rare in type B (Bussadori et al., 2000; Bussadori et al., 2001a).

The severity of PS can be graded as mild, moderate and severe according to the PG of 20-49, 50-80 and over 80 mmHg, respectively (Bussadori et al., 2000). Conventional echocardiography is an important clinical tool to evaluate successful treatment (Sisson and MacCoy, 1988; Bussadori et al., 2001a; Johnson et al., 2004; Francis et al., 2011; Buranakarl et al., 2016; Belanger et al., 2018). In an experimental rat model, PS induced longterm pressure overload rats with mild to moderate PS showed coordinated motion between IVS and LV posterior wall (LVPW) from M-mode

echocardiography. Whereas, rats wih severe PS had interventricular dyssynchrony motions between LV posterior wall (LVPW) and IVS motions during systole measured from M-mode. These rats also had markedly lower left ventricular diastolic dimension , LV volume, RVOT fractional shortening , left ventricular fractional sfhortening and CO when measuring from Doppler echocardiography (Urashima et al., 2008).

2.4 Autonomic nervous system in heart failure

ANS is a major controller of cardiovascular function. ANS consists of parasympathetic nervous system (PNS) and sympathetic nervous systems (SNS). The activation of SNS increases heart rate, contractility and total peripheral resistance leading to higher CO and systemic blood pressure, while, increased PNS activity causes the opposite effects. Normally, the heart rate fluctuation causes by the balance of these two components in order to adjust cardiovascular function to meet body requirement (Thayer et al., 2010). For instance, at rest, the cardiac PNS is more dominant than the SNS (Gregoire et al., 1996). Therefore, variations of PNS and SNS levels depend on the circadian rhythm and body status or function.

In heart failure patients, the cardiovascular functions were attenuated leading to decreased ejection fraction (EF) and CO (Meredith et al., 1993). Thus, the SNS is more activated to maintain hemodynamic stability by increasing in chronotropic, inotropic and dromotropic properties to preserve CO (Meredith et al., 1993; Kaye et

al., 1995). However, this compensation may cause unpreferable outcomes leading to higher morbidity and mortality (Thayer et al., 2010).

The SNS hyperactivity can be detected from increased plasma norepinephrine (NE) levels or NE spillover. Plasma NE concentration was reported to be higher in patients with progressive heart failure than healthy subjects (Meredith et al., 1993). However, plasma NE concentration can be altered by both increased release of NE and reduced clearance of NE (Kaye et al., 1995). Another method that can provide information on cardiac ANS activity is HRV. It can reflect the cardiac ANS alterations in cardiovascular disease patients (Thayer et al., 2010).

2.5 Heart rate variability

HRV is a measurement of the changes of beat-to-beat variation shown as RR intervals (Malik et al., 1996). HRV analysis is a non-invasive technique that can quantitatively assess the cardiac autonomic activity (Kienzle et al., 1992).

The main purposes for HRV measurement are autonomic imbalance evaluation, cardiovascular disease severity estimation and treatment response assessment (Kleiger et al., 2005). HRV analysis could indicate degree of autonomic imbalance, the risk of disease incidence and mortality in several cardiovascular diseases (Schroeder et al., 2003; Thayer et al., 2010). HRV had been used for heart failure prognosis (Binkley et al., 1991; Kienzle et al., 1992; Eaton et al., 1995) and HRV also provides information for

prescribe of drugs including β-blockers, calcium channel blockers, antiarrhythmics, psychotropic agents and cardiac glycosides on cardiovascular system (Kleiger et al., 2005). In veterinary medicine, it was used for evaluating cardiac autonomic activity (Petrie, 2005). In dogs, HRV analysis has been applied in several studies including splenectomy (Pastarapatee et al., 2017), DM (Pirintr et al., 2012) and DMVD after receives medications (Kienzle et al., 1992; Chompoosan et al., 2014; Pirintr et al., 2017).

Parameters that can be retrieved from HRV analysis are time and frequency domains. The time domain parameters include standard deviation of the NN interval (SDNN), standard deviation of the average of NN intervals in all 5 min segments of the entire recording (SDANN), the square root of the mean of the sum of the squares of differences between adjacent NN intervals (rMSSD), and percentage of number of normal-to-normal intervals with differences >50 ms divided by the total number of all NN intervals (pNN50). The frequency domain parameters include ultra low frequency (ULF), very low frequency (VLF), low frequency (LF), high frequency (HF), low frequency to high frequency ratio (LF:HF) and total power (TP) (Malik et al., 1996). SDNN reflects the total of variability in the period of records whereas rMSSD assesses the high frequency variations from the heart rate and reflects the action of vagal activity. A decrease of SDNN and rMSSD were used as independent risk factors for poor prognosis for morbidity and mortality in cardiovascular diseases (Schroeder et al., 2003; Thayer

et al., 2010). A decrease of LF:HF ratio reflects high vagal activities whereas an increase of LF:HF ratio may indicate sympathetic activation (Malik et al., 1996).

In PS patients had increase of SDNN, SDANN, and LF and had decrease of rMSSD, and HF compared with healthy subjects (Alyan et al., 2008). One month after BV, these patients showed an improvement of hemodynamic parameters measured by echocardiography (i.e., right atrial pressure and diameter). HRV parameters showed decrease of LF and LF:HF while SDNN, rMSSD, pNN50 and HF had increased. However, the HRV information in dogs with PS is not yet available.

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental animals and grouping

The client own dogs in the present study were obtained from the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University. The dogs were divided into two groups, control (CONT) and pulmonic valvular stenosis (PS) groups. The dogs in control group were client's own healthy dogs as assessed by veterinarian from history taking, complete physical examination, blood profile analysis, chest radiography, ECG and echocardiography. All control dogs had negative result for heartworm antigen and blood parasite from light microscopic examination. Control dogs had breed and aged matched with PS dogs. In PS group, PS was diagnosed with the presence of stenosis of pulmonic valve regardless of stenotic type (A or B), severity of disease (mild, moderate or severe), location (valvular, subvalvular or supravalvular) or coexisting of other congenital abnormities. The stenosis of pulmonic valves can also be at one location or multiple locations. The PS dogs with other systemic diseases and heartworm disease were excluded.

3.2 Experimental Protocol

This study consists of two parts: retrospective and prospective studies. Retrospective data were retrieved from medical history obtained from the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University during February, 2016 to September, 2018 while the prospective study was performed from September, 2018 to June, 2019.

The protocol of this study was conducted in accordance with the standard animal use guidelines and the Institutional Animal Care and Use Committees (IACUC) (protocol No 1831072). The consent forms were signed by all owners. The protocol was presented in Figure 5.

After history taking from the owners, both CONT (n=12) and PS dogs (n=13) were subjected to physical examination. Each dog was kept in a quiet room with minimal restraint while indirect blood pressure was measured three times at left forelimb using oscillometric technique. The surface ECG was recorded while the dog lays down on right lateral recumbence with four electrodes attached to all four limbs. The ECG signals were recorded for at least 30 seconds. After that, the echocardiography was performed during lateral recumbence and the 2D, M-mode and Doppler techniques including CFW, PW and CW Dopplers were performed. After echocardiography, dogs were subjected to continuous ECG recording using holter

device to assess HRV. The HRV recording was performed at least 30 min in a quiet air conditioning room with the owners.

After finishing ECG recording by holter device, two millimeters (ml) of blood samples were collected using 21 gauge needle from the cephalic or saphenous veins. The blood sample was divided and put into two tubes (0.7 ml each), ethylene diamine tetra-acetic acid (EDTA) and heparinized tubes for measurements of complete blood count and blood chemistry profiles (alkaline phosphatase; ALP, alanine aminotransferase; ALT, blood urea nitrogen; BUN, creatinine; Cr, total protein; TPr and albumin, respectively). Approximately 0.5 ml of bloods was used to examine blood parasite (BP) using light microscopic and heartworm antigen test (only in control group). All dogs in control group were also subjected to thoracic radiograph in both lateral and ventrodorsal view.

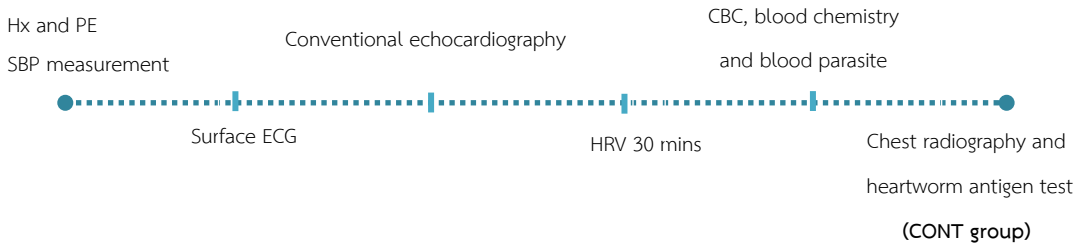

Figure 5. The experimental protocol of this study.

Abbreviations: Hx: history taking, PE: physical examination, SBP: systolic blood pressure, ECG: electrocardiography, HRV: heart rate variability, CBC: complete blood count, CONT: control and PS: pulmonic valvular stenosis

3.3 Analytical procedure and equipment

3.3.1 Blood analysis

Complete blood count analysis, including red blood cell (RBC), packed cell volume (PCV), platelet (PLT) and white blood cell (WBC) was measured using automated hematology analyzer (Mythic18, C2 Diagnostics, Montpellier, France). Blood chemistry measurements including ALT, ALP, BUN, Cr, TPr and albumin were determined using automate chemistry analyzer (The IL ILab 650 Chemistry analyzer, Diamond diagnosis, MA, USA). Blood parasites were examined using light microscopic examination and heartworm antigen test (SNAP heartworm RT test, IDEXX, ME, USA).

3.3.2 Blood pressure measurement

Indirect blood pressure was measured when dog was relaxed in a quiet room using oscillometric method (SunTech® Vet20, SunTech Medical, NC, USA). The dog was gently restrained in the right lateral recumbence position. The appropriated cuff size using approximately 40% of the limb circumference was applied on left forelimb. SBP was recorded 3 times and averaged.

3.3.3 Electrocardiographic recording

The surface ECG recording was performed according to standard veterinary procedure using ECG machine (CardiMax FX-7102, FUKUDA DENSHI, Tokyo, Japan). In general, dog was restrained in right lateral recumbence without sedation and attached with four ECG electrode clips at all four legs for the limb leads including lead I, II, III, aVR, aVL and aVF. Alcohol or ECG gel was applied at the electrode sites for electrical conduction. ECG signals were recorded for at least 30 seconds. The QTc was calculated from lead II according to Van de Water's formula: QTcV = QT - 0.087 [dutation of RR (RR) - 1000] (Van de Water et al., 1989).

3.3.4 Conventional echocardiography measurement

The echocardiography procedure was followed the standard procedure in veterinary practice. The dogs were examined by the same cardiologist to avoid inter-observer variation. In brief, the dog was restrained on table in right lateral recumbence

without sedation. ECG electrode clips were applied at three limbs for lead II recording. The functional and hemodynamic parameters of left cardiac function were obtained using an echocardiographic machine (EKO-7, Samsung Medison Co., Ltd., Gangnam-gu, Seoul, Korea) with 2-4 multi-frequency MHz phased array transducers.

The 2D echocardiography was performed for assessment of pulmonic valve abnormalities and PS type classification. The type A and type B of PS were classified as mentioned earlier (Bussadori et al., 2000).

The M-mode echocardiography was performed for evaluation of myocardial thickness and cardiac chamber diameters during systole and diastole on right PLAX view. The parameters were interventricular septum thickness during diastole (IVSd) and systole (IVSs), left ventricular internal diameter during diastole (LVIDd) and systole (LVIDs) and left ventricular posterior wall thickness during diastole (LVPWd) and systole (LVPWs) (Figure 6). FS was retrieved from echocardiographic software program. The measurement of the diameters of left atrium (LA) and aortic root (AO) and LA:AO was also performed during diastole in right PSAX view. Right ventricular thickening and RA enlargement were measured from M-mode in right PLAX view in Figure 7-8, respectively.

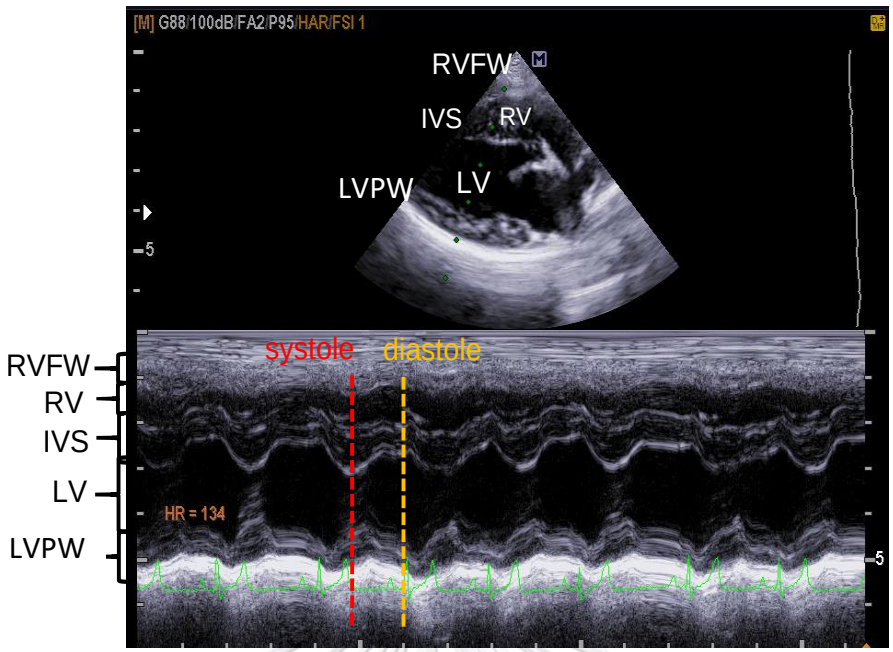

Figure 6. The example of M-mode echocardiographic image in right parasternal long axis (PLAX) view of one control dog that was used to assess the left ventricular diameter and wall thickness during systole and diastole.

Abbreviations: RVFW: right ventricular free wall, RV: right ventricle, IVS: interventricular septum, LV: left ventricle and LVPW: left ventricular posterior wall

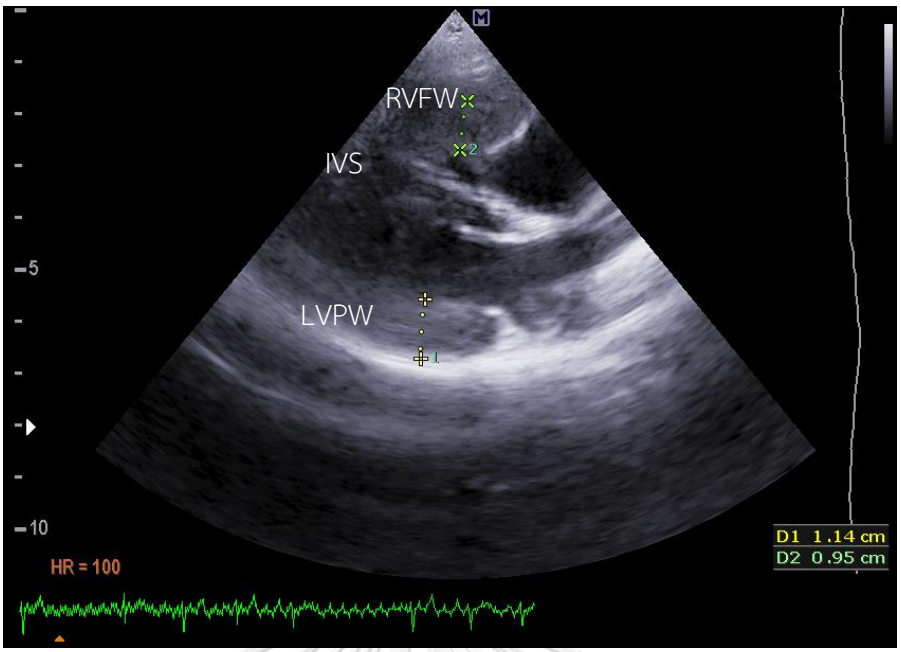

Figure 7. The example of 2D echocardiographic image in right parasternal long axis (PLAX) view of one pulmonic valvular stenosis dog that was used to evaluate right ventricular free wall thickness to left ventricular posterior wall thickness ratio (RVFW:LVPW) for the RV hypertrophy assessment. RVFW thickness (green cursor), interventricular septum (IVS) and LVPW thickness (yellow cursor) were measured using 2D.

Abbreviations: RVFW: right ventricular free wall, IVS: interventricular septum and LVPW: left ventricular posterior wall

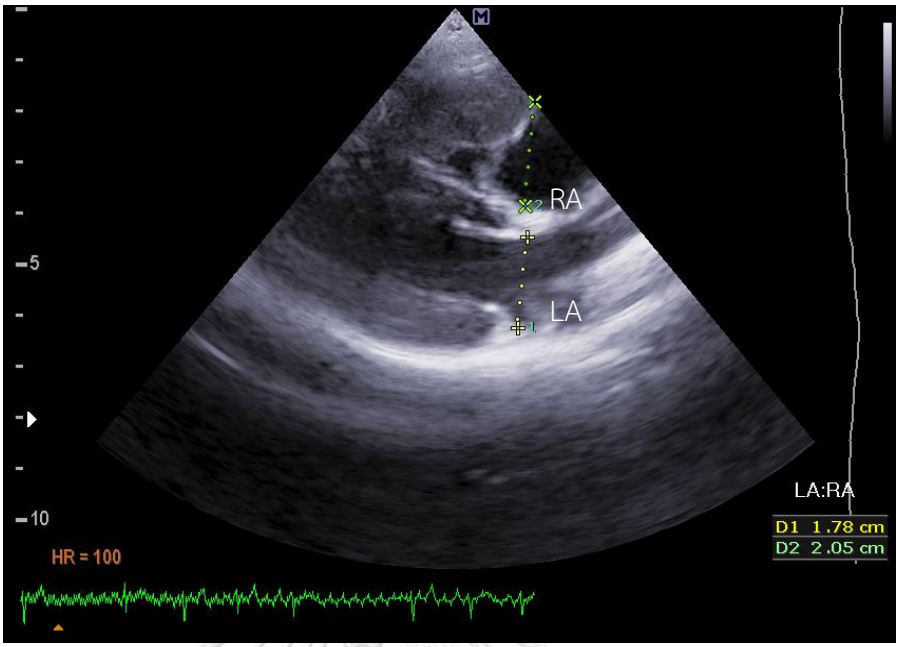

Figure 8. The example of 2D echocardiographic image in right parasternal long axis (PLAX) view of one pulmonic valvular stenosis dog that was used to measure the right atrium to left atrium diameter (RA:LA) ratio.

Abbreviations: RA: right atrium and LA: left atrium

CF Doppler echocardiography was performed to identify abnormal flow associated with turbulence flow within pulmonary artery. The PV and PG were measured on the right PSAX view at the level of pulmonic valve (Figure 9). The PG across the stenotic part was calculated by the modified Bernoulli equation for PS severity grading system (Boon, 2011). In addition, CW Doppler was used to measure VTI and STI at valvular area of the aortic or the pulmonic valves in the right PSAX view. The VTI can be calculated offline using VTI program by manually lining the VTI cursors (yellow dot) around the outlining of the blood flow across the valve (Figure 10). The

STI values including the duration of PEP and ET were generated by manually lining the starting time of PEP (yellow vertical line) and the starting to ending of ET (green vertical lines).

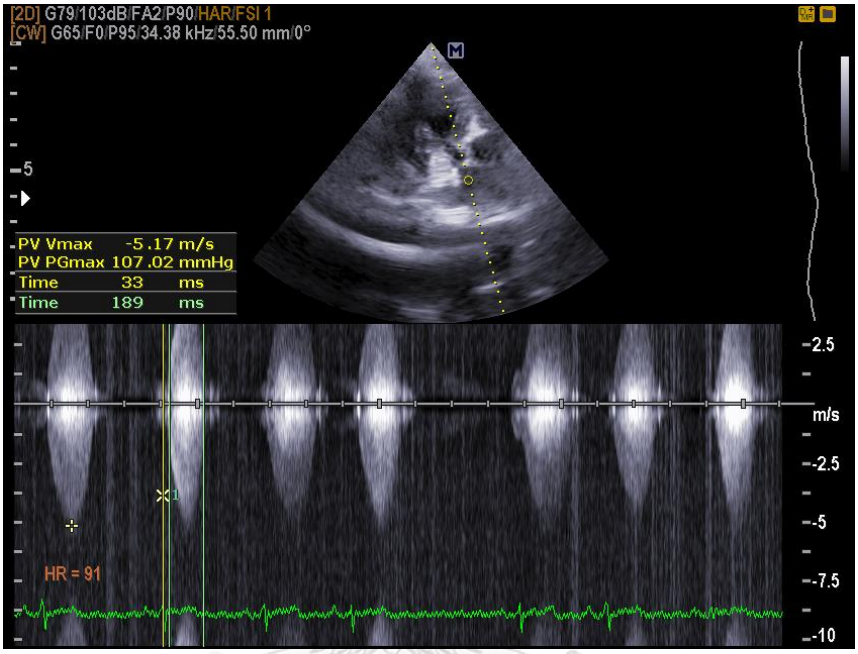

Figure 9. The continuous wave (CW) Doppler echocardiographic image that was used to assess pulmonic flow velocity (PV) and pressure gradient (PG) in right parasternal cross section view at the pulmonic valvular stenosis area. The PV was calculated by manually placed the yellow cursor at the tip of the blood flow curve.

Abbreviations: RV: right ventricle, PA: pulmonary artery, PV: pulmonic flow velocity and PG: pressure gradient

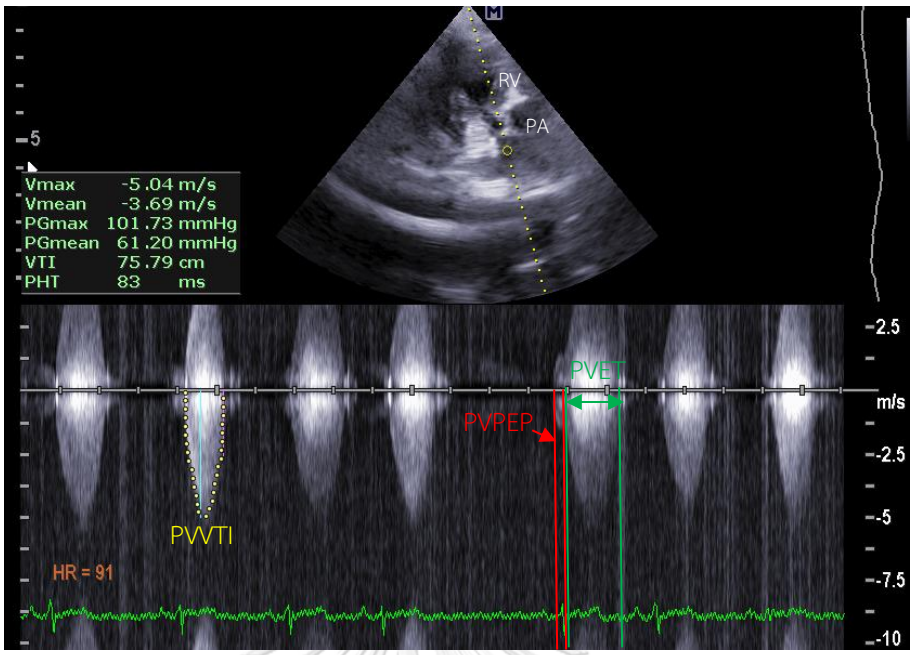

Figure 10. The example of the continuous wave (CW) Doppler echocardiographic image in the right parasternal long axis view at one pulmonic valvular stenosis dog that was used for the velocity time integral (VTI) and systolic time interval (STI) assessment. The pulmonic valve velocity time integral (PVVTI) was generated from VTI program by placing the VTI cursor (yellow dot) around the outlining of the blood flow across the pulmonic valve. The pre-ejection period (PEP) (red arrow) was measured from the starting of Q wave of ECG (red vertical line) to the pulmonic valve opening (the adjacent green vertical line) and the ejection time (ET) (green double headed arrow) was measured from the duration of the pulmonic valve opening to closing (from the first to the second vertical green lines).

Abbreviations: RV: right ventricle, PA: pulmonary artery, PVVTI: pulmonic valve velocity time integral, PVPEP: pulmonic valve pre-ejection period and PVET: pulmonic valve ejection time

3.3.5 Holter monitoring and heart rate variability (HRV) analysis

The continuous ECG recording was performed using holter device (Digital holter recorder digital walk FM-180, FUKUDA DENSHI, Tokyo, Japan). Five to seven self-adhesive electrodes (Ambu® BlueSensor P, ECG Electrodes, Ballerup, Denmark) were attached on the chest area where the skin was shaved and cleaned with alcohol in order to record two to three ECG signal channels. The electrodes were attached between the third and fifth intercostal spaces on each side of the chest. One electrode was placed at the xiphoid cartilage of the last rib to serve as a ground electrode (Figure 11). Then, all of the cables were connected with the holter device and placed together next to the scapular region where they were secured to the body using adhesive tape (Hypafix® adhesion bandage, BSN medical GmbH, Hamberg, Germany) and wrapped around the thorax using elastic bandage. The ECG was recorded for approximate 30 minutes in a quiet room with the presence of owner while data were stored in SD card for further analysis. During the recording period, dog was allowed to have some movements. However, eating, sleeping, exercise or any activities related to excitement were not allowed. All continuous ECG recordings were performed during the daytime (08.00-13.00).

Data acquisition

Data were uploaded and transferred to the computer equipped with SCM-510 software (FUKUDA DENSHI, Tokyo, Japan). The ECG signals were checked to select at least two channels that showed good quality of ECG tracings. Then, the ECG tracing was analyzed for HRV. The HRV measurement was acceptable when normal R wave was present up to 85%. All ectopic beats and waveform abnormalities were verified, corrected or deleted manually. HRV parameters were analyzed for both time and frequency domains. The time domain parameters are SDNN, SDANN, SDNN index, rMSSD and pNN50, while the frequency domain parameters are ULF: 0.00-0.004 Hz, VLF: 0.004-0.041 Hz, LF: 0.041-0.15 Hz, HF: 0.15-0.5 Hz and TP: 0-0.5 Hz (Pirintr et al., 2012; Chompoosan et al., 2014). Data obtained from 3 consecutive 10 minutes were averaged. The HF normalized and LF normalized values were calculated following the formula [LF normalized = LF/(TP-ULF-VLF) and HF normalized = HF/(TP-ULF-VLF)] (Rasmussen et al., 2012).

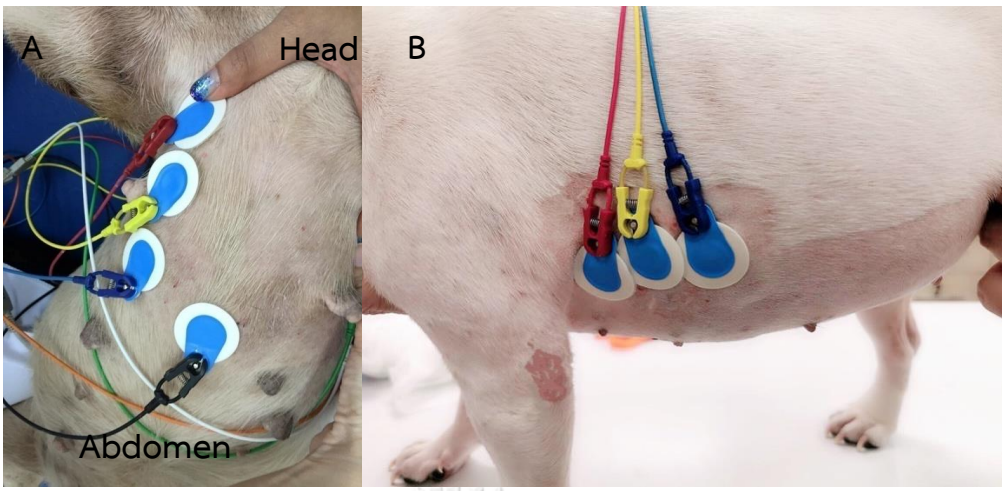

Figure 11. Locations of electrocardiography (ECG) electrode placement for holter monitoring. A: right side of the chest and B: left side of the chest

3.4 Statistic analysis

Data are presented as mean ± the standard error of the mean (SEM). Parameters obtained in the CONT dogs were compared with the PS dogs using the unpaired t-test depending on the Shapiro-Wilk normality test. The relationships between parameters were performed using the Spearman correlation. A probability value less than 0.05 is considered as statistical significance.

CHAPTER IV

RESULTS

4.1 General characteristics and clinical presentation of dogs

The dogs in this study comprised of two groups, CONT dogs and PS dogs. Twelve healthy control dogs composed of seven intact males and five intact females while, thirteen PS dogs composed of nine intact males and four intact females. The average age and body weight of CONT and PS dogs were not different and shown in Table 1. The age ranges were 0.5-5.0 years and 0.3-5.0 years while the body weight ranges were 4.1-26.0 kg and 4.4-24.0 kg in CONT and PS groups, respectively. The CONT group consisted of six French Bulldogs, two English Bulldogs, one Pomeranian, one beagle, one poodle and one shih-tzu. The PS group consisted of six French Bulldogs, two English Bulldogs, two Pomeranians, one beagle, one poodle and one shih-tzu. Other cardiac defects including atrial septal defects (ASD) and coronary aberrance were founded in PS group. Two French Bulldogs and one Pomeranian had ASD, one Bulldog had coronary aberrance and one Bulldog had both.

Table 1. General characteristics in control and pulmonic valvular stenosis dogs

Parameters	CONT group (n=12)	PS group (n=13)
Age (year)	1.56±0.36	1.76±0.38
Body weight (kg)	11.20±1.87	9.38±1.47
Sex M/F	7/5	9/4
Breed	6 French Bulldogs 2 English Bulldogs 1 Pomeranian 1 beagle 1 poodle 1 shih-tzu	6 French Bulldogs 2 English Bulldogs 2 Pomeranians 1 beagle 1 poodle 1 shih-tzu
Other congenital cardiac defects		3/13 PS with ASD 1/13 PS with coronary aberrance 1/13 PS with ASD and coronary aberrance

Data presented as mean±SEM

Abbreviations: CONT: control, PS: pulmonic valvular stenosis, M: intact male, F: intact female and ASD: atrial septal defect

All PS dogs were presented with exercise intolerance while half of them had syncope. Polycythemia was found in 2 dogs from 3 dogs that had ASD. About 30 % of dogs in PS showed signs of RCHF such as ascites and jugular distension (Table 2). Within these 4 dogs, three of them received pimobendan with other drugs approximately one week before study; one dog received pimobendan dose 0.25 mg/kg twice a day with furosemide dose 1.2 mg/kg/ twice a day, one dog received pimobendan dose 0.12 mg/kg twice a day, furosemide dose 1.3 mg/kg twice a day and ramipril dose 0.23

mg/kg twice a day, as well as, the another one received pimobendan dose 0.26 mg/kg twice a day with sildenafil dose 1 mg/kg twice a day.

Table 2. Clinical presentations and medications in pulmonic valvular stenosis dogs (n=13)

Clinical presentations and medications	Number of dogs
Clinical presentations	
Exercise intolerance	13
Syncope	6
Polycythemia	2
RCHF signs (i.e. ascites and jugular distension)	4
Medications	
Pimobendan with furosemide	1
Pimobendan with furosemide and ramipril	1
Pimobendan with sildenafil	1

Abbreviation: RCHF: right-sided heart failure

4.2 Physical examination (PE)

Complete physical examination results indicated systolic murmur grade 3 to 4 from 6 at the left heart base in all PS dogs. The average HR from auscultation were 120±7 beats per min (bpm) and 136±7 bpm, while, the average RR were 47±4 and 49±4 breaths per min in CONT and PS groups, respectively and were not different between groups. The average SBP in CONT dogs was 148±5 mmHg and in PS dogs was 150±7 mmHg which were not significantly different between groups.

4.3 Electrocardiography (ECG)

All of the CONT dogs had respiratory sinus arrhythmia (RSA). In PS group, 6 dogs had sinus tachycardia (ST). The least were RSA and normal sinus rhythm.

The examples of ECG waveforms in CONT and PS dogs were shown in Figure 12. The ECG waveform in PS dogs showed deep negative S wave. The ECG waveform amplitudes and durations in CONT and PS dogs with correlations to PG were shown in Table 3. The amplitudes of the P, S and T waves in PS group were higher than those of CONT group ($P<0.01$), while the R wave was lower than CONT group ($P<0.01$). The QRS duration was longer in PS group compared with CONT group ($P<0.001$). The P duration, PR interval, QT interval and QTc were similar between groups. All amplitudes of P, R, S and T waves and R:S ratio, as well as, QRS duration were correlated with PG. The relationship between R:S ratio and PG was shown in Figure 13. The heart rate in PS group was slightly higher without significantly different compared with CONT group.

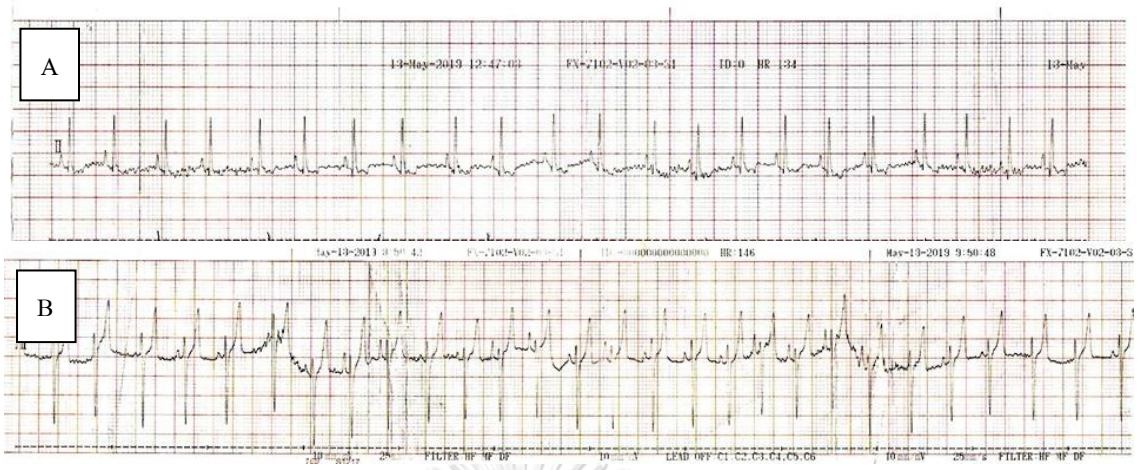

Figure 12. Examples of electrocardiogram wave forms in control (A) and pulmonic valvular stenosis (B) dogs.

Table 3. The electrocardiogram findings and waveform amplitudes and durations in control and pulmonic valvular stenosis dogs with correlations to pressure gradient

Parameters	CONT group (n=12)	PS group (n=13)	Spearman correlation coefficient (r) (n=25)	*P*-value
Rhythm (number)				
NSR	0	2		
RSA	12	5		
ST	0	6		
P wave amplitude (mV)	0.12±0.01	0.31±0.05**	0.597	<0.01
R wave amplitude (mV)	1.01±0.11	0.56±0.08**	-0.599	<0.01
S wave amplitude (mV)	0.06±0.01	0.80±0.20**	0.569	<0.01
R wave:S wave ratio	22.9±3.3	4.9±2.3***	-0.677	<0.001
T wave amplitude (mV)	0.16±0.03	0.36±0.08**	0.423	<0.05
P duration (ms)	47.4±2.5	48.1±2.7	0.057	0.784
PR interval (ms)	83.5±4.2	76.2±4.1	-0.275	0.181
QRS duration (ms)	51.1±2.3	73.8±4.4***	-0.423	<0.05
QT interval (ms)	220.4±8.1	266.1±35.5	0.126	0.544
QTc (ms)	297.0±8.4	352.1±35.5	0.271	0.188
Heart rate (bpm)	117±6	139±11	0.266	0.196

Data are presented as mean±SEM using t-test. ***P*<0.01 and ****P*<0.001

Abbreviation: CONT: control, PS: pulmonic valvular stenosis, NSR; normal sinus rhythm, RSA; respiratory sinus arrhythmia, ST; sinus tachycardia, mV: millivolt, ms: millisecond, QTc: corrected QT interval and bpm: beat per minute

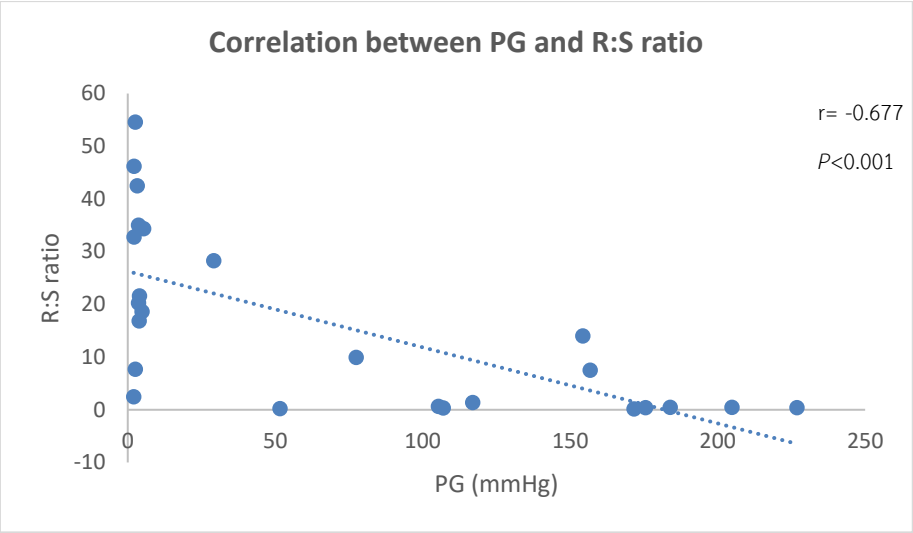

Figure 13. Relationship between pressure gradient (PG) and R wave:S wave ratio (R:S) of lead II electrocardiogram.

4.4 Echocardiographic and Doppler study

The pulmonic valve abnormalities were classified by 2D echocardiographic examination. The valvular stenosis was the most common found in PS dogs in this study (76.9% or 10/13), while combined valvular with supravalvular stenosis was appeared only 23.1% (3/13). Type A and type B of PS was observed in 7 (53.8%) and 6 PS dogs (46.2%), respectively. Echocardiographic images of normal pulmonic valve, the valvular PS both type A and B were shown in Figure 14. The severity of PS consisted of 10 severe, 2 moderate and 1 mild PS classified by PV and PG across RV and PA (Bussadori et al., 2000) from echocardiographic measurement (Figure 15).

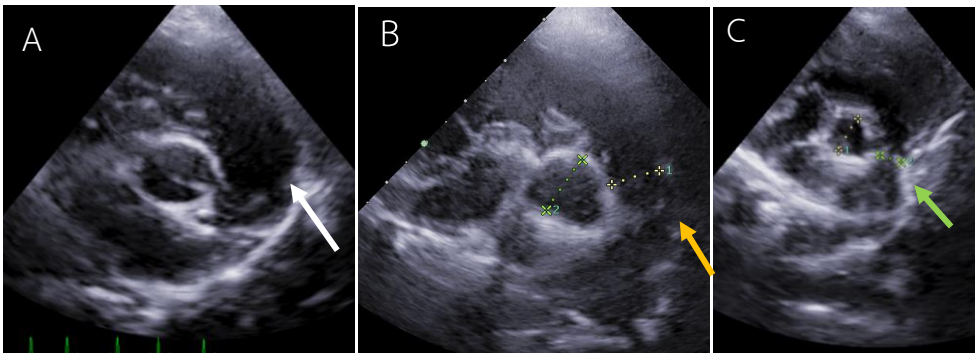

Figure 14. The 2D echocardiographic images showed normal and abnormal pulmonic valve on the right parasternal short axis (PSAX) view at pulmonic valve level. A: normal pulmonic valve (white arrow), B: valvular stenosis type A (yellow arrow) and C: valvular stenosis type B (green arrow)

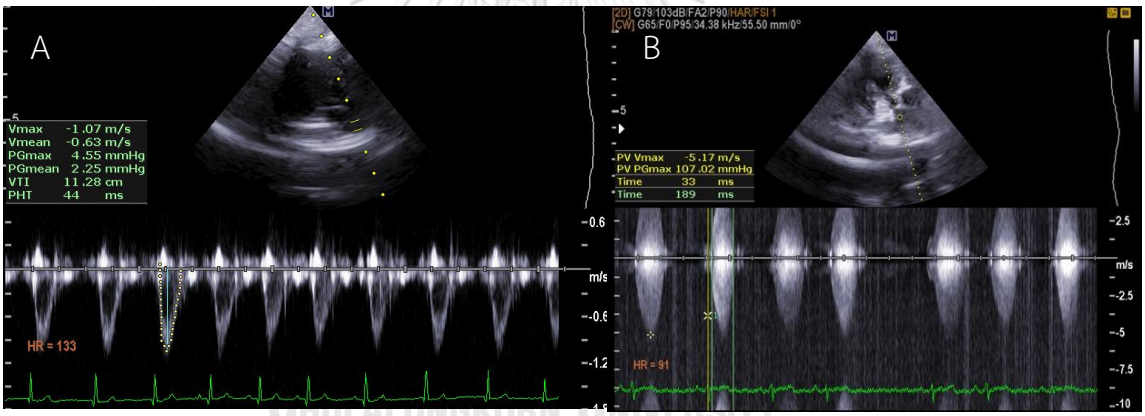

Figure 15. The pulmonic flow velocity (PV) and pressure gradient (PG) assessment on the right parasternal short axis (PSAX) view at pulmonic valve level were used to classify PS severity. A: PV and PG values in one control dog from pulse wave (PW) Doppler and B: PV and PG values in one pulmonic valvular stenosis dog from continuous wave (CW) Doppler.

The results of normalized echocardiographic parameters and correlations between PG value and echocardiographic parameters were shown in Table 4. PS group had significantly increased IVSd and IVSs thickness (P<0.01) with decreased LVIDd and LVIDs diameters (P<0.001) compared with CONT group measured from M-mode of echocardiography. CW Doppler study indicated that PS dogs had significantly higher PV and PG than CONT group (P<0.001). PG value measured form CW Doppler echocardiography in this study had significant linear correlations with cardiac dimensions including IVSdN, LVIDdN, IVSsN and LVIDsN, as well as, PV (Table 5) (Figure 16).

Table 4. The parameters of echocardiography in control and pulmonic valvular stenosis dogs and correlations between pressure gradient with other echocardiographic parameters

Parameters	CONT group (n=12)	PS group (n=13)	Spearman correlation coefficient (r) (n=25)	*P*-value
Heart rate (bpm)	134±7	142±7	0.088	0.673
IVSdN (cm)	0.47±0.02	0.60±0.03**	0.560	<0.01
LVIDdN (cm)	1.35±0.05	0.95±0.06***	-0.775	<0.001
LVPWdN (cm)	0.40±0.02	0.46±0.03	0.314	0.125
IVSsN (cm)	0.60±0.03	0.78±0.05**	0.538	<0.01
LVIDsN (cm)	0.83±0.05	0.47±0.05***	-0.801	<0.001
LVPWsN (cm)	0.60±0.02	0.66±0.03	0.251	0.222
LAN (cm)	0.80±0.04	0.83±0.05	0.021	0.919
AON (cm)	0.61±0.03	0.64±0.03	0.052	0.801
LA:AO	1.34±0.05	1.35±0.10	0.085	0.684
PV (m/s)	0.90±0.04	5.66±0.40***	1.000	<0.001
PG (mmHg)	3.30±0.32	135.45±16.60***	-	-

Data presented as mean±SEM using t-test. **P<0.01 and ***P<0.001

Abbreviations: CONT: control, PS: pulmonic valvular stenosis, bpm: beats per minute, IVSdN: interventricular septum thickness during diastole normalized by body weight, cm: centimeter, LVIDdN: left ventricular internal diameter during diastole normalized by body weight, LVPWdN: left ventricular posterior wall thickness during diastole normalized by body weight, IVSsN: interventricular septum thickness during systole normalized by body weight, LVIDsN: left ventricular internal diameter during systole normalized by body weight, LVPWsN: left ventricular posterior wall thickness during systole normalized by body weight, LAN: left atrium diameter normalized by body weight, AON: aorta diameter normalized by body weight, LA:AO: left atrium to aorta diameter ratio, PV: pulmonic valve velocity, m/s: meter per second, PG: pressure gradient and mmHg: millimeter mercury

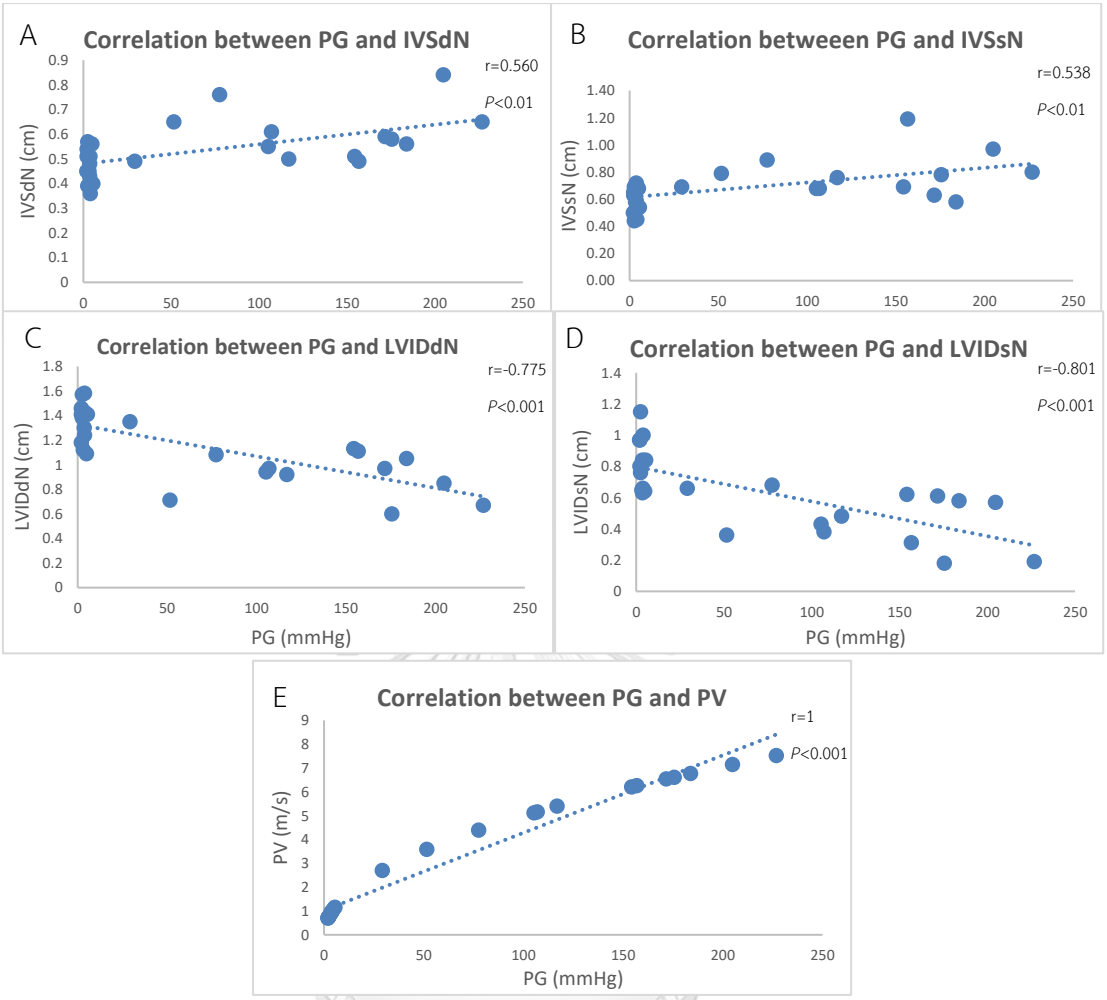

Figure 16. Spearman correlations between pressure gradient (PG) and echocardiographic parameters including interventricular septum thickness during diastole normalized by body weight (IVSdN) (A), interventricular septum thickness during systole normalized by body weight (IVSsN) (B), left ventricular internal diameter during diastole normalized by body weight (LVIDdN) (C), left ventricular internal diameter during systole normalized by body weight (LVIDsN) (D) and pulmonic valve velocity (PV) (E).

Abbreviations: PG: pressure gradient, IVSdN: interventricular septum thickness during systole normalized by body weight, IVSsN: left ventricular internal diameter during diastole normalized by body weight, LVIDdN: left ventricular internal diameter during diastole normalized by body weight, LVIDsN: left ventricular internal diameter during systole normalized by body weight and PV: pulmonic velocity

The systolic function parameters and correlations between PG value and the parameters of systolic function were shown in Table 5 while example of echocardiographic images of STI and VTI were shown in Figure 17. The right-sided systolic function of echocardiographic parameters showed that PS group had significantly increased PVVTI ($P<0.001$), pulmonic valve velocity time integral to aortic valve velocity time integral (PVVTI:AVVTI) ratio ($P<0.001$), pulmonic valve ejection time (PVET) ($P<0.05$) and PVPEP:PVET ratio ($P<0.05$) compared with CONT group. FS of left ventricle in PS group was significantly higher than in CONT group ($P<0.01$). The severity of cardiac hypertrophy and right atrium enlargement indicated that (RVFW:LVPW) and RA:LA ratios of PS dogs were significantly higher than these of CONT dogs ($P<0.001$). Also, the PG value correlated with systolic function parameters including FS, PVVTI, PVVTI:AVVTI, PVET, RVFW:LVPW and RA:LA (Table 6) (Figure 18).

There was a positive relationship between RA:LA and P wave amplitude of ECG ($P<0.01$) (n=25).

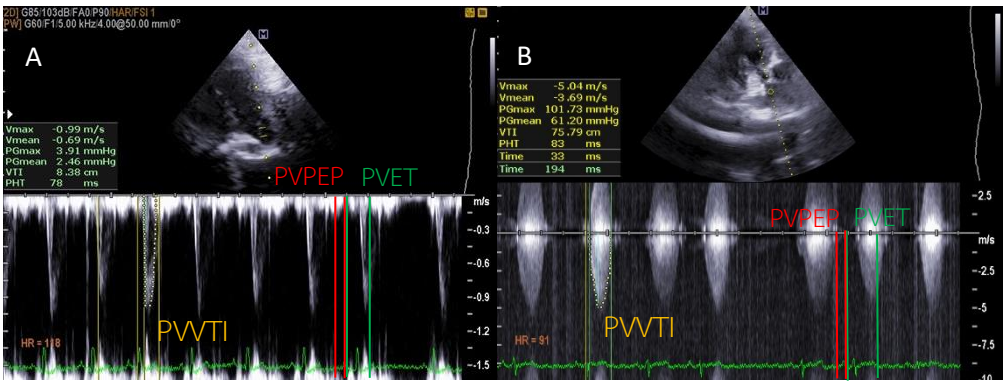

Figure 17. The echocardiography images showed pulmonic valve velocity time integral (PVVTI), pulmonic valve pre-ejection period (PVPEP) and pulmonic valve ejection time (PVET) on the parasternal short axis (PSAX) view at pulmonic valve level using Doppler. A: The echocardiographic measurement of PVVTI (yellow dot), PVPEP (red vertical line) and PVET (green vertical line) in one healthy dog and B: The echocardiographic measurement of PVVTI (yellow dot), PVPEP (red vertical line) and PVET (green vertical line) in dog with pulmonic valvular stenosis

Abbreviations: PVVTI: pulmonic valve velocity time integral, PVPEP: pulmonic valve pre-ejection time and PVET: pulmonic valve ejection time

Table 5. Systolic function, severity of the right ventricular hypertrophy and right atrium enlargement parameters in control and pulmonic valvular stenosis dogs and correlations between pressure gradient with systolic function of echocardiographic parameters

Parameters	CONT group (n=12)	PS group (n=13)	Spearman correlation coefficient (r) (n=25)	*P*-value
FS (%)	36.12±2.17	49.76±3.88**	0.506	<0.05
PVVTI (cm)	10.07±0.29	80.15±8.32***	0.812	<0.001
AVVTI (cm)	11.19±0.59	10.38±1.02	-0.307	0.134
PVVTI:AVVTI	0.93±0.05	8.67±1.22***	0.808	<0.001
PVPEP (ms)	52.75±2.04	51.00±3.79	-0.142	0.496
PVET (ms)	147.42±8.23	187.08±12.19*	0.408	<0.05
PVPEP:PVET	0.37±0.02	0.29±0.03*	-0.343	0.093
AVPEP (ms)	58.75±4.78	57.31±3.53	-0.008	0.969
AVET (ms)	123.67±7.48	129.85±5.09	-0.083	0.689
AVPEP:AVET	0.48±0.03	0.45±0.04	-0.074	0.722
RVFW:LVPW	0.66±0.03	1.26±0.12***	0.688	<0.001
RA:LA	0.83±0.01	1.32±0.07***	0.802	<0.001

Data presented as mean±SEM using t-test. *P<0.05, **P<0.01 and ***P<0.001

Abbreviations: CONT: control, PS: pulmonic valvular stenosis, FS: fractional shortening, PVVTI: pulmonic valve velocity time integral, cm: centrimeter, AVVTI: aortic valve velocity time integral, PVVTI:AVVTI: pulmonic valve velocity time integral to aortic valve velocity time integral ratio, PVPEP: pulmonic valve pre-ejection period, ms: millisecond, PVET: pulmonic valve ejection time, PVPEP:PVET; pulmonic valve velocity time integral to pulmonic valve pre-ejection period ratio, AVPEP: aortic valve pre-ejection period, AVET: aortic ejection time, AVPEP:AVET: aortic valve velocity time integral to aortic valve pre-ejection period ratio, RVFW:LVPW: right ventricular free wall thickness to left ventricular posterior wall thickness ratio and RA:LA: right atrium to left atrium diameter ratio

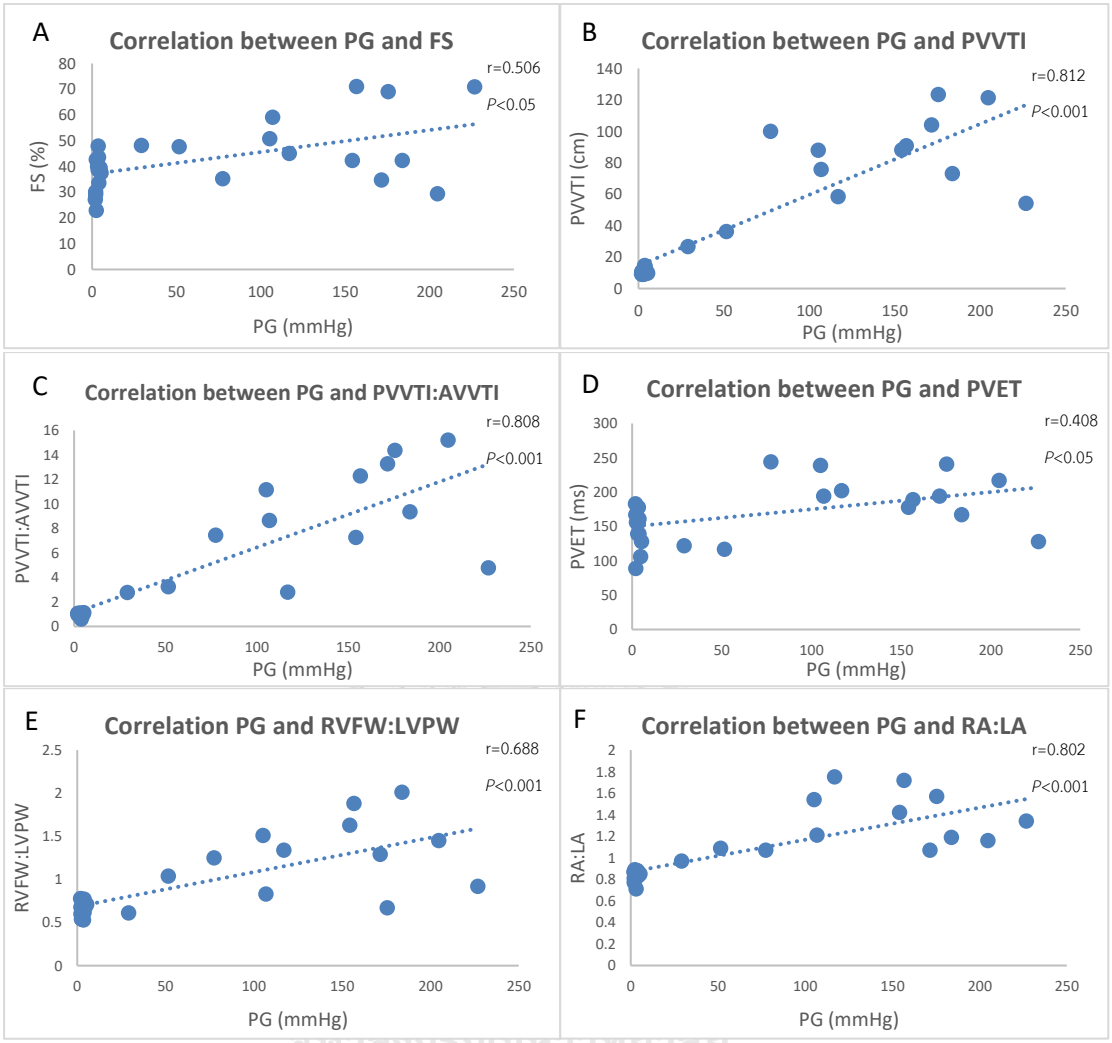

Figure 18. Spearman correlations between pressure gradient (PG) and systolic function and severity of right ventricular hypertrophy and right atrium enlargement parameters. fractional shortening (FS) (A), pulmonic valve velocity time integral (PVVTI) (B), pulmonic valve velocity time integral to aortic valve velocity time integral ratio (PVVTI:AVVTI) (C), pulmonic valve ejection time (PVET) (D), right ventricular free wall thickness to left ventricular posterior wall thickness ratio (RVFW:LVPW) (E) and right atrium to left atrium diameter ratio (RA:LA) (F)

Abbreviations: PG: pressure gradient, FS: fractional shortening, PVVTI: pulmonic valve velocity time integral, PVVTI:AVVTI: pulmonic valve velocity time integral to aortic valve velocity time integral ratio, PVET: pulmonic valve ejection time, RVFW:LVPW: right ventricular free wall thickness to left ventricular posterior wall thickness ratio and RA:LA: right atrium to left atrium diameter ratio

4.5 Heart rate variability results

The examples of spectral data in CONT and PS dogs were shown in Figure 19.

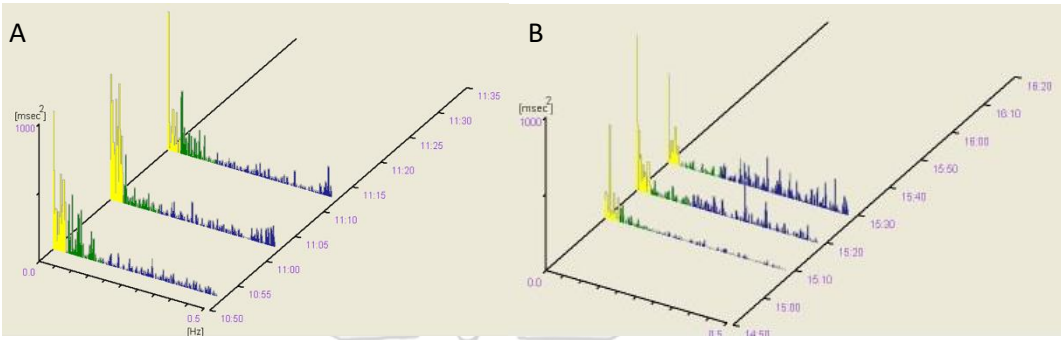

Figure 19. Examples of spectral power measured in a control dog (A) and a pulmonic valvular stenosis dog (B) for 3 consecutive 10 minutes duration.

The heart rate, time domain analysis parameters and correlations with PG values were shown in Table 6. The average heart rate was not different between groups although PS group tended to have higher heart rate. All of the time domain parameters of HRV were not different between CONT and PS groups. Additionally, no relationship between PG and time domain parameters was found.

Table 6. Time domain analysis parameters of heart rate variability and their correlations with pressure gradient in control and pulmonic valvular stenosis dogs

Parameters	CONT group (n=12)	PS group (n=13)	Spearman correlation coefficient (r) (n=25)	*P*-value
Heart rate (bpm)	108±5	120±7	0.252	0.222
SDNN (ms)	116.7±10.5	117.1±17.0	-0.127	0.539
SDANN (ms)	18.37±3.43	31.47±7.17	0.197	0.341
SDNN index (ms)	114.2±10.5	110.0±16.9	-0.166	0.423
pNN50 (ms)	44.49±5.50	31.93±6.38	-0.303	0.139
rMSSD (ms)	128.9±17.7	108.7±22.2	-0.239	0.246

Data presented as mean±SEM using t-test.

Abbreviations: CONT: control, PS: pulmonic valvular stenosis, bpm: beats per minute, SDNN: standard deviation of all NN intervals, ms: millisecond, SDANN: standard deviation of the average of NN intervals in all 5 min segments of the entire recording, SDNN index: mean of the standard deviation of NN intervals, pNN50: percentage of number of normal-to-normal intervals with differences >50 ms divided by the total number of all NN intervals and rMSSD: the square root of the mean of the sum of the squares of differences between adjacent NN intervals

The frequency domain analysis of HRV and correlations with PG values were shown in Table 7. All frequency domain analysis parameters were also not different between CONT and PS groups. The HF was slightly higher in PS group compared with CONT group. However, HF normalized showed no difference. No relationship between PG and frequency domain parameters was found.

Table 7. Frequency domain analysis parameters of heart rate variability and their correlations with pressure gradient values in control and pulmonic valvular stenosis dogs

Parameters	CONT group (n=12)	PS group (n=13)	Spearman correlation coefficient (r) (n=25)	*P*-value
ULF	721.6±119.7	1280.2±400.0	-0.034	0.870
VLF	2,698±500	3,095±759	-0.122	0.559
LF	2,213±358	1,879±550	-0.281	0.172
HF	4,803±1,035	8,125±3,642	-0.122	0.559
LF norm	0.29±0.05	0.33±0.06	0.042	0.841
HF norm	0.48±0.04	0.50±0.06	0.014	0.946
LF:HF	0.74±0.17	1.04±0.33	0.006	0.975
TP	12,755±1,974	15,953±5,191	-0.168	0.419

Data presented as mean±SEM using t-test.

Abbreviations: CONT: control, PS: pulmonic valvular stenosis, ULF: ultra low frequency, VLF: very low frequency, LF: low frequency, HF: high frequency, LF: low frequency, LF norm: LF frequency normalized, HF norm; high frequency normalized, LF:HF: low frequency to high frequency ratio and TP: total power

4.6 Blood profile analysis

Blood profile analysis and correlations between PG value were shown in Table 8. Blood profile analysis showed that PS dogs had slightly but significantly higher WBC than CONT group ($P<0.05$). PS groups showed higher both ALT and BUN compared with CONT group ($P<0.01$). The RBC content, ALP, Cr, TP and albumin concentrations showed no difference between groups. The high positive relationships were found

between either ALT (P<0.001) or BUN (P<0.05) and PG values (Figure 20). The negative relationships between PG and TP was also found (P<0.05) (Table 8).

Table 8. Complete blood count and blood chemistry profiles in control and pulmonic valvular stenosis dogs and their correlations between pressure gradient values

Parameters	CONT group (n=12)	PS group (n=13)	Spearman correlation coefficient (r) (n=25)	*P*-value
RBC ($x10^6$) (µL)	6.67±0.25	6.93±0.37	-0.023	0.911
Hb (g/dL)	17.12±0.42	16.62±0.84	-0.154	0.459
PCV (%)	44.75±1.07	44.52±2.06	-0.071	0.733
Platelet (µL)	327,000±30,369	309,231±29,491	-0.043	0.835
WBC ($x10^3$) (µL)	11.11±0.77	14.21±1.20*	0.329	0.107
Neu (%)	66.33±3.55	71.85±3.11	0.220	0.287
Eos (%)	4.93±1.06	3.65±0.69	-0.372	0.067
Lymph (%)	22.49±3.40	18.35±2.88	-0.117	0.571
Mono (%)	5.77±0.49	6.43±0.67	0.080	0.700
ALT (units)	31.83±3.07	59.08±7.16**	0.640	<0.001
ALP (IU/L)	56.50±9.79	93.85±43.35	0.262	0.204
BUN (mg%)	10.76±1.49	21.45±2.64**	0.496	<0.05
Cr (mg%)	0.88±0.04	0.98±0.05	0.243	0.238
TPr (g%)	6.36±0.18	6.10±0.21	-0.494	<0.05
Albumin (g%)	3.40±0.07	3.23±0.11	-0.354	0.082

Data presented as mean±SEM using t test. *P<0.05 and **P<0.01

Abbreviations: CONT: control, PS: pulmonic valvular stenosis, RBC: red blood cell, µl: microliter, Hb: hemoglobin, g/dL: gram per deciliter, PCV: packed cell volume, WBC: white blood cell, Neu: neutrophil, Eos: eosinophil, Lymph: lymphocyte, Mono: monocyte, ALT: alanine aminotransferase, ALP: alkaline phosphatase, IU/L: unit per liter, BUN: blood urea nitrogen, mg%: milligram percent, Cr: creatinine, TPr: total protein and g%: gram percent

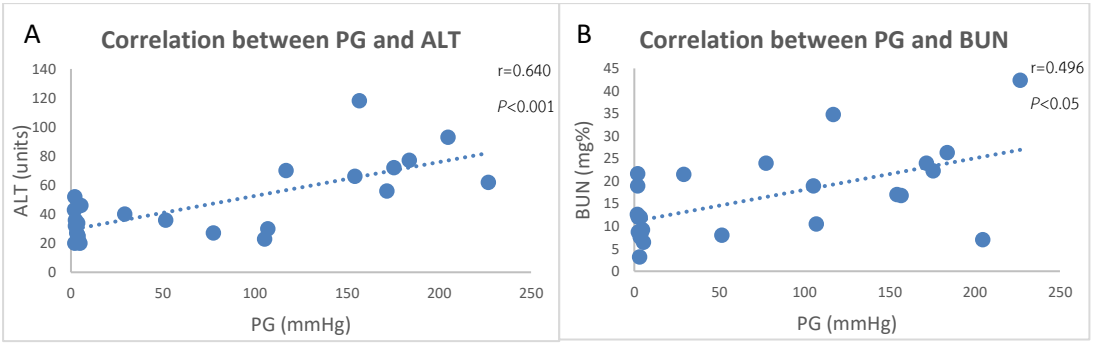

Figure 20. Spearman correlations between pressure gradient (PG) values and alanine aminotransferase (ALT) (A) or blood urea nitrogen (BUN) (B).

Abbreviations: PG: pressure gradient, ALT: alanine aminotransferase and BUN: blood urea nitrogen

CHAPTER V

DISCUSSION

PS is one of the most common congenital cardiovascular problems that have been reported in dogs, especially in young dogs (Oliveira et al., 2011). In our study, the PS dogs were presented with the average body weight as a small to medium breeds. The breeds were Bulldogs, shih Tzu, pomeranian, poodle and beagle. The Bulldogs both French and English Bulldogs were presented with PS and were the majority of PS cases. These results are consistent with the previous studies which showed the high prevalence of PS in Bulldogs (Buchanan, 1990; Oliveira et al., 2011; Kander et al., 2015; Ontiveros et al., 2019). The average age of PS dogs that showed clinical signs has some variations. However, all of the PS dogs that are brachycephalic breeds were younger than one year old. Upper respiratory obstruction commonly found in brachycephalic dogs that can depress pulmonary function may lead to more exercise intolerance and other right-sided heart failure signs (Canola et al., 2018). The male seems to have PS more than female by double that was reported earlier (Bussadori et al., 2001a). However, in other PS dog studies, equally distribution between sexes were reported (Johnson et al., 2004; Ontiveros et al., 2019). In English Bulldogs, the important anatomical variation of coronary artery is the factor that should be concerned. The aberrant coronary artery that emerged and wrapped around the pulmonary artery will

be the limitation factor for PS and the intervention is needed more consideration. Reports of English Bulldogs and Boxer with aberrant coronary artery were published with successful and unsuccessful intervention with BV (Buchanan, 1990; Kittleson et al., 1992; Johnson et al., 2004; Buranakarl et al., 2017). In the present study, one English bulldog had aberrant coronary artery and one English Bulldog had ASD and abereant coronary artery while none of French bulldog had it. Although, the aberrant coronary artery was one of the outcome factor, the intervention was successful in the previous case report (Buranakarl et al., 2017). It was interesting to see that PS can occur in concomitant with other congenital defects. This study showed 4 dogs from 13 had ASD. Two of dogs with ASD were French bulldogs and the rest were English Bulldog and Pomeranian. Previous report also showed that approximately 50% of the dogs and cats with ASD processed other congenital heart diseases including valvular defects (Chetboul et al., 2018). In terms of clinical presentation, left to right shunt of ASD can lead to right-sided volume overload and right to left shunt of ASD can cause a reduction of oxygenated blood leading to polycythemia (Maillis et al., 1974; Ikäheimo et al., 1983; Le Gloan et al., 2018). Thus, the clinical signs of PS dogs with ASD may be more severe. In the present study all 4 PS dogs with ASD had PG of severe grade with the clinical signs of exercise intolerance, while polycythemia and syncope were found in only 2 PS dogs with right to left shunt of ASD. The BV was performed in all 4 dogs while the outcomes were successful in 2 dogs, unable to reach the stenotic area in 1 dog and sudden cardiac death during BV in 1 dog.

Rather than the congenital defects, the PS was divided into type A and type B based on degree of pulmonic valvular leaflet thickening, AO:PA ratio and the presenting of pulmonary valvular hypoplasia (Bussadori et al., 2000). Our study showed that 7 from 13 PS dogs had type A while 6 PS dogs had type B. The number of type A was found more than type B in this study. This result was similar to the other reports (Bussadori et al., 2001a; Bussadori et al., 2001b; Locatelli et al., 2011; Oliveira et al., 2011). Moreover, the valvular PS was the most commonly found in this study that was also similar to other studies in both humans and dogs (Johnson et al., 2004; Oliveira et al., 2011; Kim et al., 2013). The average PG in type A and type B was 103.9±21.2 and 172.2±17.3 mmHg, respectively. It was previously shown that type A has the better outcome after BV than type B in PS dogs (Bussadori et al., 2001a; Locatelli et al., 2011). In the present study, by looking at the same grade, the clinical outcomes of type A are better than type B after BV in both PG reduction and survival time (Buranakarl et al., 2016).

It will also note that polycythemia was presented in the 2 dogs with PS and right to left shunt of ASD. The polycythemia should be a result of ASD rather than PS. The polycythemia occurred because of the lack of high oxygenated blood flow due to the mixing of low oxygenated blood from RA with high oxygenated blood in LA through right to left shunt of ASD (Magoon et al., 2018). Decreased partial pressure of oxygen (PO_2) stimulated erythropoietin (EPO) production from the kidney and sequentially

increased RBC production (Broberg et al., 2006). Many types of congenital defects such as ventricular septal defect (VSD), ASD and tetralogy of Fallot can cause secondary erythrocytosis of cyanotic congenital heart diseases (CCHD) (Maillis et al., 1974; Ikäheimo et al., 1983; Thomson et al., 1999; Gunduz et al., 2014).

Most clinical signs found in these dogs with PS were exercise intolerance and syncope as mention earlier (Bussadori et al., 2001a; Francis et al., 2011; Locatelli et al., 2011). The syncope was caused by increased right cardiac afterload causing less blood to go into the pulmonary artery. Also, increased RV pressure will limit the venous return to the left side of the heart and eventually cause low CO causing syncope. Four from thirteen PS dogs were presented with right ventricular failure, in which the ascites developed. The BV can correct many signs including exercise intolerance, syncope and fluid accumulation. The medications that were prescribed in PS dogs included positive inotrope (i.e., pimobendan), afterload reducer such as angiotensin converting enzyme inhibitors (ACEI) or sildenafil and preload reducer including furosemide. However, many dogs receiving medications without BV still had exercise intolerance and syncope. Thus, the BV is recommended for all moderate to severe PS cases in both humans (Lau and Hung, 1993; Ray et al., 1993; McCrindle, 1994) and dogs (Sisson and MacCoy, 1988; Martin et al., 1992; Bussadori et al., 2001a; Johnson et al., 2004).

The electrocardiographic patterns of all of the CONT dogs showed RSA, while almost half of the PS dogs showed ST. The ST may result from the CHF induced

sympathetic compensation, stress, anxiety, and excitement. The ECG in PS dogs showed a remarkable shape. The finding was previously described in PS dogs (McCaw and Aronson, 1984; Buranakarl et al., 2016) and dogs with right ventricular hypertrophy such as heartworm disease (Onyango, 2011). Increased S wave deflection and decreased R:S ratio were more pronounce and related to PG. The present of R:S abnormalities was found in 10 from 13 PS dogs which all of them were moderate and severe PS suggesting that these dogs had hypertrophy called RV concentric hypertrophy from increased right sided pressure overload. Concentric hypertrophy was defined as the cardiac maladaptation with increased cardiac wall thickness and cardiac myocyte mass for increased systolic function as a compensation in pressure overload situation (Samak et al., 2016). Increased hypertrophy without muscle elongation and reduced radius cause tremendous tension of the heart as described by Laplace law (Opie, 2004). Thus, the increased isometric ventricular pressure was suggested to overcome the increase in afterload at stenotic area. High shear stress at the right outflow tract may be a reason for developing the gradient between right and left sided of ventricle when the PS was progressed. Another abnormality of ECG is prolonged QRS duration. It is suggested that the depolarization of ventricle was propagated with the longer duration. The reason may be due to increased RV mass that impulse is propagated. The ECG results of PS dogs in the present study were similar to the cardiac electrical changes in patients with PS which showed increased R and S wave amplitudes with longer QRS duration and QTc than those of healthy persons (Alyan et

al., 2008). Moreover, these cardiac electrical changes showed remarkable improvement after BV and related with the hemodynamic, cardiac structural and functional improvements measured from echocardiographic parameters and cardiac biomarker using pro B-type natriuretic peptide (proBNP) (Alyan et al., 2008).

In our study, at 6-month follow up, the sudden cardiac death was reported in 2 PS cases at 6.5 and 15 months after BV. These dogs had severe PS degree with prolongation of QTc at the first diagnosis (604.5 and 666.3 ms). The prolongation of QTc was reported to relate with increased risk of ventricular tachycardia, TdP, ventricular fibrillation and cardiac death (Parisi et al., 1999; Lehmann and Morady, 2003; Straus et al., 2006). Higher P wave amplitude called P pulmonale was found in PS dogs. The P pulmonale was previously described that the depolarization of right atrium was intense. The increased amplitude may be related to the right atrial enlargement. The RA:LA > 1 that indicated RA enlargement in dogs (Serres et al., 2009; Chetboul et al., 2018) was found in most of our PS cases, except one mild PS dog. This study showed the high positive correlations between P wave amplitude and either PG or RA:LA. Thus, the right atrium enlargement was seen in PS and the severity was related to progression of PS. This RA enlargement in PS dogs was also reported to associate with cardiac death (Francis et al., 2011; Chetboul et al., 2018).

Echocardiographic data of left ventricular functions showed that PS dogs had low SV as shown by reduced both LVIDdN and LVIDsN. The increased heart rate or

increased contractility as seen by increase of FS may help to compensate low output. However, as mentioned before that the right pressure is an important factor that limit left ventricular preload. In the present study, the right ventricular hypertrophy measured from RVFW:LVPW increased in PS dogs to almost 1.3 and severe RV hypertrophy found in 4 PS dogs suggesting that the right ventricle with high volume and pressure causes the left ventricular preload reduction. The pressure at right side can transmit through interventricular septum which was thicken both during systole and diastole. Thickening of IVS indicated that the volume of left ventricle may be reduced especially when the PS was progressed as described in previous experimental study using PS mice model (Urashima et al., 2008). Therefore, higher FS may not correlate with higher CO. Moreover, these cardiac maladaptations can induce myocardial ischemia, loss of cardiac compliance, impair systolic and diastolic functions (Marino et al., 1985; Urashima et al., 2008). However, whether right atrium had an effect on venous return passing the left atrium in case of combine ASD is needed further study.

The systolic function of right and left sides of the heart was confirmed by echocardiography. Increased valve VTI on the right side more than on the left side suggested that more pressure or time was needed to eject blood to pulmonary artery. These time integral also related to PG suggesting the closing of the valve was prolonged when PS was progressed. The PEP was unchanged between PS and CONT

dogs but the PVET was prolonged. Thus, PVPEP:PVET in our PS dogs was smaller than that of CONT dogs which is in agreement with the report in patients with PS (Okamoto et al., 1981). Thus, the isovolumetric ventricular contraction period may be similar although rate of increase in pressure was high. The prolongation of ventricular ejection time may be the result of late closing of pulmonic valve. The longer ET associated with valvular stenosis had been reported in patients with aortic stenosis (Bache et al., 1973). In the case of patients with aortic stenosis, the researchers suggested that the longer LVET resulted directly from the increased outflow tract obstruction rather than the alteration of myocardial contraction capacity as this LVET showed no correlation with stroke volume and the LVET could not accurately represent stroke volume when stroke volume felt below 100 ml (Bache et al., 1973). Therefore, ET should not be used solely to interpret myocardial performance in the cases of valvular stenosis. Nevertheless, this prolonged ET is crucial for allowing enough time for right ventricular ejection in order to maintain adequate right stroke volume and increase of right ventricular ejection time may be seen by prolonged QRS complex from ECG. Increased PVET and PVVTI:AVVTI ratio compared with CONT dogs suggesting the time of abnormal pulmonic valve opening was longer in order to overcome the high right outflow tract pressure. These phenomena may be different in type B compared to type A and it may affect outcome after performing BV.

Previous reports showed that patients with PS had high ANS activity as measured from HRV analysis (Alyan et al., 2008). Sympathetic activity could be reduced by BV was suggested in PS dogs (Trikhun et al., 2019). Nevertheless, both time and frequency domains of HRV data did not show cardiac autonomic alteration in PS dogs compared with CONT dogs in the present study. The reason for unremarkable change of autonomic control may be due to the progression of PS is a long duration process that the resetting of ANS may develop was reported earlier in patients with PS (Galal et al., 1996; Alyan et al., 2008). Changes in HRV immediately after BV will be seen when abrupt change in hemodynamic occurs. Human patients with PS had the sympathetic overactivation before BV while the parasympathetic activity was significantly increased immediately 1 day after BV. These ANS alterations after BV also related with the improvement of the echocardiographic data and cardiac biomarker (Alyan et al., 2008).

Another possibility that HRV was not different. HRV was affected by many factors including excitement (Shaffer and Ginsberg, 2017) or respiratory problem (Little and Julu, 1995; Lewis et al., 2006; Roque et al., 2014). In the present study, Bulldogs had respiratory problem associated with brachycephalic breeds. Some dogs also had excitement. Thus, measurement of HRV to indicate ANS activity may be limited if it was not performed in the same animals. In clinical setting, the repeated measurement of HRV analysis may be benefit for disease follow up and ANS alteration.

Beside respiratory and excitement, there are other intrinsic factors that can affect HRV analysis such as age, breed, circadian rhythm, metabolism, renin angiotensin system, baroreceptor and endothelin (Shaffer and Ginsberg, 2017). Moreover, the short-term HRV monitoring used in this study may not be as accurate as 24 hours of HRV monitoring. Nevertheless, 24 hours of HRV monitoring has several clinical limitations i.e.; owner competences in event recording and equipment maintaining.

In the present study, the ALT and BUN were higher in PS dogs which indicated the dogs may have both hepatic and renal impairment. The right-sided heart failure causes venous congestion and low blood supply to the liver. Increased ALT, however, was still in normal range. The elevation of ALT level was reported in CHF situation either hepatic congestion from high filling pressure (backward failure) and perfusion impairment (forward failure) (Alvarez and Mukherjee, 2011). Higher BUN was also fell into the normal range but the increased BUN may indicate high intravascular volume. In dogs with right-sided heart enlargement, the increased ANP was suspected. Reported of high proNBP in dogs with both sides of congestive heart failure were found (Groenning et al., 2001; Krüger et al., 2004; Blyth et al., 2007; Ozturk et al., 2011). The increased plasma N-terminal proBNP (NT-proBNP) and a strong positive relationship between PG and plasma NT-proBNP concentration were also reported in the symptomatic PS dogs (Kobayashi et al., 2014). High pro-BNP was reported to be correlated with BUN in patients with acute decompensate heart failure (Chen et al.,

2012). Therefore, higher BUN in this study may suggest the high right ventricular preload. The relationship between BUN and PG may be suggested that BUN may be used to indicate the severity of PS.

In conclusion, the PS dogs had low left ventricular function as seen by low left ventricular preload. A decrease in load may be due to the thickening of right ventricle and interventricular septum. An increase in right ventricular ejection time compared with left was one factor to maintain pulmonary blood flow. The parameters rather than echocardiography that can be changed with PS progression is R:S wave ratio. Finally, the autonomic compensation in PS dogs were unremarkable compared with CONT dogs which may be due to chronic compensatory changes of hemodynamic in PS.

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