Original Article

Symmetric dimethylarginine in dogs with pulmonary arterial hypertension

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Abstract

Pulmonary arterial hypertension (PAH) causes impaired kidney function in humans. However, this occurrence has not been well investigated in PAH dogs. Serum symmetrical dimethylarginine (SDMA) is one of the early biomarkers of kidney impairment. The present study aims to evaluate SDMA levels of first diagnosed PAH (n=14) compared with healthy dogs (n=12) and to analyze the correlation between SDMA levels and significant echocardiographic parameters of PAH dogs. The mean SDMA of PAH dogs was statistically higher than that of healthy dogs (P = 0.003), while the mean serum creatine level had no difference among groups (P = 0.456). Precapillary PAH had a significant increase in SDMA level compared to postcapillary and healthy dogs (P = 0.010). In PAH dogs, the Pearson correlation coefficient (r value) between SDMA and echocardiographic parameters (cardiac output: CO, cardiac index: CI, stroke volume: SV, and acceleration to ejection time ratio: AT/ET) revealed a negative relationship (r_{CO} = -0.555, r_{CI} = -0.570, r_{SV} = -0.556 and $r_{AT/ET}$ = -0.537; overall $P < 0.05$), whereas a relationship between SDMA and ratio of right to left ventricular internal dimension during diastole (RVIDd/LVIDd) was positive ($r_{RVIDd/LVIDd}$ = 0.564; P = 0.018). SDMA and maximal pressure gradient of tricuspid regurgitation (TRmaxPG) had no correlation ($P > 0.05$). In conclusion, PAH dogs had a significant increase in SDMA levels, particularly precapillary PAH dogs. The results show SDMA correlated negatively with CO, CI, SV, and AT: ET and positively with RVIDd/LVIDd, suggesting that severe PAH is related to worsening kidney function. More investigations, including urinalysis, kidney ultrasonography, other kidney injury biomarkers, and GFR, are necessary to confirm this phenomenon.

Keywords: canine, echocardiography, kidney function, pulmonary arterial hypertension, SDMA

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Introduction

Pulmonary arterial hypertension (PAH), according to human literature, is defined by a mean pulmonary artery pressure greater than 25 mmHg during rest (Hoeper *et al*., 2013). PAH can be idiopathic or secondary to multiple underlying causes, including left heart disease, respiratory disease, pulmonary emboli/thrombi/thromboemboli, parasitic disease, multifactorial causes, and unclear mechanisms. In addition, PAH can be divided into two groups, precapillary and postcapillary PAH, using the detection of left atrial enlargement as a criterion for the postcapillary group (Reinero *et al*., 2020). In veterinary medicine, doppler echocardiography, a non-invasive and repeatable method, is more clinically practical than right-heart catheterization to detect PAH (Akabane *et al*., 2019). Syncope, respiratory distress, cough, exercise intolerance, ascites, and cyanotic or pale mucous membranes are the common clinical signs of PAH in dogs (Johnson *et al*., 1999).

According to the American College of Veterinary Internal Medicine (ACVIM) consensus statement guideline (Reinero *et al*., 2020), tricuspid regurgitation velocity and cardiac anatomical changes, including remodeling of both ventricles, increase in pulmonary artery diameter and flow velocity and enlargement of the right atrium and caudal vena cava have been used to assess the probability of PAH.

Cardiovascular-renal axis disorders (CvRD) or cardio-renal syndrome (CRS) refers to the pathological interaction between kidneys and the cardiovascular system. It has been categorized into three types: kidney injury originating from primarily cardiovascular dysfunction ($CvRD_H$), cardiovascular dysfunction resulting from primarily kidney injury ($CvRD_K$), and concurrent impairment of both systems or other causes affecting both kidney and cardiovascular system ($CvRD_o$) (Pouchelon *et al*., 2015). Crucial mechanisms of $CvRD_H$ are a decrease in cardiac output, which further causes activation of the neuroendocrine systems (renin-angiotensin-aldosterone system and sympathetic nervous system), generation of reactive oxygen species, and decrement of glomerular filtration rate (GFR) (McCullough *et al*., 2013; Liang *et al*., 2008). In humans, changes in pulmonary vascular resistance occurring in PAH lead to decreased cardiac output (Lai *et al*., 2014). Previously, it has been shown that PAH patients have a reduction of cardiac index, cardiac output, and mildly impaired GFR (Damman *et al*., 2007).

Estimated GFR is accepted to be the gold standard for measuring kidney function (Haller *et al*., 1998; Von Hendy-Willson and Pressler, 2011). However, practically in the clinic, serum creatinine and symmetric dimethylarginine (SDMA) are both widely used parameters for evaluating kidney function. SDMA has a moderate relationship with GFR; the sensitivity and specificity are 90% and 50%, respectively, when GFR decreases ≥ 40% (McKenna *et al*., 2020). Although CvRD has been described in both human and veterinary medicine, it is unclear whether PAH occurring in dogs affects kidney function. Therefore, the present study aims to evaluate kidney function using a clinically sensitive parameter, SDMA level, and to find the correlation between SDMA and several significant echocardiographic parameters in dogs with naturally occurring pulmonary hypertension compared to healthy dogs.

Materials and Methods

Animals: A total of 14 diagnosed pulmonary hypertensive dogs and 12 healthy dogs presented to the Veterinary Teaching Hospital, Khon Kaen University, Thailand, from October 2020 to May 2022, were included in the present study. All dog owners gave written informed consent. The study protocol was approved by the Institutional Animal Care and Use Committee of Khon Kaen University (IACUC-KKU (C) 79/66).

All dogs received health checks and cardiac evaluations consisting of physical examinations, blood pressure measurements using an oscillometric device (Vet20, SunTech Medical, USA), thoracic radiography, and standard echocardiography. Routine hematology and blood chemistry were performed in all dogs. Small-breed dogs aged ≥ seven years without cardiac abnormalities and other relevant systemic diseases were defined as the healthy group.

Pulmonary arterial hypertension was diagnosed according to the ACVIM consensus statement guidelines 2020. Briefly, the inclusion criteria were as follows: 1) clinical signs relating to PAH (*e.g*., syncope, respiratory distress, cardiogenic ascites, and exercise intolerance), 2) clinical examinations relating to PAH (*e.g*., right heart murmur with or without left heart murmur, abnormal lung sound, cyanosis mucous membrane and jugular vein distension), 3) abnormalities of thoracic radiographs (*e.g*., right heart enlargement, pulmonary lobar artery distension, pleural effusion, lung lesion due to chronic respiratory disease) and 4) abnormalities of echocardiography to assess the probability of PAH (*e.g*., peak tricuspid regurgitation velocity and echocardiographic signs of PAH from 3 anatomical sites). The echocardiographic findings categorized from anatomical sites including 1) ventricles (*e.g*., flattening of the interventricular septum, the small size of left ventricle and right ventricular hypertrophy or dilation), 2) pulmonary artery (e.g., pulmonary artery distension using pulmonary artery to aortic ratio; PA/Ao > 1, decrease of acceleration time to ejection time ratio; AT/ET < 0.30 and presence of mid-systolic notching) and 3) right atrial and caudal vena cava enlargement.

Thoracic imaging: All processes were performed without any tranquilizer, sedative, or anesthetic given to the dogs. Thoracic radiography of all dogs was done consisting of a minimum of two orthogonal views, left or right lateral and ventrodorsal (VD) or dorsoventral (DV), using a digital radiography machine (VIVIX-S 1717V, Vieworks CO., Korea). Data including vertebral heart score, heart shape, lung pattern, and characteristics of the pulmonary vessels were recorded.

Echocardiography: Echocardiography was performed by Vetus 8 (Shenzhen Mindray Animal Medical Technology Co., China) with a phased array

transducer at frequencies of 4–10, 2–8, and 2-4 MHz. for dogs weighing < 5, 5–15 and > 15 kg, respectively. All echocardiographic parameter measurements were repeated at least 2 times consecutively. Transthoracic two-dimensional, M-mode, and Doppler examinations were performed primarily by a single operator (SR) under the supervision of a board-certified veterinary specialist (TP).

Left atrium (LA) and pulmonary artery (PA) diameter were indexed to the aortic (Ao) diameter (LA: Ao and PA: Ao, respectively) via two-dimensional mode in the right parasternal short axis view. Doppler echocardiography was used to assess pulmonary flow velocity and acceleration time to ejection time ratio (AT/ET) in the same view. Furthermore, M-mode from the right parasternal short and long axis view was used to measure left ventricular values (IVSd, LVIDd, LVPWd, IVSs, LVIDs, LVPWs), the ratio of right to left ventricular internal dimension during diastole (RVIDd/LVIDd) (Visser, 2020) and left ventricular systolic function, including ejection fraction (EF), fractional shortening (FS), stroke volume (SV) and cardiac output (CO). CO was calculated using the following formula: CO = SV × HR, SV was estimated from LVIDd and LVIDs measurements by the simplified Teicholz formula.

Peak tricuspid regurgitation velocity (TRV) was measured via Doppler echocardiography using the left apical view to calculate the maximal pressure gradient of tricuspid regurgitation (TRmaxPG), according to the Bernoulli Equation (PG = 4×velocity $[m/s]^2$). We classified the probability of PAH into three levels, namely low, intermediate, and high, according to the ACVIM consensus guideline (Reinero *et al*., 2020). For the PAH group, the minimum inclusion criteria were TRV ≥ 2.8 m/s or TRmaxPG ≥ 30 mmHg.All left ventricular values were normalized using the allometric equation of Cornell's formula: normalized parameter = parameter/BW^b, whereby "BW" is body weight (kg) and "b" is a coefficient for each parameter, with 0.242 for IVSd, 0.294 for LVIDd, 0.232 for LVPWd, 0.240 for IVSs, 0.315 for LVIDs, 0.222 for LVPWs, 0.345 for LA and 0.341 for Ao. Body surface area (BSA) is calculated by the formula BSA = 0.101 × body weight $(kg)^{2/3}$ (Chun *et al*., 2006). Moreover, cardiac index (CI) and stroke volume index (SVI) were calculated by CO/BSA and CI/HR, respectively (Haskins *et al*., 2005).

SDMA measurement: Serum SDMA levels were measured using the IDEXX Catalyst One (IDEXX laboratories, USA). Each sample was duplicated to increase the accuracy of the analysis.

Statistical analysis: The data was analyzed using SPSS version 28 (KKU license). Continuous data was examined for their distribution pattern using the Shapiro-Wilk test. Normally distributed data was analyzed using the variance analysis (ANOVA) test and presented with the mean and standard deviation. Non-normally distributed variables were analyzed with the Mann-Whitney U test and shown in the form of medians and ranges (Q1, Q3). The correlations between variables were detected using the linear correlation method, and the level of correlation was shown by the Pearson correlation coefficient (r-value). For all performed analyses, a $P < 0.05$ was considered statistically significant.

Result

Animals population: Twenty-six dogs were recruited for this study, including 15 male and 11 female dogs. The average age was 9.58 ± 3.54 years, and the average weight was 6.33 ± 2.70 kg. Breeds of dogs included Shih Tzu (n = 5; 19.2%), Miniature Poodle (n = 5; 19.2%), Chihuahua (n = 5; 19.2%), Pomeranian (n = 3; 11.5%), Miniature Pinscher (n = 3; 11.5%), French Bulldog (n = 1; 3.8%), Labrador Retriever (n = 1; 3.8%), Papillon (n = 1; 3.8%), Pug (n = 1; 3.8%) and Yorkshire Terrier (n = 1; 3.8%).

Twelve healthy dogs and fourteen first-time diagnosed pulmonary arterial hypertensive (PAH) dogs without any prior treatments were included.

There were no statistical differences in sex, age, or weight between the groups ($P > 0.05$). PAH secondary to left-sided heart disease was the most diagnosed in this study, including MMVD stage B2 (n = 5; 62.5%) and MMVD stage Ca (n = 3; 37.5%). The number of dogs from each clinical classification is presented in Table 1.

Hematological and Biochemical parameters: The hematological values of all dogs were within reference ranges (Table 2). The mean hematocrit (HCT), hemoglobin (HB), and red blood cell (RBC) levels in PAH dogs were significantly lower than in healthy dogs (overall $P < 0.001$). However, white blood cells (WBC) were statistically higher in PAH dogs compared to healthy dogs ($P < 0.001$).

Blood chemistry revealed the average creatinine and ALT levels of all dogs were not different among groups (Table 2). The mean BUN, SDMA, and BUN/creatinine levels of PAH dogs (31.27 ± 16.37 mg/dL, 13.14 ± 3.98 µg/dl, 43.57 ± 21.70) were statistically higher than healthy dogs (16.97 ± 3.21 mg/dL, 9.42 ± 1.72 µg/dl, 23.95 ± 5.93) ($P < 0.01$). Moreover, the mean total protein and albumin levels were significantly lower in PAH dogs compared to healthy dogs ($P = 0.006$ and < 0.001, respectively) (Table 2).

Cardiovascular assessment: The most common clinical signs presented by pulmonary arterial hypertensive dogs were exercise intolerance and decreased appetite. Nine and three dogs displayed ascites and pulmonary edema, respectively. Moreover, murmur heart sounds at the right apical region of the heart and jugular vein distention were the most detected abnormalities by physical examination. Systolic blood pressures were not different among groups ($P = 0.36$), but mean diastolic blood pressure from PAH dogs (88.86 ± 17.66 mmHg) was statistically higher than healthy dogs (75.00 ± 10.26 mmHg) ($P = 0.023$).

Table 1 Clinical classification of PAH dogs

Cause of PAH	Number of dogs (n = 14)
Group 1 Idiopathic	-
Group 2 Left-sided heart diseases	8
Group 3 Chronic respiratory disease / Chronic hypoxia	1
Group 4 Pulmonary thromboembolism	-
Group 5 Heartworm / Lungworm	1
Group 6 Multifactorial or unclear mechanisms	
1) Chronic respiratory disease with Ehrlichiosis and MMVD stage B1	
2) Pancreatitis with cholangiohepatitis and MMVD stage B1	4
3) Ehrlichiosis and Anaplasmosis	
4) Unclear mechanism	

PAH, pulmonary arterial hypertension; MMVD, Myxomatous mitral valve disease

Table 2 Hematological and biochemical parameters of PAH and healthy dogs.

Parameter	Healthy dogs (n = 12)	PAH dogs (n = 14)	Reference value	*P*-value
HCT (%)	49.17 ± 4.22	40.50 ± 6.45	38.0-55.0	<0.001*
HB (g/dl)	16.68 ± 1.56	13.78 ± 2.37	12.0-18.0	<0.001*
RBC ($X10^6$ cell/μl)	7.51 ± 0.64	6.06 ± 0.85	6.0-9.0	<0.001*
WBC (cell/μl)	8,689.17 ± 2,844.28	14,180.00 ± 3,795.54	6,000-15,000	<0.001*
BUN (mg/dL)	16.97 ± 3.21	31.27 ± 16.37	8-26	0.004*
Creatinine (mg/dL)	0.73 ± 0.15	0.75 ± 0.41	0.5-1.8	0.456
BUN/creatinine	23.95 ± 5.93	43.57 ± 21.70	< 20	0.004*
ALT (U/L)	73.17 ± 51.7	192 ± 354	<100	0.130
Total protein (g/dl)	7.53 ± 0.98	6.12 ± 1.53	5.4-7.7	0.006*
Albumin (g/dl)	3.13 ± 0.41	2.39 ± 0.50	2.3-3.8	<0.001*
SDMA (μg/dl)	9.42 ± 1.72	13.14 ± 3.98	< 14	0.003*

Data are expressed as mean±SD.
*Significantly different indicated at $P < 0.05$.
HCT, hematocrit; HB, hemoglobin; RBC, red blood cell; WBC, white blood cell; BUN, blood urea nitrogen; BUN/creatinine, blood urea nitrogen to creatinine ratio; ALT, alanine transaminase; SDMA, symmetric dimethylarginine

Table 3 Echocardiographic parameters of PAH and control dogs

Parameter	Control (n = 12)	PAH dogs (n = 14)	*P*-value
LA: Ao	1.27 ± 0.15	1.81 ± 0.74	0.009*
PA:Ao	0.89 ± 0.08	1.43 ± 0.34	<0.001*
IVSdN (cm.)	0.38 ± 0.07	0.47 ± 0.12	0.010*
LVIDdN (cm.)	1.37 ± 0.14	1.99 ± 1.19	0.037*
LVPWdN (cm.)	0.36 ± 0.06	0.48 ± 0.13	0.005*
IVSsN (cm.)	0.58 ± 0.08	0.71 ± 0.19	0.017*
LVIDsN (cm.)	0.73 ± 0.16	1.04 ± 0.77	0.087
LVPWsN (cm.)	0.60 ± 0.10	0.78 ± 0.21	0.005*
RVIDdN/LVIDdN	0.14 ± 0.06	0.58 ± 0.62	0.010*
AT/ET	0.45 ± 0.04	0.24 ± 0.06	<0.001*
TRmaxPG (mmHg)	0	50.74 ± 17.21	<0.001*

Data are expressed as mean±SD.
*Significantly different indicated at $P < 0.05$.
LA: Ao, left atrium to aorta ratio; PA: Ao, pulmonary artery to aorta ratio; IVSdN, normalized interventricular septal diameter in diastole; LVIDdN, normalized left ventricular internal diameter in diastole; LVPWdN, normalized left ventricular posterior wall diameter in diastole; IVSsN, normalized interventricular septal diameter in systole; LVIDsN, normalized left ventricular internal diameter in systole; LVPWsN, normalized left ventricular posterior wall diameter in systole; RVIDd/LVIDd, ratio of right to left ventricular internal dimension during diastole; AT/ET, acceleration to ejection time ratio; TRmaxPG, maximal pressure gradient of tricuspid regurgitation

***Echocardiographic data*:** According to the ACVIM consensus statement guideline (Reinero *et al*., 2020), PAH dogs were divided into three levels of PAH probability: low (n = 1; 7.14%), intermediate (n = 2; 14.28%) and high (n = 11; 78.57%).

Echocardiography revealed the average of the LA: Ao ratio of dogs in the PAH group (1.81 ± 0.74) was greater than the healthy group (1.27 ± 0.15) ($P = 0.009$) (Table 3). In addition, IVSdN (0.47 ± 0.12 cm.), LVIDdN (1.99 ± 1.19 cm) and LVPWdN (0.48 ± 0.13 cm.) in the PAH group were also significantly higher than the healthy group (0.38 ± 0.07, 1.37 ± 0.14 and 0.36 ± 0.06 cm., respectively) ($P = 0.010$, 0.037 and 0.005, respectively) (Table 3).

The average of the PA: Ao ratio of PAH dogs (1.43 ± 0.34) was significantly higher than normal dogs (0.89 ± 0.08) ($P < 0.001$). All PAH dogs had tricuspid regurgitation. Tricuspid regurgitation velocity (TRV) included TRV < 3.0 m/s (n = 3; 21.43%), 3.0-3.4 m/s (n = 4; 28.57%) and > 3.4 m/s (n = 7; 50%). Moreover, a significantly higher averaged TRmaxPG and lower AT/ET (50.74 ± 17.21 mmHg, 0.24 ± 0.06) were shown when compared to healthy dogs (0 mmHg, 0.45 ± 0.04) ($P < 0.001$) (Table 3)

The LV systolic function parameters, including SV, HR, CO, and CI, were statistically increased in PAH dogs when compared to healthy dogs (Table 4) ($P = 0.043$, 0.004, 0.018, and 0.032, respectively).

In PAH dogs, The Pearson correlation analysis between SDMA and echocardiographic parameters, including CO, CI, SV, and AT/ET, showed moderate negative relations (P = 0.020, 0.017, 0.019, and 0.024, respectively), while a moderate positive correlation was revealed between RVIDd/LVIDd and SDMA (P = 0.018). There was no apparent relationship between TRmaxPG and SDMA in PAH dogs (P = 0.222). However, no relationship was found between the above parameters and SDMA in the healthy dogs (overall $P > 0.05$) (Table 5). The correlation graph is shown in Fig. 1.

When comparing PAH secondary to precapillary and postcapillary, dogs with precapillary PAH had RV size (RVIDd/LVIDd) significantly greater than postcapillary ($P < 0.001$). In precapillary PAH dogs, the LV systolic functions measured by SV, SVI, CO, and CI were significantly lower than in dogs with postcapillary PAH (overall $P < 0.001$), while BUN and SDMA levels were significantly higher ($P \leq 0.01$). However, LV systolic functions in healthy and precapillary PAH dogs were not statistically different (overall $P > 0.05$) (Table 6).

Table 4 LV Systolic function parameters by echocardiography of healthy and PAH dogs

Parameter	Healthy dogs (n = 12)	PAH dogs (n = 14)	*P*-value
FS (%)	44.83 ± 9.57	49.24 ± 13.38	0.175
EF (%)	77.01 ± 10.50	79.63 ± 12.86	0.290
SV (ml)	13.75 ± 4.87	22.00 ± 16.02	0.043*
SVI (ml/beat/m^2)	0.04 ± 0.01	0.06 ± 0.05	0.069
HR (bpm)	121.33 ± 24.23	152.39 ± 30.40	0.004*
CO (L/min)	1.67 ± 0.71	3.36 ± 2.64	0.018*
CI (liters/min/m^2)	5.24 ± 1.60	10.00 ± 8.69	0.032*
FS (%)	44.83 ± 9.57	49.24 ± 13.38	0.175

Data are expressed as mean±SD

*Significantly different indicated at $P < 0.05$

FS, fractional shortening; EF, ejection fraction; SV, stroke volume; SVI, stroke volume index; HR, heart rate; CO, cardiac output; CI, cardiac index

Table 5 Relationship between SDMA and echocardiographic parameters of PAH dogs

Parameter	r of Healthy dogs (n = 12)	*P*-value	r of PAH dogs (n = 14)	*P*-value
CO	0.184	0.284	-0.555	0.020*
CI	0.153	0.317	-0.570	0.017*
SV	0.062	0.424	-0.556	0.019*
AT/ET	-0.251	0.215	-0.537	0.024*
RVIDd/LVIDd	-0.506	0.159	0.564	0.018*
TRmaxPG	-	-	0.223	0.222

* The Pearson correlation: cut off *P*-value at 0.05 (1-tailed)

CO, cardiac output; CI, cardiac index; SV, stroke volume; AT/ET, acceleration to ejection time ratio; RVIDd/LVIDd, ratio of right to left ventricular internal dimension during diastole; TRmaxPG, maximal pressure gradient of tricuspid regurgitation; SDMA, Symmetric dimethylarginine

Table 6 Comparison of echocardiographic parameters and renal function tests between healthy and precapillary or postcapillary PAH

Parameter	Healthy (n = 12)	Precapillary PAH (n = 6)	Postcapillary PAH (n = 8)
LA: Ao	1.27 ± 0.15	1.19 ± 0.22	2.30 ± 0.65 [a]
PA:Ao	0.89 ± 0.08	1.54 ± 0.47 [b]	1.34 ± 0.19 [b]
LVIDdN (cm)	1.37 ± 0.14	1.16 ± 0.38	2.61 ± 1.22 [a]
LVIDsN (cm)	0.74 ± 0.16	0.53 ± 0.25	1.42 ± 0.82 [a]
RVIDd/LVIDd	0.12 ± 0.06	1.07 ± 0.71 [c]	0.21 ± 0.16
FS (%)	44.82 ± 9.57	53.60 ± 17.73	45.97 ± 8.92
EF (%)	77.01 ± 10.50	80.10 ± 16.83	79.27 ± 10.22
HR (bpm)	121.33 ± 24.23	149.17 ± 21.24 [b]	154.80 ± 37.12 [b]
SV (ml)	13.75 ± 4.87	8.86 ± 10.83	31.86 ± 11.58 [a]
SVI (ml/beat/m^2)	0.042 ± 0.014	0.025 ± 0.027	0.091 ± 0.036 [a]
CO (L/min)	1.67 ± 0.70	1.18 ± 1.26	5.00 ± 2.16 [a]
CI (L/min/m^2)	5.24 ± 1.60	3.40 ± 3.36	14.96 ± 8.17 [a]
BUN (mg/dL)	16.97 ± 3.21	44.92 ± 15.57 [c]	22.74 ± 10.22
Creatinine (mg/dL)	0.73 ± 0.15	0.73 ± 0.40	0.76 ± 0.45
SDMA (µg/dl)	9.42 ± 1.72	14.5 ± 4.74 [c]	12.13 ± 3.26

Data are expressed as mean±SD

[a]$P < 0.05$ as compared to healthy and precapillary PAH groups

[b]$P < 0.05$ as compared to the healthy group

[c]$P < 0.05$ as compared to healthy and postcapillary PAH groups

LA: Ao, left atrium to aorta ratio; PA: Ao, pulmonary artery to aorta ratio; LVIDdN, normalized left ventricular internal diameter in diastole; LVIDsN, normalized left ventricular internal diameter in systole; RVIDd/LVIDd, the ratio of right to left ventricular internal dimension during diastole; FS, fractional shortening; EF, ejection fraction; HR, heart rate; SV, stroke volume; SVI, stroke volume index; CO, cardiac output; CI, cardiac index; BUN, blood urea nitrogen; SDMA, symmetric dimethylarginine

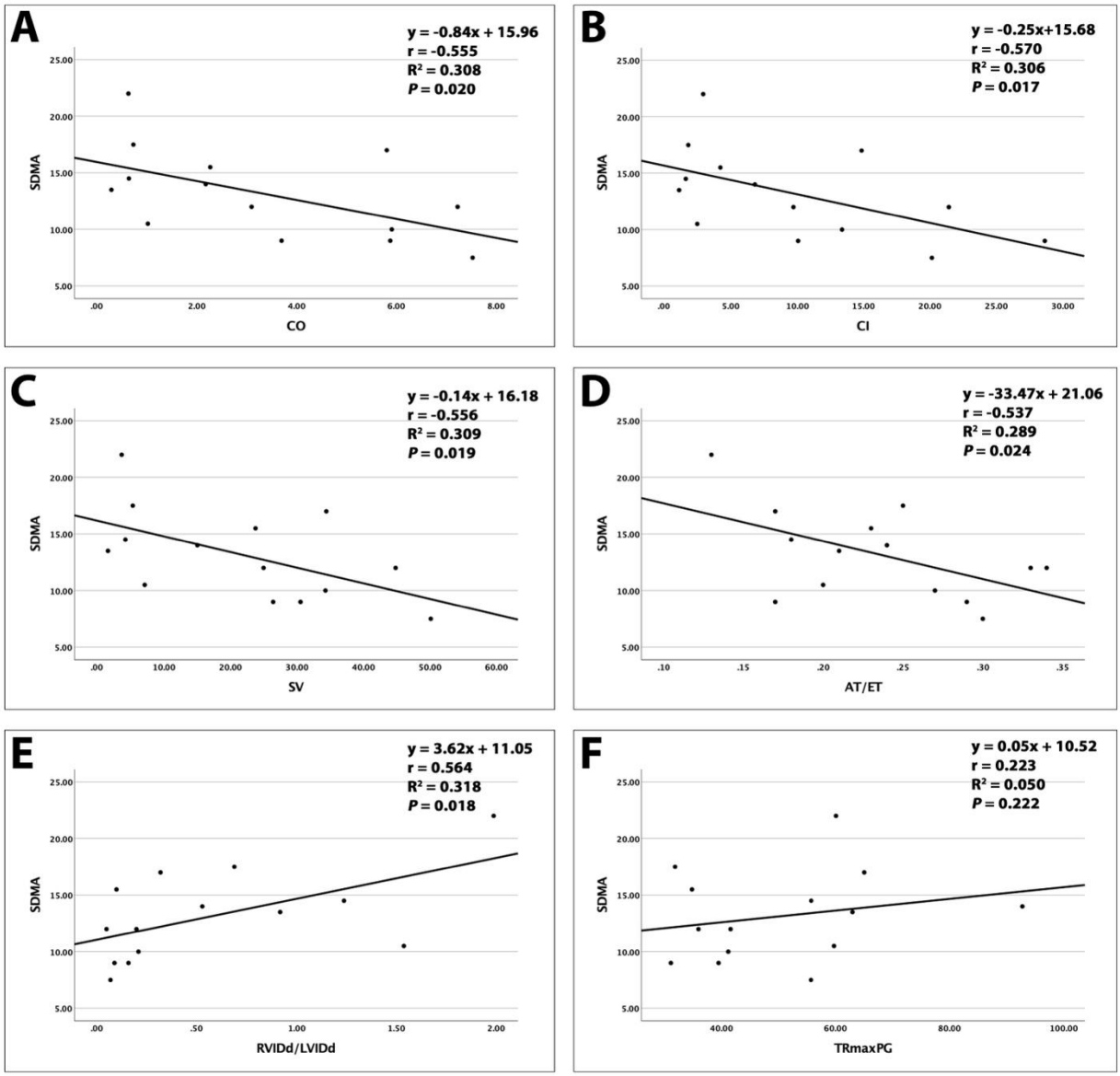

Figure 1 The analysis of linear correlations between SDMA level and echocardiographic parameters in PAH dogs: the negative relationship of CO, CI, SV, and AT/ET, respectively, $P < 0.05$ (A-D), a positive correlation between SDMA level and RVIDd/LVIDd, $P < 0.05$ (E). No correlation between SDMA level and TRmaxPG, $P > 0.05$ (F). SDMA, Symmetric dimethylarginine; CO, cardiac output; CI, cardiac index; SV, stroke volume; AT/ET, acceleration to ejection time ratio; RVIDd/LVIDd, diastolic right ventricular internal diameter to diastolic left ventricular internal diameter ratio; TRmaxPG, maximal pressure gradient of tricuspid regurgitation

Discussion

Our study is the first report to evaluate SDMA levels in newly diagnosed PAH dogs. It was found that the mean SDMA level of PAH dogs was higher than that of healthy dogs. Our result differs from Carlotta *et al*. (2020), who found no difference in SDMA levels among healthy and MMVD dogs, as well as between MMVD dogs with and without PAH. This may be because the PAH dogs were not the main population, and TRmaxPG was the only inclusion criteria used in that study. In addition, we found that BUN and BUN/creatinine ratios were higher in PAH dogs than in healthy dogs. This result is consistent with the previous studies, which reported that MMVD dogs with advanced stage disease had a decrease in GFR and an increase in BUN level (Nicolle *et al*., 2007; Valente *et al*., 2021).

The main findings of this study revealed that both SDMA and left ventricular systolic function parameters were higher in PAH dogs compared to healthy dogs. However, in PAH dogs, we also found a moderate negative correlation between the SDMA level and LV systolic function (CO, CI, SV), and AT/ET, as well as a moderate positive relationship between the SDMA level and RVIDd/LVIDd. In addition, no correlation was present between SDMA and echocardiographic parameters in healthy dogs. This indicates that right cardiomegaly and worsening of LV function affect an increment in SDMA level in PAH dogs. In line with our research, in human literature, patients with right-sided congestive heart failure and myocardial infarction with poor left ventricular systolic function had higher SDMA levels (Potočnjak *et al*., 2018; Lorin *et al*., 2017).

Non-renal diseases, such as neoplasia (particularly in lymphoma) (Coyne *et al*., 2022), diabetic mellitus (Mansour *et al*., 2023), and pancreatitis (Gori *et al*., 2020), can alter the SDMA level. However, none of the dogs in this study presented any of the aforementioned conditions. Moreover, prior to the SDMA level measurement, the PAH dogs in this study had not received any cardiovascular drugs. Therefore, treatment did not affect the SDMA level in our study.

In our study, the mean BUN and SDMA levels in precapillary PAH dogs were higher than in postcapillary PAH dogs. Moreover, right ventricular size and pulmonary diameter in precapillary PAH dogs were greater than in postcapillary PAH dogs, while LV diameter was lower. In addition, LV systolic function measured by CO, CI, SV, and SVI in precapillary PAH dogs was lower than in postcapillary PAH dogs. This can be explained by the pathophysiology of precapillary PAH, which involves the increment of pulmonary vascular resistance and right ventricular afterload, resulting in decreased pulmonary circulation and subsequently reducing blood returning to the LV (Reinero *et al*., 2020). The postcapillary PAH group had lower SDMA, which may be due to superior LV function in this group, suggesting that the heart may still be able to compensate to maintain hemodynamics better than the precapillary PAH group.

A negative relationship between SDMA and LV systolic parameters evaluated by echocardiography was only found in the PAH group, while no correlation was found in the healthy group. This finding suggests that worsening of LV systolic function may result in impaired renal function. However, the systolic functions, including SV, HR, CO, and CI, were higher in PAH dogs than in healthy dogs. This may be because most of the PAH dogs in this study were in the compensatory stage of PAH to preserve perfusion. As has been shown by Haase *et al*. (2013), people with cardiorenal syndrome type 1 are able to maintain a normal hemodynamic state by activating the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS). Nonetheless, the BUN/creatinine ratio in PAH dogs exhibited a notable rise, implying that factors such as dehydration or a high-protein diet may potentially influence the SDMA levels.

AT/ET is the echocardiographic parameter used to estimate systolic pulmonary artery pressures (PAP) (Serres *et al*., 2007; Visser *et al*., 2016; Schober, 2006). The probability of PAH increases when dogs have an AT/ET less than 0.3 (Reinero *et al*., 2020). In our study, dogs in the PAH group had averaged AT/ET equal to 0.24 ± 0.06, while the healthy group had 0.45 ± 0.04. Moreover, Pearson's correlation analysis revealed a significant negative correlation between SDMA and AT/ET. Our study findings suggest that an increased probability of PAH, as indicated by lower AT/ET values, might affect renal perfusion, resulting in deteriorating renal function. Yet, the link between AT: ET values and the severity of PAH remains undetermined.

The average TRmaxPG in PAH dogs found in this study was 50.74 ± 17.21 mmHg. There was no significant correlation between TRmaxPG and SDMA ($r = 0.223$; $P = 0.222$), similar to what has been reported by Valente *et al*. (2020). This may be due to the fact that the TRmaxPG values of the dogs in this study were mostly at low and moderate levels (TRmaxPG 30-55 mmHg (n = 7; 50%), 56-79 mmHg (n = 6; 42.86%), and > 79 mmHg (n = 1; 7.14%)). This could also be attributed to the TRmaxPG value not being a precise parameter for determining the severity of PAH. As has been shown by Reinero *et al*. (2020), PAH dogs with mild TRmaxPG can develop a congestive heart failure sign (CHF), while dogs with moderate or severe TRmaxPG may not develop CHF.

The white blood cell (WBC) count was statistically higher in dogs with PAH compared to healthy dogs; however, the values of all dogs fell within reference ranges. This could be attributed to the majority of PAH dogs having PAH secondary to MMVD. Additionally, some PAH dogs had concurrent conditions such as chronic respiratory disease, pancreatitis, cholangiohepatitis, heartworm infection, Ehrlichiosis, and Anaplasmosis. Other studies on the clinical characteristics of PAH dogs also reported mild leukocytosis in the PAH group (Johnson *et al*., 1999; Pyle *et al*., 2004). Moreover, the increased WBC may result from an increase in perivascular inflammatory cells due to pulmonary arterial remodeling in PAH dogs (Sakarin *et al*., 2023).

The limitation of this study is the inadequate number of pulmonary hypertensive dogs from each clinical classification group. Additionally, most dogs with PAH in this study exhibit low to moderate levels of TRmaxPG. Thus, if more dogs are present with high levels of TRmaxPG, the likelihood of detecting the relationship between SDMA levels and TRmaxPG might be enhanced. Moreover, all dogs were client-owned dogs, so nutritional factors could not be controlled, which may affect the BUN level. As well, in this study, PAH dogs had concurrent diseases that may alter their hydration status, including chronic respiratory disease, pancreatitis, and blood parasite infections. Finally, this study only assessed kidney function by measuring BUN, creatinine, SDMA, and blood pressure. If urinalysis, kidney ultrasonography, other early kidney biomarkers, and/or GFR could be performed, these could provide a clearer picture of the cardiovascular-renal axis disorder caused by PAH.

In conclusion, our study is the first report to measure SDMA levels in dogs with first-time diagnosed PAH without any prior treatment. Our study reveals that SDMA levels in PAH dogs, especially in precapillary PAH dogs, were significantly higher than in healthy dogs. In addition, the negative correlation between SDMA level and echocardiographic parameters of LV systolic function, as well as the positive correlation between SDMA and echocardiographic parameters indicating right heart remodeling were found, suggesting that more severe PAH may be related to more impaired renal function. Further studies using additional parameters to evaluate kidney function, such as urinalysis, kidney ultrasonography, other early biomarkers of kidney injury, and GFR, are necessary to gain more insight into cardiovascular-renal axis disorders in PAH dogs.

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