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Quantification of mitral regurgitation using proximal isovelocity surface area method in dogs

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The present study was performed to determine the accuracy and reproducibility of calculating the mitral regurgitant orifice area with the proximal isovelocity surface area (PISA) method in dogs with experimental mitral regurgitation and in canine patients with chronic mitral insufficiency and to evaluate the effect of general anesthesia on mitral regurgitation. Eight adult, Beagle dogs for experimental mitral regurgitation and 11 small breed dogs with spontaneous mitral regurgitation were used. In 8 Beagle dogs, mild mitral regurgitation was created by disrupting mitral chordae or leaflets. Effective regurgitant orifice (ERO) area was measured by the PISA method and compared with the measurements simultaneously obtained by quantitative Doppler echocardiography 4 weeks after creation of mitral regurgitation. The same procedure was performed in 11 patients with isolated mitral regurgitation and in 8 Beagle dogs under two different protocols of general anesthesia. ERO and regurgitant stroke volume (RSV) by the PISA method correlated well with values by the quantitative Doppler technique with a small error in experimental dogs ($r = 0.914$ and $r = 0.839$) and 11 patients ($r = 0.990$ and $r = 0.996$). The isoflurane anesthetic echocardiography demonstrated a significant decrease of RSV, and there was no significant change in fractional shortening (FS), ERO area, LV end-diastolic and LV end-systolic volume. ERO area showed increasing tendency after ketamine-xylazine administration, but not statistically significant. RSV, LV end-systolic and LV end-diastolic volume increased significantly ($p < 0.01$), whereas FS significantly decreased ($p < 0.01$). The PISA method is accurate and reproducible in experimental mitral regurgitation model and in a clinical setting. ERO area is considered and preferred as a hemodynamic-nondependent factor than other traditional measurements.

Key words: dog, mitral regurgitation, PISA method, color Doppler imaging

Introduction

One of the major goals of clinical cardiology is more accurate quantification of valvular regurgitation, which has proven to be a difficult task with both invasive and noninvasive methods in human medicine and veterinary practice. Color Doppler mapping, the length or area of the mitral regurgitant jet has been used as an index of severity [10,16,17,23]. However, it could be influenced not only by the severity of mitral regurgitation, but also by hemodynamics, the size of the regurgitant orifice, and the setting of instruments [3,13,22]. To overcome these limitations, a new method for analyzing the proximal isovelocity surface area (PISA) was proposed as an alternative quantitative approach. The validity of this PISA method has been reported in in vitro experiments [3,13,27] and in clinical human patients [8,26]. However, a few studies were carried out on PISA method in experimental dogs [20] and in canine patients [6,11]. The purpose of the present study was the evaluation of the feasibility and reproducibility of "PISA" method in dogs with experimentally induced mitral regurgitation, and spontaneous mitral insufficiency diagnosed by color flow Doppler echocardiography. To prove the usefulness, this method was prospectively compared with simultaneously performed quantitative Doppler and echocardiography examinations.

Mitral regurgitation may be dynamic, and regurgitant volume may be affected by variations in loading conditions. General anesthesia is known to result in alterations in

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patients heart rate, blood pressure, and systemic vascular resistance [2]. As the effects of echocardiographic alterations of mitral regurgitation accompanying general anesthesia are unknown in dogs, this study was also undertaken to evaluate the effect of general anesthesia on mitral regurgitation using color Doppler imaging in dogs with experimentally induced mitral regurgitation.

Materials and Methods

Animals

Eight adult, conditioned Beagle dogs were used. Body weights ranged from 7.7 to 13 kg with a mean of 9 kg. Preliminary data included complete physical examination with emphasis on the cardiovascular system. All dogs were examined for circulating microfilaria and had thoracic radiographs and echocardiograms. The experimental protocol was approved by the Animal Care and Use Committee at Seoul National University.

Creation of mitral regurgitation

Dogs were sedated with 0.03 mg/kg of acepromazine (Sedaject, Samu chem Co, Seoul, Korea) and 15 mg/kg of thiopental sodium (Thionyl, Daihan Pharm, Korea). After anesthesia was induced, it was maintained at least with 2% of isoflurane (Isoflurane, Rhodia, UK). During the procedure, the pulmonary arterial temperature was maintained at $38.0 \pm 0.5^{o}C$ using a circulating warm water pad. An anterior cervical site and a femoral site were shaved and aseptically prepared. With sterile technique, 1- inch cutdown were performed, and the carotid artery, external jugular vein, and femoral artery were isolated. A Swan-Ganz catheter (Cook, USA) was passed into the pulmonary artery via the external jugular vein. The measurements of pulmonary capillary wedge pressure and cardiac output were performed with the anesthetic patient monitoring system (S-3 anesthesia monitor, Datex-Ohmeda, Finland). A 14-cm, 8-Fr sheath was inserted into the carotid artery and passed into the left ventricle. A 6-Fr pigtail angiographic catheter (Pig-tail catheter, Cook, USA) was placed into the left ventricle via the femoral artery. A 5-Fr, 120-cm long, 4 prong grasping forceps (4-prong grasping forceps, ESS Inc, USA) were guided into the left ventricle via the placed sheath in carotid artery. The forceps were manipulated to engage the mitral valve chordae or mitral valve leaflets. The disruption of chordae or mitral valve leaflet was performed until there was 100% increase in pulmonary capillary wedge pressure, a grade II to VI or greater left apical holosystolic murmur, and/or a reduction in cardiac output. All manipulations of catheters and forceps were performed with fluoroscopic guidance (DXG-525RF, Dong-A X-ray, Korea). The catheters were then removed, and vascular incisions were repaired. Echocardiographic examination was performed at 1 month after creation of mitral regurgitation.

Echocardiographic imaging and analysis

Echocardiography was performed with a multifrequency sector probe (Logiq 400 pro, General Electric, USA) imaging at 6 MHz and recording Doppler at 4 MHz. The data were recorded on thermal printing paper (UP-895 MDW, Sony, Japan).

Measurement of proximal isovelocity surface area

The theoretic basis for calculating the effective regurgitant orifice (ERO) area has been described previously. The calculation was based on following formulas [9,27,28].

Flow = Area × Velocity
Regurgitant flow = ERO area × Regurgitant velocity
ERO area = Regurgitant flow/Regurgitant velocity
Integrated over the cardiac cycle,
ERO area = Regurgitant volume/Regurgitant time velocity integral

The frame rate of color Doppler imaging was 30/s and the sector arc was 30^{o}. First aliasing velocity was set to 20-50 cm/s for all examinations. Imaging was obtained from an apical four-chamber view, and color gain was adjusted to eliminate random color in areas without flow. The regurgitant orifice was imaged in the center of the echo beam and adjusted to best visualize the flow convergence region on the left ventricular side of the mitral valve. Color M-mode interrogation was set to pass through the center of the PISA, all measurements were obtained from all three beats and then averaged. The PISA radius was measured as the distance from the first alias to the leading edge of the mitral valve tracing using ultrasonographic unit internal caliper.

The regurgitant flow rate was determined by the following equation where PISA is assumed to be a hemisphere:

$$FR = 2\pi \times r^2 \times V$$

Where FR is the regurgitant flow rate (ml/s), r is the radius of the PISA (cm), and V is the aliasing velocity (cm/s). Regurgitant stroke volume (ml) using PISA method was calculated by multiplying the mean regurgitant flow rate by the regurgitant time.

Quantitative Doppler echocardiography

Quantitative Doppler study was performed as previously described [9]. The diameters of the aortic annulus in systole and the mitral annulus in diastole were measured at the point of inner edge of the leaflets. The apical 4 chamber view was used to record and digitize the pulsed wave Doppler signal at the mitral and aortic annuli, and the time-velocity integrals were computed. At least three measurements of each variable were averaged. Continuous wave Doppler echocardiography was recorded with an apical or para-apical window to obtain the maximal velocities of the regurgitant jet. Once full envelope was obtained, the outline

was digitized, and the time-velocity integral of the regurgitant jet was computed. The cross-sectional areas of the mitral and aortic annuli were calculated πR^2 formula, assuming a circular shape. The mitral and aortic stroke volumes were obtained by multiplying the cross-sectional area by the respective time-velocity integral determined by pulsed wave Doppler imaging at each specific location.

Regurgitant volume = Mitral stroke volume – Aortic stroke volume

The regurgitant fraction = Regurgitant volume/Mitral or aortic stroke volume.

Anesthetics

To assess the effects of general anesthesia and loading conditions on mitral valve function, dogs with mitral regurgitation were initially sedated with 0.03 mg/kg of acepromazine and 15 mg/kg of thiopental sodium. After tracheal intubation, anesthesia was maintained with 2% of isoflurane in oxygen at flow rate 100 ml/kg/hr. A period of 7 days was allowed to elapse following isoflurane anesthesia. Then, dogs were premedicated with 0.03 mg/kg acepromazine and 0.04 mg/kg of atropine (Daihan Pharm, Korea) following intravenous injection of 10 mg/kg of ketamine (Ketalar, Yuhan, Korea) with 2.2 mg/kg of xylazine (Rompun, Bayer Korea, Korea). Under anesthesia, M-mode and quantitative Doppler measurements were performed prior to PISA method. The latter values were measured as previously described. Preanesthetic and postanesthetic heart rates were monitored.

Clinical applications

Clinical characteristics of patients with chronic mitral insufficiency were summarized in Table 1. PISA method was utilized on 11 small breed dogs semiquantitatively assessed as having moderate to severe mitral regurgitation with physical examination, thoracic radiography, and routine echocardiography. Their ages ranged from 6 to 12 years (mean: 8.2 years) and their body weights from 2.1 to 5.8 kg (mean: 3.5 kg). Enlargement of the left atrium and left ventricle was confirmed in every animal by radiography, and vertebral heart size ranged from 10.2 to 12.5 (mean: 11.3 v). Clinical observations revealed that all dogs had a normal appetite and normal vigor. Most of the dogs had mild to severe cough, and dogs with severe cough had intolerance to exercise. Evaluations were performed by the same method on induced-mitral regurgitant group.

Statistical analysis

Measurements are expressed as the mean value ± SD. Using linear regression, the ERO area and regurgitant stroke volume determined by the PISA method were compared with that values obtained by the quantitative Doppler method. Since a wide range of values may yield a high correlation coefficient even when data are in poor agreement, the differences between pairs of measurements were additionally determined according to Bland-Altman method. To test the reproducibility of PISA calculation, measurements of the proximal accelerating flow variables were examined by the same observer after an interval of 1 week. To determine the interobserver variability, all measurements were repeated by a second independent observer on the separate day. The interobserver variability was measured by the Bland-Altman method. These were also expressed as the coefficient of variation of the repeated measurements (COVr). The COVr was calculated from the following formula: COVr = (SD of the mean differences/mean) × 100 %.

The paired samples t-test was used to assess the statistical significance of preanesthetic and postanesthetic changes in hemodynamic and Doppler echocardiographic parameters.

Results

Comparison of the PISA method with the quantitative Doppler technique

ERO area by the PISA method correlated well with values by the quantitative Doppler technique ($y = 0.641x + 3.023$, $r = 0.914$) with a small error (mean difference = 2.73 ± 2.11;

Table 1. Clinical characteristics of the patients with chronic mitral regurgitation

	Breed	BW (kg)	Clinical sign	VHS (v)	Pulmonary edema
1	Pomeranian	3.5	Cough	10.5	None
2	Maltese	2.5	Cough	11.3	None
3	Maltese	3.9	Syncope, cyanosis	12.5	Mild
4	Miniature Pinscher	4.2	Syncope	10.2	None
5	Pomeranian	2.1	Dyspnea, depression	12.1	Mild
6	Maltese	5.8	Cough, dyspnea, panting	12.0	Moderate
7	Maltese	2.5	Cyanosis, panting	10.6	Mild
8	Chihuahua	3.6	Dyspnea, hemoptysis	10.2	Moderate
9	Yorkshire Terrier	3.0	Exercise intolerance, cough	11.5	Mild
10	Maltese	3.0	Cough	12.0	Mild
11	Maltese	4.0	Cough, syncope	11.8	Mild

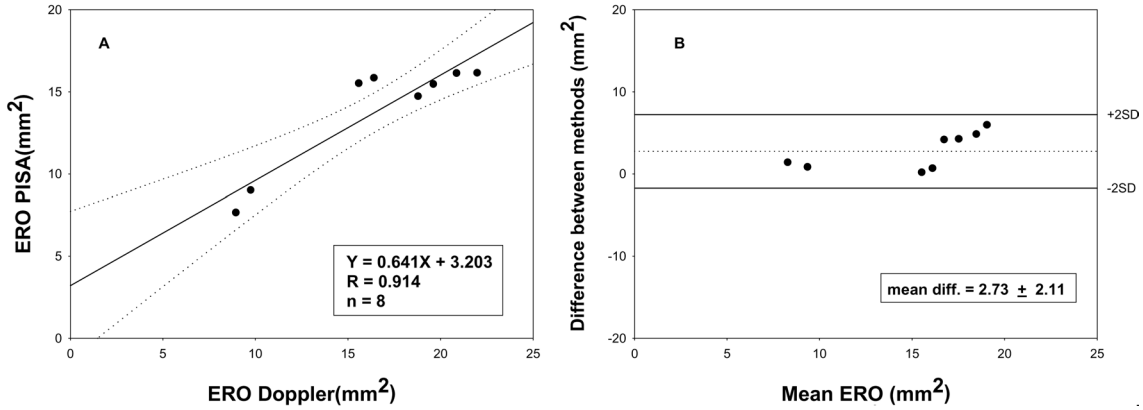

Fig. 1. Results in dogs with experimental mitral regurgitation. Correlation between the effective regurgitant orifice (ERO) area obtained by the proximal isovelocity surface area (PISA) method and by quantitative Doppler echocardiography (A). The difference between the proximal isovelocity surface area (PISA) and the Doppler values is plotted against the average of the same data. The mean difference (mean diff.) is indicated by the dashed line; the limits of agreement (continuous lines) are indicated by ± 2SDs (B).

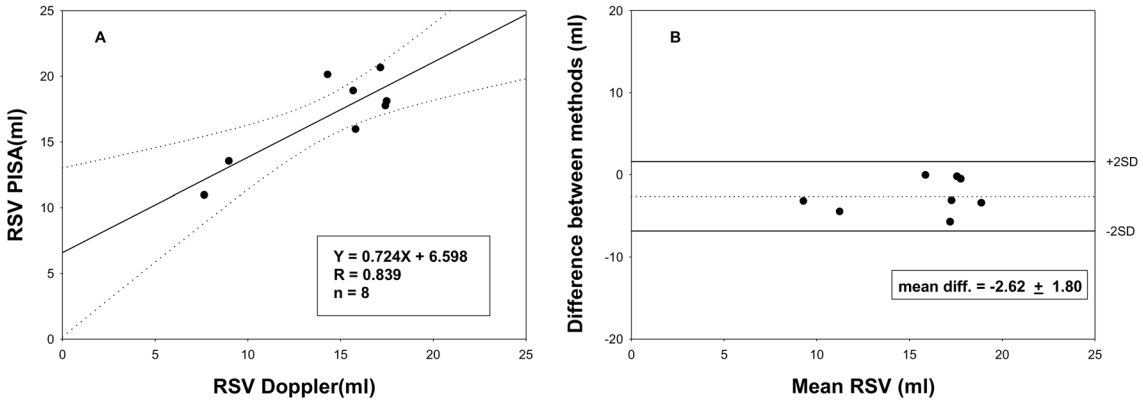

Fig. 2. Results in dogs with experimental mitral regurgitation. Correlation between the regurgitant stroke volume (RSV) obtained by the proximal isovelocity surface area (PISA) method and by quantitative Doppler echocardiography (A). The difference between the proximal isovelocity surface area (PISA) and the Doppler values is plotted against the average of the same data. The mean difference (mean diff.) is indicated by the dashed line; the limits of agreement (continuous lines) are indicated by ± 2SDs (B).

Fig. 1). A good correlation was also found between regurgitant stroke volume (RSV) by PISA and the quantitative Doppler technique ($y = 0.724x + 6.589$, $r = 0.839$) with a small error (mean difference = -2.62 ± 1.80 ml; Fig 2).

Reproducibility

The intraobserver variability was 0.101 ± 3.030 mm^2 (mean difference ± SD) with COVr = 10.63 % for ERO area and 0.631 ± 4.848 ml with 21.67% for regurgitant stroke volume. The interobserver variability was 0.58 ± 2.34 mm^2 with 12.52 %, and 1.81 ± 3.85 ml with 18.42%, respectively (Fig. 3 and 4).

The effect of anesthetics on echocardiographic parameters

There was no significant change in fractional shortening, ERO area, and LV (left ventricle) end-diastolic and LV end-systolic volume under isoflurane anesthesia (Table 2). The echocardiography under isoflurane anesthesia demonstrated a significant decrease of RSV (16.97 ± 3.33 vs 11.54 ± 4.17 ml, $p < 0.05$). ERO area showed the tendency of increase after administration of ketamine-xylazine combination, but not statistically significant (13.78 ± 3.43 vs 17.34 ± 6.69 mm^2, p = NS). RSV increased significantly from 16.97 ± 3.34 to 26.37 ± 7.19 ml ($p < 0.01$), and end-diastolic volume also increased significantly from 35.95 ± 7.72 to 53.38 ± 8.80 ml ($p = 0.01$), whereas fractional shortening significantly decreased from 37.13 ± 3.57 to 26.42 ± 3.61% ($p < 0.01$, Table 3).

Clinical applications

ERO by the PISA method correlated well with values by the quantitative Doppler technique ($y = 0.920x + 0.230$, $r = 0.99$) with a small error (mean difference = 1.886 ± 5.176; Fig. 5). A highly significant correlation was also found between RSV by PISA and the quantitative Doppler

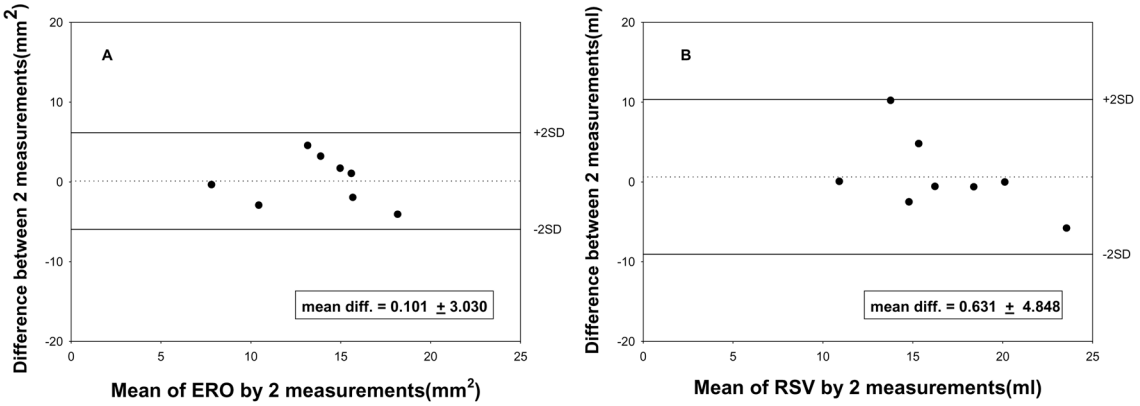

Fig. 3. Results in dogs with experimental mitral regurgitation. Scatterplots of the differences between the two measurements on the y-axis and the mean values obtained by the intraobservers on the x-axis for effective regurgitant orifice area (A) and regurgitant stroke volume (B) by the PISA method.

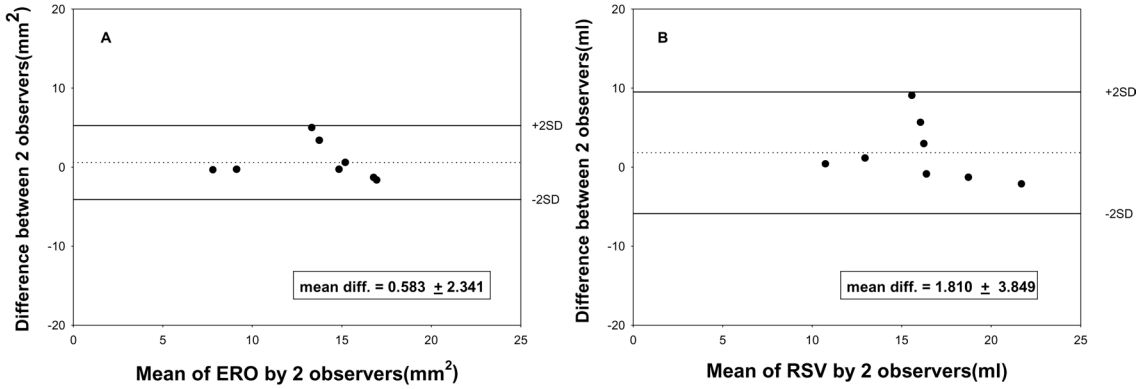

Fig. 4. Results in dogs with experimental mitral regurgitation. Scatterplots of the differences between the two observers on the y-axis and the mean values obtained by the two observers on the x-axis for effective regurgitant orifice area (A) and regurgitant stroke volume (B) by the PISA method.

technique ($y = 0.960x + 5.445$, $r = 0.996$) with a small error (mean difference = -4.505 ± 5.253 ml; Fig. 6) in spontaneous chronic mitral regurgitant patients.

Discussion

Mitral regurgitation (MR) was induced by handling the grasping forceps in left ventricle via carotid artery, in this study. An advantage of this MR model over those previously reported surgical models [5] is that it does not require a thoracotomy and thus is less invasive. Also, surgically produced models of MR may not be analogous to the volume overload seen in spontaneous MR because of the potential restrictive effects of a postoperatively thickened pericardium on the volume overloaded heart [12].

This study was investigated in a clinical setting and an experimentally induced MR the potential of the proximal flow convergence method to assess the quantitative severity of mitral regurgitation in comparison with the quantitative Doppler echocardiographic method as an established and validated standard. As shown in Fig. 1 and 2, regurgitant stroke volume (RSV) as calculated by the proximal isovelocity surface area (PISA) method showed good overall agreement with the values that were calculated by the quantitative Doppler echocardiographic method ($r = 0.839$, mean difference = -2.62 ± 1.80 ml). Similar correlations were obtained for the calculated effective regurgitant orifice (ERO) area ($r = 0.914$, mean difference = 2.73 ± 2.11 mm^2). In clinical trials, RSV and ERO as calculated by the PISA method showed highly agreement with the values that were calculated by the quantitative Doppler method ($r = 0.96$, mean difference = -4.505 ± 5.253, and $r = 0.99$, mean difference = 1.886 ± 5.176). These results were similar to those of several human studies [8,19]. Although there is a good correlation and agreement between the two methods, the tendency of underestimation was shown in ERO, while overestimation in RSV. The possible causes of these small errors include the existing intraventricular flow, which is theoretically destined to pass the left ventricular out flow tract, could superimpose the

Table 2. The effect of isoflurane anesthesia on the echocardiographic parameters

	Preanesthetic	Postanesthetic	p value
LV end-diastolic volume (ml)	35.95 ± 7.72	36.43 ± 11.35	0.879
LV end-systolic volume (ml)	11.79 ± 3.77	13.17 ± 4.76	0.358
Fractional shortening (%)	37.13 ± 3.57	33.81 ± 3.97	0.052
ERO (mm^2)	13.79 ± 7.72	11.05 ± 3.19	0.051
RSV (ml)	16.97 ± 3.33	11.54 ± 4.17	0.013

LV: left ventricle
ERO: effective regurgitant orifice area
RSV: regurgitant stroke volume

Table 3. The effect of ketamine and xylazine combination anesthesia on the echocardiographic parameters

	Preanesthetic	Postanesthetic	p value
LV end-diastolic volume (ml)	35.95 ± 7.72	53.38 ± 8.8	0.001
LV end-systolic volume (ml)	11.79 ± 3.77	25.6 ± 7.36	0.001
Fractional shortening (%)	37.13 ± 3.57	26.42 ± 3.61	0.001
ERO (mm^2)	13.79 ± 7.72	17.34 ± 6.69	0.097
RSV (ml)	16.97 ± 3.33	26.37 ± 7.19	<0.001

LV: left ventricle
ERO: effective regurgitant orifice area
RSV: regurgitant stroke volume

proximal accelerating flow through the mitral regurgitant orifice, especially when the regurgitant orifice is near the left ventricular outflow tract. The regurgitant orifice, which is close to the left ventricular wall may distort hemispheric shape of the proximal flow convergent isovelocity layers [4,15]. This may be especially true for a small left ventricular cavity during systole in small animals. All of these possibilities require further investigations. Also, higher correlation in clinical series was considered that PISA method was more accurate in chronic severe MR than mild MR estimated by semiquantitative method. Also, it seems that thick and irregular valvular margin doesn't significantly affect on measurements of PISA radius in chronic MR patients compared to experimental dogs with thin and smooth valve. Thus, ERO calculation by PISA method may be useful in dogs with chronic mitral insufficiency.

High reproducibility is important for the echocardiographic parameters, and should be evaluated. In the present study, high reproducibility was demonstrated in ERO and RSV by two observers and two measurements. These close agreement is similar to those reported in several human studies [8,19].

The authors need to discuss about some technical points used in the present study concerning accuracy of measurements of regurgitant flow rate or volume using Doppler color flow mapping of the proximal accelerating flow region. Axial and lateral resolutions of two-dimensional Doppler color flow mapping are dependent on the size and depth of the imaging area and the frequency of the transducer chosen. Whenever possible, the narrowest imaging angle, shallowest depth, highest imaging frequency and lowest pulse repetition frequency should be chosen to increase the resolutions of Doppler color mapping. The proximal accelerating field should be magnified as large as possible to minimize measurement error. The prerequisite for accurate measurement of the proximal accelerating area using two-dimensional scanning was through both standard and nonstandard imaging planes with a rotating, shifting and angulating transducer. Aotsuka et al. [1] reported that color M-mode was useful in children with small heart size because it provides color Doppler information and positional information regarding the mitral surface more clearly than B-mode color flow mapping due to its higher signal to noise ratio. The color M-mode is also thought to be useful to measure the flow convergence region in dogs, because the radius of the PISA is small and heart rate is high for color flow rate like children. The M-mode beam should be aligned center to the accelerating region and perpendicular to the regurgitant orifice plane.

One of the basic assumptions of the present study is that the shape of the PISA is a hemisphere, and calculations are based on unidirectional measurement of the PISA radius. Several experimental studies on the relationships between the shape of the PISA and machine setting or hemodynamic factors have been reported [4,18,21,24]. It was found that the contours of the PISA changed variously because of pressure gradients between the left ventricle and left atrium, the Nyquist limit, and orifice size [4,18,21,24,25]. If the orifice size and the pressure gradients between left ventricle and left atrium (almost 100 mmHg) are constant values, the Nyquist limit is an important and controllable factor that have influenced on the shape of PISA. For precise

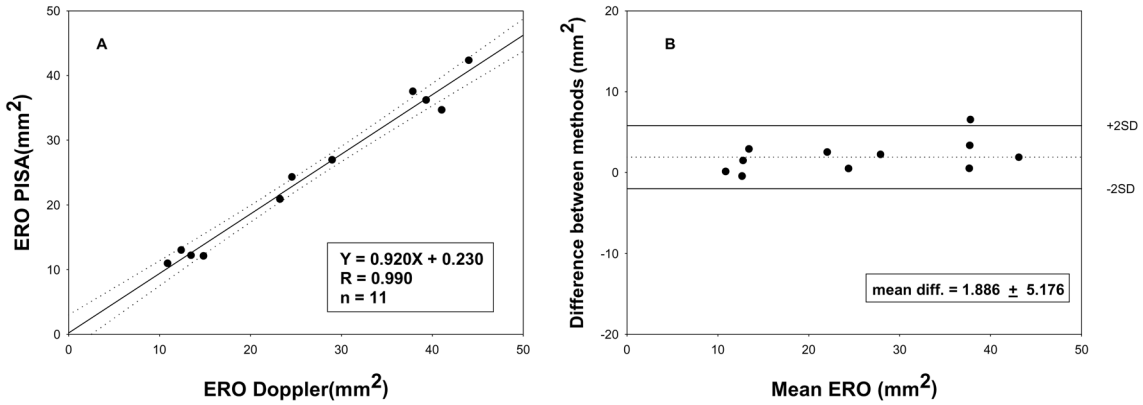

Fig. 5. Correlation between the effective regurgitant orifice (ERO) area obtained by the proximal isovelocity surface area (PISA) method and by quantitative Doppler echocardiography in patients with chronic mitral regurgitation (A). The difference of effective regurgitant orifice (ERO) area by between the PISA and Doppler methods is plotted against the average of the same data in patients with chronic mitral regurgitation. The mean difference (mean diff.) is indicated by the dashed line; the limits of agreement (continuous lines) are indicated by ± 2SDs (B).

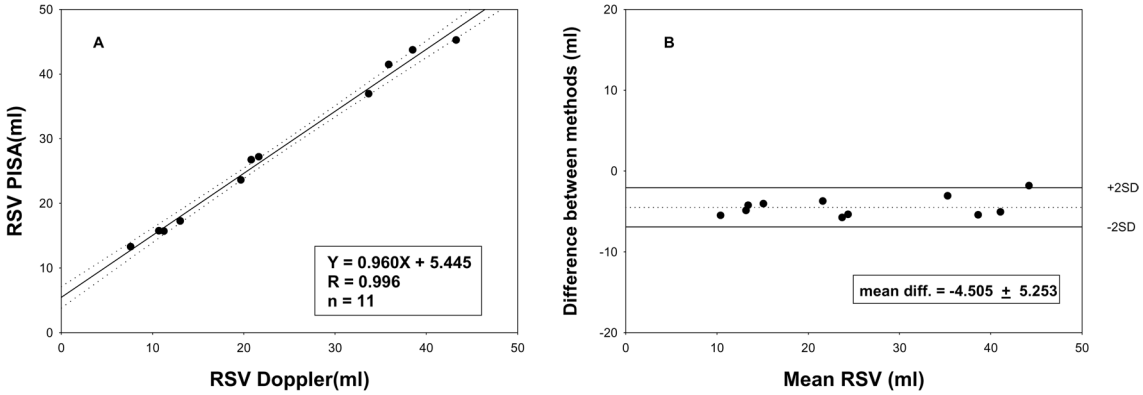

Fig. 6. Correlation between the regurgitant stroke volume (RSV) obtained by the proximal isovelocity surface area (PISA) method and by quantitative Doppler echocardiography in patients with chronic mitral regurgitation (A). The difference of regurgitant stroke volume (RSV) between the PISA and Doppler methods is plotted against the average of the same data in patients with chronic mitral regurgitation. The mean difference (mean diff.) is indicated by the dashed line; the limits of agreement (continuous lines) are indicated by ± 2SDs (B).

estimation of the regurgitant flow or RSV, the radius should be measured at the machine setting for most appropriate hemispheric assumption. Shandas *et al*. [21] reported that if the Nyquist limit is 30-55 cm/s and the pressure gradients is between 60-100 mmHg, a hemispheric model provides the best agreement between the calculated and actual flow rate. In another report, Deng *et al*. [4] indicated the optimal Nyquist limit between 30-35 cm/s is appropriate for a hemispheric assumption in most children. To minimize the error when measuring the PISA radius, it is better to set the Nyquist limit as low as possible because it tends to maximize the PISA radius; but to distinguish low intraventricular flow from true proximal accelerating flow, it should not be set the velocity too low. It was thought that setting of the Nyquist limit velocity at about 20-40 cm/s is suitable when applying the PISA method in this study. It is not strictly necessary to use the first alias to calculate flow rate since any isovelocity hemisphere should theoretically provide the same result. However, the first alias is the most apparent and reproducible region of the flow stream and is therefore most suitable for velocity estimation and measurement of radial distance.

In the present study, the dogs with induced MR were anesthetized to alter ventricular loading conditions, because general anesthesia may be a common situation that hemodynamic alteration can be occurred in old small animals such as scaling and surgery associated geriatric disease. Also, general anesthesia has profound effects on loading conditions with resulting effects on mitral valve function and regurgitant volume. In this anesthetic study, isoflurane anesthesia resulted in a non-significant change in echocardiographic parameters except regurgitant volume. Bach *et al*. [2] demonstrated general anesthesia with isoflurane altered blood pressure and LV cavity dimensions

reflecting altered loading condition. These discrepancies of the results may be due to the differences between anesthetic protocols.

All echocardiographic parameters were markedly changed except ERO area and regurgitant time in ketamine-xylazine combination anesthesia. Xylazine has cardiodepressant and arrhythmogenic effects, and induces bradycardia and a brief period of hypertension, followed by a longer-lasting decrease in cardiac output and blood pressure [25]. Xylazine-induced decreases in heart rate and cardiac output are moderated by ketamine's sympathomimetic action, while blood pressure and systemic vascular resistance are increased [14]. The increase of blood pressure and systemic vascular resistance may cause marked increase of end-systolic volume, and decrease of aortic output, thus RSV increased, while fractional shortening decreased in this study.

The change in regurgitant volume was not related to differences in heart rate, blood pressure, or technical factors in imaging, but may be related to lower systemic vascular resistance under isoflurane anesthesia and increase systemic vascular resistance under ketamine-xylazine combination. Thus, the possibility of underestimation of mitral regurgitant severity must be considered under isoflurane anesthesia, such as transesophageal echocardiography or surgery of cardiovascular system. ERO area was not changed under both isoflurane and ketamine-xylazine anesthesia that shows ERO may be hemodynamically independent factor and should be preferred as a factor reflecting the severity of mitral regurgitation.

There are several limitations in this study. First, the quantitative Doppler method was not "gold standard" to estimate the accuracy of PISA method. Direct measurement of the effective regurgitant orifice area should be performed, but such a method does not exist because of inaccuracies of measurements of flow by invasive methods [14]. Also consistent use of quantitative Doppler echocardiography has proved to be a very reliable method. Incompleteness of the PISA method has been described for measuring regurgitant flow and effective regurgitant orifice area in the previous studies. The PISA method assumes that this orifice area is roughly constant in systole, but its not true [19]. Thus we just measured instantaneous maximal PISA radius. To go beyond this limitation, total regurgitant volume might be calculated by integrating the instantaneous flow rate over time, or 3-dimensional reconstruction of the hemicircle into a hemisphere. However, these methods are not available in clinical veterinary practice. Although the theoretic problems exist, high-resolution imaging, experienced technique, and appropriate ascertainment of flow convergence allow accurate quantitation of mitral regurgitation.

Enriquez-Sarano *et al.* [7] studied the progression of MR in large clinical series. Their study suggested that regular follow-up echocardiographic examinations should be performed in patients with MR. They recommended the optimal delay for follow-up examinations. It is thought that these standards to estimate progression of MR should also be performed in veterinary clinical fields through a large clinical outcome using quantitative method. In conclusion, the feasibility of the PISA method is excellent after the initial learning phase in dogs. Flow calculations that are based on the assumption of simple hemispheric symmetry of the proximal flow field showed excellent correlation with flow values that were obtained by the more cumbersome and time-consuming Doppler two-dimensional echocardiographic method. Veterinary practitioners do not have sufficient time for gathering high quality recordings, because dogs with left heart failure may be intolerant of protracted echocardiographic examination due to severe dyspnea and cough. Thus, PISA method is especially useful in small animal practice considering its simplicity. Although refinements to the proximal convergence method are to be expected in the future, it appears to be suitable for routine echocardiographic practice in dogs.

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