Application of evaluation of left atrial phasic function via time-left atrial area curve analysis based on two-dimensional speckle tracking echocardiography in canine heart disease

（2D Speckle Tracking 心エコー図法による時間－左心房断面積曲線解析を用いた左心房相機能評価の犬心疾患への応用）

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GENERAL ABBREVIATIONS

ACEI	Angiotensin-converting enzyme inhibitors
ACVIM	American College of Veterinary Internal Medicine
AUC	Area under the receiver operating characteristic curve
A-wave	Peak velocity of late diastolic transmitral flow wave
A'-wave	Peak velocity of late diastolic wave of myocardial velocity
CI	Confidence interval
CRI	Constant rate infusion
CV	Coefficient of variation
CVHD	Chronic valvular heart disease
EAact	Left atrial active emptying area
EApass	Left atrial passive emptying area
EAtotal	Left atrial total emptying area
ECG	Electrocardiography
E-wave	Peak velocity of early diastolic transmitral flow wave

E’-wave	Peak velocity of early diastolic wave of myocardial velocity
FACact	Left atrial active fractional area change
FACpass	Left atrial passive fractional area change
FACtotal	Left atrial total fractional area change
HF	Heart failure
LA	Left atrium
LA/Ao	left atrial to aortic root ratio
LAAmax	Left atrial maximum area at ventricular end-systole
LAAmin	Left atrial minimum area at ventricular end-diastole
LAAp	Left atrial area at onset of the P wave on the ECG
LV	Left ventricle
LVEDD	Left ventricular end-diastolic diameter
LVEDDN	Left ventricular end-diastolic diameter normalized for body weight
LVESD	Left ventricular end-systolic diameter

LVESDN	Left ventricular end-systolic diameter normalized for body weight
LVFS	Left ventricular fractional shortening
PCWP	Pulmonary capillary wedge pressure
ROC	Receiver operating characteristic
SD	Standard deviation
S’-wave	Peak velocity of systolic wave of myocardial velocity
TDI	Tissue Doppler imaging

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GENERAL INTRODUCTION

Heart failure (HF) is a common clinical syndrome in both humans and dogs, wherein the ability of the heart to function as a pump to maintain a physiological circulation is compromised due to any structural and functional heart disease.[1] Echocardiography is a useful, non-invasive diagnostic tool that enables the observation of the motion and structure of the heart, and plays a pivotal role in the management of heart disease. It has been used in diagnosis of HF and evaluation of the disease severity for prognostication and treatment guide.[1]

Conventionally, in humans and dogs, echocardiographic assessment of the disease severity has been performed by determining the function and size of the left ventricle (LV), a pump ejecting blood into the systemic circulation.[2,3] In fact, it has been shown that the LV systolic and diastolic dysfunction and the increased LV size are associated with severer HF symptoms and shorter survival times in humans and dogs with heart diseases.[1,4,5]

Recently, in humans, accumulating evidence indicates that the left atrium (LA) takes an important part in the pathophysiology of heart disease by modulating LV diastolic filling through the 3 phasic functions. These include a reservoir function

(expansion associated with inflow of blood from the pulmonary veins during ventricular systole), a conduit function (passage of blood from the pulmonary veins to the LV during ventricular early diastole), and a booster pump function (augmentation of LV filling during ventricular late diastole).[6] It has been demonstrated that the LA phasic dysfunction and the increased LA sizes assessed by echocardiography are correlated with HF severity and occurrence of adverse cardiovascular events in human heart diseases.[7,8] So far, the echocardiographic evaluation of the LA phasic function has been commonly performed directly based on the calculation of fractional changes in the LA sizes (diameters, areas, or volumes) by use of the B-mode still images, or indirectly based on pulsed wave Doppler evaluation of transmitral and pulmonary venous flow, and tissue Doppler imaging (TDI).

Previously, in humans, the acoustic quantification method has been applied for determination of the time-LA area relationship for precise direct evaluation of the LA phasic function (time-LA area curve analysis).[9] This technique enables the automatic calculation of areas of interest in heart by automatically determining the blood-tissue border via software, and was applied for evaluation of the LV function at first. However, it is somewhat cumbersome to obtain reliable results on the LA function with this technique because it is difficult to exclude the presence of pulmonary veins and the LA

appendage from a region of interest.[9]

A novel method has been developed for tracking the LA wall movement via 2-D speckle tracking echocardiography, and this technology can be used to automatically derive a time-LA area or volume curve.[10] The principle of the 2-D speckle tracking method relies on the formation of speckles in echocardiographic images by reflection, scattering, and interference between the tissue and ultrasound beam. These speckles appear homogeneously distributed within the myocardium in 2-D echocardiographic images and can be tracked from frame to frame throughout the cardiac cycle. With this methodology, it is possible to exclude pulmonary veins and the LA appendage from a region of interest and to precisely perform time-LA area or volume curve analysis. In humans, time-LA area or volume curve analysis determined via 2-D speckle-tracking echocardiography is feasible and useful for evaluation of the LA phasic function in various cardiac diseases.[10,11]

On the other hand, studies on the LA phasic function have been scarce in dogs, and to our knowledge only 2 reports exist to date.[12,13] One report indicates that the reservoir and booster pump functions determined based on pulmonary venous Doppler flow were deteriorated in dogs with HF because of dilated cardiomyopathy.[12] In the other report, only the reservoir function was assessed based on the fractional changes in

the LA area and diameter, and it was impaired in HF dogs with chronic valvular heart disease (CVHD) and dilated cardiomyopathy.[13] More importantly, the application of the evaluation of the LA phasic function via time-LA area curve analysis based on 2-D speckle tracking echocardiography has not been reported in dogs.

With the above background, this study was performed in 4 stages in order to establish the clinical utility of the evaluation of the LA phasic function via time-LA area curve analysis based on 2-D speckle tracking echocardiography in canine heart disease. In the first stage, we have established the repeatability and reproducibility of indices obtained via time-LA area curve analysis. In the second stage, we have investigated the feasibility of the evaluation the LA phasic function via time-LA area curve analysis in the assessment of the disease severity of canine CVHD. The determination of the LA phasic function proved to be useful in detecting the presence of HF. With the results in clinical dogs from this stage, we then conducted experimental researches using healthy dogs in the third and fourth stages. In the third stage, we have determined the effect of various cardiovascular drugs on indices of the LA phasic function obtained via time-LA area curve analysis. Lastly, in the fourth stage, we have examined the effect of volume loading on indices of the LA phasic function assessed by time-LA area curve analysis.

CHAPTER 1

REPEATABILITY AND REPRODUCIBILITY OF INDICES OF LEFT ATRIAL PHASIC FUNCTION OBTAINED VIA TIME-LEFT ATRIAL AREA CURVE ANALYSIS BASED ON TWO-DIMENSIONAL SPECKLE TRACKING ECHOCARDIOGRAPHY IN HEALTHY DOGS

1. INTRODUCTION

So far, in dogs, the time-LA area curve analysis based on 2-D speckle tracking echocardiography has not been applied to the evaluation of LA phasic function, and therefore its feasibility, repeatability, and reproducibility remain unknown. In order to determine whether differences in data reflect clinically important changes resulting from disease progression in an individual patient, it is necessary to evaluate the repeatability and reproducibility of variables measured with this technique.

Thus, the goal of chapter 1 was to evaluate the LA phasic function in healthy dogs via 2-D speckle tracking echocardiography with time-LA area curve analysis, and to assess the feasibility, repeatability and reproducibility of measurements obtained with this modality.

2. MATERIALS AND METHODS

2.1 Animals

Six Beagles (2 males and 4 females; age, 1 year; body weight, 11.3 to 13.2 kg) that were part of a research colony owned by Hokkaido University were included. All dogs were determined to be healthy, with no mitral regurgitation or congenital cardiac abnormalities, as determined on the basis of results of a complete physical examination, electrocardiography (ECG), and standard echocardiographic examinations (including M-mode, pulsed-wave Doppler, and color-flow Doppler imaging) performed prior to the start of the study to confirm normal heart anatomy and myocardial function. All procedures were approved by the Laboratory Animal Experimentation Committee, Graduate School of Veterinary Medicine, Hokkaido University.

2.2 Study design

Echocardiographic examinations were performed on 4 days over a 2-week period (ie, 3 dogs underwent the procedure on days 1 and 7, and the remaining 3 dogs underwent the procedure on days 8 and 14). On a given day, the 3 dogs were each examined at 3 nonconsecutive times. Each variable assessed for an individual dog consisted of the mean of measurements from 3 consecutive cardiac cycles.

2.3 Two-dimensional speckle tracking echocardiography

All echocardiographic examinations were performed using commercially available ultrasound equipment (HI VISION Preirus; Hitachi Aloka Medical Ltd., Tokyo, Japan) equipped with a 3–7 MHz sector probe (EUP-S52; Hitachi Aloka Medical Ltd., Tokyo, Japan) and offline software (Left Atrial Tracking; Hitachi Aloka Medical Ltd., Tokyo, Japan). All dogs were examined while manually restrained in left lateral recumbency. No sedation was used. An apical 4-chamber view was obtained by means of second harmonic grayscale imaging, with frequency, depth, and sector width adjusted for frame-rate optimization (154 frames/s). An electrocardiography (ECG) trace (lead II) was recorded simultaneously with the echocardiographic scan.

The echocardiographic images were analyzed with the offline software mentioned above. A frame corresponding to the time of the peak R wave on the ECG was selected as indicating LV end-diastole, and the endocardium of the LA was manually traced in that frame (Figure 1). The area of the LA was then automatically calculated by the software in each subsequent frame throughout the cardiac cycle to derive a time-LA area curve. Tracking quality was assessed visually. If the tracking quality was unsatisfactory (ie, the blood-tissue border was not tracked), manual tracing of the endocardium was repeated. The LA maximum area at ventricular end-systole (LAAmax), area at onset of the P wave

on the ECG (LAAp), and minimum area at ventricular end-diastole (LAAmin) were determined by the software. Variables used as indicators of LA phasic function were calculated[10,14] as follows (Figure 2):

$$EAtotal = LAAmax - LAAmin$$

$$EApass = LAAmax - LAAp$$

$$EAact = LAAp - LAAmin$$

$$FACtotal\ (\%) = 100 \times EAtotal/LAAmax$$

$$FACpass\ (\%) = 100 \times EApass/LAAmax$$

$$FACact\ (\%) = 100 \times EAact/LAAp$$

where EAtotal, EApass, and EAact represent the LA total, passive, and active emptying areas, respectively; FACtotal, FACpass, and FACact represent the LA total, passive, and active fractional area changes, respectively. The FACtotal was calculated as an indicator of reservoir function, whereas the FACpass was determined as an indicator of conduit function. The FACact was calculated as an indicator of booster pump function. Heart rate was calculated from the R–R interval on the ECG tracing during the same cardiac cycle used for echocardiographic measurements of the LA.

2.4 Manual tracing method

The same images of the LA analyzed automatically via offline software were

analyzed via a manual tracing method. The LA endocardium was manually traced at 3 time points, and the resulting areas were calculated via automated calculation software provided with the ultrasound machine. The LAAmax was measured in a frame obtained just before mitral valve opening (ventricular end-systole), LAAp was measured in a frame obtained at the onset of the P-wave on ECG, and LAAmin was measured in a frame obtained at mitral valve closure (ventricular end-diastole). The EAtotal, EApass, EAact, FACtotal, FACpass, and FACact were calculated as previously described.

2.5 Statistical analysis

Normal distribution of the data was confirmed by means of a Shapiro-Wilk test. Results are expressed as mean ± standard deviation (SD). Statistical analyses were performed with computer software (JMP 8; SAS Institute Inc., Cary, NC, U.S.A). The following linear model was used for variables in within-day and between-day variability analysis:

$$Y_{ijk} = \mu + day_i + dog_j + (day \times dog)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the kth value measured for dog j on day i, μ is the general mean, day_i is the differential effect of day i, dog_j is the differential effect of dog j, $(day \times dog)_{ij}$ is the interaction term between day and dog, and ε_{ijk} is the model error. The SD of repeatability was estimated as the residual SD of the model and the SD of

reproducibility as the SD of the differential effect of day. The corresponding coefficients of variation (CV) were determined by dividing each SD by the mean. Following suggestions from reports of previous studies,[15,16] clinical acceptability was defined as a CV ≤ 20%.

A Bland-Altman analysis with modifications for repeated measures was performed to assess agreement between measurements for variables determined automatically with speckle tracking echocardiography and those obtained by manual tracing; mean values for measurements obtained from the same images via the 2 methods were plotted against the difference (the value determined via speckle tracking echocardiography minus the value determined by manual tracing).[17] The mean difference (bias) as well as SD, 95% CI, and *P* values of the differences were calculated. For all statistical comparisons, values of $P < 0.05$ were accepted as significant.

3. RESULTS

In the apical 4-chamber view, movement of the LA wall was tracked from frame to frame throughout the cardiac cycle with 2-D speckle tracking echocardiography. All of the obtained images from 108 cardiac cycles of 6 dogs were analyzed to derive time-LA area curves, and all required variables were determined for each curve (Figure 1). Subjective assessment of time-LA area curves revealed that LA area increased during LV systole (reservoir phase), decreasing passively during LV early diastole (conduit phase), and remaining relatively stable during LV diastasis before decreasing actively during atrial contraction (booster pump phase).

Within-day and between-day CVs for variables obtained via speckle tracking echocardiography of the LA were summarized (Table 1). The CVs were < 20% (range, 0.41% to 16.4%) for all variables. Overall, the total emptying fraction was 49.8 ± 3.5%, passive emptying fraction was 27.7 ± 4.0%, and active emptying fraction was 30.5 ± 4.3%.

The dispersion of differences for values determined via speckle tracking echocardiography and the manual tracing method was evaluated by use of Bland-Altman analyses with modifications for repeated measures (Table 2). Agreement

was good (ie, the differences were nonsignificant [$P > 0.05$ for all comparisons]) between the 2 methods for all variables.

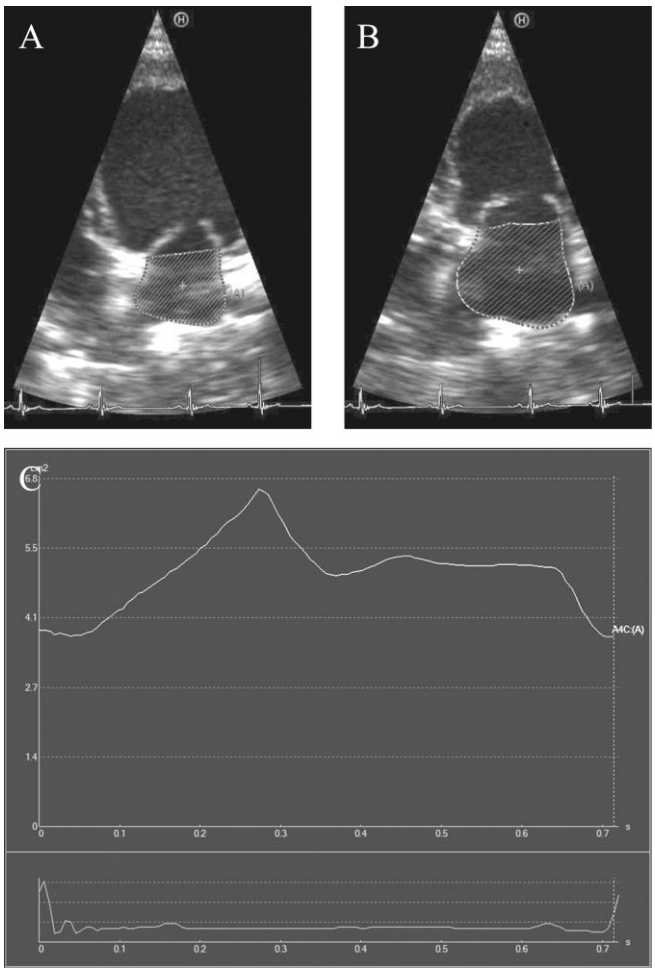

Figure 1. Representative 2-D echocardiographic images of the LA in the apical 4-chamber view of a healthy 1-year-old Beagle and a computer-generated time-LA area curve for a single cardiac cycle in the same dog. (A) Image obtained at the time of peak R wave on the ECG (LV end-diastole). When manually tracing the endocardial border of the LA, the LA appendage and the confluence of the pulmonary veins were excluded from the measurement, and a horizontal line was drawn across the mitral annular plane. (B) Image obtained at LV end-systole. The endocardial borders of the LA were

automatically tracked in each frame throughout the cardiac cycle with speckle-tracking software. (C) Area of the LA in each frame throughout the cardiac cycle was automatically calculated to derive the time-LA area curve via software. Time (in seconds) is shown on the x-axis and area (in square centimeters) is shown on the y-axis. The simultaneous ECG for the recorded cardiac cycle appears below the curve.

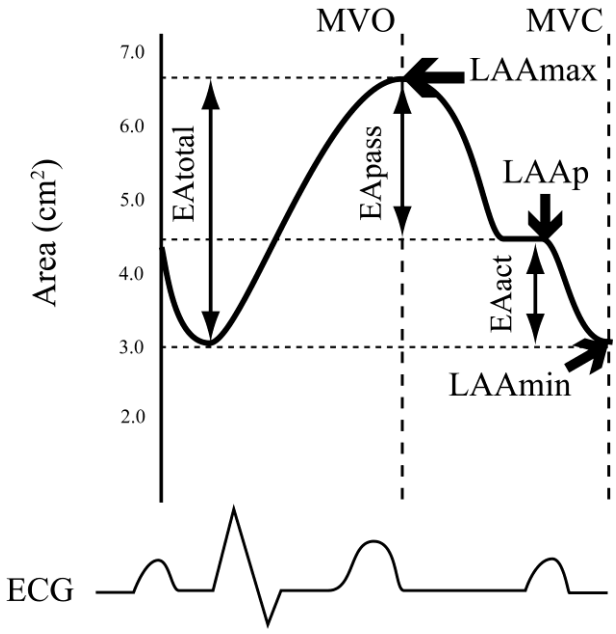

Figure 2. Schematic representation of the time-LA area curve (top) generated during a single cardiac cycle (represented as an ECG tracing; bottom). Measurements of LA area are shown. From the onset of ventricular systole, atrial area progressively increases, reaching its maximal dimension at ventricular end-systole. After mitral valve opening, atrial area rapidly decreases during early ventricular diastole. During diastasis, LA area remains constant or is slightly increased. At the end of diastasis, atrial contraction begins, causing atrial area to decrease to its minimal dimension. MVC = Mitral valve closure. MVO = Mitral valve opening.

Table 1. Mean values with within-day and between-day SDs and CVs for heart rate and LA variables determined via 2-D speckle tracking echocardiography in 6 healthy Beagles.

Variable	Mean ± SD	Within-day		Between-day	
		SD	CV (%)	SD	CV (%)
Heart rate (beats/min)	112 ± 18	14.1	12.5	3.4	3.1
LAAmax (cm^2)	6.73 ± 0.79	0.38	5.72	0.03	0.41
LAAp (cm^2)	4.87 ± 0.65	0.23	4.77	0.11	2.15
LAAmin (cm^2)	3.38 ± 0.48	0.23	6.88	0.14	4.08
Emptying area (cm^2)					
Total	3.35 ± 0.31	0.29	8.70	0.17	4.99
Passive	1.86 ± 0.33	0.26	14.0	0.08	4.16
Active	1.49 ± 0.44	0.13	9.25	0.25	16.4
Fractional area change (%)					
Total	49.8 ± 3.5	2.65	5.31	2.06	4.14
Passive	27.7 ± 4.0	2.76	9.98	0.95	3.44
Active	30.5 ± 4.3	2.61	8.57	3.97	13.0

Table 2. Results of Bland-Altman analysis of agreement between measurements for LA variables determined automatically via speckle-tracking echocardiography and by use of a manual tracing method for the same 6 dogs in Table 1.

Variable	**Bias**	**SD**	**95% CI**
LAAmax (cm^2)	0.014	0.54	–0.16 to 0.20
LAAp (cm^2)	–0.0019	0.44	–0.15 to 0.14
LAAmin (cm^2)	0.0037	0.28	–0.093 to 0.010
Emptying area(cm^2)			
Total	0.0051	0.50	–0.16 to 0.17
Passive	0.011	0.47	–0.15 to 0.17
Active	–0.0056	0.26	–0.093 to 0.083
Fractional area change (%)			
Total	–0.11	4.92	–1.78 to 1.55
Passive	–0.049	6.01	–2.08 to 1.98
Active	–0.10	2.95	–1.10 to 0.89

The difference between methods was nonsignificant ($P > 0.05$) for all variables.

4. DISCUSSION

Results of this study indicated that the use of time-LA area curve analysis based on 2-D speckle tracking echocardiography was feasible in dogs for evaluation of the LA phasic functions. The repeatability and reproducibility of measurements obtained with this method were considered clinically acceptable for use in healthy dogs. All of the obtained images were of sufficient quality to allow analysis for derivation of time-LA area curves, and agreement between the speckle tracking echocardiography method and manual tracing was considered good for all variables. In addition, the within-day and between-day CVs for all variables measured via speckle tracking echocardiography were considered clinically acceptable (< 20%).

The indirect evaluation of LA phasic function by use of the Doppler echocardiographic variables for transmitral blood flow, pulmonary venous blood flow, and myocardial tissue velocities has the limitation of clinically insufficient repeatability and reproducibility in dogs,[18,19] despite the reported usefulness in detecting the LA dysfunction in canine heart disease.[12] Actually, some parameters of transmitral and pulmonary venous Doppler flow and myocardial TDI were not adequately repeatable and reproducible (ie, within- and between-day CVs > 20%).[18,19] This is mainly because

the measurements of the Doppler-based techniques are affected by the angle between the wave source and receiver[20] and translational movement of the heart.[21]

In contrast, the good repeatability of measurements obtained with time-LA area curve analysis with speckle tracking echocardiography in this study may be partially attributed to the fact that the speckle tracking technique is independent of the angle between the wave source and receiver.[22] In humans, the variability of measurements of time-LA area curve analysis based on 2-D speckle tracking echocardiography is adequate for routine clinical use.[10,11] Also, a previous study in canine CVHD showed that the repeatability and reproducibility of measurements of LV strain and strain rate obtained by means of 2-D speckle tracking echocardiography are clinically acceptable.[23] In the study, measurements of LV strain and strain rate in dogs revealed within-day CVs between 7.1% and 18.3% and between-day CVs between 4.2% and 12.7%.[23] In this study, within-day and between-day CVs for all variables (0.41% to 16.4%) were considered comparable to those findings.

To our knowledge, this study provides the first description of LA phasic function assessed with time-LA area curve analysis in dogs. Thus there are no available reference intervals for areas of the LA during various functional phases, or LA emptying areas or fractional area changes measured with any technique in healthy dogs. However,

the mean values of total, passive, and active fractional area changes (49.8%, 27.7%, and 30.5%, respectively) in this study were fairly similar to previously reported values (47%, 31%, and 25%, respectively) determined via the acoustic quantification method in healthy adult humans.[24]

In this study, the apical 4-chamber view was obtained for analysis, and other views were not evaluated. However, in the right parasternal short-axis view, it is impossible to exclude the presence of the LA appendage from a region of interest when manually tracing the outline of the LA. Also, in the right parasternal long-axis view, it is difficult to adjust the sector width of images for frame rate optimization. Further, the apical 2-chamber view is not routinely used in veterinary medicine.

The time-LA area curves, rather than the time-LA volume curves, were obtained for the evaluation of the LA phasic function in this study, although LA volumes can be calculated by the method of Simpson et al[25] by use of the same type of offline software[10,11] and would provide a more accurate estimate of LA size than do LA areas.[25] However, the LA is a complicated structure and, to our knowledge, the calculation of LA volumes in dogs by the method of Simpson et al[25] has not been evaluated against a gold standard technique such as MRI.

In conclusion, time-LA area curve analysis based on 2-D speckle tracking

echocardiography provides repeatable and reproducible quantitative measurements in the evaluation of the LA phasic function in healthy dogs. In chapter 2, with these findings, we will investigate the feasibility of this modality in the assessment of the disease severity of canine CVHD, the most common heart disease in dogs.

5. SUMMARY

In this chapter, we have established the feasibility, repeatability, and reproducibility of variables obtained via time-LA area curve analysis based on 2-D speckle tracking echocardiography in healthy dogs. All of the obtained echocardiographic images were of sufficient quality to allow time-LA area curve analysis, and agreement between this modality and manual tracing was good for all variables. In addition, the within-day and between-day CVs for all variables measured with this technique were clinically acceptable.

CHAPTER 2

LEFT ATRIAL PHASIC FUNCTION IN DIFFERENT STAGES OF DISEASE SEVERITY IN CANINE CHRONIC VALVULAR HEART DISEASE

1. INTRODUCTION

The CVHD is the most common heart disease in dogs. Its prevalence depends on the breed, but may reach almost 100% in geriatric small breed dogs.[26] This disorder is characterized by progressive myxomatous valve degeneration, which mainly involves the mitral valve, causing incomplete coaptation of the leaflets with valvular regurgitation.[26] Although many affected dogs remain asymptomatic for years or even for life, its progression can cause severe left-sided volume overload and the elevation of LV filling and LA pressures, ultimately leading to congestive HF and death.[3,5,26]

The evaluation of disease severity is important for prognostication and treatment guide. To date, echocardiographic severity markers useful for prognostication in canine CVHD include LA and LV dilation, and the increased peak velocity of early diastolic transmitral flow wave (E-wave).[3–5] On the contrary, to our knowledge, the utility of the LA phasic function indices via time-LA area curve analysis as clinical severity markers in canine CVHD is still unknown.

Thus, the goal of chapter 2 was to elucidate the LA phasic function in different stages of disease severity in canine CVHD to determine its usefulness as a clinical severity marker.

2. MATERIALS AND METHODS

2.1 Animals

The study population was partially retrospectively (between October 2010 and July 2012, 17 dogs) and prospectively (between August 2012 and November 2013, 26 dogs) recruited from client-owned dogs with CVHD in the Veterinary Teaching Hospital of Graduate School of Veterinary Medicine, Hokkaido University. Informed owner consent was obtained. Each dog was included only once in the study. Diagnostic criteria for CVHD were the combination of the presence of mitral valve prolapse, any degree of mitral valve leaflet thickening by 2-D echocardiography, and the identification of any degree of mitral valve regurgitation by color Doppler examination, with or without mitral valve thickening.[5] Dogs were excluded if they had congenital heart disease, dilated cardiomyopathy, or clinically relevant concurrent systemic diseases.

Owner interviews and physical examinations were performed to provide the following data: age, sex, body weight, and medical history.

2.2 Conventional echocardiography

The echocardiographic equipment used in this chapter was as described in chapter 1. No dogs were sedated for echocardiographic tests. All data were stored

digitally and analyzed off-line. The mean of 3 cardiac cycles was calculated in all variables.

From a right parasternal short-axis view, M-mode variables of the LV including LV end-diastolic diameter (LVEDD) and end-systolic diameter (LVESD) were obtained and LV fractional shortening (LVFS) was calculated. The LVEDD was normalized for the body weight (LVEDDN) using the following formula: LVEDD/[body weight (kg)]$^{0.294}$.[27] The LVESD was normalized for the body weight (LVESDN) using the following formula: LVESD/[body weight (kg)]$^{0.315}$.[27] These normalizations were based on the results of regression analyses reported by Cornell et al.[27] The left atrial to aortic root ratio (LA/Ao) was obtained from a 2-D right parasternal short axis view.[28]

Pulsed-wave Doppler echocardiography was used to measure the transmitral flow velocity from a left apical 4-chamber view. Peak velocities of the early diastolic transmitral flow wave (E-wave) and late diastolic transmitral flow wave (A-wave) were measured, and the ratio of the E-wave to the A-wave was calculated. When the early and late diastolic transmitral waves were completely or partially fused, the E- and A-waves, and the ratio of the E-wave to the A-wave were not determined.

The TDI velocities of myocardial motion were recorded from the left apical 4-chamber view with the sample volume positioned at the septal mitral annulus.[29] The

peak velocities of the early diastolic wave (E'-wave), late diastolic wave (A'-wave), and systolic wave (S'-wave) of the myocardial velocity were measured, and the ratio of the E′-wave to the A′-wave and ratio of the E-wave to the E′-wave were calculated. When the early and late diastolic waves of the myocardial velocity were completely or partially fused, the E'- and A'-waves, the ratio of the E'-wave to the A'-wave, and the ratio of the E-wave to the E'-wave were not measured.

2.3 Left atrial phasic function indices

Two-dimensional speckle tracking echocardiography and the evaluation of the LA phasic function are as detailed in chapter 1. When a time-LA area curve had no diastasis portion, indices of the LA phasic function were not determined. The LAAmax, LAAp, and LAAmin were indexed for the body surface area calculated with the use of the formula [$(10.1 \times \text{body weight}^{2/3})/10^4$].

2.4 Clinical classification of dogs

In each dog, based on the presence of clinical signs and echocardiographic examinations and thoracic radiographs, CVHD was classified as stage B1, B2, C, or D according to the American College of Veterinary Internal Medicine (ACVIM) consensus.[30] Stage B1 includes dogs without clinical signs due to CVHD and echocardiographic evidence of cardiomegaly (ie, LA/Ao < 1.6 and LVEDDN <

1.85).[27,28,30,31] In this study, stage B1 dogs were regarded as a hospital control (control group) because: (1) it would be difficult to recruit demographically matched healthy controls for stage B2 to D dogs considering their demographics and the prevalence of CVHD, and (2) stage B1 dogs were deemed healthy except for having hemodynamically nonsignificant mitral regurgitation and expected to be relatively matched for demographics with stage B2 to D dogs. Stage B2 includes animals with echocardiographic evidence of cardiomegaly (ie, LA/Ao > 1.6 or LVEDDN > 1.85), but without clinical signs caused by CVHD (group B2). Stages C and D include dogs with clinical and radiographical signs of left-sided HF (ie, pulmonary edema). Stage C and D dogs were combined in one group (group C/D) due to the small sample size.

2.5 Statistical Analysis

Statistical analysis was performed with the use of commercially available software (JMP Pro 11.0; SAS Institute Inc., Cary, NC, U.S.A.). The level of significance was set at $P < 0.05$. The normal distribution of the data was confirmed by means of a Shapiro-Wilk test. For parametric data, the overall difference between groups (control, B2, and C/D) was determined by one-way ANOVA, and then post-hoc multiple comparisons were made using the Tukey-Kramer test. For non-parametric data, the overall difference between groups was determined with the Kruskal-Wallis test, and

then post-hoc multiple comparisons were made using the Steel-Dwass test.

The receiver operating characteristic (ROC) analysis with the Mann-Whitney *U* test with Bonferroni correction for multiple testing was used to assess the ability of echocardiographic variables to detect dogs with left-sided HF (ie, stage C/D) among dogs with hemodynamically significant mitral regurgitation and cardiomegaly (ie, stage B2 and C/D). The area under the ROC curve (AUC) and its 95% CI were calculated for each variable. The most optimal cutoff value was determined by the one with the highest Youden index. The cutoff values were applied and sensitivities and specificities were calculated for each variable.

3. RESULTS

A total of 43 dogs with CVHD, including 14 in the control group (stage B1), 17 in group B2, and 12 in group C/D were recruited (Table 3). Age was significantly different between the control and group C/D (P = 0.016, Table 3). No significant differences were observed among the groups in the body weight, or sex distribution. The most commonly represented breed was Chihuahua (n = 8), followed by Shih-tzu (n = 6), Cavalier King Charles Spaniel (n = 4), miniature Dachshund (n = 4), mixed breed (n = 4), miniature Schnauzer (n =3), Beagle (n = 2), Maltese (n = 2), and Pomeranian (n = 2). In addition, 8 other small- and medium-sized breeds with 1 dog each were enrolled. All dogs in the control group except for 2 dogs receiving angiotensin-converting enzyme inhibitors (ACEI) were administered no cardiac medications (Table 3). Some group B2 dogs were prescribed ACEI. The majority of group C/D dogs were treated with ACEI, pimobendan, and diuretics (loop diuretics and/or spironolactone).

The LVEDDN values increased significantly with disease severity (control versus group B2, P = 0.0013; control versus group C/D, P = 0.0001; group B2 versus group C/D, P = 0.029), while the LVESDN was not significantly different among the groups (Table 4). The median LVFS was significantly higher in group C/D than in the

control (P = 0.018). The LA/Ao values increased significantly with disease severity (control versus group B2, $P < 0.0001$; control versus group C/D, $P < 0.0001$; group B2 versus group C/D, $P = 0.0015$, Table 4).

Transmitral flow velocities could be determined in all except 1 dog (fusion of E- and A-waves due to high heart rate). The E-wave was higher in group C/D compared with the control and group B2 (group C/D versus control, $P < 0.0001$; group C/D versus group B2, $P = 0.0001$), as well as the ratio of the E-wave to the A-wave (group C/D versus control, $P < 0.0001$; group C/D versus group B2, $P = 0.0001$, Table 4). The A-wave was not significantly different among the groups.

The E'- and A'-waves, and the ratio of the E-wave to the E'-wave could be determined in all except 2 dogs (1 dog, fusion of E'- and A'-waves due to high heart rate; 1 dog, missing data), while the S'-wave could be measured in all except 1 dog (missing data). The E'-wave was significantly higher in group C/D than in the control (P = 0.010), whereas the A'- and S'-waves were not significantly different among the groups. The ratio of the E-wave to the E'-wave was higher in group C/D when compared to the control (P = 0.014), but not significantly different between group B2 and each of the control or group C/D (Table 4).

The LA phasic function indices could be calculated in all except 3 dogs (2

dogs, time-LA area curves without diastasis portion due to high heart rates; 1 dog, poor image quality). Significant increases with disease severity were observed in the body surface area-indexed LAAmax (control versus group B2, P = 0.002; control versus group C/D, P = 0.0001; group B2 versus group C/D, P = 0.0005, Table 4), LAAp (control versus group B2, P = 0.0036; control versus group C/D, P = 0.0001; group B2 versus group C/D, P = 0.0012), and LAAmin (control versus group B2, P = 0.0027; control versus group C/D, P = 0.0001; group B2 versus group C/D, P = 0.0005). There were no significant differences in the FACtotal among the groups. The FACpass was significantly higher in group C/D compared with the control (P = 0.0055). The FACact decreased significantly with disease severity (control versus group B2, P = 0.031; control versus group C/D, P = 0.0010; group B2 versus group C/D, P = 0.0058).

Table 5 shows the results of the ROC analysis. The LA/Ao, E-wave, the ratio of the E-wave to the A-wave, and the FACact had the ability to detect dogs with HF (group C/D) among dogs in groups B2 and C/D (Bonferroni-corrected $P < 0.05$). The LA/Ao > 2.25 resulted in sensitivity of 83% and specificity of 88% in detecting the presence of HF (Table 5). The E-wave > 1.27 m/s resulted in sensitivity of 92% and specificity of 94%, while the ratio of the E-wave to the A-wave resulted in sensitivity of 67% and specificity of 100%. The FACact resulted in sensitivity of 73% and specificity of 100%.

Table 3. Demographic variables and medical history among disease stages in 43 dogs with CVHD.

Variable	Control (Stage B1, n = 14)	Stage B2 (n = 17)	Stage C/D (n = 12)	Overall *P* value
Age (years)	9.5 (4–14)[b]	11 (5–15)[a,b]	12.5 (8–15)[a]	0.017 (A)
Weight (kg)	6.0 (2.2–10.4)[a]	7.0 (2.2–23.6)[a]	4.1 (2.0–11.9)[a]	0.14 (K)
Sex (No. of intact)				
Male	6 (2)	14 (4)	6 (3)	
Female	8 (5)	3 (2)	6 (3)	
Administered drugs				
ACEI	2	7	8	
Pimobendan	0	0	7	
Loop diuretics	0	0	6	
Spironolactone	0	2	3	
Digoxin	0	0	2	
Nitrates	0	0	2	
Beta-blocker	0	0	1	

Continuous data are expressed as the median (range). Values with different superscript letters indicate significant differences among groups. A = ANOVA. K = Kruskal-Wallis test.

Table 4. Echocardiographic parameters among disease stages in 43 dogs with CVHD.

Variable	Control (Stage B1, n = 14)	Stage B2 (n = 17)	Stage C/D (n = 12)	Overall *P* value
LVEDDN	15.0 (12.3–18.0)[c]	17.5 (14.1–22.3)[b]	20.1 (15.6–25.9)[a]	< 0.0001 (K)
LVESDN	8.2 (7.3–10.0)[a]	9.3 (5.6–12.4)[a]	9.5 (5.5–12.3)[a]	0.30 (A)
LVFS (%)	44.5 (31.0–51.1)[b]	47.1 (27.0–69.5)[a,b]	50.7 (44.8–65.4)[a]	0.023 (A)
LA/Ao	1.37 (1.09–1.54)[c]	1.90 (1.45–3.06)[b]	2.49 (1.92–3.74)[a]	< 0.0001 (K)
Transmitral flow (n = 42)*				
E-wave (m/s)	0.71 (0.51–1.03)[b]	0.74 (0.53–1.30)[b]	1.38 (1.06–1.79)[a]	< 0.0001 (K)
A-wave (m/s)	0.67 (0.44–0.99)[a]	0.87 (0.52–1.04)[a]	0.75 (0.43–1.26)[a]	0.097 (A)
E-wave: A-wave	1.09 (0.61–1.61)[b]	1.00 (0.60–1.69)[b]	1.96 (1.26–3.12)[a]	< 0.0001 (A)
TDI*				
E'-wave (cm/s, n = 41)	5.8 (3.9–9.7)[b]	6.2 (3.7–11.0)[a,b]	8.6 (4.1–10.8)[a]	0.0257 (A)
A'-wave (cm/s, n =41)	6.9 (5.1–11.8)[a]	7.4 (6.0–11.5)[a]	7.2 (4.3–11.0)[a]	0.26 (A)
S'-wave (cm/s, n = 42)	6.9 (5.0–12.2)[a]	8.9 (4.5–12.4)[a]	9.2 (5.4–20.2)[a]	0.045 (K)
E-wave: E'-wave (n = 41)	12.3 (6.5–18.2)[b]	14.3 (6.4–23.9)[a,b]	17.1 (12.6–34.4)[a]	0.014 (K)
LA phasic area indexed for body surface area (cm^2/m^2, n =40)*				
LAAmax	11.3 (7.7–16.4)[c]	18.5 (10.9–27.0)[b]	28.9 (22.2–65.2)[a]	< 0.0001 (K)
LAAp	9.1 (6.4–13.8)[c]	13.4 (7.9–21.3)[b]	19.8 (14.1–55.0)[a]	< 0.0001 (K)
LAAmin	6.2 (4.0–10.1)[c]	9.6 (5.9–15.4)[b]	16.0 (10.2–51.7)[a]	< 0.0001 (K)
LA fractional area change (%, n = 40)*				
Total	46.1 (37.4–58.0)[a]	45.1 (37.1–61.9)[a]	41.0 (20.7–57.8)[a]	0.37 (A)
Passive	18.1 (10.6–32.8)[b]	24.8 (10.8–46.5)[a,b]	28.5 (15.5–41.7)[a]	0.0076 (A)
Active	31.3 (26.9–47.5)[a]	28.4 (20.2–42.2)[b]	17.6 (6.1–32.2)[c]	< 0.0001 (K)

See Table 3 for key. *These variables were not recorded in all dogs.

Table 5. The AUCs with their 95% CIs and optimal diagnostic cutoffs for the detection of dogs with HF (group C/D) among dogs in groups B2 and C/D.

Variable	AUC	95% CI	Cutoff	Sensitivity	Specificity
LVEDDN	0.78	0.56–0.91	17.9	0.92	0.59
LVESDN	0.56	0.33–0.76	9.5	0.67	0.59
LVFS	0.67	0.45–0.83	44.8	1.00	0.41
LA/Ao	0.89†	0.69–0.97	2.25	0.83	0.88
Transmitral flow					
E-wave	0.97†	0.85–1.00	1.27	0.92	0.94
A-wave	0.64	0.38–0.83	0.78	0.75	0.69
E-wave: A-wave	0.89†	0.70–0.97	1.72	0.67	1.00
TDI					
E'-wave	0.74	0.49–0.90	8.4	0.67	0.93
A'-wave	0.66	0.43–0.84	5.8	0.33	1.00
S'-wave	0.55	0.33–0.76	12.5	0.25	1.00
E-wave: E'-wave	0.71	0.48–0.87	17.0	0.58	0.80
LA fractional area change					
Total	0.64	0.38–0.84	41.0	0.55	0.88
Passive	0.67	0.44–0.84	23.7	0.90	0.50
Active	0.86†	0.60–0.96	19.8	0.73	1.00

†These variables had the ability to detect the presence of HF (Bonferroni-corrected $P < 0.05$).

4. DISCUSSION

Results of this study indicate that the LA phasic function assessed by use of time-LA area curve analysis is impaired in advanced stages, especially in HF stage, and can be a useful clinical severity marker in canine CVHD. The FACact, the index of the LA booster pump function, was lower in stage B2 when compared to the control, and further reduced in stage C/D in comparison with stage B2. In addition, the FACact had the ability to detect dogs with left-sided congestive HF among dogs with hemodynamically significant mitral regurgitation.

In dogs with CVHD, the LA size, LV end-diastolic size, and E-wave have been shown as useful echocardiographic severity markers for detection of left-sided congestive HF and prognostication.[3,4,13,31,32] The LV and LA enlargement indicates the presence of chronic and hemodynamically significant mitral regurgitation, which can cause the elevation of the LV filling and LA pressures and left-sided congestive HF.[3] Also, the increased early diastolic pressure gradient between the LV and LA caused by the increased LA pressure causes the elevation of the E-wave.[3] Actually, in this study, the LA/Ao, LVEDDN, and E-wave were higher in dogs in advanced stages, particularly in HF stage, in accordance with previous studies in dogs with CVHD.[4,13]

However, in humans with mitral regurgitation, the evaluation of disease severity by use of the above-mentioned echocardiographic variables sometimes has not been accurate because of their several limitations in the presence of mitral regurgitation. The LV and LA sizes are increased to compensate for the increase in the LV filling and LA pressures, respectively, and also because of dysrhythmia (eg, bradycardia, atrial fibrillation). Thus, the LV and LA enlargement can be associated with normal or slightly elevated LV filling and LA pressures.[33] Also, the E-wave is determined by the LA stroke volume as well as the pressure gradient between the LV and LA during ventricular early diastole. Therefore, the E-wave can be elevated by the increased LA stroke volume associated with mitral regurgitation, despite a normal LA pressure.[33]

With this background, many recent studies in humans with mitral regurgitation have focused on whether the LA phasic function can be a more accurate severity marker than conventional ones.[7,34] Interestingly, a previous study has shown that in patients with mitral regurgitation, the LA dysfunction was the only independent predictor of long-term mortality after mitral valve surgery.[34] Therefore, also in dogs with CVHD, it needs to be clarified whether the LA phasic function can provide more accurate information regarding disease severity.

During LV systole (a reservoir phase), the LA is distended by blood inflow

from the pulmonary veins and stores energy in the form of pressure for the LV filling.[6,34] The LA reservoir function is determined by LA relaxation and stiffness (compliance), LV contraction leading to descent of the LV base, and right ventricular systole that modulates the pulmonary circulation.[6,34,35] In mitral regurgitation, there are several factors that can both enhance and inhibit the reservoir function. On one hand, the reservoir function may be enhanced associated with the increased LA compliance, which is initially observed in mitral regurgitation and because of LA eccentric hypertrophy,[34,36,37] and the LV hypercontraction caused by LV volume overload,[34] evidenced by the increase in the LVFS in group C/D. On the other hand, the reservoir function may be impaired by the decreased LA compliance because of ultrastructural changes of the LA myocardium including interstitial fibrosis and myocardial hypertrophy caused by chronic mitral regurgitation and LA volume overload.[34] Taken together, it is likely that the FACtotal was not different among the groups because these opposing factors could have counteracted each other.

During ventricular early diastole following the reservoir phase, the LA acts as a conduit allowing a passive blood transfer from the LA and pulmonary veins to the LV.[6,34] Determinants of the conduit function include LA recoil, LV relaxation, and the pressure gradient between the LV and LA during ventricular early diastole.[6,34] In the

presence of mitral regurgitation, the increased atrioventricular pressure gradient during LV early diastole can cause the enhanced conduit function.[34,37] On the contrary, ultrastructural changes of LA and LV wall may impair the LA recoil and LV relaxation, respectively, leading to the diminishment of the conduit function.[34] Collectively, in this study, it is possible that the FACpass was higher in group C/D compared with the control because enhancing effects of mitral regurgitation on the conduit function could have surpassed its inhibiting effects.

During LV late diastole (a booster pump phase), the LA contracts actively to finalize the LV filling.[6,34] The LA booster pump function depends on LA afterload (LV compliance and end-diastolic pressure), LA preload (the LA size before starting active contraction), and LA intrinsic contractility.[6,34,37] When mitral regurgitation is mild, the booster pump function is enhanced via the Frank-Starling mechanism associated with the increased LA preload,[6,34,37] which was supported by the increase in the LAAp indexed for body surface area in dogs in groups B2 and C/D. However, when mitral regurgitation becomes severe, the booster pump function is impaired due to the exhaustion of the Frank-Starling mechanism, the increased LA afterload (LV end-diastolic pressure), and the deteriorated intrinsic LA contractility associated with ultrastructural changes of the LA myocardium (eg, interstitial fibrosis and myocardial

degeneration).[34,37] The result on the FACact in this study is in agreement with that reported in a previous study in humans, where the LA booster pump function was impaired in patients with severe asymptomatic mitral regurgitation and was further reduced in those with severe symptomatic mitral regurgitation when compared to those with moderate or mild mitral regurgitation.[38]

Because this study was an observational study, medical treatments in each dog were not standardized. Cardiovascular drugs used in medical treatment can cause the changes in the intrinsic LA property and LA loading conditions (preload and afterload), and this could have affected the indices of the LA phasic function in this study. Therefore, in chapters 3 and 4, we will conduct experimental studies using healthy dogs to elucidate the effect of cardiovascular drugs and preload (volume load) on these indices.

This study had a few limitations. Firstly, it lacked invasive cardiovascular procedures and cardiac pathology, which would provide information about the LV filling and LA pressures, and LA intrinsic properties. Secondly, the sample size was small, which might have led to a type-2 error. Thirdly, this study was a cross-sectional study, and therefore the findings here do not prove causality between the LA dysfunction and the onset of left-sided HF.

In conclusion, the LA phasic function evaluated with time-LA area curve analysis based on 2-D speckle tracking echocardiography is impaired in advanced stages, especially in HF stage, of canine CVHD. Furthermore, the LA phasic function can be a useful severity marker for detection of left-sided congestive HF.

5. SUMMARY

In this chapter, we investigated the LA phasic function evaluated with time-LA area curve analysis based on 2-D speckle tracking echocardiography in different stages of disease severity in dogs with CVHD. Client-owned dogs with CVHD were recruited and classified into the control (stage B1), group B2 (stage B2), or group C/D (stages C or D) according to the ACVIM consensus. Among indices of the LA phasic function, the index of the booster pump function was lower in group B2 when compared to the control, and further reduced in group C/D in comparison with group B2. In addition, the index of the booster pump function had the ability to detect dogs with left-sided congestive HF. These results indicate that the LA phasic function assessed by time-LA area curve analysis is impaired in advanced stages, especially in HF stage, and can be a useful clinical severity marker in canine CVHD.

CHAPTER 3

EFFECTS OF VARIOUS CARDIOVASCULAR DRUGS ON INDICIES OF LEFT ATRIAL PHASIC FUNCTION IN HEALTHY DOGS

1. INTRODUCTION

In veterinary practice, medical treatment by use of cardiovascular drugs is the mainstay of the management of HF. Cardiovascular agents can modulate cardiac loading conditions (ie, preload and afterload), myocardial intrinsic performance (ie, contractility and lusitropy), cardiac electrophysiological properties, and/or neurohormonal factors (eg, the renin-angiotensin-aldosterone system).[39] Therefore, cardiovascular medications can change cardiac mechanical function by modulating these factors, and this would not only allow us to monitor treatment efficacy based on the assessment of cardiac function, but also may complicate the interpretation of the cardiac function.

Before indices of LA phasic function determined from time-LA area curve analysis can be used to evaluate disease severity or guide treatments in real clinical practice of canine heart disease, it is necessary to understand how each index is influenced by cardiovascular drugs. To our knowledge, basic information on the effects of cardiovascular agents on indices of LA phasic function in dogs is lacking.

Thus, the goal of chapter 3 was to elucidate the effects of 4 intravenous cardiovascular drugs (positive and negative inotropes, an inodilator, and a vasopressor) on the LA phasic function of dogs.

2. MATERIALS AND METHODS

2.1 Animals

Nine Beagles (3 males and 6 females; age, 1 to 3 years; body weight, 9.5 to 13.0 kg) that were part of a research colony owned by our laboratory were included in the study. All dogs were determined to be healthy with no cardiac abnormalities on the basis of results of a physical examination, ECG, and standard echocardiographic examinations (including M-mode and pulsed-wave and color-flow Doppler imaging) performed prior to study initiation. All procedures were approved by the Laboratory Animal Experimentation Committee of Graduate School of Veterinary Medicine at Hokkaido University.

2.2 Study protocol

Each dog was administered 1 cardiovascular drug among dobutamine hydrochloride (Dobutrex; Shionogi & Co. Ltd., Osaka, Japan), esmolol hydrochloride (Brevibloc; Maruishi Pharmaceutical Co. Ltd., Osaka, Japan), milrinone lactate (Milrinone; Takata Seiyaku Co. Ltd., Tokyo, Japan), or phenylephrine hydrochloride (Neosynesin Kowa; Kowa Pharmaceutical Co. Ltd., Tokyo, Japan) on an experimental day, and there was at least 14 days between experimental days. Each drug was given to

6 dogs; however, some dogs did not receive all 4 drugs because they were involved in other experiments and were unavailable on one or more experimental days (ie, dobutamine and esmolol were administered to dogs 1, 2, 3, 4, 5, and 6, and milrinone and phenylephrine were administered to dogs 4, 5, 6, 7, 8, and 9).

Each dog was sedated with intravenous propofol (Propofol Mylan; Mylan Inc., Canonsburg, PA, U.S.A.) at an induction dosage of 3 to 6 mg/kg and a maintenance rate of 0.4 to 0.6 mg/kg/min. The maintenance rate of propofol was adjusted as necessary to maintain sedation and spontaneous breathing. The dog was positioned in left or right lateral recumbency as necessary. Systolic, diastolic, and mean arterial blood pressures were monitored noninvasively with an oscillometric technique (BSM-5192; Nihon Kohden Co., Tokyo, Japan). The heart rate was monitored by an ECG-equipped (lead II) ultrasonographic unit (HI VISION Preirus; Hitachi Aloka Medical Ltd., Tokyo, Japan). Baseline echocardiographic measurements were obtained after blood pressure and heart rate stabilized, which was approximately 5 minutes after the initiation of the propofol infusion. Then the designated cardiovascular drug was administered, and the hemodynamic variables were allowed to stabilize (approximately 20 to 30 minutes after initiation of propofol administration) before the echocardiographic examination was repeated. Dogs were allowed to recover from sedation following completion of the

second echocardiographic examination.

2.3 Cardiovascular drugs

Dobutamine (5.0 μg/kg/min), esmolol (500 μg/kg/min), and phenylephrine (2.0 μg/kg/min) were administered intravenously as constant rate infusions (CRIs).[29,40,41] Milrinone (25 μg/kg) was administered as a slow intravenous bolus for 5 minutes followed by a CRI of 0.5 μg/kg/min.[42,43] All doses were determined on the basis of results of preliminary studies.

2.4 Conventional echocardiography

The echocardiographic equipment used in this chapter was as described in chapter 1. Echocardiographic measurements were made irrespective of the respiratory phase. All data were stored digitally and analyzed off-line. The mean of 3 consecutive cardiac cycles was calculated for all variables including those obtained by 2-D speckle tracking echocardiography.

As detailed in chapter 2, the LVEDD and LVESD were obtained, and the LVFS was calculated. The E- and A-waves were measured and the ratio of the E-wave to the A-wave was calculated, as described in chapter 2. The stroke volume was calculated by multiplying the time velocity integral, which was measured by tracing the Doppler aortic flow profile from the left apical 5-chamber view, with the luminal area of the

aorta, which was calculated by tracing the aortic lumen on the right parasternal transverse view.[44,45] The cardiac output was then calculated by multiplying the stroke volume by the heart rate during evaluation of the Doppler aortic flow profile. As detailed in chapter 2, the TDI variables including the S′-, E’-, and A’-waves were measured. The ratio of the E′-wave to the A′-wave and ratio of the E-wave to the E′-wave were also calculated.

2.5 Left atrial phasic function indices

Two-dimensional speckle tracking echocardiography and the evaluation of the LA phasic function are as detailed in chapter 1.

2.6 Statistical analysis

Normal distribution of the data was confirmed by means of a Shapiro-Wilk test. A commercially available statistical software program (JMP Pro 10.0; SAS Institute Inc., Cary, NC, U.S.A.) was used to develop a mixed linear model, with state (baseline versus after drug administration), drug (dobutamine, esmolol, milrinone, or phenylephrine), and the interaction between state and drug included as categorical fixed effects, and dog identification included as a random effect. The effects of state and drug on the values of the measured variables were assessed by the *F* test. For each drug, pairwise comparisons of measurements between baseline and after drug administration were

performed by calculation of the least squares means and use of the Bonferroni correction to account for multiple comparisons. For all analyses, values of $P < 0.05$ were considered significant.

3. RESULTS

Hemodynamic (Table 6), conventional echocardiographic (Table 7), and LA phasic function (Table 8) indices before (baseline) and after administration of dobutamine, esmolol, milrinone, and phenylephrine were summarized. There was a significant ($P < 0.05$) interaction between state (baseline or after drug administration) and drug for all variables except the A′-wave, the ratio of the E′-wave to the A′-wave, the ratio of the E-wave to the E′-wave, LAAmin, EApass, FACpass, and FACact.

Following administration of dobutamine, systolic and mean arterial pressures, LVFS, stroke volume, cardiac output, E- and A-waves, E'- and S'- waves, LAAmax, EAtotal, EAact, and FACtotal were significantly increased from baseline values. Conversely, the heart rate did not change significantly after dobutamine administration, whereas the LVESD was significantly decreased from the baseline.

Following administration of esmolol, the heart rate, LA phasic function indices, E- and A-waves, and E′- and A′-waves did not differ significantly from baseline values. Mean arterial pressure, cardiac output, stroke volume, LVFS, and S′-wave were significantly decreased and the LVESD was significantly increased from baseline values after esmolol administration.

Following administration of milrinone, there were no significant changes in the heart rate, systolic, diastolic, and mean arterial pressures, or any of the LA phasic function indices. The LVEDD and LVESD were significantly decreased, whereas the LVFS and S′-wave were significantly increased, compared with baseline values.

Following administration of phenylephrine, systolic, diastolic, and mean arterial pressures, LAAp, LVEDD, LVESD, and the ratio of the E-wave to the A-wave were significantly increased from baseline values. Conversely, the heart rate, cardiac output, LVFS, A-wave, and S′-wave were significantly decreased from baseline values. The LA emptying areas and fractional area changes were not altered significantly after phenylephrine administration.

Table 6. Least squares mean values (95% CI) for hemodynamic variables for 6 healthy adult Beagles before (baseline) and after administration of dobutamine, esmolol, milrinone, or phenylephrine.

Variable	Drug	Baseline	After drug administration
Heart rate (beats/min)	D	99 (88–109)	89 (79–100)
	E	105 (94–115)	110 (99–120)
	M	94 (83–105)	101 (90–111)
	P	94 (89–110)	62 (51–72)*
SAP (mmHg)	D	102 (92–112)	121 (111–132)*
	E	118 (107–128)	103 (92–113)
	M	105 (95–116)	92 (81–102)
	P	107 (97–118)	133 (123–144)*
DAP (mmHg)	D	48 (41–55)	59 (51–66)
	E	56 (48–63)	46 (38–53)
	M	48 (41–58)	41 (33–48)
	P	54 (46–61)	81 (73–88)*
MAP (mmHg)	D	73 (64–82)	88 (79–97)*
	E	88 (79–97)	72 (63–81)*
	M	76 (67–85)	67 (58–76)
	P	78 (69–87)	109 (100–118)*

D = Dobutamine. E = Esmolol. M = Milrinone. P = Phenylephrine. SAP = Systolic arterial pressure. DAP = Diastolic arterial pressure. MAP = Mean arterial pressure. *Value differs significantly ($P < 0.05$) from the corresponding baseline value.

Table 7. Least squares mean values (95% CI) for conventional echocardiographic variables for the dogs of Table 6.

Variable	Drug	Baseline	After drug administration
LVEDD (mm)	D	30.4 (28.7–32.1)	31.9 (30.2–33.6)
	E	30.1 (28.4–31.8)	31.3 (29.6–33.0)
	M	32.0 (30.3–33.7)	28.6 (26.9–30.3)*
	P	31.2 (29.5–32.9)	34.6 (32.9–36.3)*
LVESD (mm)	D	23.3 (21.3–25.3)	20.3 (18.3–22.3)*
	E	22.1 (20.1–24.1)	25.4 (23.4–27.4)*
	M	23.1 (21.1–25.1)	18.7 (16.7–20.7)*
	P	22.6 (20.6–24.6)	27.6 (25.6–29.6)*
LVFS (%)	D	23.5 (19.0–28.0)	36.2 (31.7–40.7)*
	E	26.9 (22.4–31.4)	18.6 (14.1–23.1)*
	M	27.9 (23.4–32.4)	35.2 (30.7–39.7)*
	P	27.7 (23.2–32.2)	20.0 (15.5–24.5)*
Stroke volume (mL)	D	27.7 (22.8–32.6)	35.8 (30.9–40.7)*
	E	28.3 (23.6–33.0)	22.5 (17.8–27.2)*
	M	27.1 (22.4–31.8)	24.1 (19.4–28.8)
	P	26.4 (21.7–31.8)	26.4 (21.7–31.1)
Cardiac output (L/min)	D	2.79 (2.38–3.19)	3.44 (3.04–3.84)*
	E	3.01 (2.62–3.39)	2.52 (2.13–2.91)*
	M	2.59 (2.20–2.98)	2.27 (1.88–2.66)
	P	2.43 (2.04–2.82)	1.78 (1.40–2.17)*
E-wave (m/s)	D	0.72 (0.64–0.80)	0.90 (0.82–0.98)*
	E	0.69 (0.61–0.77)	0.62 (0.54–0.70)
	M	0.70 (0.62–0.78)	0.67 (0.59–0.75)
	P	0.61 (0.53–0.69)	0.58 (0.50–0.66)
A-wave (m/s)	D	0.47 (0.40–0.54)	0.64 (0.57–0.70)*
	E	0.49 (0.42–0.55)	0.47 (0.40–0.53)
	M	0.47 (0.40–0.54)	0.40 (0.33–0.46)
	P	0.48 (0.41–0.54)	0.34 (0.27–0.41)*

E-wave: A-wave	D	1.61 (1.34–1.88)	1.47 (1.20–1.74)
	E	1.48 (1.20–1.75)	1.34 (1.07–1.61)
	M	1.52 (1.25–1.79)	1.71 (1.44–1.98)
	P	1.28 (1.01–1.55)	1.78 (1.50–2.05)*
E’-wave (cm/s)	D	5.0 (4.0–6.1)	7.4 (6.4–8.4)*
	E	5.5 (4.5–6.6)	4.7 (3.7–5.7)
	M	6.2 (5.1–7.2)	6.2 (5.2–7.2)
	P	5.8 (4.8–6.8)	5.6 (4.6–6.6)
A’-wave (cm/s)	D	4.9 (4.0–5.9)	6.1 (5.2–7.0)
	E	5.3 (4.4–6.3)	5.3 (4.3–6.2)
	M	5.1 (4.1–6.0)	4.2 (3.3–5.1)
	P	4.7 (3.7–5.6)	4.4 (3.5–5.3)
S’-wave (cm/s)	D	7.5 (6.4–8.5)	10.2 (9.2–11.3)*
	E	8.0 (7.0–9.0)	5.9 (4.9–6.9)*
	M	7.0 (5.9–8.0)	8.7 (7.7–9.8)*
	P	6.9 (5.9–7.9)	4.5 (3.5–5.5)*
E’-wave: A’-wave	D	1.12 (0.97–1.27)	1.12 (0.97–1.27)
	E	1.09 (0.94–1.24)	0.96 (0.81–1.11)
	M	1.19 (1.04–1.34)	1.33 (1.18–1.48)
	P	1.19 (1.04–1.34)	1.20 (1.05–1.35)
E-wave: E’-wave	D	13.9 (11.7–16.2)	13.4 (11.1–15.7)
	E	12.7 (10.5–15.0)	13.3 (11.0–15.6)
	M	12.5 (10.2–14.7)	12.1 (9.8–14.3)
	P	11.5 (9.2–13.7)	11.0 (8.7–13.3)

See Table 6 for key.

Table 8. Least squares mean values (95% CI) for LA phasic function indices obtained by 2-D speckle tracking echocardiography for the dogs of Table 6.

Variable	Drug	Baseline	After drug administration
LAAmax (cm^2)	D	5.71 (4.83–6.59)	6.94 (6.05–7.82)*
	E	5.65 (4.77–6.54)	5.42 (4.53–6.30)
	M	5.66 (4.77–6.54)	5.07 (4.18–5.95)
	P	5.35 (4.47–6.23)	6.44 (5.56–7.32)
LAAp (cm^2)	D	3.95 (3.31–4.59)	4.69 (4.05–5.33)
	E	4.07 (3.43–4.71)	4.08 (3.44–4.72)
	M	4.40 (3.76–5.04)	3.87 (3.24–4.51)
	P	4.15 (3.51–4.79)	5.16 (4.52–5.80)*
LAAmin (cm^2)	D	2.83 (2.34–3.32)	3.07 (2.58–3.56)
	E	3.01 (2.52–3.50)	3.03 (2.54–3.52)
	M	3.18 (2.68–3.66)	2.82 (2.33–3.31)
	P	3.18 (2.69–3.66)	3.79 (3.30–4.28)
Emptying area (cm^2)			
Total	D	2.88 (2.37–3.39)	3.87 (3.36–4.38)*
	E	2.64 (2.14–3.15)	2.39 (1.88–2.89)
	M	2.48 (1.98–2.99)	2.25 (1.74–2.75)
	P	2.17 (1.67–2.68)	2.65 (2.14–3.15)
Passive			
	D	1.72 (1.38–2.06)	2.20 (1.86–2.55)
	E	1.54 (1.19–1.88)	1.29 (0.95–1.64)
	M	1.29 (0.95–1.95)	1.24 (0.90–1.58)
	P	1.25 (0.91–1.59)	1.33 (0.99–1.67)
Active			
	D	1.14 (0.85–1.43)	1.64 (1.35–1.93)*
	E	1.08 (0.79–1.37)	1.07 (0.78–1.36)
	M	1.22 (0.92–1.51)	1.03 (0.74–1.32)
	P	0.95 (0.66–1.24)	1.34 (1.05–1.63)

Fractional area change (%)			
Total	D	50.2 (46.3–54.1)	56.3 (52.5–60.2)*
	E	45.9 (42.0–49.8)	43.1 (39.2–46.9)
	M	44.4 (40.5–48.2)	44.0 (40.1–47.8)
	P	41.7 (37.8–45.5)	41.8 (37.9–45.6)
Passive			
	D	30.2 (26.6–33.7)	32.1 (28.6–35.6)
	E	26.8 (23.3–30.4)	23.5 (20.0–27.0)
	M	23.3 (19.8–26.9)	24.0 (20.5–27.5)
	P	23.4 (19.8–26.9)	20.9 (17.4–24.5)
Active			
	D	28.2 (23.5–32.9)	35.3 (30.6–40.0)
	E	25.8 (21.1–30.5)	25.3 (20.6–30.0)
	M	27.6 (22.9–32.3)	26.4 (21.7–31.1)
	P	23.9 (19.2–28.6)	26.3 (21.6–31.0)

See Table 6 for key.

4. DISCUSSION

Results of this study demonstrated that echocardiographic measurements of LA phasic function did not change in parallel with those of the LV and were fairly stable following the administration of dobutamine, esmolol, milrinone, or phenylephrine in healthy dogs. To our knowledge, this was the first study conducted in dogs to evaluate the effects of various cardiovascular drugs on LA phasic function indices as measured by time-LA area curve analysis based on 2-D speckle tracking echocardiography.

The 4 drugs used in this study were chosen because they are commonly used in veterinary practice and have different mechanisms of action, which allowed us to elucidate the effects of each drug on LA phasic function indices and investigate the potential usefulness of those indices for guiding treatment decisions in clinically ill dogs. Dobutamine is a predominantly β-1 adrenergic agonist with positive inotropic and lusitropic effects and is used for the treatment of dogs with cardiogenic shock or acute pulmonary edema caused by CVHD or dilated cardiomyopathy.[46] Milrinone is a phosphodiesterase inhibitor with positive inotropic and lusitropic effects and vasodilatory activity (an inodilator),[42,46–48] which can be administered intravenously. Inodilators such as pimobendan are used to treat dogs with congestive HF, cardiogenic

shock associated with CVHD, or dilated cardiomyopathy.[46] Esmolol is an ultra-short acting, selective β-1 adrenergic antagonist with negative inotropic and lusitropic effects[29,40,49–52] and was selected because its effects were expected to contrast with those of dobutamine. Phenylephrine is an α-1 adrenergic agonist with vasoconstrictive effects[53] and was selected because its effects were expected to contrast with those of milrinone.

Changes in the 3 LA phasic functions are determined by cardiovascular factors other than intrinsic LA inotropy and lusitropy. The reservoir function is modulated by LV contraction that leads to descent of the LV base, right ventricular systolic pressure that is transmitted through the pulmonary circulation, and LA intrinsic relaxation and chamber stiffness.[6,42] The conduit function is modulated by LA recoil, LV relaxation, and the early diastolic pressure gradient between the LV and LA.[6] The booster pump function is modulated by LV compliance and end-diastolic pressure (ie, LA afterload) and LA intrinsic contractility.[6] Additionally, an increase in the blood volume of venous return (ie, LA preload) will enhance all 3 LA phasic functions,[6,49,54] and it is possible that drug-induced changes in LA intrinsic contractility and relaxation will not affect the indices of LA phasic function.

In the dogs of this study, administration of dobutamine caused a significant

increase in indices of LA reservoir and booster pump functions in a manner similar to that observed in healthy humans,[55,56] whereas administration of esmolol caused no significant change in LA phasic function, which was similar to the effects of esmolol administration to healthy cats.[57] This discrepancy in the effects of dobutamine and esmolol on LA phasic function could have several explanations. The negative inotropic and lusitropic effects of esmolol could have been offset by a compensatory response to drug-induced hypotension, which resulted in baroreflex-mediated vasoconstriction and an increase in venous return.[58] Also, healthy dogs have fewer β-adrenergic receptors in the LA than in the LV,[59] thus, the negative inotropic and lusitropic effects of esmolol on the LA might have been inadequate to cause a significant decrease in LA function, especially in the presence of the concurrent drug-induced effects on the LV.

To our knowledge, prior to this study, the effect of milrinone on LA function indices had not been investigated in healthy humans or animals. Although milrinone, like dobutamine, has positive inotropic and lusitropic actions,[42,46–48] its administration did not significantly affect LA phasic function of the dogs of the present study. It is possible that milrinone-induced vasodilation[42] caused a decrease in venous return and LA preload, which counteracted the drug's positive inotropic and lusitropic effects.[6,49,54] Indeed, in this study, a decrease in venous return following milrinone administration

was supported by a decrease in LVEDD.

Similar to milrinone, administration of phenylephrine did not significantly affect LA phasic function in the dogs of the present study. However, phenylephrine had the opposite effect of milrinone on LV function. Contrary to milrinone, phenylephrine might have suppressive effects on LA contractility and lusitropy in addition to its vasoconstrictive effect. A phenylephrine-induced increase in systemic blood pressure could impair LV systolic function, which in turn could adversely affect LA reservoir function. Impairment of LV contractility may have adverse effects on LV relaxation because of a reduction in suction effects, which then impairs conduit function.[60–62] Additionally, booster pump function may be adversely affected when LV end-diastolic pressure increases subsequent to an increase in venous return associated with vasoconstriction and a baroreflex-mediated decrease in the heart rate.[6,49,53–54] In this study, LVEDD was significantly increased from its baseline value following phenylephrine administration, which suggested that the increase in venous return associated with phenylephrine-induced vasoconstriction and the subsequent decrease in heart rate might augment all 3 LA phasic functions.[6,49,54,60] Thus, it is likely that the opposing effects of phenylephrine administration on LA phasic function counterbalanced each other in this study.

Results of this study suggested that indices of LA phasic function obtained by time–LA curve analysis were less sensitive than were indices of LV function for monitoring the efficacy of inotropic and lusitropic drugs. However, the present study involved only healthy dogs. It is possible that my findings cannot be extrapolated to dogs with clinical cardiac disease, associated with increased sympathetic activation and parasympathetic withdrawal, and a volume overloaded state,[36,62,63] and monitoring LA phasic function indices could provide useful guidance for the adjustment of cardiovascular drug doses in those dogs. Indeed, results of another study[64] indicate that administration of an inodilator improves LA reservoir and booster pump functions in human patients with HF.

The present study had a few limitations. Firstly, invasive cardiovascular procedures were not performed; LA pressure-volume loop analysis, an invasive procedure that is the gold standard for evaluation of LA phasic function, might have detected changes in LA intrinsic properties. Assumptions regarding venous return were made solely on the basis of echocardiographic variables. Secondly, the sample size was small, and not all dogs were available to receive all 4 cardiovascular drugs, which might have led to a type-2 error. Thirdly, the effects of sedation on the cardiac function could not be eliminated, and the study did not include a placebo, or control, treatment in

which dogs were sedated for echocardiographic examination without concurrent administration of a cardiovascular drug. We sedated the dogs with propofol because the level of sedation could be easily adjusted to minimize its effect on autonomic activity and mental status; however, propofol can impair LA and LV contractility and lusitropy[65,66] and cause vasodilation, which results in a reduction in preload.[66] Therefore, the positive inotropic and lusitropic and vasoconstrictive effects of the cardiovascular drugs used in this study might have been partially counterbalanced by the concurrent administration of propofol.

In conclusion, LA phasic function indices obtained via time–LA area curve analysis based on 2-D speckle tracking echocardiography were fairly stable and did not parallel changes in LV function indices following administration of dobutamine, esmolol, milrinone, or phenylephrine to healthy dogs.

5. SUMMARY

In this chapter, we investigated the effects of 4 intravenous cardiovascular drugs (dobutamine, esmolol, milrinone, and phenylephrine) on the LA phasic function indices obtained via time–LA area curve analysis based on 2-D speckle tracking echocardiography in healthy dogs. Compared with baseline values, indices for LA reservoir and booster pump functions and LV contractility and lusitropy were increased following dobutamine administration; indices for LA phasic function and LV lusitropy were unchanged and indices for LV contractility were impaired following esmolol administration; indices for LA phasic function and LV relaxation were unchanged and indices for LV systolic function were augmented following milrinone administration; and indices for LA phasic function and LV lusitropy were unchanged and indices of LV contractility were impaired following phenylephrine administration. These results indicate that echocardiographic measurements of LA phasic function do not change in parallel with those of the LV and are fairly stable following the administration of dobutamine, esmolol, milrinone, or phenylephrine in dogs.

CHAPTER 4

EFFECTS OF ACUTE VOLUME LOADING ON INDICIES OF LEFT ATRIAL PHASIC FUNCTION IN HEALTHY DOGS

1. INTRODUCTION

In advanced heart diseases, a volume overload states occurs as a result of sodium and water retention caused by neurohormonal alternations in compensation for arterial underfilling associated with reduced cardiac output. This compensatory mechanism temporarily plays a role in maintaining the cardiac output via the Frank-Starling mechanism, but eventually causes excessive elevation of pulmonary and systemic venous pressures, leading to congestive left and right HF, respectively.[39]

Accumulating findings in dogs show that volume load can affect LV systolic and diastolic functions via the Frank-Starling mechanism, and this may complicate echocardiographic evaluation of the LV function.[3] On the other hand, to our knowledge, basic findings regarding the effect of volume loading on indices of the LA phasic function based on time-LA area curve analysis in dogs are lacking. Such findings are necessary in order to use these indices to evaluate the disease severity and guide treatment options in clinical dogs in the future.

Therefore, the goal of chapter 4 was to elucidate the relationship between volume loading and indices of the LA phasic function determined by time-LA area curve analysis in dogs.

2. MATERIALS AND METHODS

2.1 Animals

Six healthy Beagles (3 males and 3 females; age, 1 to 3 years; body weight, 8.8 to 11.4 kg) that were part of a research colony owned by our laboratory were included in the present study. Each dog was determined to be healthy, with no cardiac abnormalities, as determined on the basis of the results of a complete physical examination, ECG, and standard echocardiographic examinations (including M-mode, pulsed-wave Doppler, and color flow Doppler imaging) performed prior to the start of the study to confirm normal cardiac anatomy and function. All procedures were approved by the Laboratory Animal Experimentation Committee, Graduate School of Veterinary Medicine, Hokkaido University.

2.2 Study protocol

In each dog, 20-gauge over-the-needle catheters were placed in the left and right cephalic veins for intravenous infusion, and a 24-gauge over-the-needle catheter was placed in the left or right dorsal pedal artery for direct arterial pressure monitoring. Each dog was administered atropine sulfate (0.05 mg/kg, subcutaneously; Atropine Sulfate Injection; Mitsubishi Tanabe Pharma Corp., Osaka, Japan), cefazolin sodium

hydrate (20 mg/kg, intravenously; Cefamezin α; Astellas Pharma Inc., Tokyo, Japan), and heparin sodium (100 U/kg, intravenously; Heparin Sodium Injection; Ajinomoto Pharmaceuticals Co. Ltd., Tokyo, Japan), and sedated with butorphanol tartrate (0.2 mg/kg, intravenously; Vetorphale; Meiji Seika Pharma Co. Ltd., Tokyo, Japan) and midazolam hydrochloride (0.1 mg/kg, intravenously; Dormicum Injection; Astellas Pharma Inc., Tokyo, Japan). Anesthesia was then induced by the administration of propofol (6 mg/kg, intravenously; Propofol Mylan; Mylan Inc., Canonsburg, PA, U.S.A.). Following endotracheal intubation, anesthesia was maintained with isoflurane (1.75 to 2.0%; Isoflu; DS Pharma Animal Health Co. Ltd., Osaka, Japan) in 100% oxygen. The end-tidal partial pressure of CO_2 was monitored and maintained between 35 and 45 mmHg by use of mechanical ventilation with a tidal volume of 10 to 15 mL/kg and a respiratory rate of 10 to 12 breaths/min. The heart rate and arterial pressure via arterial catheterization were monitored and recorded continuously with the use of a commercially available polygraph instrument (RMC-4000; Nihon Kohden Co., Tokyo, Japan).

Each dog was positioned in left lateral recumbency. A 6-F, 12-cm introducer sheath (FAST-CATH Hemostasis Introducers; St. Jude Medical Inc., Minnetonka, MN, U.S.A.) was percutaneously inserted into the right external jugular vein using the

Seldinger technique, and fluoroscopic guidance was used to advance a 5-F, 75-cm Swan-Ganz catheter (Swan-Ganz thermodilution catheter; Edwards Lifesciences Corp., Irvine, CA, U.S.A.) into the pulmonary artery. The catheter was connected to the polygraph equipment for the acquisition of hemodynamic data.

Following a stabilization period of about 10 minutes, baseline recordings of hemodynamic and echocardiographic variables were performed. After baseline measurements, preload was increased by the intravenous infusion of warmed lactated Ringer's solution (Solulact; Terumo Corp., Tokyo, Japan) at 150 mL/kg/h for 90 minutes. This dose was a modification of the dose reported in previous studies[67,68] and determined based on the results of preliminary studies. After starting infusion, hemodynamic and echocardiographic assessments were performed every 15 minutes. At each timepoint, hemodynamic recordings were conducted before echocardiographic examinations. After the final echocardiographic examination was completed, each dog was given furosemide (4 to 6 mg/kg, intravenously; Lasix Injection; Sanofi K. K., Tokyo, Japan) and allowed to recover from anesthesia.

2.3 Hemodynamic evaluation

Hemodynamic parameters were recorded and stored digitally during the brief cessation of mechanical ventilation. Parameters included the heart rate, mean arterial

pressure, mean pulmonary arterial pressure, mean pulmonary capillary wedge pressure (PCWP), mean right atrial pressure, and cardiac output. The pulmonary arterial and right atrial pressures were measured using the distal and proximal ports of a Swan-Ganz catheter, respectively. The PCWP was determined when the balloon at the end of a Swan-Ganz catheter was inflated to be wedged in a pulmonary capillary. Following pressure recordings, the cardiac output was determined by the thermodilution technique. Briefly, a bolus of 5 mL of cold saline was injected into the right atrium through the proximal port of a Swan-Ganz catheter, and the pulmonary arterial blood temperature was recorded by the thermistor at the catheter tip. The cardiac output was calculated from the area under the curve for the resulting blood temperature curve by the cardiac output computer equipped on the polygraph instrument. The mean of 5 consecutive cardiac cycles was calculated in pressures; and the mean of 4 measurements was determined for cardiac output.

2.4 Conventional echocardiography

The echocardiographic equipment used in this chapter was as described in chapter 1. Simultaneously with echocardiographic imaging, an ECG trace (lead II) was recorded by an ECG equipped on the ultrasonographic device, besides that on the polygraph instrument. All data were stored digitally and analyzed off-line. The mean of

3 consecutive cardiac cycles was calculated in all measurements.

As detailed in chapter 2, the E- and A-waves were measured, and the ratio of the E-wave to the A-wave was calculated. When the E- and A-waves were completely or partially fused, these variables were not determined. From the left apical 5-chamber view, the aortic Doppler flow profile was obtained with the sample volume placed immediately below the aortic valve. The LV ejection time was measured from the onset to the end of the aortic flow. The LV pre-ejection period was also measured as the interval from the start of the QRS complex to the beginning of aortic flow, and the ratio of the LV pre-ejection time to ejection time was calculated.[44,69] The pulsed Doppler-derived myocardial performance index was calculated with the use of the formula (a – b)/b, where "a" represents the interval from the cessation to the onset of transmitral flow and "b" represents the LV ejection time.[44,69]

The TDI parameters including S'-, E'-, and A'-waves were measured as in chapter 2. The ratio of the E'-wave to the A'-wave and ratio of the E-wave to the E'-wave were also calculated. In addition, the TDI-derived myocardial performance index was determined with the use of the following equation: (isovolumic contraction time + isovolumic relaxation time)/duration of systolic wave of myocardial velocity.[44,68] The isovolumic contraction time was the interval from the end of the late diastolic wave

to the onset of the systolic wave of myocardial velocity. The isovolumic relaxation time was the interval from the end of the systolic wave to the onset of the early diastolic wave of myocardial velocity. The duration of the systolic wave of myocardial velocity was the interval from the onset to the end of the systolic wave of myocardial velocity. The E'- and A'-waves, the ratio of the E'-wave to the A'-wave, and the ratio of the E-wave to the E'-wave were not measured when the E'- and A'-waves were completely or partially fused.

2.5 Left atrial phasic function indices

Two-dimensional speckle tracking echocardiography and the evaluation of the LA phasic function are as detailed in chapter 1. When a time-LA area curve had no diastasis portion, the LAAp, EApass, EAact, FACpass, and FACact were not determined.

2.6 Statistical analysis

Commercially available statistical software (JMP Pro, version 10.0; SAS Institute Inc., Cary, NC, U.S.A.) were used. For all analyses, the level of significance was set at $P < 0.05$. The normal distribution of the data was confirmed by means of a Shapiro-Wilk test. A linear mixed model was developed with time (baseline, 15, 30, 45, 60, 75, and 90 minutes) as a categorical fixed effect, and dog identity as a random effect.

The effect of time on the values of the measured parameters was assessed by the *F* test. Pairwise comparisons between the baseline and each timepoint were performed by obtaining least squares means and using Bonferroni correction to account for multiple comparisons.

In order to investigate the relationship between the PCWP and each of the indices of the LA phasic function, multiple regression analysis was performed with each of the LA function parameters as the dependent variable. In model 1, the PCWP and dummy coding of the enrolled dogs were included as covariates (linear regression model). In model 2, the linear and quadratic terms of the PCWP, and dummy coding of the enrolled dogs were entered as covariates (quadratic regression model). For each LA function parameter, model 2 was accepted if the effect of the quadratic term of the PCWP was significant, and a log-likelihood ratio Chi-square test revealed that model 2 significantly improved the fit when compared to model 1. Otherwise, model 1 was accepted for each parameter.

3. RESULTS

Hemodynamic data and echocardiographic parameters including indices of the LA phasic function at the baseline and during volume overloading are summarized in Tables 9–11. Significant time effects ($P < 0.05$) were detected for all hemodynamic and echocardiographic parameters except for 6 parameters (mean arterial pressure, the ratio of the E-wave to the A-wave, the S'-wave, the ratio of the E'-wave to the A' -wave, the ratio of the E-wave to the E'-wave, and the isovolumic relaxation time.

The mean PCWP was significantly increased at 15 to 90 minutes after starting infusion compared with the value at the baseline, and reached approximately 10 mmHg higher than the baseline value after about 45 to 60 minutes (Table 9). The heart rate was significantly higher at 60 minutes when compared to the value at the baseline. Furthermore, volume loading induced a significant increase in the mean pulmonary arterial pressure, right atrial pressure, and cardiac output at 15 to 90 minutes, compared with the baseline values.

The E- and A-waves, and the ratio of the E-wave to the A-wave were determined without fusion of the E- and A-waves in all dogs at the baseline and at 15 and 30 minutes, in 4 dogs at 45 and 90 minutes, and in 3 dogs at 60 and 75 minutes after

starting volume loading. There were significant increases in the E- and A-waves, and the LV ejection time, when compared to the values obtained at the baseline (Table 10). Also, compared with the baseline values, there were significant decreases in the LV pre-ejection period, the ratio of the LV ejection time to pre-ejection period, and the pulsed Doppler-derived myocardial performance index at 15 to 90 minutes.

The E'- and A'-waves, and the ratio of the E'-wave to the A'-wave were determined without fusion of the E'- and A'-waves in all dogs at the baseline and at 15 and 30 minutes, in 5 dogs at 45 minutes, in 3 dogs at 60 minutes, and in 4 dogs at 75 and 90 minutes after starting volume loading. Also, the ratio of the E-wave to the E'-wave was obtained in all dogs at the baseline and at 15 and 30 minutes, in 4 at 45 and 90 minutes, and in 3 at 60 and 75 minutes. Volume loading induced significant increases in the E'- and A'-waves, and the duration of the systolic wave of myocardial velocity at 15 to 90 minutes, when compared to the values at the baseline (Table 10). Furthermore, the TDI-derived myocardial performance index was significantly decreased at 15 to 90 minutes compared with the value obtained at the baseline. The isovolumic relaxation time was significantly decreased at 45 to 90 minutes when compared to the baseline value.

The LAAp, EApass, EAact, FACpass, and FACact were measured from

time-LA area curves with the diastasis portion in all dogs at the baseline and at 15 and 30 minutes, in 5 dogs at 45 minutes, in 4 dogs at 60 and 75 minutes, and in 3 dogs at 90 minutes after starting infusion. Volume loading caused significant increases in the LAAmax, LAAp, and total, passive, and active emptying areas and fractional area changes at 15 to 90 minutes, compared with the values obtained at the baseline (Table 11). The LAAmin was significantly increased at 30 to 90 minutes when compared to the baseline value.

In a multiple regression analysis with a log-likelihood ratio Chi-square test, model 2 (quadratic regression model) was accepted for the FACtotal and FACact. For the FACpass, model 1 (linear regression model) was accepted (Table 12, Figure 3).

Table 9. Least squares mean values (95% CI) obtained from the linear mixed model for hemodynamic data before (baseline) and during volume loading in 6 Beagles.

	Volume loading (min)						
Variable	**Baseline**	**15**	**30**	**45**	**60**	**75**	**90**
Heart rate (beats/min)	108 (102–113)	103 (98–109)	108 (103–114)	111 (105–116)	116 (110–121)†	111 (105–117)	108 (103–114)
Mean PAP (mmHg)	9.5 (7.4–11.6)	12.7 (10.5–14.8)*	15.0 (12.9–17.1)*	16.3 (14.2–18.5)*	17.3 (15.2–19.5)*	17.5 (15.3–19.6)*	17.5 (15.4–19.6)*
Mean PCWP (mmHg)	3.3 (1.3–5.4)	8.0 (6.0–10.0)*	10.2 (8.1–12.2)*	11.3 (9.3–13.4)*	12.7 (10.6–14.7)*	13.2 (11.1–15.2)*	14.0 (12.0–16.0)*
Mean RAP (mmHg)	0.3 (-0.9–1.6)	5.3 (4.1–6.6)*	6.3 (5.1–7.6)*	7.2 (5.9–8.4)*	7.3 (6.1–8.6)*	8.2 (6.9–9.4)*	8.3 (7.1–9.6)*
MAP (mmHg)	56 (49–62)	54 (48–61)	57 (50–63)	58 (52–65)	60 (53–66)	60 (54–67)	59 (53–66)
Cardiac output (L/min)	2.1 (1.9–2.4)	2.5 (2.3–2.8)*	2.8 (2.6–3.1)*	2.9 (2.7–3.2)*	3.0 (2.7–3.2)*	3.1 (2.9–3.4)*	3.1 (2.8–3.3)*

PAP = Pulmonary arterial pressure. RAP = Right atrial pressure. MAP = Mean arterial pressure. *,†Significant (*$P < 0.01$; †$P < 0.05$) time effect when compared to the baseline.

Table 10. Least squares mean values (95% CI) obtained from the linear mixed model for conventional echocardiographic parameters before (baseline) and during volume loading in 6 Beagles.

	Volume loading (min)						
Variable	**Baseline**	**15**	**30**	**45**	**60**	**75**	**90**
E-wave (m/s)§	0.64 (0.58–0.70)	0.81 (0.74–0.87)*	0.80 (0.74–0.86)*	0.80 (0.73–.087)*	0.85 (0.77–0.93)*	0.83 (0.75–0.91)*	0.78 (0.70–0.86)*
A-wave (m/s)§	0.34 (0.28–0.40)	0.45 (0.39–0.51)*	0.47 (0.41–0.53)*	0.49 (0.42–0.55)*	0.47 (0.39–0.54)*	0.50 (0.43–0.57)*	0.51 (0.44–0.58)*
E-wave: A-wave§	2.02 (1.69–2.35)	1.83 (1.51–2.16)	1.74 (1.42–2.07)	1.69 (1.31–2.06)	1.84 (1.42–2.27)	1.65 (1.23–2.08)	1.54 (1.16–1.91)
PEP (ms)	69 (63–74)	57 (51–62)*	52 (47–58)*	52 (47–58)*	52 (47–58)*	53 (48–59)*	53 (47–59)*
ET (ms)	182 (170–193)	226 (214–238)*	240 (229–252)*	248 (236–260)*	252 (240–264)*	260 (248–272)*	266 (254–278)*
PEP: ET	0.38 (0.35–0.41)	0.25 (0.22–0.28)*	0.22 (0.19–0.25)*	0.21 (0.18–0.24)*	0.21 (0.18–0.24)*	0.21 (0.18–0.24)*	0.20 (0.17–0.23)*
PD-MPI	0.49 (0.40–0.57)	0.35 (0.27–0.43)†	0.29 (0.21–0.38)*	0.29 (0.21–0.38)*	0.32 (0.23–0.40)*	0.30 (0.21–0.38)*	0.32 (0.23–0.40)*
E'-wave (cm/s)‖	6.3 (5.2–7.5)	9.2 (8.1–10.3)*	9.7 (8.5–10.8)*	9.4 (8.2–10.6)*	9.0 (7.8–10.3)*	8.8 (7.5–10.0)*	8.5 (7.3–9.7)*
A'-wave (cm/s)‖	3.6 (2.8–4.3)	4.7 (4.0–5.5)†	5.0 (4.3–5.8)*	5.3 (4.5–6.1)*	5.5 (4.6–6.4)*	4.9 (4.1–5.7)*	4.8 (4.0–5.7)†
S'-wave (cm/s)	5.4 (4.7–6.2)	5.3 (4.5–6.0)	5.5 (4.7–6.2)	5.4 (4.6–6.1)	5.5 (4.7–6.3)	5.4 (4.6–6.2)	5.3 (4.5–6.1)
E'-wave: A'-wave‖	1.81 (1.40–2.22)	2.04 (1.62–2.45)	2.00 (1.54–2.37)	2.00 (1.56–2.44)	1.61 (1.14–2.07)	1.87 (1.40–2.33)	1.81 (1.37–2.25)
E-wave: E'-wave§	10.3 (9.1–11.5)	8.9 (7.7–10.1)	8.5 (7.3–9.7)	8.6 (7.3–10.0)	9.5 (8.0–10.9)	9.5 (8.0–11.0)	9.3 (7.9–10.6)
IVCT (ms)	64 (50–79)	59 (45–74)	58 (43–72)	50 (35–64)*	43 (29–58)*	45 (31–60)*	43 (28–57)*
IVRT (ms)	57 (47–67)	58 (48–68)	58 (48–68)	59 (49–69)	62 (52–72)	56 (46–66)	59 (49–69)
S' duration (ms)	173 (162–183)	215 (205–226)*	226 (216–237)*	237 (226–247)	235 (224–246)*	243 (233–254)*	253 (242–263)*
TDI-MPI	0.71 (0.62–0.80)	0.55 (0.46–0.64)*	0.51 (0.42–0.61)*	0.46 (0.37–0.56)*	0.45 (0.36–0.54)*	0.42 (0.33–0.51)*	0.40 (0.31–0.39)*

PEP = LV pre-ejection period. ET = LV ejection time. PD-MPI = Pulsed Doppler-derived myocardial performance index. IVCT = Isovolumic contraction time. IVRT = Isovolumic relaxation time. S' duration = Duration of systolic wave of myocardial velocity. TDI-MPI = TDI-derived myocardial performance index. §Evaluation of all dogs at the baseline and at 15 and 30 minutes; 4 dogs at 45 and 90 minutes; 3 dogs at 60 and 75 minutes; flow patterns with fused E- and A-waves were discarded. ‖Evaluation of all dogs at the baseline and at 15 and 30 minutes; 5 dogs at 45 minutes; 3 dogs at 60 minutes; 4 dogs at 75 and 90 minutes; TDI with fused E'- and A'-waves were discarded. *See* Table 9 for reminder key.

Table 11. Least squares mean values (95% CI) obtained from the linear mixed model for LA phasic function indices obtained by 2-D speckle tracking echocardiography before (baseline) and during volume loading in 6 Beagles.

	Volume loading (min)						
Variable	**Baseline**	**15**	**30**	**45**	**60**	**75**	**90**
LAAmax (cm^2)	5.34 (4.65–6.02)	7.06 (6.37–7.75)*	7.76 (7.07–8.45)*	7.88 (7.19–8.57)*	7.92 (7.23–8.61)*	8.14 (7.45–8.83)*	8.13 (7.44–8.81)*
LAAp (cm^2)¶	3.91 (3.51–4.32)	4.70 (4.29–5.10)*	5.03 (4.62–5.43)*	4.97 (4.56–5.38)*	4.74 (4.31–5.16)*	4.84 (4.42–5.27)*	4.92 (4.48–5.36)*
LAAmin (cm^2)	3.34 (2.89–3.80)	3.70 (3.24–4.16)	3.88 (3.43–4.34)*	3.49 (3.48–4.39)*	3.89 (3.43–4.35)*	4.01 (3.55–4.47)*	4.00 (3.54–4.46)*
Emptying area (cm^2)							
Total	2.00 (1.62–2.38)	3.36 (2.98–3.74)*	3.87 (3.49–4.25)*	3.95 (3.57–4.33)*	4.03 (3.65–4.41)*	4.13 (3.75–4.51)*	4.13 (3.75–4.51)*
Passive¶	1.43 (1.08–1.77)	2.37 (2.02–2.71)*	2.73 (2.39–3.07)*	2.74 (2.37–3.10)*	3.00 (2.61–3.40)*	3.49 (3.10–3.89)*	3.16 (2.73–3.59)*
Active¶	0.57 (0.34–0.79)	0.99 (0.77–1.22)*	1.14 (0.92–1.37)*	1.09 (0.86–1.32)*	1.02 (0.77–1.27)*	1.03 (0.79–1.28)*	1.06 (0.80–1.33)*
Fractional area change (%)							
Total	37.3 (34.1–40.5)	47.5 (44.3–50.7)*	50.0 (46.7–53.2)*	50.0 (46.8–53.2)*	51.0 (47.8–54.2)*	51.0 (47.7–54.2)*	51.0 (47.8–54.2)*
Passive¶	26.6 (23.6–29.5)	33.4 (30.5–36.3)*	35.3 (32.4–38.2)*	35.4 (32.3–38.5)*	38.9 (35.6–42.3)*	39.3 (36.0–42.7)*	38.9 (35.2–42.6)*
Active¶	14.6 (10.3–18.9)	21.1 (16.8–25.4)*	22.6 (18.3–26.9)*	22.0 (17.6–26.5)*	21.7 (17.0–26.3)*	21.4 (16.8–26.0)*	21.7 (16.8–26.5)*

¶Evaluation of all dogs at the baseline and at 15 and 30 minutes; 5 dogs at 45 minutes; 4 dogs at 60 and 75 minutes; 3 dogs at 90 minutes; from time-LA area curves without the diastasis portion, only LAAmax, LAAmin, and total emptying area and fractional area change were calculated. *See* Table 9 for reminder key.

Table 12. Maximum likelihood estimates (95% CI), adjusted coefficients of determination (R^2), and log-likelihood ratio Chi-square tests of multiple linear and quadratic regression models with LA phasic function indices as dependent variables.

	Model 1 (Linear regression)		Model 2 (Quadratic regression)			Log-likelihood ratio Chi-square test	
Variable (Number of observations)	**PCWP**	**R^2**	**PCWP**	**$PCWP^2$**	**R^2**	**Accepted**	***P* value**
Fractional area change							
Total (42)	1.01 (0.69–1.34)#	0.57	3.23 (2.51–3.94)#	-0.11 (-0.15– -0.079)#	0.80	Q	< 0.0001
Passive (34¶)	1.24 (0.94–1.54)#	0.76	1.81 (0.66–2.96)#	-0.035 (-0.10–0.033)	0.76	L	0.30
Active (34¶)	0.81 (0.49–1.12)#	0.75	2.21 (1.15–3.28)#	-0.086 (-0.15– -0.023)#	0.80	Q	0.0038

$PCWP^2$ = Quadratic term of PCWP. L = Linear regression model (model 1). Q = Quadratic regression model (model 2). #Significant ($P < 0.05$) effect in a model. *See* Table 11 for reminder key.

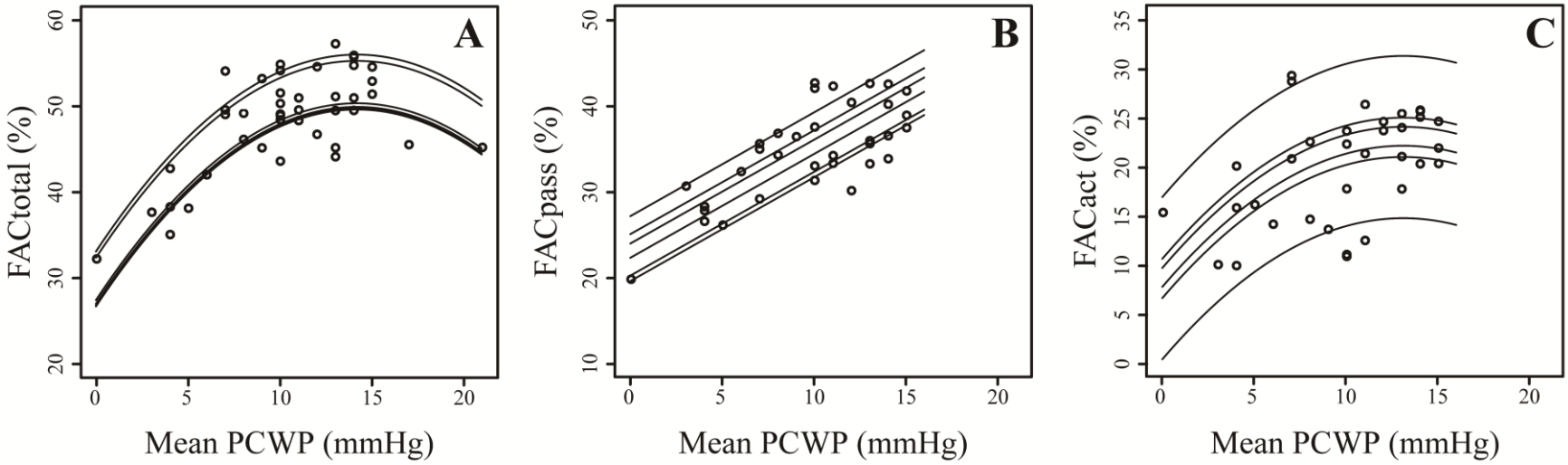

Figure 3. Relationships between the PCWP and indices of the LA phasic function in 6 Beagles: the FACtotal (A), FACpass (B), and FACact (C). The regression lines in each of the 6 dogs (solid lines) are indicated for each panel. Quadratic regressions were fitted better to the relationships between the PCWP and each of the FACtotal and FACact, compared with linear regressions. Linear regressions were fitted better for predicting the relation between the PCWP and FACpass.

4. DISCUSSION

The goal of this study was to obtain basic findings in dogs, regarding changes in indices of the LA phasic function assessed by means of time-LA area curve analysis in response to volume loading. This is in order to elucidate the relationship between these indices and changes in the volume load caused by disease progression or cardiovascular medications in clinical dogs in the future. Findings here show that the LA phasic function determined based on this technique augments during volume loading in dogs. In addition, results of this study may indicate that the relationships between volume loading and each of the reservoir and booster pump functions are quadratic rather than linear, while the conduit function enhances linearly with volume loading.

Volume overload could both enhance and impair the LA phasic function. Volume loading augments the booster pump function by the Frank-Starling mechanism associated with an increased LA preload,[6,49,54] as evidenced by the increase in the LAAp in this study. Also, the increase in volume load enhances the reservoir function by augmenting the LA booster pump function, the LV systolic function, as supported by the decreases in the ratio of the LV pre-ejection time to ejection time and the LV isovolumic

contraction time in this study, and the cardiac output, by the Frank-Starling mechanism.[6,49,54] Furthermore, volume loading augments the conduit function by increasing the LV relaxation and early diastolic pressure gradient between the LV and LA,[6] as suggested by the increases in the E- and E'-waves in this study as well as in previous studies.[67,68] On the contrary, the increased LV end-diastolic pressure (ie, LA afterload), as evidenced by the increase in the PCWP in this study, could impair the booster pump function (ie, afterload mismatch). Also, a previous experimental study in healthy dogs showed that volume loading decreases the LA compliance, which could suppress the reservoir function.[54] Furthermore, when the LA and LV are markedly dilated and operate on the descending limb of the Frank-Starling curve during volume loading, their contractility will decrease, causing the impairment of reservoir and booster pump functions.[6,38] Taken together, in this study, it is indicated that the enhancing effect of volume loading on the 3 phasic functions could have surpassed its suppressive effect. In addition, it is also suggested that during later phases of volume loading, its enhancing effect on the reservoir and booster pump functions could have been offset by its suppressive effect.

The findings here have several clinical implications. Firstly, these data indicate that the detection of the LA dysfunction based on indices of the LA phasic areas might

be precluded by the enhancing effect of volume loading on the LA phasic function. This could be of concern in dogs with CVHD, especially that associated with a milder severity where the optimal Frank-Starling relationship is not exceeded in the LA and LV, and the afterload mismatch does not occur in the LA. Secondly, considering the quadratic relationships between volume loading and parameters of reservoir and booster pump functions, it is possible that they are more useful in evaluating the disease severity when compared to those of the conduit function. Actually, in humans with asymptomatic mitral regurgitation, it has been demonstrated that all of the LA reservoir, conduit, and booster pump functions evaluated based on the LA phasic volumes are enhanced.[37] On the other hand, in people with severe mitral regurgitation requiring mitral surgery, it has been reported that the LA reservoir and booster pump functions are deteriorated, while the conduit function is not. [34]

Furthermore, the results of this study suggest that the assessment of hemodynamic improvement with cardiovascular medications based on parameters of the LA phasic areas can be obscured because the decrease in the volume load might mask the augmentation of the LA phasic function. This could be of particular concern in treatments with diuretics and inodilators. However, this assumption based on findings here in healthy dogs may not be extrapolated to clinical dogs with changes in the LA

intrinsic properties and severe volume overload. Actually, it has been reported that an inodilator enhances LA reservoir and booster pump functions despite a decrease in the LA volume load in human patients with heart failure.[64]

This study had several limitations. Firstly, these data could not be extrapolated to dogs with higher levels of PCWP. In a previous study of healthy dogs given an infusion of dextran solution, indices of LA reservoir and booster pump functions calculated by the LA diameters determined by a sonomicrometer were decreased to baseline levels at mean LA pressures above about 15 mmHg.[54] Secondly, a complete autonomic block was not used in this study. Especially, in dogs, it has been reported that acute volume loading by fluid infusion can cause the Bainbridge reflex, by which the withdrawal of vagal tone and an associated increase in the heart rate can occur.[70] This might have led to a state of sympathetic dominance, augmenting the LA function. Thirdly, the effects of anesthesia on the cardiac function could not be eliminated. Isoflurane can cause the impairment of myocardial contractility and lusitropy in the LV and LA.[71] Thus, the enhancing effect of volume loading on the LA function might have been blunted by the usage of isoflurane in this study.

In conclusion, the LA phasic function determined with the time-LA area curve analysis based on 2-D speckle tracking echocardiography was enhanced during volume

loading in healthy dogs. The effect of the volume load should be considered when evaluating the LA phasic function by use of this technique in dogs with heart diseases.

5. SUMMARY

In this chapter, we investigated the relationship between volume loading and the LA phasic function indices determined with time-LA area curve analysis based on 2-D speckle tracking echocardiography in healthy dogs. Compared with baseline values, indices for LA reservoir, conduit, and booster pump functions were increased following volume loading by use of intravenous fluid administration. In addition, the relationships between volume loading and each of the indices of the reservoir and booster pump functions are quadratic, whereas the index of the conduit function enhances linearly with volume loading. These results indicate that the LA phasic function assessed with time-LA area curve analysis augments during volume loading in dogs.

GENERAL CONCLUSION

The goal of this study was to establish the clinical usefulness of the evaluation of the LA phasic function via time-LA area curve analysis based on 2-D speckle tracking echocardiography in canine heart disease. The findings of the present study indicate that this technique can be used for the assessment of the clinical severity in dogs with heart disease. Furthermore, we have obtained basic findings in healthy dogs about the relationships between the LA phasic function and the changes in hemodynamics caused by cardiovascular drugs and volume loading. These will allow for the elucidation of the interrelations between the LA phasic function and the hemodynamic changes observed in clinical dogs with heart disease in the future.

In chapter 1, we have determined the feasibility, repeatability, and reproducibility of LA phasic function indices based on time-LA area curve analysis in healthy dogs. All of the obtained echocardiographic images could be used for time-LA area curve analysis with sufficient quality, and agreement between this technique and manual tracing was good for all variables. Further, the within- and between-day CVs for all variables including the FACtotal (reservoir function), FACpass (conduit function), FACact (booster pump function) determined via this technique were clinically

acceptable. Thus, these findings demonstrate that time-LA area curve analysis can be applied for the evaluation of the LA phasic function in dogs.

In chapter 2, we have investigated the LA phasic function in different stages of disease severity in dogs with CVHD. Client-owned CVHD dogs were recruited and classified into 3 groups based on the ACVIM consensus statement: the control (stage B1), group B2 (stage B2), or group C/D (stage C or D). Among the LA phasic function indices, the FACact was lower in group B2 compared with the control, and further reduced in group C/D in comparison with group B2. Furthermore, the FACact had the ability for the detection of dogs with left-sided congestive HF. Therefore, these data demonstrate that the LA phasic function assessed with time-LA area curve analysis is impaired in advanced stages, in particular in HF stage, and can be used as a clinical severity marker in dogs with CVHD.

In chapter 3, we have investigated the effects of 4 intravenous cardiovascular drugs (ie, dobutamine, esmolol, milrinone, and phenylephrine) on the LA phasic function indices in healthy dogs. Compared with baseline values, the FACtotal, FACact, and indices for LV contractility and lusitropy were increased by dobutamine infusion; all LA phasic function indices (ie, FACtotal, FACpass, FACact) and those for LV lusitropy were unchanged and indices for LV contractility were decreased by esmolol

infusion; indices for all of the LA phasic functions and LV relaxation were unchanged and indices for LV systolic function were increased by milrinone infusion; and indices for all LA phasic functions and LV lusitropy were unchanged and indices for LV contractility were decreased by phenylephrine infusion. Thus, these results demonstrate that the LA phasic function evaluated by use of time-LA area curve analysis does not parallel the changes in the LV function and are fairly stable after the administration of dobutamine, esmolol, milrinone, or phenylephrine in dogs.

In chapter 4, we have investigated the effect of volume loading on the LA phasic function indices in healthy dogs. Compared with baseline values, all LA phasic function indices (ie, FACtotal, FACpass, and FACact) were increased following volume loading with intravenous fluid administration. In addition, the relationships between volume loading and each of the FACtotal and FACact were quadratic, whereas the FACpass increased linearly with volume loading. Therefore, these findings demonstrate that the LA phasic function assessed with time-LA area curve analysis enhances during volume loading in dogs.

In order to clarify the role of the LA phasic function in the onset of HF in dogs with heart disease in the future, longitudinal follow-up studies that can confirm associations between the onset of HF and the changes in the LA phasic function in

clinical dogs are needed. In addition, it is necessary to investigate the relationship between the invasive hemodynamic measurements, the gold standard for the objective assessment of HF status, and the LA phasic function in canine heart failure model. Also, further studies that can determine the changes in the LA phasic function during medical treatments of dogs with heart disease are needed to establish the usefulness of the evaluation of the LA phasic function in guiding treatments by use of cardiovascular medications. Furthermore, future studies should include dogs with heart diseases other than CVHD, such as cardiomyopathies and congenital heart diseases.

In conclusion, through this study we were able to establish the applicability of the evaluation of the LA phasic function via time-LA area curve analysis with 2-D speckle tracking echocardiography in assessing disease severity of canine heart disease. Additionally, we were able to clarify basic findings in healthy dogs on the relationships between the LA phasic function and hemodynamics. The LA phasic function determined with time-LA area curve analysis is potentially useful as a novel severity maker in dogs with heart disease.

JAPANESE SUMMARY（要旨）

Application of evaluation of left atrial phasic function via time-left atrial area curve analysis based on two-dimensional speckle tracking echocardiography in canine heart disease

（2D Speckle Tracking 心エコー図法による時間－左心房断面積曲線解析を用いた左心房相機能評価の犬心疾患への応用）

心エコー図検査による心疾患の重症度評価は、予後推定や治療方針決定のためにヒト、犬の双方において必須なものとなっている。重症度評価の従来の評価対象は、全身に血液を拍出するその重要性と多くの心疾患の主病変であることから、左心室の形態や機能（収縮能・拡張能）であった。近年医学では、評価対象として、左心室の拡張能を調節する役割を持つ左心房の３相からなる機械的機能（左心房相機能：Reservoir、Conduit、Booster pump 機能）が注目され、予後推定等におけるその有用性が確立されている。とりわけ現在は、新たな技術である 2D Speckle Tracking 心エコー図法による左心房相機能評価が盛んに研究され、重症度評価における有用性が報告され始めている。一方、犬の心疾患においては、左心房相機能についての研究は限られておりその有用性は不

明である。さらには、2D Speckle Tracking 心エコー図法を用いた左心房相機能評価についてはこれまで応用されていない。そこで本研究では、2D Speckle Tracking 心エコー図法による左心房相機能評価の犬心疾患における臨床的有用性を確立するために４段階からなる実験を行った。

第１段階として、2D Speckle Tracking 心エコー図法による左心房相機能評価の犬への応用可能性を検討した。健常犬に対して本法を実施することにより時間-左心房断面積曲線を作成し、相機能を表す 3 つの断面積変化率（FACtotal : Reservoir 機能、FACpass : Conduit 機能、FACact : Booster pump 機能）の算出を試みた。その結果、本法実施のために取得した心エコー動画の全てにおいて全３指標の算出が可能であり、また、同動画を用いて用手法により算出した３指標の数値とも良好に一致した。さらには、各３指標の日内・日間変動係数は全て２０％以下であり、検査再現性は犬への臨床応用に適したものであった。以上の結果から、本法による左心房相機能評価が犬において応用可能であることが示された。

続いて第２段階として、本法による左心房相機能評価の犬心疾患の重症度評価への応用可能性を検討した。犬において最も多い心疾患である慢性僧帽弁疾患の臨床例を包含し、アメリカ獣医内科学会（ACVIM）のガイドラインに基づいてコントロール群（Stage B1：心拡大なし）、B2 群（Stage B2：心拡大あ

り、うっ血性左心不全なし)、C/D 群（Stage C または D : うっ血性左心不全あり）の３群に分類した。各症例において本法により左心房相機能評価を行ったところ、相機能を表す３指標の中で、FACact はコントロール群と比較して B2 群において低下しており、さらには B2 群と比較して C/D 群においてさらに低下していた。加えて ROC 解析の結果、FACact はうっ血性左心不全の検出に有用であることが示された。以上の結果より、本法による左心房相機能評価は犬心疾患の重症度評価において有用であることが示唆された。

次いで第３段階として、左心房相機能評価が臨床例における薬物療法の効果判定に利用できるかを検討するための基礎的実験として、健常犬を用いて心血管薬投与による血行動態の変化が左心房相機能へ及ぼす影響を研究した。心血管薬としてはドブタミン（陽性変力薬)、エスモロール（陰性変力薬)、ミルリノン（強心性血管拡張薬)、フェニレフリン（血管収縮薬）を用いた。ドブタミン投与により FACtotal、FACact、左室収縮能指標、左室拡張能指標は機能亢進側へ変化した。エスモロール投与により左心房相機能指標（FACtotal、FACpass、FACact)、左室拡張能指標は変化を示さず、左室収縮能指標は機能低下側へ変化した。ミルリノン投与により左心房相機能指標、左室拡張能指標は変化を示さず、左室収縮能指標は機能亢進側へ変化した。フェニレフリン投与により左心房相機能指標、左室拡張能指標は変化を示さず、左室収縮能指標は

機能低下側へ変化した。これらの結果から、犬において左心房相機能は左心室機能とは異なり、心血管薬による血行動態の変化に対し変化を示さず安定的であることが示された。

さらに第４段階として、犬の心疾患症例において左心房相機能と容量負荷の関係性を解析するための基礎的実験として、健常犬を用いて過剰静脈内輸液による容量負荷が左心房相機能へ及ぼす影響を研究した。容量負荷により左心房相機能を表す３指標全てが機能亢進側へ変化した。さらに、FACtotal および FACact は容量負荷により上昇した後に低下し始める二次曲線的変化を示したのに対し、FACpass は容量負荷により上昇し続ける一次直線的変化を示した。以上の結果から、犬において左心房相機能は容量負荷により亢進することが明らかとなった。

今後明らかにすべき研究課題の１つとして、犬心疾患における心不全発現と左心房相機能の間の因果関係の検討が挙げられる。そのためには、心疾患症例の左心房相機能の経時的変化を観察する追跡研究や、心不全モデル犬において左心房相機能と侵襲的な血行動態指標との関係性を検討する実験研究が必要である。加えて、犬心疾患の内科療法の治療効果判定における左心房相機能評価の有用性についても明らかにしていきたい。そのためには、内科療法中の症例において左心房相機能の変化を経時的に観察していく必要がある。さらに

は、慢性僧帽弁疾患のみならず心筋症や先天性心疾患などの他の心疾患の重症度評価における左心房相機能評価の有用性も明らかにしたいと考えている。

最後に、本研究により 2D Speckle Tracking 法を用いた左心房相機能評価が犬心疾患の重症度評価に有用である可能性が示された。また、種々の血行動態の変化と左心房相機能の関係性についての健常犬における基礎的知見を集積することができた。今後、左心房相機能評価による正確な重症度評価を通した、犬心疾患のより適切な管理・治療の実現が期待される。

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