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Assessment of Vertebral Left Atrial Size and C-reactive Protein in Dogs With Myxomatous Mitral Valve Disease

Sun-Hwee Hwang and Kun Ho Song[1]

College of Veterinary Medicine, Chungnam National University, Daejeon 34134, South Korea

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Abstract : Recently, a new method of evaluating left atrial size called vertebral left atrial size (VLAS) was introduced in dogs. Total 155 dogs were examined at the Veterinary Medical Teaching Hospital of Chungnam National University. In this study, myxomatous mitral valve disease (MMVD) stage and VLAS showed a significant correlation in those dogs. Also, the relationship between C-reactive protein (CRP) and VLAS has yet to be examined. We found a strong positive correlation between VLAS and CRP—a significant increase in CRP was observed with increasing VLAS values. Thus, it would be beneficial to measure VLAS besides employing the current radiological and echocardiographic methods when evaluating heart size. Measuring VLAS could be an additional diagnostic tool for diagnosing MMVD in dogs.

Key words **:** myxomatous mitral valve disease, vertebral left atrial size, c-reactive protein, dog.

Introduction

Myxomatous mitral valve disease (MMVD) is the most common acquired heart disease in dogs (11,24). It is characterized by slow and gradual degeneration of the mitral valve due to unknown cause. Also, the degeneration leads to subclinical period during which several factors (ex; murmur, heart size) seriously changes as valve regurgitation progresses (5). Among many factors, left atrial enlargement (LAE) is a reliable indicator for predicting the progression and severity of heart disease (22).

It is important to use it to accurately diagnose the disease, to evaluate the prognosis, and to administer the appropriate medications prior to the onset of congestive heart failure (CHF) (4,6). Therefore, assessment of left atrial size is considered as an important factor in dogs with high risk of congestive heart failure (9,23). Although the assessment of cardiac size by echocardiography is still the most useful method, it has several limitations. It requires an expert who can competently handle echocardiography and make an accurate assessment, and it takes a lot of time and money (1). Thoracic radiography is effective diagnostic evaluation for patient with MMVD (16). Besides, it is considered the gold-standard tool for diagnosing pulmonary edema (12,16).

The ACVIM guidelines, updated in 2019, present criteria for determining MMVD Stage B. Stage B can be further classified into stages B1 or B2 according to several criteria (16).

Patients in stage B2 must satisfy the following criteria: murmur intensity ≥ 3/6, an echocardiographic left atrial-to-aortic ratio (LA/Ao) ≥ 1.6 (25), normalized left ventricular internal diameter in diastole (LVIDDN) ≥ 1.7 (10), and a thoracic radiographic vertebral heart score (VHS) > 10.5 (18). If echocardiography is unavailable, VHS ≥ 10.5, or evidence of a rising interval change revealed by thoracic radiography can replace echocardiography to confirm stage B2 (16). Recently, a new radiographic indicator of left atrium size, the vertebral left atrial size (VLAS), was proposed (16,19,20). VLAS is the length between the most ventral part of the carina and the caudal point of the left atrium where it crosses the most dorsal aspect of the caudal vena cava. Following the VHS measurement method, the measured distance is compared using vertebral body units (19). The recently updated consensus guidelines state that VLAS values of ≥ 3 likely confirm MMVD stage B2 (16,19). There have been several studies on VLAS; however, no studies have explored correlations among breeds. We focused on VLAS in three breeds of dogs in which MMVD is prevalent in South Korea.

Inflammation is a significant part of the progression and pathogenesis of diverse forms of CHF. In veterinary medicine, C-reactive protein (CRP), an acute-phase protein produced by the liver, is used as a marker of inflammation (8,13,14,21). CRP concentrations are elevated by overexpression of myocardial cytokine in the atria and ventricles in dogs with heart failure induced by various cardiac diseases, including MMVD (21). Based on the VLAS cutoff value presented in a study published in 2020 (20), this study evaluated CRP by dividing the patients into groups according to the highest sensitivity value of 2.6 and the highest specificity value of 3.1.

The purpose of this study, therefore, is as follows: First, to compare VLAS values according to the ACVIM stage. Second, to compare the VLAS values of three breeds (Maltese, Shih-Tzu, and Poodle) with MMVD. Third, to confirm the correlation between CRP and VLAS in dogs with MMVD.

[1]Corresponding author.
E-mail : songkh@cnu.ac.kr

Materials and Methods

Animals

This study involved a total of 155 dogs with MMVD. All dogs were examined at the Veterinary Medical Teaching Hospital of Chungnam National University between December 2017 and August 2020. Before initiating the experiment, the clients informed consent was obtained. MMVD was diagnosed based on physical examinations, thoracic radiography, and echocardiography. All of the patients were classified as MMVD Stage B1, B2, or C-D according to the ACVIM guidelines (16). Also, the VHS, LA/Ao ratios, LVIDDN, and VLAS values of the species of Maltese ($n = 67$), Shih-Tzu ($n = 51$), and Poodle ($n = 37$) were compared. Thirteen patients with postural errors and abnormal vertebrae and patients whose accurate readings were difficult due to distinct pulmonary edema were excluded.

Besides, 23 dogs were included to investigate CRP values in relation to VLAS. All the patients had cardiovascular symptoms at the time of their visit. The patients were divided into three groups according to a study on the VLAS cutoff value. This was based on the highest sensitivity value of 2.6 and the highest specificity value of 3.1 (20). Patients in which other diseases may have affected CRP were excluded as much as was possible.

Thoracic radiography

Thoracic radiography was conducted to obtain dorsoventral views, right lateral views, and left lateral views in all of the patients. VHS and VLAS values were measured for each dog. Both VLAS and VHS were measured using a digital caliper in the right lateral view. The method of measuring VLAS was as follows: A line was drawn and measured from the most ventral side of the carina to the most dorsal side of the caudal vena cava where it crossed the left atrium. A line equal in length was then drawn beginning at the fourth thoracic vertebra and presented in vertebral body units (7,19).

Echocardiography

Echocardiography was conducted using an iU22® (Phillips, Bothwell WA, 98041 USA) to identify mitral valve degeneration and mitral regurgitation. Mitral valve prolapses were identified by color doppler imaging in the left apical 4-chamber view and two-dimensional echocardiography. Left ventricular internal diameter in diastole (LVIDD) was obtained in M-mode. Normalized left ventricular internal diameter (LVIDDN) was evaluated using the formula (LVIDDN = LVIDD (cm)/weight (kg)$^{0.294}$). The LA/Ao ratio was measured in the right parasternal two-dimensional short-axis view (9,10).

Blood sampling

Blood samples of 2 ml were obtained from the cephalic vein or the jugular vein of the fasted dogs. Following collection, the samples were left to coagulate. The blood samples were then centrifuged at 8,000 g for 10 minutes, and centrifuged serum was separated into Eppendorf tubes.

The biochemical profiles were measured using a biochemical analyzer (Mindray, BS-330, LinkedIn, China). The CRP reference of this machine is from 0 to 2; if CRP measures 2 or higher, the level is considered to be elevated.

Statistical analysis

Statistical analysis was performed using a commercial computer-based software program (IBM SPSS statistics 24.0.0, SPSS Inc., USA). The values of each group, including age, weight, VHS, VLAS, LA/Ao ratio, and LVIDDN were reported as medians and interquartile ranges (IQR). The values among the three groups were compared using the Shapiro-Wilk test, and post hoc tests were conducted using the Bonferroni correction. Additionally, the Kruskal-Wallis test was conducted to evaluate the correlation between VLAS and CRP. A p value of < 0.05 can be interpreted as statistically significant.

Results

The study population comprised 155 dogs, classified by two criteria. The first was to classify the dogs according to their ACVIM stage. The second was to classify the dogs by breed. Age, weight, and sex, with detailed data for each stage, were summarized (Table 1). The stage B1 group consisted of 88 dogs, and the Stage B2 and C-D groups comprised 46 dogs and 21 dogs, respectively.

The illustrative data of the echocardiographic and radio-

Table 1. Descriptive data of radiographic and echocardiographic values according to ACVIM stage

Variable	Stage B1	Stage B2	Stage C-D
Total (n = 155)	88	46	21
Age (yr)	12.00 (8.13-14.75)	12.00 (8.38-14.00)	13.00 (11.00-16.50)
Weight (kg)	3.83 (3.00-5.38)	4.25 (3.15-5.19)	3.80 (2.70-5.70)
Sex (M/F)	(35) / (53)	(22) / (24)	(11) / (10)
VHS	10.00 (9.80-10.28)	11.25 (10.90-11.73)*	11.60 (11.00-12.25)†
VLAS	2.20 (2.10-2.30)	2.80 (2.70-2.83)*	3.00 (2.90-3.10)†, ††
LA/Ao ratio	1.51 (1.33-1.70)	2.08 (1.83-2.38)*	2.18 (1.94-2.50)†
LVIDDN	1.37 (1.26-1.50)	1.85 (1.74-1.95)*	1.97 (1.81-2.10)†

ACVIM, American College of Veterinary Internal Medicine; M, male; F, Female; VHS, Vertebral heart size; VLAS, vertebral left atrial size; LA/Ao ratio, left atrial-to-aortic ratio; LVIDDN, normalized left ventricular internal diameter in diastole.
*$P < 0.05$; significant difference between stage B1 and stage B2, †$P < 0.05$; significant difference between stage B1 and stage C-D, ††$P < 0.05$; significant difference between stage B2 and stage C-D.

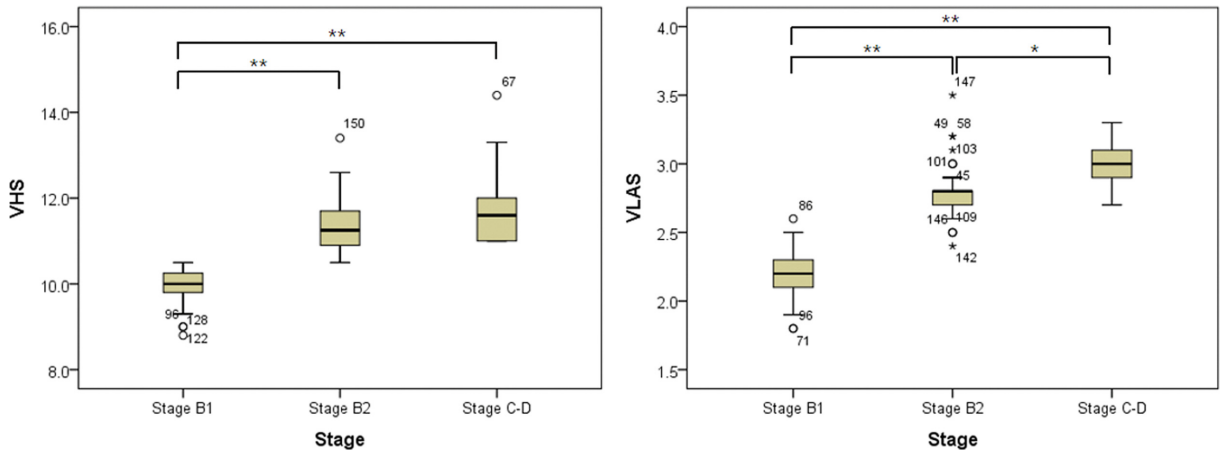

Fig 1. Evaluation of the correlation between VHS and VLAS according to the ACVIM stage. VHS; Vertebral heart size, VLAS; vertebral left atrial size, ACVIM; American College of Veterinary Internal Medicine. $^{*}P < 0.05$, $^{**}P < 0.01$.

graphic values in each stage were summarized (Table 1). When comparing the values such as VHS, LA/Ao ratio, LVIDDN, and VLAS between each group, there were significant differences between the B1 group and the B2 group ($p < 0.05$), and the B1 group and the C-D group ($p < 0.05$). When comparing the B2 group with the C-D group, only VLAS was observed to be significantly different ($p < 0.05$) (Table 1, Fig 1).

The dogs in this study were classified by breed as follows: Maltese ($n = 67$), Shih-Tzu ($n = 51$), Poodle ($n = 37$). Characteristic data such as age, weight, and sex for each breed, and radiographic and echocardiographic values according to breed were summarized (Table 2).

The VLAS values were 2.4 for the Maltese, 2.5 for the Shih-Tzus, and 2.5 for the Poodles, showing that there was no significant difference among the breeds. Additionally,

Table 2. Descriptive data of radiographic and echocardiographic values according to breed with MMVD

Variable	Maltese	Shih-tzu	Poodle
Total (n = 155)	67	51	37
Age (yr)	11.00 (8.00-13.00)	14.75 (11.00-17.00)	11.00 (8.00-12.20)
Weight (kg)	3.40 (2.50-4.00)	5.40 (4.50-6.50)	3.60 (3.00-4.73)
Sex (M/F)	(29) / (38)	(25) / (26)	(14) / (23)
VHS	10.50 (10.00-11.20)	10.40 (10.00-11.00)	10.30 (9.95-11.20)
VLAS	2.40 (2.20-2.80)	2.50 (2.20-2.80)	2.40 (2.10-2.80)
LA/Ao ratio	1.70 (1.50-2.20)	1.78 (1.54-2.09)	1.67 (1.32-2.10)
LVIDDN	1.62 (1.32-1.87)	1.61 (1.40-1.84)	1.46 (1.32-1.79)

M, male; F, Female; VHS, Vertebral heart size; VLAS, vertebral left atrial size; LA/Ao ratio, left atrial-to-aortic ratio; LVIDDN, normalized left ventricular internal diameter in diastole.

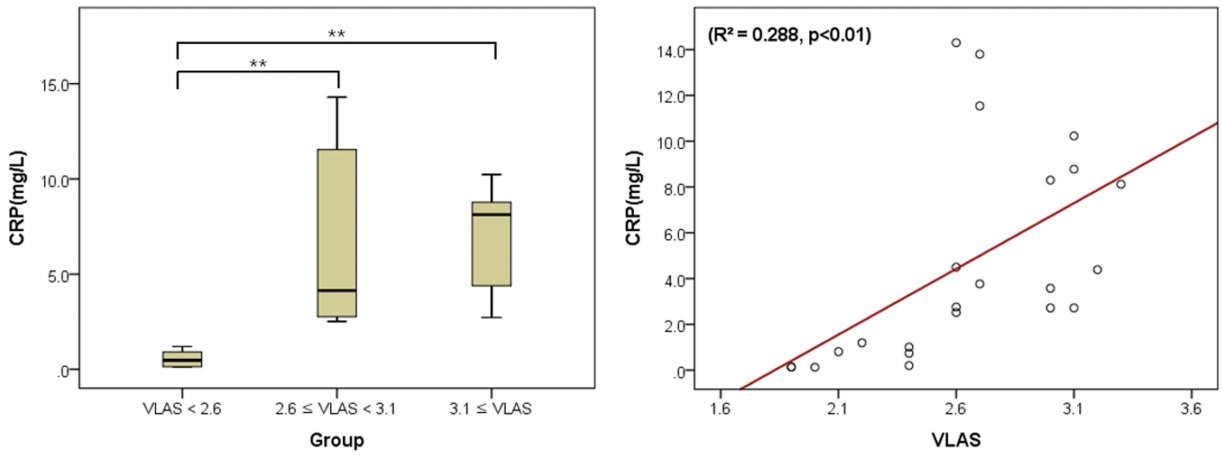

Fig 2. Evaluation of the correlation between VLAS and CRP in three groups. VLAS; vertebral left atrial size, CRP; C-reactive protein. $^{**}p < 0.01$.

VHS, LA/Ao ratio, and LVIDDN values showed no significant difference (Table 2).

An evaluation of the correlation between VLAS and CRP in the three groups is summarized in Fig 2. We found a strong positive correlation between VLAS and CRP—a significant increase in CRP was observed with increasing VLAS values. Twenty-three dogs were classified into three groups: a VLAS < 2.6 group, a 2.6 ≤ VLAS < 3.1 group, and a VLAS ≥ 3.1 group. The CRP values were analyzed for each group.

There was a significant difference between the VLAS < 2.6 group and the 2.6 ≤ VLAS < 3.1 group ($p < 0.01$). Also, there was a significant difference between the VLAS < 2.6 group and the VLAS ≥ 3.1 group ($p < 0.01$). However, there was no significance between the 2.6 ≤ VLAS < 3.1 group and the VLAS ≥ 3.1 group (Fig 2).

Discussion

In this study, we found that VLAS has no significant association with these breeds. MMVD stage and VLAS showed a significant correlation in those dogs. Also, we found a strong positive correlation between VLAS and CRP—a significant increase in CRP was observed with increasing VLAS values.

LAE is an important diagnostic factor for dogs with MMVD; therefore, LA/Ao ratios obtained from short-axis and long-axis echocardiographic images were examined. Because identifying LAE is essential in assessing the severity of MMVD, it is evaluated using a variety of methods. The 2019 ACVIM consensus is based on LA/Ao ratios, LVIDDN, and VHS for the evaluation of cardiac enlargement (16). In addition, a new radiographic technique for quantifying LA size, VLAS, has been introduced in veterinary medicine (19). Several studies have already demonstrated the diagnostic value of predicting cardiac remodeling (17,20). In a previous study, VLAS was measured in several breeds of healthy dogs (26). In our study, we focused on the VLAS values of three breeds with a predisposition to MMVD in South Korea. When we evaluated each breed, the median VLAS value of the Maltese was 2.40, the Shih-Tzu was 2.50, and the Poodle was 2.40. There was no significant difference among the breeds. In a study by Malcolm *et al*. (19), it was suggested that VLAS ≥ 2.3 is a reliable indicator of left atrial enlargement. In our study, each group with MMVD had a VLAS value ≥ 2.3.

In a study measuring VLAS in healthy adult dogs, the median value of VLAS was measured as 1.9, and the reference range of VLAS in healthy adult dogs was measured from 1.4 to 2.2 (26). Due to the limitations of that study, it was suggested that further studies on various dog breeds affected by diseases are needed (26). In this study, there was no significant difference among the three breeds regarding VHS, LA/Ao ratios, LVIDDN, and VLAS. Mikawa *et al.* (20) focused on the sensitivity and specificity of the cutoff value of VLAS concerning ACVIM Stage B2. It was found that 2.6 appeared to be the most clinically relevant VLAS cutoff value for ACVIM stage B2. The sensitivity and specificity were 95% and 84%, respectively; both of which were high (20).

CRP indicates an inflammatory state in the body, and various studies have demonstrated increased CRP in patients with CHF (13,14,21). In patients with CHF, CRP is elevated by overexpression of cytokines in cardiomyocytes (21). Elevated CRP does not necessarily indicate the severity of heart disease, but it can be used to determine a patient's condition in the early stages of CHF (21). In this study, there were significant CRP differences between the VLAS < 2.6 group and the 2.6 ≤ VLAS < 3.1 and VLAS ≥ 3.1 groups. There was no significant difference between the 2.6 ≤ VLAS < 3.1 group and the VLAS ≥ 3.1 group. We found that CRP values increased with increasing VLAS.

Kim *et al*. (17), reported that VLAS in the B2 and the C-D groups was significantly higher than in the B1 group according to the ACVIM stage. Those results are supported by the findings in this study; similar results were observed between the stage B1 group and the B2 and C-D groups.

There are two limitations to this study. First, the effects of cardiac medications such as pimobendan, diuretics, and angiotensin-converting enzyme inhibitor (ACEi) were not considered. Several studies have shown that these drugs affect the size of the heart in patients with MMVD (3,15). Thus, it is necessary to conduct research with sufficient consideration given to drugs that reduce VLAS. Second, we excluded diseases that could increase CRP as much as possible; however, this study did not exclude all diseases that could increase CRP.

In conclusion, there was no significant difference among the three breeds examined regarding VLAS. However, VLAS in the B2 and the C-D groups was significantly higher than in the B1 group according to the ACVIM stage. Furthermore, CRP increased proportionally as VLAS increased. In evaluating the size of the heart, it would be beneficial to measure VLAS in addition to the existing radiological and echocardiographic methods, thereby employing it as an additional diagnostic tool.

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