Echocardiographic Estimation of Left Atrial Pressure in Beagle Dogs with Experimentally-Induced Mitral Valve Regurgitation

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ABSTRACT. Non-invasive and immediate estimation of left atrial pressure (LAP) is very useful for the management of mitral regurgitation (MR), and many reports have assessed echocardiographic estimations of LAP to date. However, it has been unclear of which examination and evaluate article possess the best accuracy for the MR severity. The present research aims to establish the echocardiographic estimation equation of LAP that is well applicable for clinical MR dogs. After the chordae tendineae rupture was experimentally induced via left atriotomy in six healthy beagle dogs (three males and three females, two years old, weighing between 9.8 to 12.8 kg), a radio telemetry transmitter catheter was inserted, which allows the continuous recordings of LAP without the use of sedation. Approximately 5 weeks after the surgery, echocardiographic examination, indirect blood pressure measurement, measurement of plasma atrial natriuretic peptide (ANP) and LAP measurement by way of the radio telemetry system was performed simultaneously. Subsequently, simple linear regression equations between LAP and each variable were obtained, and the equations were evaluated whether to be applicable for clinical MR dogs. As a result, the ratio of early diastolic mitral flow to early diastolic lateral mitral annulus velocity (E/Ea) had the strongest correlation as maximum LAP=7.03*(E/Ea)–54.86 (r=0.74), and as mean LAP=4.94*(E/Ea)–40.37 (r=0.70) among the all variables. Therefore, these two equations associated with E/Ea should bring more precise and instant estimations of maximum and mean LAP in clinical MR dogs.

KEY WORDS: atrial natriuretic peptide, mitral annulus velocity, mitral inflow, radio telemetry system.

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Mitral valve regurgitation (MR), secondary to degenerative mitral valve apparatus disease (MVD), is one of the most common cardiac disorders in dogs [1]. Although surgical interventions such as mitral valve repair [11] and replacement [3] have been reported, most owners do not pursue these surgical options due to the cost or the small number of facilities able to perform such interventions. Therefore, most patients follow appropriate drug therapy on the basis of adequate evaluation of clinical symptoms.

In dogs with moderate to severe MR, left atrial pressure (LAP) rises markedly as a result of volume overload and regurgitation [1, 27]. Echocardiography has been one of the most valuable modalities for evaluating left atrial and ventricular dilation and slower MR velocity which is caused by the elevated LAP [7]. A number of reports have described the echocardiographic estimation of LAP and its alternative, which is the left ventricular filling pressure (LVFP), in humans [5, 6, 20] and in veterinary medicine [21, 26]. Recently, the usefulness of isovolumic ventricular relaxation time (IVRT) [24], pulmonary venous flow (PVF) [14], plasma atrial natriuretic peptide (ANP) concentration [2, 17] and tissue Doppler imaging (TDI) [10] have also been reported as noninvasive assessment of LAP in volume overload models and clinical MR patients including human and dogs. However, there were problems with dogs that were used in these studies.

Some studies used healthy dogs under acute volume-overload condition [20, 24], which may differ from the clinical cases in the pathophysiology. For example, the *v* wave, which should be higher than the *a* wave in the LAP cycle in dogs with MR, might be lower than *a* wave in these acute volume-overload models because there is no regurgitation at the time when the *v* wave appears. Therefore, since the peak of the early mitral inflow (E wave) velocity is induced by the *v* wave during the diastolic period, the observed E wave in the acute volume-overload model should be lower than that observed in clinical MR. The volume-overload models should not be used as the imitation of clinical MR dogs.

Additionally, even when dogs with MR were used, the studies were performed under general anesthesia [21]. Any anesthesia has more or less effects on hemodynamic status including LAP, which obviously indicates that LAP measurement under awaking condition is more desired. In these studies, LAP was recorded shortly after the rupture of the chordae tendineae [21]. Failing hearts in clinical settings gradually shows the structural remodeling due to the compensation effects with time, which suggests strongly that the recordings shortly after MR creation is inappropriate to apply in the clinical cases.

Therefore, these echocardiographic evaluations should be investigated in conscious dogs with chronic MR if we wish to apply the equations in a clinical setting.

In the present study, the radio telemetry system, which

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enables continuous recording of LAP without the use of any anesthesia, was used in six beagle dogs, approximately five weeks after the creation of experimentally-induced MR. Simultaneously with LAP measurement, echocardiographic examinations, blood pressure measurements and measurement of plasma ANP concentration were also performed. Regression equations were established on the basis of those evaluations, and their utility in clinical MR patients is discussed on the basis of the extrapolation method.

MATERIALS AND METHODS

Study 1. Constructing regression equations between LAP and echocardiographic and ANP values in experimentally-induced MR dogs

Animals: Six 2-year-old beagle dogs (three males, three females) weighing 9.8 to 12.8 kg [11.1 ± 0.98 kg (mean ± SD)] were used. Dogs were housed in individual metal cages (size: W 90 cm × D 100 cm × H 110 cm) in an air-conditioned room (temperature: 22 ± 2°C, humidity: 50 ± 10%). Fresh drinking water was freely accessible and the dogs were fed commercial dry food (Healthy Label, Nisshin Pet Food Inc., Tokyo, Japan) twice daily. This study was approved by the Tokyo University of Agriculture and Technology (Approval number: 20–70), and these dogs were managed and cared for in accordance with the standards established by Tokyo University of Agriculture and Technology (TUAT) and described in its "Guide for the Care and Use of Laboratory Animals". In addition, all the records during the whole experimental period were also used to maintain the dogs' conditions.

Preparation of MR model dogs and transmitter implantation: Surgical and postoperative care procedures were as described in the previous report [15]. Follow-up care included the auscultation of lung/cardiac sound and blood test (complete blood cell count, blood urea nitrogen, creatinine, alkaline phosphatase and electrolytes). Thoracic radiography (FCR CAPSULA-2V CAPSULA V VIEW, FUJIFILM Co., Ltd., Tokyo, Japan) and echocardiography (SSD-5000, Aloka Co., Ltd., Tokyo, Japan) were performed to evaluate pulmonary venous congestion and cardiac dilation.

LAP recordings: Apart from the routine echocardiography for the postoperative management, investigation of echocardiography, BP measurement (BP-100D, Fukuda ME, Tokyo, Japan) and ANP measurement was not performed for at least 5 weeks following the implantation of the telemetry transmitter (TA11PA-D70, Data Sciences International, St. Paul, MN, U.S.A.). This is because the anatomical shape and the function of the heart were observed by echocardiography to change gradually for the compensation in this period. Using the radio telemetry system (PhysioTel, Data Sciences International), we measured LAP during the whole period of the echocardiographic examinations while the animals were conscious. The maximum, mean and minimum LAP were obtained as the average of 10-sec recorded segments from continuous waveform

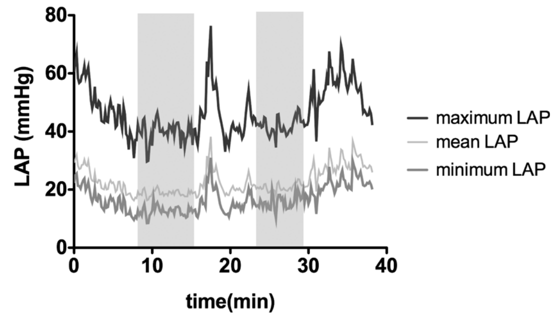

Fig. 1. Fluctuations of each LAP recorded during echocardiography in dog number 6. We confirmed stable LAP recordings the time zone (shaded region) during all the time and recorded echocardiography along with BP measurement for validating the correlation equations. In this recording, the abrupt elevation of LAP at 16-min after starting the recording was thought to be associated with position change of echocardiography. Abrupt LAP elevation at mid-term between two shaded regions indicates the timing of position changes.

recordings (DSI Dataquest A.R.T.™, Data Sciences International). The stability of LAP was confirmed in order to minimize the influences such as excitements, we performed echocardiography and BP measurement (Fig. 1). The average values of LAP during the selected period were used to validate linear regressions between LAP and each variable at last. In the present study, we investigated a total of 37 separate examinations from 6 dogs. The intervals between each investigation in the same dog were set to be more than one week. The individual 37 recordings were performed in about 9 months after the surgery.

Echocardiography: Transthoracic conventional echocardiography, 2-dimensional, M-mode, spectral Doppler and tissue Doppler echocardiography were performed by a single investigator (TI). Each dog was positioned in left and right recumbency, and echocardiographic examinations were performed using a digital ultrasonographic system with a 5.0-MHz sector transducer. Sweep speed during the Doppler or M-mode recordings was 150 to 200 mm/sec. Optimized right parasternal projections (long-axis and short-axis) were used to measure cardiac dimensions. Ratio of left atrial dimension to aortic root dimension (LA/Ao) was assessed in a short-axis M-mode at heart base level and in a long-axis M-mode [7]. For assessing the scale of LA enlargement by dual projections, in order to minimize the effect of intra- and inter- physician errors, we used two indexes partially corrected for BW. The LA dilation index was calculated from measured LAD/standard LAD (where LAD is actual left atrial diameter, and standard LAD is based on BW). LA/Ao was calculated from measured LAD/standard AoD (where AoD is the actual aortic diameter, and standard AoD is based on BW). Standard LAD and AoD were calculated from the equations standard $LAD=0.9*BW^{0.3}$ and standard $AoD=0.72*BW^{0.35}$, these being supported by meta-analysis of past reports [16]. Based on the scale of LV enlargement using short-axis M-mode projection, we used LVEDD (left ventricular end diastolic diameter), LVEDI (left ventricular end diastolic index: left ventricular end-diastolic volume/BSA based on BW) and LVEDDI (left ventricular end-diastolic dilation index: measured LVEDD/standard LVEDD based on BW). BSA and standard LVEDD were calculated from the equations $BSA=10.1*BW^{0.67}$ and standard $LVEDD=1.44*BW^{0.32}$, these being supported by meta-analysis of past reports [16]. Optimized left apical parasternal projections of the left ventricular inflow tracts were used for assessing mitral inflow and MR flow in the two-chamber view, and those of outflow tracts were used for the assessment of aortic flow in the five-chamber view [7]. A 6-mm sample volume was used. The same mitral inflow tract view as the two-chamber view was used to evaluate lateral mitral annulus velocity (Ea) with pulsed TDI, and a 2-mm sample volume was used [9]. With the Doppler signals of the mitral inflow, each peak velocity (peak E and peak A) was measured, and then E/A and E/Ea were calculated. IVRT was calculated by subtracting the interval between the R wave and the cessation of left ventricular outflow from the interval between the R wave and the onset of mitral inflow. These intervals were measured from the individually recorded images [13]. MR flow was recorded with a high-intensity continuous wave spectral Doppler signal and we calculated MR pressure gradient (MRPG) on the basis of a modified Bernoulli's equation ($PG=4v^2$, v: velocity). $T_{(E-Ea)}$ was calculated by subtracting the onset of mitral inflow from the onset of mitral annulus movement, and then $T_{(E-Ea)}$/IVRT was calculated by dividing $T_{(E-Ea)}$ by IVRT. These echocardiographic profiles were obtained from consecutive 3 beats and the averages were calculated. During echocardiography, a monitoring ECG was used and recorded for timing of measurements. All the echocardiographic recordings were stored on the internal hard drive of the echocardiography and transmitted to the DICOM server online (DICOM server, ImageONE Co., Ltd., Tokyo, Japan).

Blood pressure measurements: All indirect arterial BP values were measured by the oscillometric method. A cuff size appropriate for tail circumference was selected and measured simultaneously with MR velocity measurements by echocardiography, and 3 consecutive measurements were averaged for each dog. Then we subtracted MRPG from SBP and treated the value as SBP-MRPG.

Plasma canine ANP measurement: Blood samples were obtained for storage and subsequent measurement of ANP were performed to evaluate left atrial wall stress. Approximately 3 m*l* of blood was obtained via the external jugular vein, and immediately placed into a tube containing ethylenediamine tetraacetic acid (EDTA) and aprotinin for measurement of plasma ANP concentration. Within 15 min of collection, plasma was harvested by centrifugation at 2,000 rpm for 10 min at 5°C. The plasma was then separated within 30 min of collection and preserved at –80°C until analysis. Measurement of plasma canine ANP was performed by an EIA method using a commercially available kit [ANP, alpha (1–28) (Human, Ovine, Canine) - EIA kit, Phoenix Pharmaceuticals, INC, Burlingame, CA, U.S.A.].

Study 2. Clinical application of the regression equations

Animals: To evaluate the clinical value of the regression equation, variables were obtained from a total of 257 dogs with clinical MR (7.7 ± 3.2 kg, 140 males, 117 females) were enrolled in this study. All dogs were referred to the Animal Medical Center of the Tokyo University of Agriculture and Technology between February 2006 and August 2008. A MVD diagnosis was based on the physical examination, electrocardiogram, thoracic radiography and echocardiographic evidence of nodular thickening of the mitral valve, regurgitant flow into the LA and mitral valvular prolapse. The authors evaluated the severity of the heart failure, the intensity of cardiac murmurs, the degrees of cardiac enlargement on thoracic radiography and the echocardiography. Dogs with ventricular premature contraction and any type of atrioventricular block were excluded from this study. The 257 dogs with MR were divided into 5 groups on the basis of the International Small Animal Cardiac Health Council (ISACHC) classification: ISACHC 1a (n=11), ISACHC 1b (n=116), ISACHC 2 (n=115), ISACHC 3a (n=12), ISACHC 3b (n=3), as shown in Table 1. The number of dogs classified ISACHC 3b was restricted because of the higher risks associated with clinical examinations.

Echocardiography and blood pressure measurement: Echocardiographic measurements were conducted in the same way as in the previous section except that echocardiographic evaluation was performed by 4 investigators. All the echocardiographic recordings were stored on the internal hard drive of the echocardiograph and the DICOM server. Indirect arterial BP was also measured during echocardiographic examinations in the same way as described in the study 1 section. A cuff appropriate for tail or forelimb circumferences were selected and BP was measured for each dog. 3 consecutive measurements were averaged for each patient and the averages were used.

Plasma canine ANP measurement: For measurements of plasma ANP concentration, blood samples were collected and measured in the same way as described in the study 1 section.

The data are expressed as mean values ± standard deviation (SD). For validating the correlation between LAP and the echocardiographic values, we used a simple linear regression analysis. Bias between the observed LAP values and SBP-MRPG was determined by Bland-Altman analysis. Kruskal-Wallis test was used to compare LAP between ISACHC classifications. Statistical significance was defined as $P<0.05$. GraphPad Prism version 5.0a (GraphPad, San Diego, CA, U.S.A.) was used to perform these statistical analyses.

RESULTS

Study 1. Constructing regression equations between LAP and echocardiographic and ANP values in experimental MR dogs

The individual 37 recordings were divided into 4 groups based on the International Small Animal Cardiac Health

Table 1. The equations obtained from the 37 surgically-induced MR dogs

Variable	No. of observations	LAP	Correlation coefficients	Simple linear regression equation
LVEDD (cm)	37	Maximum*	0.477	Y=58.11X–229.3
		Mean*	0.571	Y=29.97X–115.7
		Minimum*	0.447	Y=26.68X–105.2
LVEDDI (cm/cm)	37	Maximum*	0.464	Y=174.6X–218.3
		Mean*	0.581	Y=87.84X–107.1
		Minimum*	0.549	Y=71.37X–87.95
LVEDI (cm^3/m^2)	37	Maximum*	0.59	Y=0.49X–57.13
		Mean*	0.612	Y=0.25X–26.88
		Minimum*	0.558	Y=0.21X–23.33
Peak E velocity (m/sec)	37	Maximum*	0.7	Y=69.04X–74.15
		Mean*	0.67	Y=39.82X–41.76
		Minimum*	0.485	Y=37.17X–42.01
E/A	37	Maximum*	0.338	Y=39.96X–72.09
		Mean*	0.377	Y=21.37X–36.49
		Minimum*	0.351	Y=17.62X–31.46
E/Ea	37	Maximum*	0.741	Y=7.034X–54.86
		Mean*	0.695	Y=4.935X–40.37
		Minimum*	0.32	Y=4.400X–38.52
Longitudinal LA dilation index	37	Maximum*	0.39	Y=145.7X–166.3
		Mean*	0.474	Y=75.01X–83.06
		Minimum*	0.569	Y=54.35X–59.89
Longitudinal LA/Ao (cm/cm)	37	Maximum*	0.394	Y=130.5X–164.5
		Mean*	0.481	Y=66.99X–81.86
		Minimum*	0.583	Y=48.28X–58.64
Transversal LA dilation index	37	Maximum*	0.617	Y=50.63X–43.40
		Mean*	0.646	Y=28.01X–22.44
		Minimum*	0.707	Y=21.11X–17.11
Transversal LA/Ao (cm/cm)	37	Maximum*	0.619	Y=45.69X–43.21
		Mean*	0.651	Y=25.22X–22.28
		Minimum*	0.718	Y=18.93X–16.88
IVRT (sec)	30	Maximum	0.081	Y= –584.4X +31.34
		Mean	0.047	Y= –254.8X +18.67
		Minimum	0.048	Y= –174.8X +13.30
E/IVRT (m/sec^2)	30	Maximum	0.039	Y=0.0073X +22.85
		Mean	0.006	Y=0.0016X +15.56
		Minimum	0.005	Y=0.0010X +11.18
$T_{(E-Ea)}$/IVRT (sec/sec)	30	Maximum	0.004	Y=1.213X +24.37
		Mean	0.016	Y=1.315X +14.88
		Minimum	0.01	Y=0.7304X +10.87

* Each variable and LAP showed the significant linear regression with $P<0.05$.

Council (ISACHC) classifications (1a: n=9, 1b: n=11, 2: n=13, 3a: n=4). At the beginning of the measurement (5 weeks after MR creation), 6 dogs were classified as ISACHC 1a, 1b and 2. Four dogs classified in ISACHC 1a and 1b were unchanged or worsen by little (to 1b or 2), while the others classified in ISACHC 2 were worsen (to 3a) with time with some coughing. Thoracic radiography indicated clear lung fields in dogs 1–4, whereas left atrial dilatation and interstitial lung opacity, alveolar lung opacity, or both, indicating pulmonary edema, were identified in dogs 5 and 6. Six dogs did not show any improvement for 9 months. Each LAP at the time of measurements is summarized in Fig 2. These inter-classificational LAP values differed significantly in almost all comparisons in each LAP.

1) Echocardiographic examination: Table 2 summarizes the value of echocardiographic variables, correlation equations and correlation coefficients on the basis of simple linear regressions between each LAP (maximum, mean and minimum) and echocardiographic values. IVRT showed a negative value in 7 of 37 samples because the period from electrocardiogram R-wave to the beginning of the mitral inflow exceeded the period from electrocardiogram R-wave

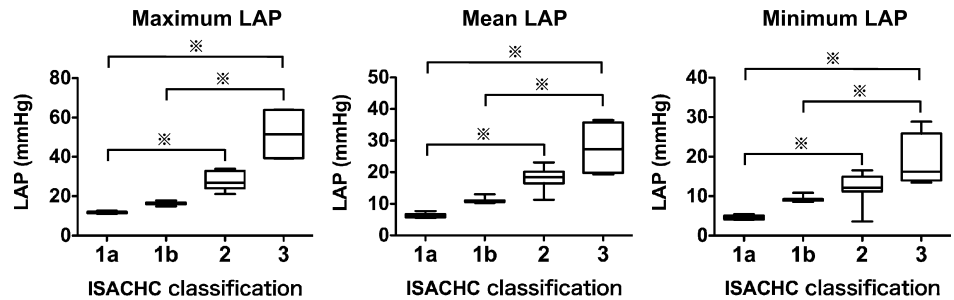

Fig. 2. The profile of each LAP recorded in all 37 investigations. ISACHC 1a: n=9, 1b: n=11, 2: n=13, 3: n=4. *: LAP significantly differs among ISACHC groups with P<0.001.

Table 2. Clinical profiles of 257 MR dogs enrolled in the study 2

ISACHC Class	1a	1b	2	3a	3b
n	11	116	115	12	3
Age	5.3 ± 1.8	7.9 ± 3.2	8.5 ± 2.9	8.1 ± 3.1	8.3 ± 3.3
Sex	M (6), F (5)	M (63), F (53)	M (59), F(56)	M (9), F (3)	M (3), F(0)
BW	14.3 ± 6.3	6.9 ± 4.2	6.9 ± 3.4	8.0 ± 3.6	11.3 ± 4.8
Echocardiography					
LA/Ao	1.32 ± 0.23	1.56 ± 0.29	1.79 ± 0.30	1.97 ± 0.41	2.24 ± 0.49
LVEDI (ml/m^2)	88.1 ± 22.3	118.4 ± 34.4	153.1 ± 32.9	183.5 ± 19.8	347.8 ± 16.3
%FS	43.1 ± 5.23	41.9 ± 4.38	45.4 ± 5.52	43.9 ± 6.19	47.9 ± 4.81
E wave velocity (m/sec)	0.71 ± 0.12	0.82 ± 0.22	1.03 ± 0.24	1.33 ± 0.28	1.76 ± 0.34
E/Ea	6.98 ± 1.32	8.54 ± 2.56	10.6 ± 3.12	14.9 ± 3.16	15.2 ± 4.74

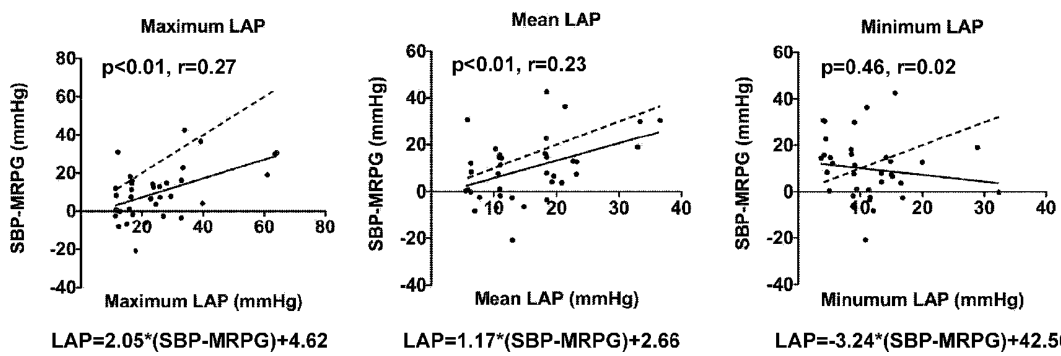

Fig. 3. Comparisons between recorded LAP and SBP-MRPG values in all 37 investigations. The simple linear regressions in maximum and mean LAP were significant with P<0.05. The continuous line represents the simple linear regression between the two values, whereas the dashed line is the equality line of which the slope is 1.0 and the y-intercept is 0.

to the end of the aortic flow. The remaining 30 samples were used for assessing the utility of IVRT, whereas all 37 samples were used for regression among the other variables. Except for IVRT, E/IVRT and $T_{(E\text{-}Ea)}$/IVRT, linear regression of each samples shows significant correlations, with P<0.05. For the morphological evaluations, each LAP showed better correlations with short-axis LA/Ao and short-axis LA dilation index than long-axis LA view and left ventricular diameter. When compared around transmitral flow parameters, the mitral E wave velocity and E/Ea ratio showed better correlations than E/A ratio and the morphological measurements including the scale of LA and LV (Table 2 and Fig. 4). LVEDD, mitral E wave velocity and E/Ea showed better correlations with maximum and mean LAP than with minimum LAP. In contrast, left atrial morphological parameters (short-axis and long-axis LA view) showed better correlations with minimum LAP than with maximum and mean LAP.

Echocardiographic-blood pressure estimation: Maximum and mean LAP tended to be higher than SBP-MRPG as MR severity increased, when compared to recorded LAP with SBP-MRPG values, as shown in Fig 3. Although the correlations between maximum and mean LAP were significant (r=0.27, 0.23 as maximum, mean LAP), the correlation coefficients were far weaker than for the other echocardiographic variables. Using the Bland-Altman analysis to vali-

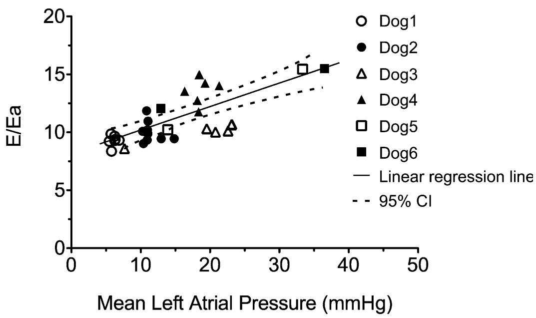

Fig. 4. Simple linear regression line showing positive correlation between recorded mean LAP value and E/Ea, which was obtained from individual 37 investigations. The dashed lines on either side of the continuous line represents 95% confidence intervals.

date the utility of SBP - MRPG, the bias ± SD was 14.97 ± 14.71 mmHg with limits of agreement of –13.87 to 43.80 mmHg in maximum LAP, whereas the mean ± SD bias was –5.60 ± 12.30 mmHg with limits of agreement of –29.72 to 18.51 mmHg in mean LAP, as shown in Fig 5.

3) Plasma ANP concentration estimation: When comparing each LAP value with plasma ANP concentrations measured by EIA[i], significant correlations were found in all simple linear regressions with $P<0.05$ (Fig. 6). However, the correlation coefficients were weaker than for the echocardiographic variables (r=0.44, 0.31 and 0.42 as maximum, mean and minimum LAP).

Study 2. Clinical application of the regression equations

On the basis of the correlation equations shown in Table 2, maximum, mean and minimum LAP were estimated (Tables 3, 4 and 5) in a total of 257 clinical cases. Some estimated values in ISACHC 1a and 3b were incomplete due to the low number of patients. Estimated values based on mitral inflow and scale of LV enlargement were lower than those estimated in experimental MR dogs, whereas estimated values based on plasma ANP concentration, SBP - MRPG and the scale of LA enlargement were higher than those estimated in experimental MR dogs.

1) Echocardiographic estimation: When comparing the averages of each estimated LAP between ISACHC classifications, the estimated LAP, excluding short-axis LA/Ao and short-axis LA dilation index, had a tendency to increase depending on ISACHC classification. In addition, many of the estimated LAP values were negative, especially in ISACHC 1a and 1b in lower estimated parameters. There were

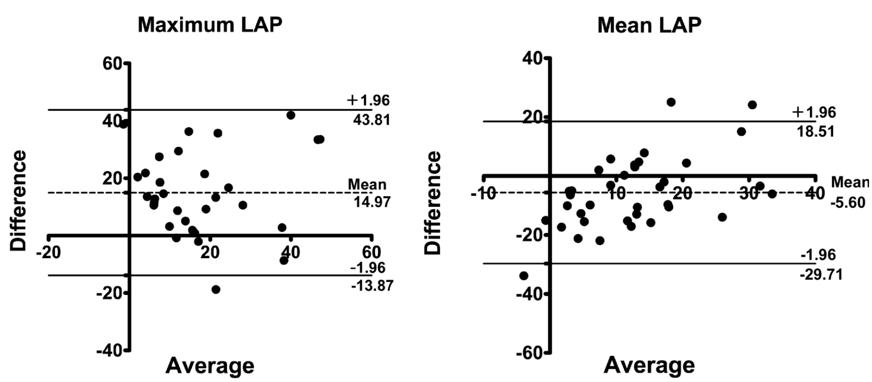

Fig. 5. Bland-Altman plot illustrating bias in the estimation based on SBP-MRPG. The mean difference is indicated by a dashed horizontal line, and limits of agreement (mean ± 1.96 SD) are indicated by continuous horizontal lines.

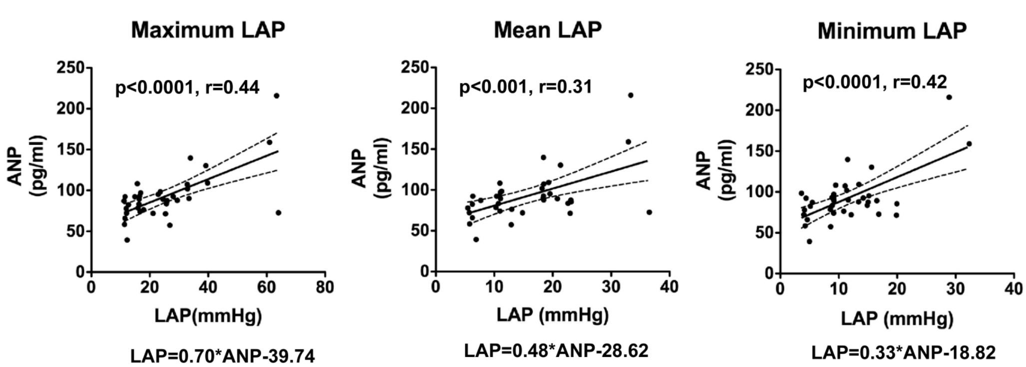

Fig. 6. Simple linear regression line showing positive correlation between recorded LAP values and plasma ANP concentration, which was obtained from individual 37 investigations. The dashed lines on either side of the continuous line represents 95% confidence intervals.

Table 3. Comparisons of maximum LAP values in a total of 251 clinical cases, which were estimated using the equations obtained in study 1

Variable	All n=251	ISACHC1a n=7	ISACHC1b n=116	ISACHC2 n=113	ISACHC3a n=12	ISACHC3b n=3
LVEDI	10.24 ± 35.69	–13.82 ± 30.15[c,d)]	0.86 ± 27.83[b,c,d)]	17.94 ± 40.06[a,d)]	32.69 ± 22.10*[b,d)]	113.49 ± 27.95*[a,b,c)]
E wave	–7.49 ± 19.88	–24.33 ± 8.87[b,c,d)]	–16.83 ± 15.36[b,c,d)]	–2.37 ± 16.07[a,c,d)]	18.30 ± 19.10*[a,b,d)]	48.27 ± 23.59*[a,b,c)]
E/A	–13.49 ± 24.12	–22.15 ± 1.96[c)]	–19.96 ± 21.54[d)]	–11.87 ± 21.76[c)]	12.79 ± 40.91*[b)]	13.12 ± 16.30[a)]
E/Ea	13.41 ± 23.95	–6.61 ± 2.98[b,c,d)]	2.42 ± 16.97[b,c,d)]	18.06 ± 22.37[a,c,d)]	48.52 ± 23.49*[a,b)]	51.10 ± 16.41*[a,b)]
Longitudinal LA dilation index	–16.52 ± 42.31		–34.49 ± 30.90[b,c,d)]	–10.26 ± 47.32[a)]	18.23± 7.05[a)]	43.11 ± 31.34[a)]
Longitudinal LA/Ao	54.33 ± 62.78		27.67 ± 45.83[d)]	63.35 ± 70.13[a)]	107.57 ± 10.27[a)]	143.96 ± 43.18[a)]
Transversal LA dilation index	9.41 ± 12.58		4.62 ± 11.13[b)]	11.91 ± 12.67[a)]	8.07 ± 9.79	
Transversal LA/Ao	34.80 ± 18.78		27.84 ± 16.43[b)]	38.44 ± 19.02[a)]	32.74 ± 15.14	
SBP-MRPG	46.98 ± 50.69	31.71 ± 13.37[c)]	42.82± 49.59[c)]	44.16 ± 46.88[c)]	91.96 ± 43.44*[a,b)]	

*: The estimated LAP increased significant, compared to that in the ISACHC 1a. a); Increased significantly, compared to ISACHC 1b, b); Increased significantly, compared to ISACHC 2, c); Increased significantly, compared to ISACHC 3, a, d); Increased significantly, compared to ISACHC 3b.

Table 4. Comparisons of mean LAP values in a total of 251 clinical cases, which were estimated using the equations obtained in study 1

Variable	All n=251	ISACHC1ba n=7	ISACHC1b n=116	ISACHC2 n=113	ISACHC3a n=12	ISACHC3b n=3
LVEDI	7.86 ± 17.61	–5.59 ± 15.51	3.83 ± 14.31	11.89 ± 17.01	18.33 ± 11.37	59.90 ± 14.38
E wave	–3.21 ± 10.75	–12.31 ± 4.58	–7.93 ± 8.30	–0.18 ± 9.04	10.73 ± 10.32	26.39 ± 12.75
E/A	–5.52 ± 12.30	–9.71 ± 1.43	–8.70 ± 10.99	–4.28 ± 11.23	8.11 ± 20.87	8.28 ± 8.32
E/Ea	7.99 ± 13.23	–3.06 ± 1.50	2.90 ± 9.37	10.56 ± 12.35	27.38 ± 12.97	28.80 ± 9.06
Longitudinal LA dilation index	–5.03 ± 17.12		–14.84 ± 9.13	–1.55 ± 18.43	10.27 ± 3.74	23.48 ± 16.63
Longitudinal LA/Ao	31.96 ± 29.62		15.33 ± 24.26	39.03 ± 27.23	57.62 ± 5.44	76.88 ± 22.86
Transversal LA dilation index	6.32 ± 6.43		3.80 ± 5.99	7.63 ± 5.39	4.94 ± 5.27	
Transversal LA/Ao	20.34 ± 9.65		16.68 ± 8.83	22.21 ± 8.12	18.21 ± 8.14	
SBP-MRPG	32.27 ± 24.73	26.28 ± 7.67	30.09 ± 22.77	31.61 ± 25.26	60.85 ± 24.93	

also large deviations in many categories. For the LV-morphological estimations, the estimated values in ISACHC 3b were much higher than in experimental MR dogs, whereas the LA-morphologically estimated values were much higher than those estimated in experimental MR dogs in all ISACHC classifications. In Fig. 7, maximum and mean LAP values extrapolated from E/Ea are indicated, and the values significantly increase in proportion to ISACHC classification, with $P<0.05$.

2) Echocardiographic-blood pressure estimation: The increase of maximum and mean LAP estimated by SBP - MRPG was significantly accompanied by the ISACHC classification ($P<0.05$), whereas minimum LAP did not show this tendency. The inter-classificational difference was far less than the differences among other parameters. These estimated values were higher than those estimated in experimental MR dogs.

3) Plasma ANP concentration estimation: The increase of each LAP estimated by plasma ANP concentration was significantly accompanied by the ISACHC classification with $P<0.05$. These estimated values were higher compared to values measured in experimental MR dogs.

DISCUSSION

In this present study, we used radio telemetry system to perform continuous LAP recording under conscious conditions in dogs with MR, and obtained the echocardiographic estimation equations, which would bring more precise and clinically-applicable equations. Although PCWP measurement is the most reliable method for estimating preload including LAP, the use of cardiac catheter examinations is restricted in small-animal medicine due to catheter-related infections and therefore, a noninvasive echocardiographic

Table 5. Comparisons of minimum LAP values in a total of 251 clinical cases, which were estimated using the equations obtained in study 1

Variable	All n=251	ISACHC1a n=7	ISACHC1b n=116	ISACHC2 n=113	ISACHC3a n=12	ISACHC3b n=3
LVEDI	5.56 ± 14.07	–5.19 ± 12.40	0.93 ± 11.47	8.767 ± 13.66	13.94 ± 8.66	47.16 ± 9.95
E wave	–6.00 ± 10.12	–14.58 ± 4.31	–10.75 ± 7.82	–3.39 ± 8.18	7.13 ± 9.31	22.39 ± 10.40
E/A	–6.10 ± 10.28	–9.60 ± 1.19	–8.67 ± 9.19	–5.22 ± 9.28	5.30 ± 16.71	5.44 ± 6.02
E/Ea	5.17 ± 10.69	–3.77 ± 1.21	0.26 ± 7.58	7.25 ± 9.99	20.85 ± 9.81	22.00 ± 5.98
Longitudinal LA dilation index	–3.27 ± 12.29		–10.31 ± 6.56	–0.44 ± 13.45	7.72 ± 2.49	17.19 ± 9.75
Longitudinal LA/Ao	23.20 ± 21.13		11.33 ± 17.30	28.76 ± 19.73	41.50 ± 3.56	55.24 ± 13.31
Transversal LA dilation index	4.64 ± 4.78		2.22 ± 4.46	5.61 ± 4.01	3.60 ± 3.20	
Transversal LA/Ao	15.04 ± 7.04		11.51 ± 6.54	16.43 ± 6.01	13.46 ± 4.92	
SBP-MRPG	5.34 ± 6.49	6.92 ± 2.01	5.92 ± 5.98	5.51 ± 6.63	–2.16 ± 6.06	

Table 6. The equation profiles to estimate LAP values in dogs with MR or human with heart failure

	Ishikawa	Oyama, 2004 (21)	Karston, 2008 (14)	Dokainish, 2004 (6)
Model	Rupture of tendinous cord	Rupture of tendinous cord	Acute volume-overload	Various cardiac diseases (human)
Ill period	Subchronic	Acute	Acute	Chronic
Anesthesia	Unanesthetized	General anesthesia	General anesthesia	Only local anesthesia
P value	Significant <0.0001	Significant unreported	Insignificant	Significant <0.001
r value	0.695	r^2=0.83	–	0.69
Equations	4.94*(E/Ea)–40.37	6.38*(E/Ea)–28.3	–	0.90*(E/Ea)+6.00

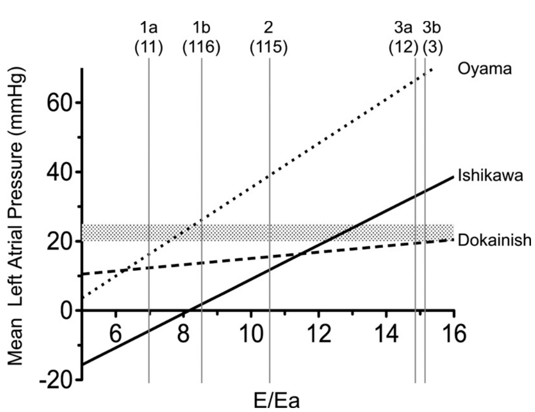

Fig. 7. Comparisons of LAP estimation equations in clinical MR dogs. Number of dogs for each ISACHC classification is indicated in the parenthesis at the top of the perpendicular lines. A dotted area indicates 20–25 mmHg of mean LAP, which is the threshold level of pulmonary edema.

estimation of LAP would be a valuable tool. Nonetheless, there have not been reports well applicable for clinical patients.

Elevated LAP would be expected to cause left atrial dilation, and estimation of LA size seems viable. However, inter-physician and intra-physician variations may greatly influence the measurements. In the experimental MR model, the correlation coefficients of LA morphological scales were higher than in the clinical cases, because they were measured by a single physician. Estimated LAP in ISACHC 3a in clinical cases was comparatively similar to the values recorded in the experimental MR model using short-axis LA/Ao and short-axis LA dilation index. However, estimated LAP did not correlate with recorded values in other ISACHC classifications. Consequently, it seems that LAP estimation based on an LA morphological scale is more difficult than initially expected. We usually evaluate the LAD using LA/Ao with optimized right parasternal projections at the time of ventricular end-systole, when the *v* wave appears as maximum LAP in dogs with MR. However, minimum LAP had the best correlation with LA morphological scales and there is no obvious explanation based on ECG-related pressure variations. The pathophysiological significance of minimum LAP is incompletely understood at present, and it is still arguable whether measuring minimum LAP has any importance.

In human cardiovascular medicine, IVRT has been used for noninvasive assessment of diastolic function [22, 28, 29]. However, many reports have indicated that IVRT is also influenced by age, sex, preload, afterload and HR [18, 25]. In past reports, good correlations between left ventricular filling pressure (almost the same as mean LAP), IVRT and its modified parameters (ratio of peak E wave to IVRT) were indicated without any consideration to the stability of R-R interval under general anesthesia [22, 29]. In this present study, it was very difficult to calculate IVRT in telemetrized dogs due to the instability of the R-R interval resulting from respiratory arrhythmia, and the r values were negative in 7 out of 37 cases. As a result, the correlation coefficients were much lower, contrary to the past reports [26].

Mean LAP estimation calculated from SBP-MRPG is the classical and statistically-warranted method for estimating mean PCWP in human MR patients, whose mean PCWP values are around 10 mmHg [8, 10]. However, the significance of this method in MR dogs, whose mean LAP was higher than human patients, has not been confirmed. Contrary to LAP variations, LV pressure (LVP) variations during mid-systolic period are relatively stable with a narrow range when the peak MR velocity is recorded. However, considering the timing of LAP variations, the slope of the higher *v* wave derived from higher LAP is much more steep in proportion to MR severity, and the timing of measurement of peak MR velocity is defined by the pressure gradient between LAP and LVP. In addition, in order to perform indirect arterial BP measured by the oscillometric method, we always have to consider over-excitement, which affects the reliability of SBP-MRPG easily. In clinical settings, the increases derived from tension are much greater than our experimental model dogs, and varies among patients. It is therefore concluded that the usefulness of SBP-MRPG for the assessment of LAP in dogs with MR is doubtful.

Measurements of mitral inflow pattern, such as E/A and deceleration time of E wave, provide useful information for noninvasive assessment of left ventricular diastolic function [19]. However, abnormally elevated LAP accelerates E wave velocity in the presence of MR, and many reports have indicated that human mitral inflow velocity and their patterns are no longer taken to assess left ventricular diastolic function in MR patients except for mild regurgitation [23]. In veterinary medicine, CHF dogs often show abnormal relaxation patterns showing higher peak A wave velocity than peak E wave velocity, and therefore we can diagnose as abnormal left ventricular relaxation in dogs with cardiac disorders including MR. In comparison with human MR patients, elevated LAP due to regurgitation is markedly higher, and the theory that mitral inflow should be determined by not LV diastolic function but LAP would be also more generally applicable in veterinary medicine. In fact, peak E wave velocity and E/Ea have good correlation coefficients with maximum and mean LAP in the study 1, indicating that E wave velocity was mainly due to LAP and that LV diastolic failure was less advanced in almost all dogs. In the study 1, considering the highly maintained Ea [12.4 ± 0.9 cm/sec (mean ± SD), data not shown] and the shortened illness period compared to clinical cases, our results might be more or less different from clinical settings.

E/Ea has been already reported to estimate preload in veterinary medicine. Schober *et al.* could not obtain significant linear regression with not MR but an acute volume overload model and, and denied the utility of E/Ea (Table 6) [24]. In contrast, Oyama *et al.* examined with an acute MR model, and achieved a significant linear regression equation with good correlation [21]. However, the clinical utility of the equation was not validated in the paper, and we noticed a propensity for overestimation when the equation is applied in clinical patients (Fig. 7).

Our estimated value for mean LAP on the basis of E/Ea in dogs belonging to ISACHC 1b was 2.9 mmHg, while averaged mean LAP values estimated from Dokainish *et al.* and Oyama *et al.* were 13.7 and 26.2 mmHg respectively (Fig. 7) [6, 21]. When LAP or PCWP reaches above 20–25 mmHg, pulmonary edema and cardiac cough are likely to be present and recognized as life-threatening condition. Generally speaking, MR dogs in ISACHC 1b don't show any symptoms, and the estimated LAP by Oyama's equation would be clearly overestimated, although the values based on our equation might be underestimated by little. Unfortunately, the true LAP values based on catheter examination were not measured in clinical patients enrolled in this experiment, and therefore we cannot confirm the true validity of the estimated LAP.

Limitations: The present study had some limitations. Some additional experiments on quality control are desirable to justify conclusions from these equations validated in this experiment. Although the dogs were well trained to be calm during echocardiography and blood pressure measurement, continuous recordings of LAP without any fluctuations is very difficult to perform under unsedated and unanesthized condition.

In addition, we must discuss animal model. MVD generally shows chronic disease process, and the adaptation process to MVD plays a key role in the pathophysiology. In this study, we ruptured mitral chordae tendineae, and the acute hemodynamic changes were the strongest factor in the pathogenic process of this model, indicating the possibility that our results might be no longer applicable for MR clinical patients. On the other hand, we observed gradual changes of the function and morphology of their hearts by weekly telemetry recordings and echocardiography, suggestive of compensation mechanisms for chordae tendineae rupture. Although a 5-week period is long enough to be defined as subchronic or chronic period, our models might be close to chronic models and clinical patients. Further clinical evaluation of the equations should be performed with a large population of MR patients in near future study.

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