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Use of vertebral left atrial size for staging of dogs with myxomatous valve disease

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Abstract *Introduction/Objectives:* The American College of Veterinary Internal Medicine (ACVIM) guidelines suggest that pimobendan should be initiated in dogs which meet all criteria of stage B2 myxomatous mitral valve disease (MMVD): murmur intensity $\geq$ 3/6, left atrial-to-aortic ratio $\geq$ 1.6, normalized left ventricular internal diameter in diastole $\geq$ 1.7, and vertebral heart size $>$ 10.5. Recently, a new radiographic index for left atrial enlargement, vertebral left atrial size (VLAS), was proposed. The objective of the present study was to evaluate whether VLAS is useful in staging MMVD and if it can distinguish between ACVIM stages B1 and B2.
Animals: Ninety-seven client-owned dogs with MMVD were evaluated and classified as ACVIM stage B1, B2, or C-D.
Materials and Methods: The echocardiographs and radiographs of all the dogs were retrospectively evaluated to obtain left atrial-to-aortic ratio, normalized left ventricular internal diameter in diastole, and VLAS values. The data were analyzed to assess the correlation between these measurements and VLAS, and the optimal cutoff value of VLAS was determined.
Results: A VLAS cutoff value of 2.6 provided the greatest diagnostic accuracy for identification of dogs with ACVIM stage B2 MMVD (area under the curve, 0.96; sensitivity, 95%; specificity, 84%). A VLAS $\geq$2.5 exhibited the highest sensitivity

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(sensitivity, 100%; specificity, 78%), and a VLAS $\geq$ 3.1 exhibited the highest specificity (sensitivity, 47%; specificity, 100%).
Conclusions: VLAS is a helpful index for monitoring MMVD using radiography. A VLAS cutoff value of 2.5 could be used to identify dogs that may benefit from echocardiography to determine if they have reached ACVIM stage B2.

Abbreviations

ACVIM	American College of Veterinary Internal Medicine
AUC	Area under the curve
ICC	Intraclass correlation coefficient
LA:Ao	Left atrial-to-aortic ratio
LVIDDN	Normalized left ventricular internal diameter in diastole
MMVD	Myxomatous mitral valve disease
VHS	Vertebral heart size
VLAS	Vertebral left atrial size

Introduction

Myxomatous mitral valve disease (MMVD) is the commonest heart disease in middle-aged and older small-breed dogs [1]. In some dogs, MMVD progression induces congestive heart failure and fatal pulmonary edema, thus effective treatments are required. Pimobendan, a calcium sensitizer and a phosphodiesterase 3 inhibitor, is widely used to treat dogs with MMVD and cardiac remodeling. In previous studies, administration of pimobendan significantly improved the general condition and survival time of dogs with MMVD [2—4] and prolonged the time until recurrence of congestive heart failure [5]. However, one study reported that long-term administration of pimobendan may aggravate asymptomatic MMVD [6].

The American College of Veterinary Internal Medicine (ACVIM) guidelines, updated in 2019, recommend initiating pimobendan at MMVD stage B2 [7]. Dogs in this category should meet all of the following criteria: murmur intensity $\geq$3/6, left atrial-to-aortic ratio (LA:Ao) $\geq$ 1.6, normalized left ventricular internal diameter in diastole (LVIDDN) $\geq$ 1.7, and vertebral heart size (VHS) $>$ 10.5. These criteria are derived from the Evaluation of Pimobendan In dogs with Cardiomegaly (EPIC) Study [8], a large prospective clinical trial of pimobendan in dogs with MMVD. However, two of the remodeling criteria, LA:Ao and LVIDDN, must be measured by echocardiography. When echocardiography is unavailable, a VHS $\geq$11.5 or evidence of increasing interval change of radiographic cardiac enlargement has been proposed as alternative criteria for stage B2 [7], but definitive radiographic criteria are yet to be determined.

Recently, a new radiographic index, vertebral left atrial size (VLAS), was proposed as a useful radiographic measurement for the detection of left atrial enlargement in dogs with MMVD [9]. VLAS is the distance between the ventral border of the carina and the point where left atrium intersects with the dorsal border of the caudal vena cava, expressed in vertebral body units, and a VLAS $\geq$2.3 was shown to detect left atrial enlargement in a previous study [9]. The updated guidelines also state that VLAS values $\geq$ 3 likely identify stage B2 MMVD [7]; however, there has been no research confirming VLAS use for MMVD staging thus far. The purpose of the present study was to evaluate whether VLAS can be useful in staging MMVD and distinguish between ACVIM stages B1 and B2.

Animals, materials, and methods

Study population

The medical records of dogs with MMVD were evaluated retrospectively. All dogs were examined at the Cardiovascular Service of the Animal Medical Center, Nippon Veterinary and Life Sciences University, between January 2012 and August 2018. To be included, the dogs had to have undergone echocardiography and right lateral thoracic radiography on the same day. Dogs with unavailable images, congenital heart diseases, cardiomyopathy, severe pulmonary edema, cardiac tumors, pericardial effusion, or thoracic deformities were excluded. All the dogs were classified as stage B1, B2, or C-D according to the

ACVIM guidelines [7]. The diagnostic criteria for designation as ACVIM stage B2 included presence of all of the following: murmur intensity $\geq$3/6, LA:Ao $\geq$ 1.6, LVIDDN $\geq$1.7, and VHS $>$10.5. Dogs with current or past clinical signs of pulmonary edema were classified as ACVIM stage C-D. Dogs previously diagnosed with pulmonary edema at another hospital were defined as stage C and classified as stage C-D.

Echocardiography

One veterinary cardiologist (N.T.) performed all the echocardiographic examinations without anesthesia. LA:Ao and LVIDDN values were obtained. LA:Ao was determined from the B-mode image of the right parasternal window short axis view using a previously described method [10]. LVIDDN was measured using the M-mode at the right parasternal window short axis view [11]. These values were obtained from the medical records database at the Cardiovascular Service of the Animal Medical Center. LVIDDN was normalized to body weight using the following formula [12]: LVIDDN = left ventricular internal diameter in diastole (cm)/(body weight (kg))$^{0.294}$.

Thoracic radiography

All dogs underwent thoracic radiography without anesthesia. The right lateral radiographic images were collected, and VHS and VLAS values were obtained for each dog. VHS was calculated as the sum of the long and short axes of the heart as previously described [13]. The long axis was measured from the ventral border of the carina to the apex of the heart, and the short axis, perpendicular to the long axis, was defined as the line with maximum dimension of the heart in the central third region including the right atrium and left heart chambers. VLAS was measured as previously described [9]. A line was drawn and measured from the ventral border of the carina to the dorsal border of the caudal vena cava where it intersected with the left atrium. The same line length was drawn beginning at the cranial edge of the fourth thoracic vertebra and expressed in vertebral body units to the nearest 0.1 vertebra as VLAS. These measurements were performed by the same investigator (S.M.), who was blinded to the echocardiographic measurements of all of the dogs, using ImageJ image analysis software (version 1.50i, National Institutes of Health, Bethesda, MD, USA) [14,15].

Determination of the intraobserver and interobserver variability in VLAS measurements

To determine the intraobserver variability of VLAS measurements, the investigator (S.M.) measured the VLAS of 20 dogs on three separate occasions. To determine the interobserver variability of VLAS measurements, five other investigators (M.N., R.A., A.S., M.O., and H.M.) measured the VLAS of the same 20 dogs. All investigators were blinded to the echocardiographic measurements of the dogs. The 20 dogs were randomly selected from the study population, and the images were analyzed in a random order.

Statistical analysis

Statistical analysis was performed using Easy R (EZR) statistical software (version 1.40, Saitama Medical Center, Jichi Medical University, Shimotsuke, Japan) [16]. The normality of the data was evaluated using the Shapiro–Wilk test. Normally distributed data were presented as mean $\pm$ standard deviation of the sample, whereas non-normally distributed data were presented as medians (range: min–max). The normally distributed parameters were compared between ACVIM stage groups using one-way analysis of variance followed by *t*-tests with the Bonferroni correction for multiple comparisons. The non-normally distributed parameters were compared between the ACVIM stage groups using the Kruskal–Wallis test followed by the Man–Whitney *U* test with the Bonferroni correction for multiple comparisons. Nominal and categorical variables were compared using Pearson's chi-squared test. Spearman's rank correlation coefficient (*r*) was calculated to determine the associations between VLAS and echocardiographic parameters. The sensitivity and specificity of VLAS in the detection of each parameter were plotted to generate a receiver operating characteristic curve, and the optimal cutoff value was defined as the point on the curve with greatest Youden index (sensitivity - [1 - specificity]). The area under the curve (AUC) was used to evaluate the diagnostic accuracy of the test. The intraclass correlation coefficient (ICC) was calculated to evaluate the intraobserver and interobserver variabilities in VLAS measurements, as described previously [17]. Values of ICCs greater than 0.75 were considered reliable. A *p*-value less than 0.05 was considered statistically significant.

Table 1 Characteristics, echocardiographic parameters, and medication records of the dogs according to ACVIM stage.

Variable	ACVIM stage			*p* value
	Stage B1	Stage B2	Stage C-D	
Number of dogs	64	19	14	—
Age (years)	12.2 (4.5—17.3)	10.8 (7.0—15.2)	12.4 (8.6—15.7)	0.342
Body weight (kg)	4.5 (1.9—16.6)	4.6 (2.4—13.1)	4.1 (1.8—8.8)	0.723
Sex (male/female)	35/29	7/12	5/9	0.243
Murmur intensity (scale, 1—6)	3 (1—6)	6 (3—6)	6 (4—6)	—
LA:Ao	1.54 (0.94—2.61)	2.93 (1.62—4.08)	2.67 (1.31—4.33)	—
LVIDDN (cm/kg$^{0.294}$)	1.65 ± 0.27	2.24 ± 0.31	2.15 ± 0.44	—
VHS (v)	10.2 (8.5—13.6)	12.2 (10.9—14.8)	11.7 (10.5—14.8)	—
VLAS (v)	2.1 (1.5—3.0)	3.0 (2.5—3.8)*	3.3 (2.2—3.8)*	<0.001
Drug				
None	24/64	2/19	0/14	—
ACE inhibitor	39/64	17/19	14/14	—
Pimobendan	15/64	14/19	11/14	—
Loop diuretic	4/64	3/19	9/14	—
Isosorbide dinitrate	6/64	5/19	6/14	—

ACE: angiotensin-converting enzyme; ACVIM: American College of Veterinary Internal Medicine; LA:Ao: left atrial-to-aortic ratio; LVIDDN: normalized left ventricular internal diameter in diastole; VHS: vertebral heart size; VLAS: vertebral left atrial size. Normally distributed data are presented as the mean ± SD. Data that were not normally distributed are presented as the median (range). — = not determined

*$p < 0.05$ vs. stage B1 group.

Results

Dogs

Ninety-seven dogs (47 males, 50 females) were included in the present study. The mean age was 11.4 ± 2.8 years, and the median body weight was 4.5 kg (range, 1.8—16.6). Overall, ACVIM stage B1, B2, and C-D groups included 64, 19, and 14 dogs, respectively. The stage B1 group included 16 Chihuahuas, 11 Maltese, 7 Shih Tzus, 6 miniature dachshunds, 4 Cavalier King Charles spaniels, 4 miniature schnauzers, 4 mixed-breed dogs, 3 toy poodles, 2 Pomeranians, 2 Yorkshire terriers, and 1 beagle, Chinese crested, spitz, West Highland white terrier, and wire fox terrier. The stage B2 group included 4 Maltese, 3 Chihuahuas, 2 Cavalier King Charles spaniels, 2 papillons, 2 toy poodles, and 1 American cocker spaniel, Jack Russell terrier, mixed-breed dog, miniature dachshund, Pomeranian, and Shih Tzu. The stage C-D group included 8 Chihuahuas, 3 Cavalier King Charles spaniels, 2 toy poodles, and 1 Pomeranian. The median age, median body weight, and sex distribution did not differ significantly among the three groups. Seventy-one dogs had been administered medication for MMVD (Table 1), but none of the dogs were taking these medicines during the examinations in the hospital. As expected, the murmur intensity, LA:Ao, LVIDDN, and VHS of the dogs increased with increasing ACVIM stage. VLAS also increased with increasing ACVIM stage. However, VLAS was not significantly different between the stage B2 and C-D groups.

Correlation analyses

We analyzed the correlations between VLAS and echocardiographic parameters in all dogs. VLAS significantly positively correlated with LA:Ao ($r = 0.68$ [95% confidence interval {CI}, 0.55 to 0.77], $p < 0.001$; Fig. 1A) and LVIDDN ($r = 0.61$ [95% CI, 0.47 to 0.72], $p < 0.001$; Fig. 1B).

Distinguishing ACVIM stages B1 and B2 using VLAS

To evaluate whether VLAS can be used to distinguish between ACVIM stages B1 and B2, we analyzed the dogs with stage B MMVD (n = 83). The sensitivity and specificity of VLAS in detecting the echocardiographic criteria for cardiac remodeling, LA:Ao ≥ 1.6 and/or LVIDDN ≥1.7, are shown in Table 2. The diagnostic accuracy of VLAS in detecting dogs with a LA:Ao ≥ 1.6 (AUC, 0.80 [95% CI, 0.70 to 0.89]) or LVIDDN ≥ 1.7 (AUC, 0.80 [95% CI, 0.71 to 0.90]) was moderate. When a VLAS cutoff value of 2.5 was used, specificities greater

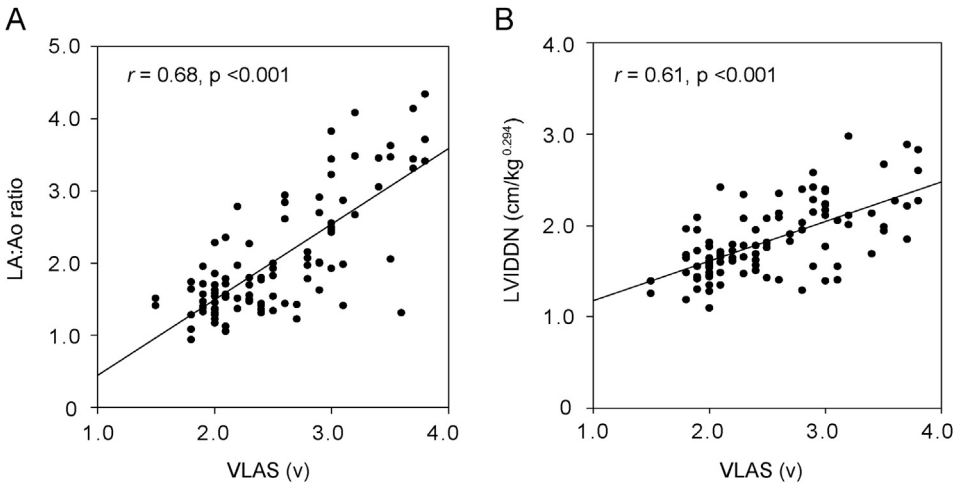

Fig. 1 Correlations between VLAS and echocardiographic parameters. (A) LA:Ao and (B) LVIDDN significantly correlated with VLAS. LA:Ao: left atrial-to-aortic ratio; LVIDDN: normalized left ventricular internal diameter in diastole; VLAS: vertebral left atrial size; *r*: Spearman's rank correlation coefficient.

Table 2 Accuracy of VLAS for detecting echocardiographic parameters.

Echocardiographic criteria for cardiac remodeling	Number of dogs meeting the criteria	AUC (95% CI)	VLAS cutoff value	Sensitivity (95% CI)	Specificity (95% CI)	Youden index
LA:Ao ≥1.6	45/83	0.80 (0.70—0.89)	≥2.5	62% (48—75%)	87% (73—94%)	49
LVIDDN ≥1.7	44/83	0.80 (0.71—0.90)	≥2.5	64% (49—76%)	87% (73—94%)	51
LA:Ao ≥1.6 and LVIDDN ≥1.7	28/83	0.87 (0.77—0.97)	≥2.5	86% (69—94%)	84% (72—91%)	69

AUC: area under the curve; CI: confidence interval; LA:Ao: left atrial-to-aortic ratio; LVIDDN: normalized left ventricular internal diameter in diastole; VLAS: vertebral left atrial size.

Table 3 Accuracy of VLAS for distinguishing between ACVIM stages B1 and B2.

Patients	AUC (95% CI)	VLAS cutoff value	Cutoff type	Sensitivity (95% CI)	Specificity (95% CI)	Youden index	Positive predictive value (95% CI)	Negative predictive value (95% CI)
ACVIM stage B2	0.96 (0.93—1.00)	≥2.5	Maximum sensitivity	100% (83—100%)	78% (67—87%)	78	58% (41—73%)	100% (93—100%)
		≥2.6	Maximum Youden index	95% (75—99%)	84% (74—91%)	79	64% (46—79%)	98% (90—100%)
		≥2.7		84% (62—95%)	88% (77—94%)	72	67% (47—82%)	95% (86—98%)
		≥2.8		84% (62—95%)	91% (81—96%)	75	73% (52—87%)	95% (87—98%)
		≥2.9		79% (57—92%)	94% (85—98%)	73	79% (57—92%)	94% (85—98%)
		≥3.0		63% (41—81%)	97% (89—99%)	60	86% (60—96%)	90% (81—95%)
		≥3.1	Maximum specificity	47% (27—68%)	100% (94—100%)	47	100% (70—100%)	87% (77—93%)

ACVIM: American College of Veterinary Internal Medicine; AUC: area under the curve; CI: confidence interval; VLAS: vertebral left atrial size.

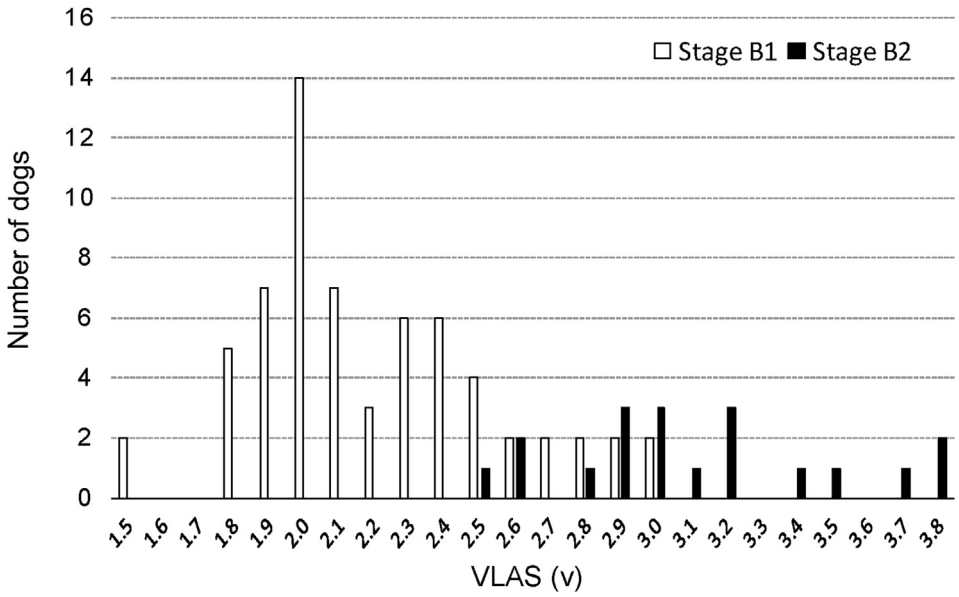

Fig. 2 Distribution of VLAS values in the American College of Veterinary Internal Medicine stage B1 and B2 groups. All dogs with stage B2 had a VLAS $\geq$2.5, and no dog with stage B1 had a VLAS $\geq$3.1. VLAS: vertebral left atrial size.

than 80% were noted, but the observed sensitivities were not high. On the other hand, the diagnostic accuracy of VLAS in detecting dogs satisfying both echocardiographic criteria, LA:Ao $\geq$ 1.6 and LVIDDN $\geq$1.7, was slightly higher (AUC, 0.87 [95% CI, 0.77 to 0.97]), with improved sensitivity and specificity. The Pearson's chi-squared test revealed that VLAS $\geq$2.5 was significantly associated with LA:Ao $\geq$ 1.6 and LVIDDN $\geq$1.7 ($p < 0.001$). However, LA:Ao $\geq$ 1.6 and LVIDDN $\geq$1.7 were independent ($p = 0.108$). Table 3 shows the sensitivity and specificity of VLAS in detecting dogs with ACVIM stage B2, defined as dogs with murmur intensity $\geq$3/6, LA:Ao $\geq$ 1.6, LVIDDN $\geq$1.7, and VHS >10.5. When a VLAS cutoff value of 2.6 was used, the diagnostic accuracy of VLAS in identifying dogs with ACVIM stage B2 was higher (AUC, 0.96 [95% CI, 0.93 to 1.00]), and the sensitivity and specificity were 95% (95% CI, 75—99%) and 84% (95% CI, 74—91%), respectively (Table 3). The distribution of VLAS values in ACVIM stage B1 and B2 groups is shown in Figure 2. A VLAS cutoff value of 2.5 exhibited the highest sensitivity, and a VLAS cutoff value of 3.1 exhibited the highest specificity.

Intraobserver and interobserver variability in VLAS measurements

To determine the reliability of the VLAS measurements, the intraobserver and interobserver ICCs were calculated. The intraobserver and interobserver ICCs were 0.97 (95% CI, 0.94 to 0.99) and 0.89 (95% CI, 0.82 to 0.95), respectively. Because both ICC values were greater than 0.75, the obtained VLAS measurements were deemed to be reliable.

Discussion

VLAS was recently proposed as a new radiographic measurement by Malcolm et al. [9]. They suggested that a VLAS $\geq$2.3 is a useful marker of left atrial enlargement. They measured LA:Ao values from short-axis and long-axis echocardiographic images, and left atrial enlargement was defined as a short-axis LA:Ao $\geq$ 1.6 or a long-axis LA:Ao $\geq$ 2.6. In that study, short-axis LA:Ao positively correlated with VLAS, and the observed correlation coefficient was 0.70. In the present study, we observed a similar correlation coefficient between LA:Ao and VLAS ($r = 0.68$). The optimal VLAS cutoff value for detecting a short-axis LA:Ao $\geq$ 1.6 in dogs with ACVIM stage B was 2.5 (62% sensitivity and 87% specificity), which was consistent with the previous study (cutoff value of 2.5: 67% sensitivity and 84% specificity).

Moreover, we showed that VLAS also correlated with LVIDDN, an echocardiographic parameter reflecting left ventricular enlargement. Because the measurement of VLAS does not include the radiographic projection of the left ventricle, its increase can only be indirectly associated with left

ventricular enlargement [13,18]. Left ventricular enlargement with MMVD suggests considerable left heart strain. Because left heart strain inevitably expands the left atrium, VLAS may therefore reflect this phenomenon. However, LA:Ao $\geq$ 1.6 and LVIDDN $\geq$1.7 were independent in Pearson's chi-squared test, and LVIDDN was independently associated with VLAS. These results suggest that VLAS reflects enlargement of the entire left side of the heart, rather than only left atrial enlargement. VLAS represents the distance between the carina and the caudal border of the left atrium where it intersects with the caudal cava. Therefore, VLAS may increase because the trachea is elevated when cardiac enlargement occurs, so the distance from the vena cava to the carina increases. Therefore, we suggest that VLAS may be a sensitive marker of enlargement of the left side of the heart.

The present study indicates that VLAS can distinguish between dogs with ACVIM stages B1 and B2 with high accuracy. The receiver operating characteristic analysis shows that the most clinically relevant VLAS cutoff value was 2.6, but the predictive value depends on the prevalence rate. The prevalence rate of ACVIM stage B2 in the present study was 23%. In previous studies, 39% [9], 44% [19], and 54% [20] of dogs with ACVIM stage B were stage B2. However, these studies were published before the ACVIM guidelines were updated in 2019, and the diagnostic criteria for stage B2 were more lenient than those in the current guidelines. The low prevalence of stage B2 in this study is thought to reflect this. Since no studies have categorized populations according to the new diagnostic criteria, the validity of the prevalence of stage B2 in this study needs to be supported by future studies. Based on the prevalence of stage B2 in this study, if pimobendan was administered to all dogs with a VLAS $\geq$2.6, ten stage B1 dogs would receive this medication. In addition, one stage B2 dog would not receive pimobendan. This means that a total of 11 dogs (17%) would be managed inappropriately as currently outlined by the ACVIM consensus guidelines. Therefore, it is not recommended to determine pimobendan initiation based on VLAS measurement only. In the present study, VLAS $\geq$3.1 distinguished stage B2 dogs with 100% specificity. This result may suggest that VLAS values of $\geq$3.1 likely identify stage B2 MMVD in the absence of echocardiography. VLAS values $\geq$ 2.5 identified dogs with stage B2 with 100% sensitivity. Therefore, we propose that a VLAS cutoff value of 2.5 should be used to recommend echocardiography to identify whether MMVD in stage B1 dogs has progressed to ACVIM stage B2. All dogs included in the present study had previously been diagnosed with MMVD. Consequently, dogs not yet diagnosed with MMVD should have echocardiography performed regardless of the VLAS value.

Because this was a retrospective cross-sectional study, it was not possible to assess the effects of medication on VLAS. In the present study, almost all the dogs had already been administered cardiac medication because they were at a secondary care facility. A previous study indicated that pimobendan reduced VHS in dogs with MMVD [5,21]. Therefore, it is possible that these medications could affect VLAS, although further prospective longitudinal studies on the effect of medication on VLAS are required.

Conclusions

VLAS appears to be valuable for the identification of left-sided cardiomegaly and in distinguishing between dogs with stages B1 and B2 MMVD. A VLAS cutoff value of 2.5 could be used to identify dogs which require echocardiography for further cardiac evaluation and a cutoff value of 3.1 may be useful to identify dogs that have likely reached stage B2 MMVD, particularly in cases where echocardiography is not possible. Thus, measurement of VLAS may be helpful in the optimization of care for dogs with heart murmurs and cardiac enlargement.

Conflict of Interest Statement

The authors do not have any conflicts of interest to disclose.

References

[1] Serres FJ, Chetboul V, Tissier R, Carlos Sampedrano C, Gouni V, Nicolle AP, Pouchelon JL. Doppler echocardiography-derived evidence of pulmonary arterial hypertension in dogs with degenerative mitral valve disease: 86 cases (2001-2005). J Am Vet Med Assoc 2006;229:1772—8.

[2] Lombard CW, Jons O, Bussadori CM. Clinical efficacy of pimobendan versus benazepril for the treatment of acquired atrioventricular valvular disease in dogs. J Am Anim Hosp Assoc 2006;42:249—61.

[3] Mizuno M, Yamano S, Chimura S, Hirakawa A, Takusagawa Y, Sawada T, Maetani S, Takahashi A, Mizuno T, Harada K, Shinoda A, Uchida S, Takeuchi J, Mizukoshi T, Endo M, Uechi M. Efficacy of pimobendan on

survival and reoccurrence of pulmonary edema in canine congestive heart failure. J Vet Med Sci 2017;79:29—34.
[4] Atkins CE, Haggstrom J. Pharmacologic management of myxomatous mitral valve disease in dogs. J Vet Cardiol 2012;14:165—84.
[5] Haggstrom J, Boswood A, O'Grady M, Jons O, Smith S, Swift S, Borgarelli M, Gavaghan B, Kresken JG, Patteson M, Ablad B, Bussadori CM, Glaus T, Kovacevic A, Rapp M, Santilli RA, Tidholm A, Eriksson A, Belanger MC, Deinert M, Little CJ, Kvart C, French A, Ronn-Landbo M, Wess G, Eggertsdottir A, Lynne O'Sullivan M, Schneider M, Lombard CW, Dukes-McEwan J, Willis R, Louvet A, DiFruscia R. Longitudinal analysis of quality of life, clinical, radiographic, echocardiographic, and laboratory variables in dogs with myxomatous mitral valve disease receiving pimobendan or benazepril: the QUEST study. J Vet Intern Med 2013;27:1441—51.
[6] Chetboul V, Lefebvre HP, Sampedrano CC, Gouni V, Saponaro V, Serres F, Concordet D, Nicolle AP, Pouchelon JL. Comparative adverse cardiac effects of pimobendan and benazepril monotherapy in dogs with mild degenerative mitral valve disease: a prospective, controlled, blinded, and randomized study. J Vet Intern Med 2007;21:742—53.
[7] Keene BW, Atkins CE, Bonagura JD, Fox PR, Haggstrom J, Fuentes VL, Oyama MA, Rush JE, Stepien R, Uechi M. ACVIM consensus guidelines for the diagnosis and treatment of myxomatous mitral valve disease in dogs. J Vet Intern Med 2019;33:1127—40.
[8] Boswood A, Haggstrom J, Gordon SG, Wess G, Stepien RL, Oyama MA, Keene BW, Bonagura J, MacDonald KA, Patteson M, Smith S, Fox PR, Sanderson K, Woolley R, Szatmari V, Menaut P, Church WM, O'Sullivan ML, Jaudon JP, Kresken JG, Rush J, Barrett KA, Rosenthal SL, Saunders AB, Ljungvall I, Deinert M, Bomassi E, Estrada AH, Fernandez Del Palacio MJ, Moise NS, Abbott JA, Fujii Y, Spier A, Luethy MW, Santilli RA, Uechi M, Tidholm A, Watson P. Effect of pimobendan in dogs with preclinical myxomatous mitral valve disease and cardiomegaly: the EPIC study-A randomized clinical trial. J Vet Intern Med 2016;30:1765—79.
[9] Malcolm EL, Visser LC, Phillips KL, Johnson LR. Diagnostic value of vertebral left atrial size as determined from thoracic radiographs for assessment of left atrial size in dogs with myxomatous mitral valve disease. J Am Vet Med Assoc 2018;253:1038—45.
[10] Hansson K, Haggstrom J, Kvart C, Lord P. Left atrial to aortic root indices using two-dimensional and M-mode echocardiography in cavalier King Charles spaniels with and without left atrial enlargement. Vet Radiol Ultrasound 2002;43:568—75.
[11] Thomas WP, Gaber CE, Jacobs GJ, Kaplan PM, Lombard CW, Moise NS, Moses BL. Recommendations for standards in transthoracic two-dimensional echocardiography in the dog and cat. Echocardiography committee of the specialty of cardiology, American College of veterinary internal medicine. J Vet Intern Med 1993;7:247—52.
[12] Cornell CC, Kittleson MD, Della Torre P, Haggstrom J, Lombard CW, Pedersen HD, Vollmar A, Wey A. Allometric scaling of M-mode cardiac measurements in normal adult dogs. J Vet Intern Med 2004;18:311—21.
[13] Buchanan JW, Bucheler J. Vertebral scale system to measure canine heart size in radiographs. J Am Vet Med Assoc 1995;206:194—9.
[14] Rasband WS. ImageJ. Bethesda, Maryland, USA: U. S. National Institutes of Health; 1997-2012. http://imagej.nih.gov/ij/.
[15] Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 2012;9:671—5.
[16] Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 2013;48:452—8.
[17] Trevethan R. Intraclass correlation coefficients: clearing the air, extending some cautions, and making some requests. Health Serv Outcome Res Methodol 2017;17:127—43.
[18] Henjes CR, Hungerbuhler S, Bojarski IB, Nolte I, Wefstaedt P. Comparison of multi-detector row computed tomography with echocardiography for assessment of left ventricular function in healthy dogs. Am J Vet Res 2012;73:393—403.
[19] Park JS, Park JH, Seo KW, Song KH. Correlation between NT-proBNP and lipase levels according to the severity of chronic mitral valve disease in dogs. J Vet Sci 2019;20:e43.
[20] Vezzosi T, Mannucci T, Pistoresi A, Toma F, Tognetti R, Zini E, Domenech O, Auriemma E, Citi S. Assessment of lung ultrasound B-lines in dogs with different stages of chronic valvular heart disease. J Vet Intern Med 2017;31:700—4.
[21] Boswood A, Gordon SG, Haggstrom J, Wess G, Stepien RL, Oyama MA, Keene BW, Bonagura J, MacDonald KA, Patteson M, Smith S, Fox PR, Sanderson K, Woolley R, Szatmari V, Menaut P, Church WM, O'Sullivan ML, Jaudon JP, Kresken JG, Rush J, Barrett KA, Rosenthal SL, Saunders AB, Ljungvall I, Deinert M, Bomassi E, Estrada AH, Fernandez Del Palacio MJ, Moise NS, Abbott JA, Fujii Y, Spier A, Luethy MW, Santilli RA, Uechi M, Tidholm A, Schummer C, Watson P. Longitudinal analysis of quality of life, clinical, radiographic, echocardiographic, and laboratory variables in dogs with preclinical myxomatous mitral valve disease receiving pimobendan or placebo: the EPIC study. J Vet Intern Med 2018;32:72—85.