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Predictive model for the detection of pulmonary hypertension in dogs with myxomatous mitral valve disease

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ABSTRACT. Pulmonary hypertension (PH) often occurs due to a left heart disease, such as myxomatous mitral valve disease (MMVD), in dogs and is diagnosed using Doppler echocardiography and estimated pulmonary arterial pressure. Diagnosis of PH in dogs requires expertise in echocardiography: however, the examination for PH is difficult to perform in a clinical setting. Thus, simple and reliable methods are required for the diagnosis of PH in dogs. The purpose of this study was to develop models using multiple logistic regression analysis to detect PH due to left heart disease in dogs with MMVD without echocardiography. The medical records of dogs with MMVD were retrospectively reviewed, and 81 dogs were included in this study and classified into PH and non-PH groups. Bivariate analysis was performed to compare all parameters between the groups, and variables with P values of <0.25 in bivariate analysis were included in multiple logistic regression analysis to develop models for the detection of PH. In multiple logistic regression analysis, the model included a vertebral heart scale short axis of >5.2 v, and a length of sternal contact of >3.3 v was considered suitable for the detection of PH. The predictive accuracy of this model (85.9%) was judged statistically adequate, and therefore, this model may be useful to screen for PH due to left heart disease in dogs with MMVD without echocardiography.

KEY WORDS: canine, multiple logistic regression analysis, myxomatous mitral valve disease, pulmonary hypertension

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Pulmonary hypertension (PH) is defined as an increase in pulmonary arterial pressure and induces pressure overload of the right ventricular and ultimately causes clinical signs of right-sided heart failure, including hepatomegaly and ascites. Left heart disease is one of the causes of PH, and "PH due to left heart disease" is classified as group 2 in The Dana Point 2008 Updated Clinical Classification system of the World Health Organization [1]. In humans, PH due to left heart disease often occurs secondary to valvular disease [1]. Similarly, in dogs, PH due to left heart disease is caused by valvular disease, especially myxomatous mitral valve disease (MMVD) [12, 13, 32].

MMVD is the most common heart disease in dogs, and middle-aged and older small dogs are most commonly affected [31]. MMVD often causes PH due to left heart disease as a consequence of a chronic increase in left atrial pressure. Therefore, PH is generally described as a complication of advanced MMVD in dogs [20, 31].

In humans, the gold standard for diagnosis of PH is cardiac catheterization [1, 10, 12]. However, in dogs, this test is uncommonly performed in clinical settings, because it requires general anesthesia. In veterinary medicine, PH is usually diagnosed by estimated pulmonary arterial pressure with the modified Bernoulli equation using the velocity of tricuspid and/or pulmonary regurgitation obtained by Doppler echocardiography [10, 31, 33]. These methods, however, require expertise in echocardiography. Therefore, more simple and reliable diagnostic methods are required to appropriately detect dogs with PH in clinical settings. The purpose of the present study was to develop models using multiple logistic regression analysis for the detection of PH without echocardiography in dogs with MMVD.

MATERIALS AND METHODS

Animals: We conducted a retrospective review of the medical records of dogs with MMVD that were admitted to the Cardiovascular Service of the Animal Medical Center, Nippon Veterinary and Life Sciences University, between April 2007 and March 2012. The inclusion criteria included dogs with a body weight of ≤15 kg that were normal chested and had undergone echocardiography, including the Doppler method. Dogs with other diseases causing PH (i.e., congenital heart diseases, cardiomyopathy, canine heartworm disease and pulmonary diseases [10]) and without follow-up data were excluded.

Review of medical records: The profile, history, clinical findings and echocardiography findings of the dogs were collected. If records for complete blood count (CBC), serum chemistry, blood pressure, radiograph and electrocardiography were available, they were reviewed. In this study, PH

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was diagnosed by Doppler estimation of systolic and/or diastolic pulmonary pressure gradient. The modified Bernnoulli equation was used to estimate pulmonary pressure gradient.

Pulmonary pressure gradient =4 × (peak flow velocity of tricuspid regurgitation or pulmonary artery regulation)^2

Velocity of tricuspid regurgitation (TRV) and pulmonary artery regulation (PRV) were obtained with the apical 4-chamber view or at the right ventricular outflow tract level of the parasternal short axis view. Estimated systolic and diastolic pulmonary pressures were calculated by pulmonary pressure gradient plus the estimated right ventricular pressure. The estimated right atrial pressure was 5 mmHg in dogs with a non-enlarged right atrium or 10 mmHg in dogs with an enlarged right atrium. In theory, dogs with an estimated systolic pulmonary pressure ≥30 mmHg and/or diastolic pulmonary pressure ≥19 mmHg would be considered to have PH [8, 9, 11, 18]. However, in human patients with PH, Doppler echocardiographic estimates of pulmonary pressures have been reported to be inaccurate [26], and the above criteria may underestimate pulmonary pressures. Therefore, in the present study, TRV ≥3.1 m/s and PRV ≥2.8 m/s were considered to indicate PH (PH group) in accordance with recent studies [10, 23, 30]. Dogs with TRV <3.1 m/s and/or PRV <2.8 m/s were assigned to the non-PH group. The severity of PH was classified as mild (31–50 mmHg), moderate (51–75 mmHg) or severe (>75 mmHg) by systolic pulmonary pressure [31]. All dogs were also classified according to International Small Animal Cardiac Health Council (ISACHC) class (i.e., Ia, Ib, II, IIIa and IIIb). In the non-PH group, all data were obtained from medical records acquired at the final visit, and in the PH group, the data were obtained when the dogs were diagnosed with PH.

The clinical findings reviewed included body temperature, heart and respiratory rates, presence and grade of heart murmur (Levine 1–6), point of maximal intensity, presence of clinical signs related to congestive heart failure (including coughing, pulmonary edema, ascites, hepatomegaly, syncope, cyanosis and exercise intolerance) and systemic blood pressure (systolic, mean and diastolic) values. Serum chemistry included measures of plasma N-terminal pro-B-type natriuretic peptide (NT-proBNP), total bilirubin (Tbil), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), creatinine (Cre), glucose (Glu), total protein (TP), inorganic phosphorus (IP) and sodium (Na). Radiographic findings included vertebral heart scale (VHS), length of sternal contact, diameter of the caudal vena cava and presence of tracheal elevation on lateral radiographs. Dogs with primary lung disease based on radiographic findings were excluded. VHS was calculated as the sum of the short axis (S-ax) and long axis (L-ax) of the heart, length of sternal contact and diameter of the caudal vena cava for comparison with the number of vertebral bodies starting at the fourth thoracic vertebra (T4) [2, 15, 21]. Figure 1 shows that L-ax is measured from the ventral border of the carina to the most distant ventral contour of the heart and that S-ax is determined perpendicular to the L-ax at the level of the caudal vena cava [2]. Interobserver variation in sternal contact measurement was assessed using 5 radiographic images acquired on different days from 5 dogs with stable MMVD without PH. Intra-assay variation was evaluated using the same radiographic images, and analyses were repeated 10 times to calculate the coefficient of variation. Correlations between echocardiographic parameters and radiographic parameters were investigated. Electrocardiographic findings included the type of pathological arrhythmia; the amplitudes (mV) of the P, Q, R and S waves; and the durations of the P wave (msec), QRS complex (msec), QT interval (msec), corrected QT interval (QTc, msec) and RR interval (msec). All measurements were recorded as the mean of 3 stable waves in lead II. The corrected QT interval was calculated using Fridericia's formula (QTc=QT/RR$^{1/3}$) [10, 18].

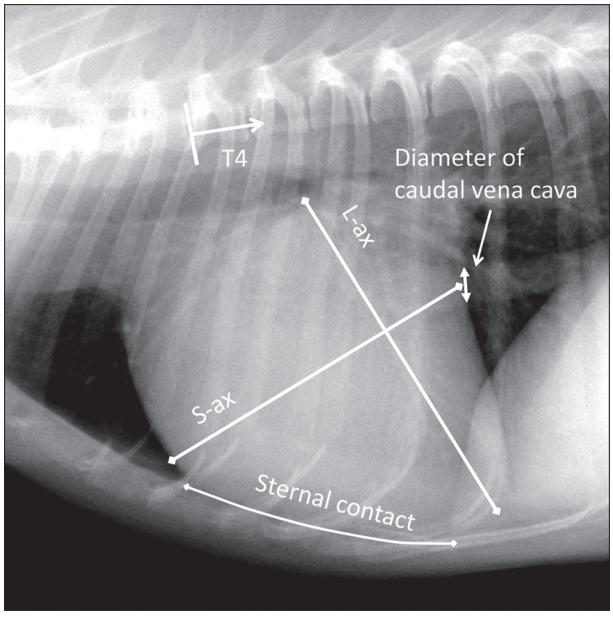

Fig. 1. Method for measurement of radiographic findings. S-ax is the short axis of the vertebral heart scale (VHS). L-ax is the long axis of the VHS. VHS is the sum of S-ax and L-ax. All parameters were measured for comparison with the number of vertebral bodies, starting at the fourth thoracic vertebra (T4).

Statistical analysis: The Shapiro–Wilk test was applied to determine whether the data were distributed normally. Normally distributed data are presented as the mean ± standard deviation (SD), whereas data that were not normally distributed are presented as the median (range). All data in the PH and non-PH groups were compared using the Student's *t* test or Mann–Whitney *U* test for continuous variables and the χ^2 test for categorical variables. The sensitivity and specificity for detection of PH were evaluated using a receiver operating characteristic (ROC) curve, if there was a significant difference in the continuous variable. Multiple logistic regression analysis was performed to create models for detection of PH. The inclusion criterion for model selection in a covariate set was defined as $P<0.25$ according to bivariate analysis. The model was validated using the Hosmer–Lemeshow goodness-of-fit test. In multiple logistic regression analysis, continuous variables were converted to dual category data

Table 1. Characteristics of dogs with myxomatous mitral valve disease included in this study

	PH group	Non-PH group
Number of dogs (n)	17	64
Age (years)	11.5 (8.8–13.2)	11.3 (3.2–15.5)
Weight (kg)	6.4 (2.3–12.6)	4.5 (1.6–14.2)
Sex (n)		
Intact male	9	23
Neutered male	2	13
Intact female	1	14
Neutered female	5	14
ISACHC cardiac function (n)		
Ia	0	26
Ib	2	13
II	7	23
IIIa	8	2

Data are presented as the median (range). Values without a range indicated in parentheses indicate the number of dogs.

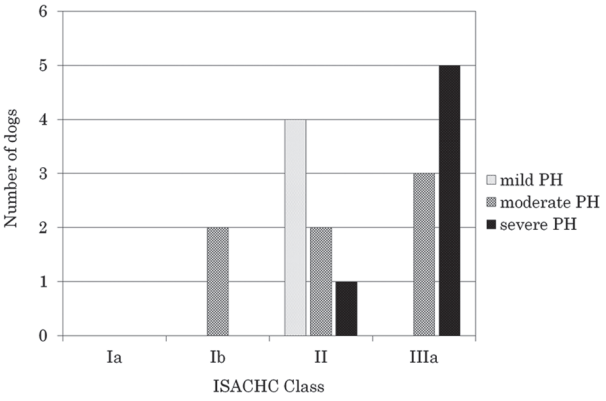

Fig. 2. The severity of PH and ISACHC classification in the PH group. The severity of PH was classified into mild (31–50 mmHg), moderate (51–75 mmHg) or severe (>75 mmHg) by systolic pulmonary pressure.

using the cutoff value of each for the detection of PH using an ROC curve. *P* value of <0.05 was considered significant. Statistical analysis was performed using Dr. SPSS II for Windows (SPSS Japan, Inc., Tokyo, Japan).

RESULTS

In the present study, 81 dogs were included. There were 9 mixed-breed dogs, and the remaining 72 dogs were purebreds, consisting of 13 Maltese, 11 Shih Tzus, 10 Cavalier King Charles spaniels, 9 Chihuahuas, 7 Pomeranians, 5 Yorkshire terriers, 4 Shetland Sheepdogs, 3 miniature dachshunds, 2 American cocker spaniels, 2 miniature schnauzers, and 1 dog each of the following breeds: toy poodle, West Highland white terrier, Japanese Shiba Inu, Spitz, Japanese Chin and Pekinese. The PH group included 17 dogs, and the non-PH group included 64 dogs (Table 1). There were no dogs classified as ISACHC class IIIb. The distributions of age, body weight and gender were not significantly different between the groups ($P \geq 0.05$). The morbidity rate for PH in these dogs was 21.0%, which was increased according to ISACHC classification ($P<0.001$). Morbidity rates of 0, 13.3, 23.3 and 80.0% were detected for ISACHC classes Ia, Ib, II and IIIa, respectively. The severity of PH is shown in Fig. 2, and it increased significantly according to the ISACHC class ($P=0.030$).

All data in the PH and non-PH groups are listed in Table 2. Arrhythmias included sinus tachycardia (n=3), Mobitz type II second-degree atrioventricular block (n=3), first-degree atrioventricular block (n=2), atrial premature contraction (n=2), atrial fibrillation (n=2), sinus arrest (n=2), ventricular premature beat (n=1), Mobitz type I second-degree atrioventricular block (n=1), third-degree atrioventricular block (n=1) and parasystole (n=1). Table 3 indicates the correlation coefficients between radiographic and echocardiographic parameters. S-ax was correlated with the left ventricular end-diastolic dimension (LVDd, r=0.448, $P<0.01$), but sternal contact was not (r=0.122, $P=0.291$). Sternal contact was significantly correlated with the left atrial (LA)/ aortic (Ao) root ratio (LA/Ao) (r=0.386, $P<0.01$). The interobserver and intra-assay variations for sternal contact were 6.9% and 2.5%, respectively. $P<0.25$ for 31 parameters in the bivariate analysis (Table 2) and so these parameters were included in multiple logistic regression analysis.

One model for detection of PH in dogs with MMVD was generated from multiple logistic regression analysis using these 31 variables (Table 4). The model included S-ax and sternal contact (odds ratios, 22.9 and 15.3; $P=0.005$ and 0.015, respectively), and it predicted the presence of PH with an accuracy of 87.5% (Table 5). The correlation coefficient between S-ax and sternal contact was 0.586, and the coefficient of dispersion expansion was 1.580.

DISCUSSION

To the best of our knowledge, this is the first report describing multiple logistic regression analysis to evaluate a statistical model for the detection of PH in dogs with MMVD. This model included values for S-ax and sternal contact. The predictive accuracy of this model was 85.9%, and it is suitable for detection of PH in dogs. However, given the population in this study, this model has limited applicability to dogs (i.e., normal shallow-chested and small-breed dogs with MMVD).

In the present study, 9 of 17 dogs with PH indicated mild-to-moderate chronic heart disease (ISACHC classes Ib and II). It was reported that dogs classified into ISACHC classes Ia, Ib and II could have PH, suggesting that dogs with left heart disease exhibiting non-to-mild heart failure symptoms may have PH; therefore, screening and evaluation of PH should be performed in such dogs [31]. The predictive model in the present study uses parameters easily obtained by radiography, and it may be useful for screening of PH if normal shallow-chested and small-breed dogs with MMVD are suspected of having PH due to left heart disease.

In an earlier study, S-ax was associated with cardiac en-

Table 2. Comparison of values of variables between the PH and non-PH groups

	Total	PH		non-PH		*P*	
		Value	Dogs with available data (n)	Value	Dogs with available data (n)		
Physical examination findings							
Body temperature (°C)	9	38.6	1	39.1 ± 0.7	8	–	
Heart rate (bpm)	80	140 (42–180)	17	120 (56–216)	63	>0.25	
Respiratory rate (breaths/min)	15	30	1	30 (20–66)	14	–	
Cardiac murmur findings							
Left sound intensity (Levine)	43	5 (2–6)	6	4 (2–6)	37	>0.25	
Right sound intensity (Levine)	34	4 (2–6)	6	3 (1–6)	28	0.23	
Symptoms of heart failure							
Coughing (n)	81	14	17	31	64	0.012	*
Pulmonary edema (n)	81	3	17	9	64	>0.25	
Ascites (n)	81	5	17	1	64	0.001	*
Hepatomegalia (n)	81	3	17	0	64	0.008	*
Syncope (n)	81	5	17	2	64	0.004	*
Cyanosis (n)	81	2	17	1	64	0.110	
Exercise intolerance (n)	81	6	17	6	64	0.015	*
X-ray examination							
VHS (v)	79	13.0 ± 1.6	17	10.7 ± 1.0	62	<0.001	*
S-ax (v)	79	6.2 ± 0.8	17	4.9 ± 0.6	62	<0.001	*
L-ax (v)	79	6.8 ± 0.9	17	5.8 ± 0.6	62	<0.001	*
Sternal contact (v)	78	4.3 ± 0.8	16	3.0 ± 1.1	62	<0.001	*
Caudal vena cava (v)	78	1.0 ± 0.1	16	0.9 ± 0.1	62	0.002	*
Tracheal elevation (n)	79	17	17	38	62	0.002	*
Electrocardiography							
Heart rate (bpm)	69	151 (38–174)	12	122 (43–227)	57	0.027	*
Arrhythmia (n)	72	7	14	11	58	0.034	*
R (mV)	70	2.75 ± 0.87	13	2.38 ± 0.88	57	0.174	
QTc (msec)	65	260 (250–280)	10	244 (210–320)	55	0.010	*
RR interval (msec)	65	410 (350–840)	10	447 (260–700)	55	0.193	
Blood test values							
NT-proBNP (pmol/*l*)	81	2210.9 (130.7–13924.0)	17	1298.7 (41.2–12605.0)	64	0.072	
Tbil (mg/d*l*)	18	0.20 (0.10–0.40)	3	0.10 (0.00–0.70)	15	0.049	*
ALT (U/*l*)	29	136 (48–217)	5	48 (13–192)	24	0.043	*
GGT (U/*l*)	17	25.5 (11–40)	2	7 (11–40)	15	0.098	
Cre (mg/d*l*)	56	0.69 (0.40–2.30)	12	0.85 (0.20–3.70)	44	0.177	
Glu (mg/d*l*)	26	93.0 ± 8.8	4	102.8 ± 11.9	22	0.130	
TP (g/d*l*)	39	5.3 (4.1–6.8)	8	6.1 (3.6–7.5)	31	0.036	*
IP (mg/d*l*)	48	4.3 ± 0.9	10	3.9 ± 1.0	38	0.237	
Na (mEq/*l*)	51	145 (134–153)	12	146 (135–152)	39	0.209	
Platelets ($\times 10^4/\mu l$)	24	58.2 ± 18.1	5	41.9 ± 23.4	19	0.165	
WBC ($\times 10^2/\mu l$)	23	111.0 (100.0–148.0)	5	85.5 (61.0–632.0)	18	0.118	
Neutrophils ($\times 10^2/\mu l$)	19	94.4 (74.2–125.1)	5	67.4 (41.5–57.2)	14	0.116	
Systemic blood pressure							
Systolic (mmHg)	51	135 (106–169)	7	137 (102–202)	44	>0.25	
Mean (mmHg)	50	98 ± 18	7	104 ± 23	43	>0.25	
Diastolic (mmHg)	51	77 ± 19	7	88 ± 22	44	0.229	

Parametric data are presented as the mean ± SD. Nonparametric data are presented as the median (range). * $P<0.05$.

largement caused by PH, not L-ax [2]. In the present study, S-ax was included in the detection model. PH due to left heart disease is caused by an increased left atrial pressure and subsequently increased pulmonary pressure. Overload of the pulmonary artery leads to right ventricular dilatation [10]. Anatomically, L-ax may be predominantly affected by the size of the left ventricular, whereas S-ax may be associated with the size of the left and right ventricles. Dogs with PH due to left heart disease should have left and right ventricular dilatation; therefore, S-ax, which reflects left and

Table 3. Correlation coefficients between radiographic and echocardiographic parameters

	VHS	L-ax	S-ax	Sternal contact	LA	Ao	LA/Ao	LVSd	LVDd	LVPWd
VHS	-	0.926**	0.929**	0.61**	0.515**	–0.059	0.657**	–0.079	0.474**	–0.016
L-ax	-	-	0.744**	0.554**	0.515**	–0.011	0.591**	0.007	0.451**	–0.015
S-ax	-	-	-	0.586**	0.478**	–0.059	0.634**	–0.129	0.448**	–0.029
Sternal contact	-	-	-	-	0.256*	–0.207	0.386**	–0.214	0.122	–0.062

LVSd: Left ventricular end-systolic dimension. LVPWd: Left ventricular posterior wall dimensions. * P<0.05 ** P<0.01.

Table 4. Prediction model generated using multiple logistics regression analysis

	Variable	Coefficient	OR (95% CI)	P
Model	S-ax >5.2 v	3.129	22.9 (2.6–199.5)	0.005
(n=78)	Sternal contact >3.3 v	2.730	15.3 (1.7–137.1)	0.015
	Constant	–5.389		
Prediction formula	core=3.129 (with/without S-ax >5.2 v) + 2.730 (with/without sternal contact >3.3 v) − 5.389			

In the prediction formula, the contents within the parentheses are replaced with 1 or 0 depending on the presence or absence of the variable. The score calculated in the prediction formula was substituted with P'=1/1 + exp (−1 × score), and PH was determined if P'>0.5. OR: Odds ratio.

Table 5. The predictive value of the model

	Positive predictive value	Negative predictive value	Predictive accuracy for PH
Model (n=78)	87.5% (14/16)	85.5% (53/62)	85.9%

right ventricular dilatation, might be included in the detection model for PH due to left heart disease.

The present study included sternal contact as a variable for the detection of PH, but this variable was not useful to detection of PH in an earlier study [3]. In a previous study, an increase in sternal contact was considered to reflect only right ventricular enlargement [17], but one study demonstrated that the increase in sternal contact may be affected by both left and right ventricular enlargement [3]. In the present study, sternal contact was found to be correlated with S-ax, and this suggested that sternal contact reflects left and right ventricular enlargement. However, LVDd was not significantly related to sternal contact. Therefore, sternal contact may better reflect right ventricular enlargement than left ventricular enlargement. This opinion is in accordance with why the detection model included S-ax and not L-ax. Therefore, sternal contact might be included in the detection model for PH due to left heart disease.

Both S-ax and sternal contact are radiographic parameters indicating cardiac size and probably reflect right ventricular and left atrial dilatation. The correlation coefficient between these variables was 0.586, but the coefficient of dispersion expansion was 1.580, indicating the absence of multicollinearity. It is unknown if there is any interbreed variation in S-ax and sternal contact. However, VHS is known to vary according to breed [7, 15]. Therefore, S-ax and sternal contact might also be affected by breeds. This study included dogs with a body weight of ≤15 kg to minimize the influence of breed on S-ax and sternal contact. Sternal contact has been an uncommon parameter assessed on radiograph. In deep-chested dogs, sternal contact may be underestimated because the heart is distant from the sternum, and thus, this parameter is thought to be unusable in these dogs. Moreover, the reference range of sternal contact is unknown. Despite the limitation of breed differences, VHS has often been used as a tool to assess mitral valve disease and heart size [5, 21]. Radiography can be performed more easily than echocardiography, and S-ax and sternal contact can be measured easily. In dogs that have a body weight of ≤15 kg and are normal-chested, both S-ax and sternal contact were useful for detection of PH due to left heart disease in dogs with MMVD.

Studies in humans and rats have indicated that prolongation of QTc was related to PH and right ventricular hypertrophy, and QTc was considered an independent predictor of worse clinical outcomes of PH [24, 27]. In dogs, one study demonstrated that QTc increased according to the New York Heart Association functional classification [14]. In the present study, QTc was significantly prolonged in dogs with PH in the bivariate analysis, but the odds ratio with 95% confidence interval for QTc was not statistically significant in the multiple logistic regression analysis. Right ventricular hypertrophy is often observed in groups other than group 2 in the Dana Point classification of PH [1]. Therefore, QTc might be included in the detection model for PH due to left heart disease.

In humans, atrial fibrillation often occurs in PH caused by left-sided heart failure and reflects the severity of heart failure [29]. However, the relationship between atrial fibrillation and PH in dogs is unknown. The relationship could not been

assessed in the present study, because atrial fibrillation was only recorded in 2 dogs with PH and MMVD classified as ISACHC class IIIa.

In humans, the plasma NT-proBNP concentration is a prognostic marker of PH [16]. The plasma NT-proBNP concentration is significantly correlated with pulmonary arterial pressure in dogs [6]. In the present study, however, the plasma NT-proBNP concentration of the PH group was not significantly higher than that of the non-PH group, and this variable was not included in the model generated from the multiple logistic regression analysis. In humans, the plasma NT-proBNP concentration is also affected by the glomerular filtration rate, gender and obesity [16, 22]. In dogs, the NT-proBNP concentration was affected by the glomerular filtration rate and gender [18, 25]. These confounding factors might have affected the NT-proBNP concentration, so it was not included the detection model.

There are several limitations in the present study. Dogs were excluded from this study, if there was any evidence of congenital heart diseases, cardiomyopathy, canine heartworm disease, hypoxia or pulmonary diseases in their histories, clinical findings, echocardiograms or radiographs in order to select dogs with PH due to left heart disease caused by MMVD. The above conditions may cause another type of PH (i.e., due to congenital heart disease, lung disease, hypoxia or chronic thromboembolic) that is different from PH due to left heart disease, and these forms of PH were excluded from this study. However, idiopathic PH might not be entirely excluded, because it was diagnosed by exclusion [4]. Secondly, the effects of any treatment on these variables were not assessed. Therapeutic agents for heart failure due to MMVD, such as isosorbide dinitrate, hydralazine hydrochloride, angiotensin-converting enzyme inhibitor, diuretics and phosphodiesterase inhibitors, may have affected the pulmonary pressure and underestimated PH. In humans, moreover, not all patients with PH develop significant tricuspid regurgitation [19, 28]; thus, the detection model in the present study may not be useable in dogs without tricuspid regurgitation or pulmonary artery regulation. This model targeted only small-breed dogs with a normal shallow chest and MMVD. However, it is thought that MMVD is a quite common heart disease in small-breed dogs and often leads to PH; therefore, this model is useful.

In conclusion, the model developed in this study was sufficiently useful for screening PH due to left heart disease in many small-breed dogs. Further research that includes more detailed data is required to increase the accuracy and determine the effect of limitations, such as drugs and breeds, on diagnostic models of PH in dogs with MMVD.

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