Journal of Veterinary Internal Medicine
Open Access

Standard Article

J Vet Intern Med 2016;30:1806–1815

Red Blood Cell Distribution Width, Hematology, and Serum Biochemistry in Dogs with Echocardiographically Estimated Precapillary and Postcapillary Pulmonary Arterial Hypertension

E. Mazzotta, C. Guglielmini, G. Menciotti, B. Contiero, M. BaronToaldo, M. Berlanda, and H. Poser

Background: Red blood cell distribution width (RDW) is a quantitative measurement of anisocytosis. RDW has prognostic value in humans with different cardiovascular and systemic disorders, but few studies have investigated this biomarker in dogs.

Objectives: To compare the RDW in dogs with precapillary and postcapillary pulmonary hypertension (PH) and a control population of dogs and to correlate RDW with demographic, echocardiographic, and laboratory variables.

Animals: One hundred and twenty-seven client-owned dogs including 19 healthy dogs, 82 dogs with myxomatous mitral valve disease (50 dogs without PH and 32 dogs with postcapillary PH), and 26 dogs with precapillary PH.

Methods: Prospective study. Dogs were allocated to groups according to clinical and echocardiographic evaluation. RDW and selected laboratory and echocardiographic variables were compared among dog groups. Associations between RDW and demographic, laboratory, and echocardiographic variables were analyzed using correlation and multiple regression analysis.

Results: Median RDW in dogs with precapillary PH (13.8%, interquartile range 13.2–14.9%) and postcapillary PH (13.7, 13.2–14.7%) was significantly increased compared to healthy dogs (13.3, 12.3–13.7%; $P < .05$ for both comparisons), but only dogs with severe PH had significantly increased RDW compared to dogs without PH ($P < .05$). Peak tricuspid regurgitation pressure gradient was significantly associated with increased RDW (rho = 0.263, $P = .007$). Serum urea concentration, hematocrit, age, and white blood cell number were significantly associated with RDW in the multivariate analysis.

Conclusions and Clinical Importance: Underlying pathophysiologic processes associated with PH instead of severity of PH are likely responsible for increased RDW in dogs with PH.

Key words: Azotemia; Canine; Cardiac biomarker; Cardiovascular disease; Echocardiography.

Red blood cell distribution width (RDW) is a measurement of the size variation as well as an index of the heterogeneity of the circulating erythrocytes.[1–3] The RDW is a component of a CBC and is automatically calculated by modern cell counters by dividing the standard deviation of erythrocyte volume by the mean corpuscular volume (MCV).[4] Evaluation of RDW, in combination with MCV, is conventionally used in the differential diagnosis of anemia and has been traditionally employed to discriminate between regenerative and nonregenerative anemia in the dog.[1,2] A high value of RDW was identified as a negative prognostic indicator in humans with different cardiovascular and thrombotic disorders, including pulmonary arterial hypertension[5,6] and pulmonary embolism.[7] The value of RDW in dogs with myxomatous mitral valve disease (MMVD) and compensated and decompensated heart failure has been

Abbreviations:

Ao	aortic diameter
BW	body weight
cTnI	cardiac troponin I
E-max	transmitral early diastolic velocity
FS	fractional shortening
LA:Ao	left atrium to aortic diameter
LA	left atrial diameter
LVDD	left ventricular diastolic diameter
LVDDn	left ventricular diastolic diameter normalized for body weight
LVSD	left ventricular systolic diameter
LVSDn	left ventricular systolic diameter normalized for body weight
MCV	mean corpuscular volume
MMVD	myxomatous mitral valve disease
PH-MMVD	pulmonary hypertension associated with myxomatous mitral valve disease
PH	pulmonary hypertension
PIPG	pulmonic insufficiency pressure gradient
PTE	pulmonary thromboembolism
RDW	red blood cell distribution width
TR Vmax	peak tricuspid regurgitation velocity
TRPG	tricuspid regurgitation pressure gradient
TR	tricuspid regurgitation
WBC	white blood cell number

From the Department of Animal Medicine, Production and Health, University of Padova, Legnaro, (Mazzotta, Guglielmini, Menciotti, Contiero, Berlanda, and Poser); and the Department of Veterinary Medical Sciences, Alma Mater Studiorum-University of Bologna, Bologna, Italy (BaronToaldo).

Dr. Menciotti's present address is Department of Small Animal Clinical Sciences, VA-MD College of Veterinary Medicine, Virginia Tech, Blacksburg

This work was carried out at the Department of Animal Medicine, Production and Health, University of Padova.

Not supported by a Grant.

Preliminary results were presented at the 23rd ESVIM-CA Congress, Liverpool, UK, 12-14 September 2013.

Corresponding author: C. Guglielmini, Department of Animal Medicine, Production and Health, Viale dell'Università 16, Agripolis, 35020 Legnaro, PD, Italy; e-mail: carlo.guglielmini@unipd.it.

Submitted March 4, 2016; Revised August 29, 2016; Accepted September 13, 2016.

DOI: 10.1111/jvim.14596

recently investigated, and it was neither associated with disease severity nor to heart failure.[8]

Pulmonary hypertension (PH) refers to an increase in pulmonary artery pressure that is usually secondary to various cardiovascular, respiratory, or systemic diseases in dogs.[9,10] In humans, 5 types of PH are recognized according to the underlying pathophysiological mechanism.[11] A more simplified classification commonly employed in dogs distinguishes precapillary PH from postcapillary PH, the latter being PH associated with left-sided cardiac diseases.[9,10] The diagnosis of PH can be accurately obtained by direct pulmonary pressure measurement via cardiac catheterization, but this technique is invasive and rarely performed in the clinical canine practice.[12,13] Noninvasive estimation of pulmonary artery systolic and diastolic pressure may be obtained by Doppler echocardiography.[10,12,14]

Different circulating markers of cardiovascular damage have been investigated in dogs with PH, including cardiac troponins and natriuretic peptides.[14–16] In a recent retrospective study, an increased RDW was found in a group of dogs with PH of different etiologies compared to control dogs but no information has been provided on the mechanism underlying this increase.[17] Red blood cell distribution width is routinely included in the clinical work up of dogs and is therefore a cost-effective variable compared to other more expensive cardiac biomarkers. Although 2 recent retrospective studies have investigated the RDW in dogs with different cardiovascular disorders,[8,17] more information about the effect of PH or underling diseases on RDW may better clarify its clinical usefulness. The aim of this study was to compare RDW between dogs with echocardiographically estimated precapillary and postcapillary PH and a control population and to determine whether RDW is correlated with clinical, laboratory, and echocardiographic variables.

Materials and Methods

Animals

The protocol of this prospective study was approved by the Ethics Committee of the University of Padua. Client-owned dogs presented to the cardiology service of the Veterinary Teaching Hospital of the University of Padua from January 2013 to November 2015 were eligible for entering the study. During the same time period, healthy dogs owned by students were also enrolled. After the owner signed a written consent form, each dog underwent physical examination, ECG, thoracic radiography, echocardiographic examination (two-dimensional real time, M-mode, and echo-Doppler), and blood sampling for CBC and serum biochemical profile. A single investigator (CG) evaluated both the thoracic radiographs and echocardiograms. Based on results of these examinations, dogs were assigned to the different groups according to the following criteria:

(1) Clinically healthy dogs (control group): dogs with normal physical examination and unremarkable ECG, thoracic radiography, echocardiographic, echo-Doppler, CBC, and serum biochemical profile findings.
(2) Dogs with MMVD without PH (MMVD group): dogs with an audible systolic murmur; thickened mitral valve leaflets on two-dimensional echocardiography; mild-to-severe mitral valve regurgitation; and low velocity tricuspid regurgitation (TR) (i.e., < 2.8 m/s, corresponding to a peak tricuspid regurgitation pressure gradient [TRPG] < 31 mmHg), end-diastolic pulmonic insufficiency, or both (i.e., < 2.2 m/s, corresponding to a pulmonic insufficiency pressure gradient [PIPG] < 19 mmHg) on spectral Doppler interrogation. Dogs with MMVD but without detectable TR, pulmonic insufficiency, or both were excluded from the study.
(3) Postcapillary PH associated with MMVD (PH-MMVD group): dogs with an audible systolic murmur; thickened mitral valve leaflets on two-dimensional echocardiography; moderate-to-severe mitral valve regurgitation associated with left atrial enlargement (LA:Ao $\geq$1.6); peak TR velocity (TR Vmax) $\geq$2.8 m/s (TRPG $\geq$31 mmHg), end-diastolic pulmonic insufficiency $\geq$2.2 m/s (PIPG $\geq$19 mmHg), or both without echocardiographic and echo-Doppler evidence of right ventricular outflow obstruction; and high transmitral early diastolic flow velocity (E-max > 1.2 m/s) on Doppler echocardiography. Dogs with PH-MMVD and other diseases potentially associated with precapillary PH (e.g., overt respiratory disease, heartworm disease, or hyperadrenocorticism) were excluded from the study.
(4) Precapillary PH (precapillary PH group): dogs with clinical signs of dyspnea, tachypnea, syncope, ascites, lethargy, or exercise intolerance and a right-sided systolic murmur; and peak TR Vmax $\geq$ 2.8 m/s (TRPG $\geq$ 31 mmHg), end-diastolic pulmonic insufficiency $\geq$2.2 m/s (PIPG $\geq$ 19 mmHg), or both on spectral Doppler echocardiography without echocardiographic and echo-Doppler evidence of right ventricular outflow obstruction and other congenital or acquired heart diseases.

Echocardiographic Examination

Echocardiographic and echo-Doppler examinations were performed in awake dogs without sedation with commercial ultrasound units[a,b] equipped with different phased-array transducers and continuous ECG monitoring. Standard echocardiographic scan planes were used in each dog.[18] Ventricular measurements were obtained from the right parasternal window, short axis view by two-dimensional guided M-mode echocardiography. Measurements of the left atrial (LA) and aortic diameter (Ao) were obtained using a two-dimensional method from the right parasternal short axis view at the level of the aortic root.[19] The following echocardiographic parameters were measured: left ventricular diastolic diameter (LVDD), left ventricular systolic diameter (LVSD), LA, and Ao. The diameters of the left ventricle were normalized for the effect of the body weight (BW) using the following equations: normalized LVDD (LVDDn) = LVDD/[$BW^{0.294}$] and normalized LVSD (LVSDn) = LVSD/[$BW^{0.315}$], as previously described.[20] Other echocardiographic indices included fractional shortening (FS), and LA:Ao.[19,21] Measurements of transmitral E-max were obtained with pulsed-wave Doppler from the left apical four-chamber view.

In dogs with PH, the TRPG was calculated based on the Doppler-derived systolic right ventricle to right atrial pressure gradient. The latter was calculated applying the modified Bernoulli equation ($\Delta p = 4 \times$ velocity^2) to the TR Vmax measured from the left parasternal four-chamber view. A peak TR Vmax $\geq$ 2.8 m/s (TRPG $\geq$ 31 mmHg) was considered to be indicative of PH.[16,17] Dogs with peak TR Vmax of 2.8–3.5 m/s, corresponding to TRPG of 31–50 mmHg, were considered to have mild PH, dogs with peak TR Vmax of 3.6–4.3 m/s, corresponding to TRPG of 51–75 mmHg, were considered to have moderate PH, and dogs with peak TR Vmax $\geq$ 4.4 m/s, corresponding to TRPG $\geq$ 76 mmHg, were considered to have severe PH.[16,17]

Laboratory Evaluation

CBC and serum biochemical analyses were performed within 24 hours on blood samples collected from dogs fasted for 12 hours beforehand. The RDW and other hematologic variables were measured by an automated CBC analyzer[c] previously validated for canine hematology.[2,22] Serum biochemical variables were evaluated by a commercial analyzer.[d] The internal quality controls provided by the manufacturers "Test Point Normal Control" and "Normal and Pathologic" were run daily for hematology and clinical chemistry analysis, respectively. The external quality control was performed for both analyzers using human control material every week[e] and following the EQA-RQAS (External Quality Assessment-Randox International Quality Assessment Scheme) monthly.

Reference intervals for RDW, hematocrit, and serum urea and creatinine concentrations, in the laboratory where the analyses were performed, were 11.9 to 14.5%, 38 to 57%, 20 to 50 mg/dL, and 0.5 to 1.5 mg/dL, respectively. Dogs were considered anemic when hematocrit was ≤37%. Dogs were considered to have mild anemia when hematocrit was ≥30 and ≤37%, and moderate-to-severe anemia when hematocrit was ≤29%.[23] Dogs were considered azotemic when the serum urea and creatinine concentrations were >50 mg/dL and >1.5 mg/dL, respectively.

Statistical Analysis

Data are reported as median and interquartile ranges. The nonparametric Kruskal-Wallis test was used to analyze equality of medians among groups according to the presence and type of PH as well as PH severity. When the factors were significant, a *posthoc* test with a Bonferroni correction was applied. For nominal data (sex), differences were evaluated by the chi-squared test.

Associations between continuous variables and RDW were investigated by Spearman correlation coefficient. After testing for collinearity, variables significantly associated with RDW were included in a multiple regression analysis performed in a stepwise manner. The relative importance of the included variables was assessed by order of entry into the model as well as by the change in the model R^2 (ΔR^2).

All statistical analyses were performed with a statistical software program.[f] The level of significance was set at $P < .05$.

Results

Study Population

The study population included 127 dogs of various breeds, with 65 males and 62 females. Among these, 19 dogs were clinically healthy, 50 dogs had MMVD without PH, 32 dogs had PH-MMVD, and 26 dogs had precapillary PH. The demographic data of each patient group are shown in Table 1. Among the 58 dogs with PH, 26 (19 with PH-MMVD and 7 with precapillary PH), 20 (10 with PH-MMVD and 10 with precapillary PH), and 12 (3 with PH-MMVD and 9 with precapillary PH) dogs had mild, moderate, and severe PH, respectively. Dogs with PH-MMVD and precapillary PH were significantly older compared to control dogs ($P < .001$ for each comparison). Dogs with PH-MMVD had significantly lower BW compared to control dogs ($P < .05$).

Among dogs with precapillary PH, 10 dogs had chronic respiratory disorders (interstitial lung disease, 7 dogs; pulmonary neoplasia, 1 dog; chronic bronchitis, 1 dog; and tracheal collapse, 1 dog); 6 dogs had pulmonary arteries parasitic disease (heartworm disease, 5 dogs; angiostrongylosis, 1 dog); 3 dogs had suspected pulmonary thromboembolism (PTE) because of a compatible clinical presentation and a diagnosed predisposing condition (i.e., hyperadrenocorticism, 2 dogs) or echocardiographic evidence of a large thrombus inside the right pulmonary artery (1 dog). In 5 dogs, left-sided cardiac disease was excluded but the cause of PH could not be determined. Anemia was diagnosed in 3.1% (1 of 32) and 11.5% (3 of 26) of dogs with PH-MMVD and precapillary PH, respectively, and anemia was mild in the former and moderate/severe in the latter. Only 1 dog with anemia associated with precapillary PH showed mild signs of regenerative anemia. Azotemia was diagnosed in 24% (12 of 50) and 57.7% (15 of 26) of dogs with PH-MMVD and precapillary PH, respectively. Anemia associated with azotemia was found in 3.8% (1 of 26) of dogs with precapillary PH, but no dog with PH-MMVD had anemia associated with azotemia.

Thirty-one dogs were treated before visit, and the drugs prescribed by the attending clinician are listed in Table 1. In particular, 16% (8 of 50), 62.5% (20 of 32), and 11.5 (3 of 26) dogs with MMVD, PH-MMVD, and precapillary PH, respectively, were receiving 1 or more drugs at the moment of blood sampling.

Laboratory and Echocardiographic Variables

Laboratory and echocardiographic data of the enrolled dogs grouped according to type or severity of PH are shown in Tables 2 and 3, respectively. The median white blood cell number (WBC) was significantly higher in dogs with PH-MMVD and precapillary PH compared to that of healthy dogs ($P < .001$ for both comparisons) and dogs with MMVD ($P < .01$ and $P < .001$, respectively); also, it was higher in dogs with mild, moderate, and severe PH compared to that of dogs without PH ($P < .05$, $P < .001$ and $P < .001$, respectively). The median serum urea concentration of dogs with MMVD, PH-MMVD, and precapillary PH was significantly higher than that of control dogs ($P < .05$, $P < .001$ and $P < .001$, respectively), and the median serum urea concentration of dogs with PH-MMVD and precapillary PH was significantly higher compared to that of dogs with MMVD ($P < .05$ for both comparisons). Furthermore, the median serum urea concentration of dogs with mild, moderate, and severe PH was significantly higher compared to that of dogs without PH ($P < .05$, $P < .05$, and $P < .001$, respectively), and the median serum urea concentration of dogs with severe PH was significantly higher compared to that of dogs with mild PH ($P < .05$).

Dogs with precapillary PH had lower LVDDn and LVSDn compared to dogs with MMVD and PH-MMVD ($P < .001$ for both variables) and to normal dogs ($P < .05$), and lower LA:Ao compared to dogs with PH-MMVD ($P < .001$). Dogs with precapillary PH had also significantly higher TR Vmax and TRPG compared to both dogs with MMVD ($P < .001$ for both variables) and dogs with PH-MMVD ($P < .01$ for both variables) (Table 2).

Table 1. Clinical data in 127 dogs.

	Healthy (n = 19)	MMVD (n = 50)	PH-MMVD (n = 32)	Precapillary PH (n = 26)	Overall *P*-Value
Age (years)	8.5 (7.1–10.4)	10.8 (7.0–13.4)	12.0 (11.0–14.1)***	12.0 (10.0–14.0)***	<.001
Body weight (kg)	14.5 (9.0–28.0)	9.9 (7.8–14.1)	8.7 (7.0–10.9)*	10.5 (7.0–20.0)	.028
Sex (male/female)	7/12	28/22	20/12	10/16	.15
Drugs received (n)	NA	P (6)	ACE-I (17)	ACE-I (2)	
		ACE-I (5)	Fu (12)	P (2)	
		Fu (4)	P (7)	Ma (1)	
		Di (4)	Sp (1)	F (1)	
		Sp (4)		Si (1)	
		Dt (1)		St (1)	

MMVD, Myxomatous mitral valve disease without pulmonary hypertension; PH-MMVD, pulmonary hypertension associated with myxomatous mitral valve disease; PH = pulmonary hypertension; n, number of dogs; NA, not applicable; P, pimobendan; ACE-I, angiotensin-converting enzyme inhibitor; Fu, furosemide; Di, digoxin; Sp, spironolactone; Dt, diltiazem; Ma, maropitant; Si, sildenafil; St, steroids.

*$P < .05$ compared with healthy dogs.

**$P < .01$ compared with healthy dogs.

***$P < .001$ compared with healthy dogs.

Data are expressed as median (interquartile range).

Table 2. Laboratory and echocardiographic data in 127 dogs.

		MMVD			
	Healthy (n = 19)	MMVD (n = 50)	PH-MMVD (n = 32)	Precapillary PH (n = 26)	Overall *P*-Value
Hematology					
RDW (%)	13.3 (12.3–13.7)	13.5 (12.7–14.2)	13.7 (13.2–14.7)*	13.8 (13.2–14.9)*	.011
Hematocrit (%)	48.6 (45.6–50.5)	45.5 (42.2–50.5)	48.2 (41.2–51.8)	48 (45–51.2)	.58
Hemoglobin (g/dL)	168 (160–172)	157 (144–171)	164 (141–175)	162 (148–173)	.81
MCV (fL)	69.2 (66.7–70.7)	68.3 (64.6–70.4)	68.2 (65.0–70.3)	68.9 (65.4–71.5)	.76
WBC (10^9/L)	7.4 (6.2–10.5)	9.6 (7.5–11.0)	12.2 (9.3–18.1)***,††	15.1 (10.0–25.3)***,†††	<.001
Platelets (10^9/L)	355 (262–403)	339 (284–449)	372 (315–460)	405 (270–493)	.32
Biochemistry					
Urea (mg/dL)	29.0 (17.8–34.0)	38.5 (28.0–44.0)*	47.0 (37.0–81.0)***,†	59.8 (33.0–81.5)***,†	<.001
Creatinine (mg/dL)	0.94 (0.76–1.07)	0.94 (0.81–1.11)	1.23 (0.85–1.42)	0.98 (0.83–1.26)	.11
Total proteins (g/L)	65.7 (62.6–71.2)	61.4 (58.7–67.0)	62.1 (57.5–68.5)	69.2 (61.8–75.8)	.11
Echo					
LVDDn	1.54 (1.4–1.57)	1.59 (1.46–1.72)	2.16 (2.0–2.26)***,†††	1.13 (0.87–1.43)*,†††,###	<.001
LVSDn	0.97 (0.93–1.00)	0.91 (0.8–1.09)*	1.1 (0.94–1.23)	0.66 (0.6–0.86)*,†††,###	<.001
LA:Ao	1.30 (1.20–1.40)	1.50 (1.33–1.66)***	2.83 (2.45–3.36)***,†††	1.38 (1.30–1.55)###	<.001
FS (%)	34 (31–39)	39 (33–44)	48 (41–54)***,†††	41 (27–46)	<.001
E-max (m/s)	0.70 (0.58–0.83)	0.74 (0.60–0.94)	1.62 (1.39–1.81)***,†††	0.57 (0.41–0.63)**,†††,###	<.001
TR Vmax (m/s)	NA	2.34 (1.92–2.62)	3.46 (3.25–3.69)†††	4.05 (3.46–4.61)†††,##	<.001
TRPG (mmHg)	NA	21.9 (14.7–27.4)	47.9± (40.1–54.0)†††	65.8 (47.8–85.0)†††,##	<.001

RDW, Red blood cell distribution width; MCV, mean corpuscular volume; WBC, white blood cell number; LVDDn, left ventricular diastolic diameter normalized for body weight; LVSDn, left ventricular systolic diameter normalized for body weight; LA, left atrial diameter; Ao, aortic diameter; FS, fractional shortening; E-max, transmitral peak E-wave velocity; TR Vmax, peak velocity of tricuspid regurgitation; TRPG, tricuspid regurgitation pressure gradient; n, number of dogs; MMVD, myxomatous mitral valve disease; PH-MMVD, pulmonary hypertension associated with MMVD.

*$P < .05$ compared with healthy dogs.

**$P < .01$ compared with healthy dogs.

***$P \leq .001$ compared with healthy dogs.

†$P < .05$ compared with dogs with MMVD.

††$P < .01$ compared with dogs with MMVD.

†††$P \leq .001$ compared with dogs with MMVD.

#$P < .05$ compared with dogs with PH-MMVD.

##$P < .01$ compared with dogs with PH-MMVD.

###$P \leq .001$ compared with dogs with PH-MMVD.

Data are expressed as median (interquartile range).

When dogs were grouped according to the severity of PH (Table 3), dogs with mild PH had significantly higher LVDDn, LA:Ao, FS, and E-max compared to dogs without PH ($P < .01$, $P < .001$, $P < .05$, and $P < .001$, respectively), whereas dogs with severe PH had significantly lower LVDDn and LVSDn compared

Table 3. Laboratory and echocardiographic data in 69 dogs without pulmonary hypertension (No PH), including healthy dogs and dogs with myxomatous mitral valve disease without PH, and 58 dogs with different degree of PH.

		PH			
	No PH (n = 69)	Mild (n = 26)	Moderate (n = 20)	Severe (n = 12)	Overall *P*-Value
Hematology					
RDW (%)	13.4 (12.7–14.0)	13.7 (13.2–14.5)	13.5 (13.0–14.8)	14.5 (13.6–17.3)*	.009
Hematocrit (%)	47.4 (44.3–50.5)	49.7 (45.0–53.0)	46.7 (40.4–51.4)	48.0 (43.4–50.2)	.36
Hemoglobin (g/dL)	161 (146–171)	166 (150–177)	157 (134–173)	163 (149–172)	.55
MCV (fL)	68.3 (65.5–70.6)	68.2 (66.0–70.5)	69.0 (65.3–70.9)	67.7 (64.5–69.6)	.82
WBC (10^9/L)	8.7 (7.2–10.5)	10.6 (8.7–17.3)*	15.9 (10.2–20.4)***	14.4 (11.7–37.0)***	<.001
Platelets (10^9/L)	341 (277–429)	399 (314–493)	372 (270–491)	385 (297–415)	.34
Biochemistry					
Urea (mg/dL)	34.0 (26.0–42.0)	44.0 (30.4–68.0)*	48.0 (35.0–83.0)*	81.5 (51.1–138.0)***,†	<.001
Creatinine (mg/dL)	0.94 (0.80–1.11)	1.16 (0.85–1.31)	0.91 (0.79–1.41)	1.15 (0.91–1.44)	.086
Total proteins (g/L)	64.0 (59.3–69.0)	65.7 (59.0–71.0)	62.7 (56.3–74.5)	67.0 (60.9–69.7)	.75
Echo					
LVDDn	1.57 (1.43–1.72)	2.03 (1.55–2.19)**	1.83 (0.96–2.15)	1.13 (0.84–1.66)†	.001
LVSDn	0.93 (0.80–1.04)	1.05 (0.88–1.17)	0.77 (0.64–1.18)	0.63 (0.43–0.71)**,††	<.001
LA:Ao	1.41 (1.27–1.58)	2.45 (1.79–2.89)***	1.94 (1.30–2.93)	1.57 (1.30–2.23)	<.001
FS (%)	36 (32–42)	42 (39–48)*	45 (34–49)	52 (41–59)**	.001
E-max (m/s)	0.72 (0.60–0.86)	1.38 (0.70–1.73)***	0.97 (0.54–1.63)	0.68 (0.45–1.25)	.001
TR Vmax (m/s)	2.34 (1.92–2.62)[a]	3.28 (3.12–3.45)***	3.77 (3.66–4.06)***,†††	4.97 (4.57–5.28)***†††,###	<.001
TRPG (mmHg)	21.9 (14.7–27.4)[a]	43.0 (38.9–47.6)***	56.8 ± (53.4–65.8)***,†††	98.6 (83.4–111.3)***,†††,###	<.001

RDW, Red blood cell distribution width; MCV, mean corpuscular volume; WBC, white blood cell number; LVDDn, left ventricular diastolic diameter normalized for body weight; LVSDn, left ventricular systolic diameter normalized for body weight; LA, left atrial diameter; Ao, aortic diameter; FS, fractional shortening; E-max, transmitral peak E-wave velocity; TR Vmax, peak velocity of tricuspid regurgitation; TRPG, tricuspid regurgitation pressure gradient; n, number of dogs.

*$P < .05$ compared with dogs without PH.
**$P \leq .01$ compared with dogs without PH.
***$P < .001$ compared with dogs without PH.
†$P < .05$ compared with dogs with mild PH.
††$P < .01$ compared with dogs with mild PH.
†††$P < .001$ compared with dogs with mild PH.
#$P < .05$ compared with dogs with moderate PH.
##$P < .01$ compared with dogs with moderate PH.
###$P < .001$ compared with dogs with moderate PH.
[a]Values obtained from 50 dogs with TR.

to dogs with mild PH ($P < .05$ and $P < .01$, respectively), and significantly lower LVSDn and higher FS compared to dogs without PH ($P < .01$ for both comparisons).

RDW

In healthy dogs, the median (interquartile range) RDW was 13.3% (12.3–13.7%). Seven of 50 (14%) dogs with MMVD, 9 of 32 (28.1%) dogs with PH-MMVD, and 8 of 26 (30.8%) dogs with precapillary PH had RDW greater than the upper reference limit of 14.5%. In particular, dogs with precapillary PH and increased RDW included 4 dogs with chronic respiratory disorders, 2 dogs with heartworm disease, and 1 dog each with PTE and indeterminate PH etiology. The 17 dogs with PH and increased RDW (9 with PH-MMVD and 8 with precapillary PH) were quite equally distributed among the different PH groups, with 6 dogs each in the mild and moderate PH group, and 5 dogs in the severe PH group.

The median (interquartile range) of RDW in dogs with PH-MMVD and precapillary PH was 13.7% (13.2–14.7%) and 13.8% (13.2–14.9%), respectively, and was significantly higher compared to that of control dogs ($P < .05$ for both comparisons). No significant difference was found in the RDW between control dogs and dogs with MMVD (Table 2 and Fig 1A). The median RDW in dogs with severe PH was 14.5% (13.6–17.3%) and was significantly higher compared to that of dogs without PH ($P < .05$) (Table 3 and Fig 1B). The RDW of dogs with mild and moderate PH was not significantly different from that of dogs without PH.

Correlation and Multiple Regression Analysis

Spearman's coefficient of correlation between RDW and demographic, laboratory, and echocardiographic variables is shown in Table 4. The RDW was significantly positively associated with increasing age, white blood cell number (WBC), serum urea concentration, TR Vmax, and TRPG and significantly negatively associated with hematocrit and hemoglobin. Scatterplots of RDW and the independent variables age, WBC, hematocrit, serum urea concentration, and TRPG are depicted in Figure 2.

To identify important contributors to RDW, multivariate stepwise regression models were constructed including age, WBC, hematocrit, serum urea concentration, and TRPG as independent variables (Table 5). Hemoglobin and TR Vmax were excluded from the model after testing for collinearity. Based on the order of entry, serum urea concentration had the highest explanatory power in the model and explained about 12% of the total variation of RDW. The estimated regression coefficient was about 0.01, which means that for every unitary increase of serum urea, RDW increased of 0.01%. The other variables included in the model showed an average incremental contribution of R^2 of about 6%. The final R^2 of the model was 30%. Only hematocrit showed a negative regression coefficient. The variance inflation factors were about 1 for every predictor which means that there was no correlation among the variables. Therefore, the variance of estimated regression coefficients was not inflated at all. TRPG was the only predictor not included in the model.

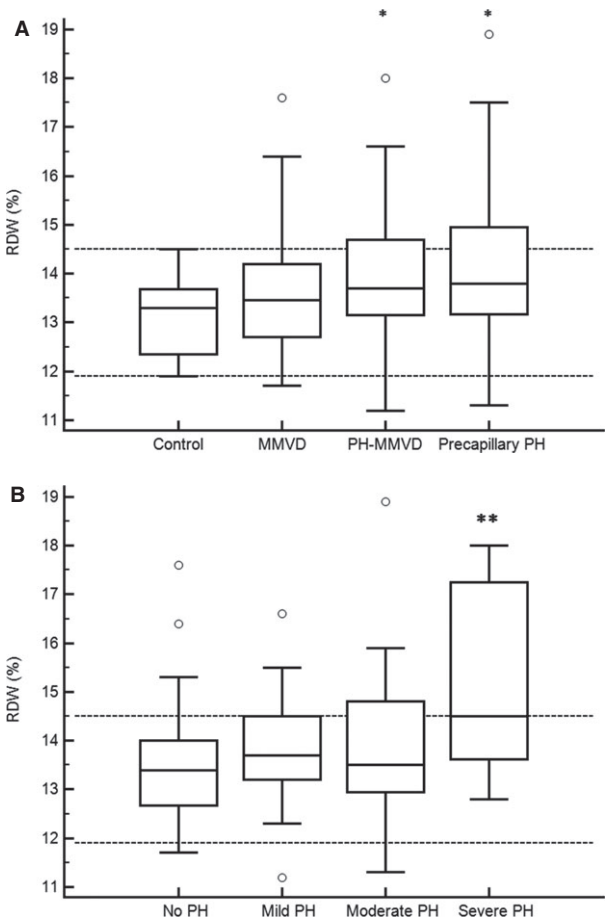

Fig 1. Box and whisker plots of the RDW obtained from (**A**) 19 healthy dogs (control), 50 dogs with myxomatous mitral valve disease without pulmonary hypertension (MMVD), 32 dogs with postcapillary pulmonary hypertension associated with myxomatous mitral valve disease (PH-MMVD), and 26 dogs with precapillary pulmonary hypertension (precapillary PH) and (**B**) 59 dogs without PH (No PH) including healthy dogs and dogs with MMVD without PH, 26 dogs with mild PH (19 with PH-MMVD and 7 with precapillary PH), 20 dogs with moderate PH (10 with PH-MMVD and 10 with precapillary PH), and 12 dogs with severe PH (3 with PH-MMVD and 9 with precapillary PH). Dashed lines indicate RDW reference interval. Outliers are plotted separately as white dots. Median (horizontal lines), inter quartile range (boxes) and 95% CI (whiskers) are also represented. * Statistical difference ($P < .05$) with control dogs. ** Statistical difference ($P < .01$) with dogs without PH.

Discussion

The results of the present study revealed that RDW was increased in both dogs with PH-MMVD and dogs with precapillary PH without concomitant changes of MCV, but only dogs with severe PH had increased RDW. Multivariate analyses showed that age, hematocrit, WBC, and serum urea concentration, but not Doppler-estimated TRPG, namely PH severity, were significantly associated with increased RDW in the dog.

Dogs with precapillary PH had a higher value of TR Vmax and, consequently, TRPG, compared to dogs with PH-MMVD. These findings support that precapillary PH is usually associated with a greater increase in pulmonary arterial pressure compared to postcapillary PH in dogs,[15,24,25] although they might be the consequence of a more advanced disease in dogs with precapillary PH. Hence, dogs with precapillary PH are often evaluated after the onset of clinical signs, whereas dogs with MMVD can be evaluated at different disease stages, even when clinical signs of PH are not present.

Table 4. Spearman's correlation coefficients between red blood cell distribution width and demographic, laboratory, and echocardiographic variables in 127 dogs.

	Spearman's Correlation Coefficient	*P*-Value
Age	0.327	<.001
Weight	−0.058	.52
Hematocrit	−0.225	.011
Hemoglobin	−0.227	.010
MCV	−0.077	.39
WBC	0.319	<.001
Platelets	0.057	.53
Urea	0.311	<.001
Creatinine	0.128	.16
Total proteins	−0.068	.47
LVDDn	0.004	.96
LVSDn	−0.023	.80
LA:Ao	0.163	.07
FS	−0.002	.98
E-max	−0.023	.80
TR Vmax	0.263	.007
TRPG	0.263	.007

MCV, Mean corpuscular volume; WBC, white blood cells number; LVDDn, left ventricular diastolic diameter normalized for body weight; LVSDn, left ventricular systolic diameter normalized for body weight; LA, left atrial diameter; Ao, aortic diameter; FS, fractional shortening; E-max, transmitral peak E-wave velocity; TR Vmax, peak velocity of tricuspid regurgitation; TRPG, tricuspid regurgitation pressure gradient.

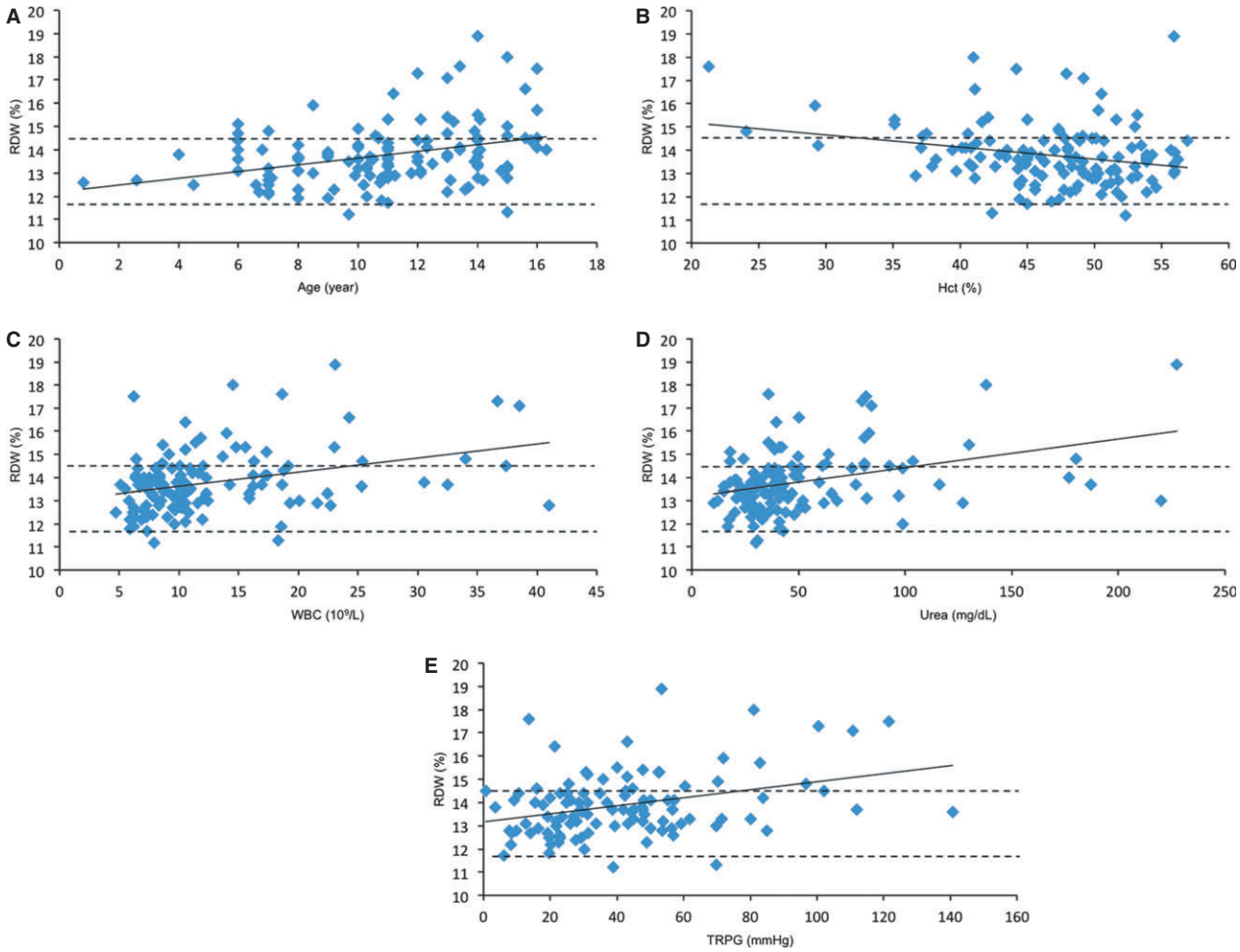

Fig 2. Scatterplots of the relationship between red blood cell distribution width (RDW) and (**A**) age, (**B**) hematocrit (Hct), (**C**) white blood cell number (WBC), and (**D**) serum urea concentration in 69 dogs without pulmonary hypertension (PH) and 58 dogs with PH, and between RDW and (**E**) tricuspid regurgitation pressure gradient (TRPG) in 108 dogs with tricuspid regurgitation. Dashed lines indicate RDW reference interval.

Table 5. Multivariate regression equations showing the relationship between red blood cell distribution width and demographic, laboratory, and echocardiographic variables in 127 dogs.

Order of Entry	Variable	Partial R^2	Model R^2	Coefficient	±SE	*P*-Value	Variance Inflation Factor
1	Urea	0.117	0.117	0.01	0.003	<.001	1.338
2	Hematocrit	0.117	0.234	−0.07	0.019	<.001	1.038
3	Age	0.039	0.273	0.086	0.04	.025	1.148
4	WBC	0.030	0.304	0.034	0.017	.044	1.219

SE, standard error; WBC, white blood cells number.

According to both inclusion criteria (i.e., increased LA and E-max, which are echocardiographic indices associated with more severe prognosis in dogs with MMVD)[26–28] and the observed increased of left ventricular dimension, dogs with PH-MMVD showed more severe left-sided cardiac remodeling compared to dogs with MMVD without PH, as previously reported.[15,29–31] Conversely, dogs with precapillary PH had reduced left ventricular dimension compared to dogs of all the other groups, likely as a consequence of reduced venous return to the left heart.[25,32,33]

More than one quarter and one-third of dogs with PH-MMVD and precapillary PH had RDW greater than the reference interval, respectively, and their median RDW was significantly increased compared to that of control dogs. However, only dogs with severe PH, regardless of the underlying pathophysiologic mechanism, had increased RDW compared to dogs without PH or dogs with mild or moderate PH. Increased RDW in dogs with precapillary PH but not in dogs with postcapillary PH compared to healthy dogs has been recently reported.[17] Differences in study design

and cardiac diseases associated with postcapillary PH might explain different results between this study and that previous study. Chronic respiratory disorders, heartworm disease, and PTE, in addition to MMVD, were most frequently associated with increased RDW in dogs with PH reported here. Increased RDW has been reported as an early predictor of RV failure and an independent negative prognostic factor in human patients with chronic pulmonary disorders,[34,35] and acute and chronic thrombotic and thromboembolic pulmonary disorders.[7,36–39]

In dogs of the present study, age was positively associated with increased RDW in the multiple regression model. A gradual increase of RDW with aging has been reported in the human scientific literature.[40] In the dog, a recent study evidenced no significant difference of RDW between puppies and adult dogs,[41] but no information is available regarding changes of RDW in aged dogs. RDW was negatively associated with hematocrit and hemoglobin concentration. However, no correlation was found between RDW and MCV, differently from previous studies both in humans and dogs.[5,8,17] The reasons of this discrepancy remain unclear. The biologic mechanisms underlying the association between high RDW and different human cardiovascular diseases including myocardial infarction, coronary artery disease, atrial fibrillation, stroke, peripheral artery occlusive disease, and PH, as well as other systemic disorders (e.g., cancer, diabetes, kidney, or liver disease), are still largely unclear.[3,5,6,40,42] In people, it has not yet been well elucidated whether RDW might be an independent cardiovascular risk factor or whether it rather only represents a simple marker of a concomitant disorder, such as an underlying inflammatory state that impairs erythrocyte maturation; impaired renal function and consequent inadequate production of erythropoietin; malnutrition (i.e., deficiencies in nutrients, such as iron, vitamin B12, and folate); or oxidative damage.[3,5,36,40,42] Inflammation, oxidative stress, or both have been proposed as the important determinants of RDW in some forms of PH in humans.[3,6] The increased WBC concentration we found in dogs with PH-MMVD and precapillary PH, as well as in dogs with moderate and severe PH, and the significant association of WBC and RDW suggest that inflammation may be associated with anisocytosis in dogs with PH. However, increased WBC in these dogs might also represent a stress response for the underlying disease, hospitalization, or both[43] Increased urea concentration but not creatinine concentration was found in dogs of the present study with PH-MMVD and precapillary PH as well as in dogs with moderate and severe PH. Furthermore, RDW had a significant positive association with serum urea but not creatinine concentration suggesting a possible relationship between RDW and prerenal azotemia. In humans, a strong, graded, and independent association between RDW and estimated glomerular filtration rate has been demonstrated as well as a parallel increase of RDW and stages of chronic kidney disease.[44,45] However, because of the lack of urinalysis results, we cannot draw firm conclusions about the actual origin of azotemia in dogs of the present study. Moreover, serum urea values could have been affected by drugs employed in some dogs at inclusion (e.g., furosemide and ACE-inhibitors),[46,47] but this effect could not be quantified. Other variables with a significant association with RDW were hematocrit and hemoglobin, as well as TR Vmax and associated TRPG. However, in addition to age and hematocrit, only serum urea concentration and WBC remained significantly associated with increased RDW after multivariate analyses. These findings suggest that RDW is likely a marker of concomitant disorders in dogs with PH because the association between RDW and severity of PH was not confirmed in the multivariate analyses.

Few studies have been specifically focused on cardiac biomarkers in dogs with naturally occurring PH including evaluation of cardiac troponin I (cTnI) and natriuretic peptides concentration in dogs with precapillary PH, PH-MMVD, and experimentally induced chronic embolic PH.[14–16,48] Because of differences in study design and patient population, some disparities exist among results of these studies but increased levels of cTnI and brain natriuretic peptide were observed in dogs with precapillary PH, particularly in those with more severe PH, PTE, or both.[15,16] Although the RDW, which is a routine part of the CBC, would be a cost-effective alternative to more expensive laboratory evaluations, its diagnostic and prognostic utility in dogs with PH appears limited according to the results of the present and a recent study,[17] respectively.

There were various limitations to the present study. Healthy dogs were age-matched with dogs with MMVD but not with dogs with PH-MMVD and precapillary PH, and a positive association between RDW and age was found in dogs of the present study. The diagnosis of PH was based on Doppler echocardiographic findings of TR Vmax, and neither cardiac catheterization nor other echocardiographic and echo-Doppler parameters were employed for the direct and indirect confirmation or exclusion of PH.[31,49,50] Doppler evaluation of TR Vmax is the most commonly employed technique for the diagnosis of canine PH in the clinical setting,[12,13] and the presence of PH was considered very unlikely in clinically healthy dogs without clinical, radiographic, and echocardiographic evidence of cardiorespiratory disorders, although a measurable TR was not available in these dogs. Furthermore, no assessment of right ventricular function was carried out. Thus, underestimation of PH severity in dogs with right ventricular dysfunction cannot be excluded. Some of the dogs classified with PH-MMVD could have concomitant undetectable chronic bronchial or pulmonary disease. In these dogs, PH was considered postcapillary but a precapillary component cannot be completely excluded. Dogs with precapillary PH had higher TRPG compared to dogs with PH-MMVD, but this probably reflects a different disease stage that could have biased the study results. Absolute reticulocyte count was not available. These data would have added additional useful information on the

pathophysiology of the observed difference in RDW and would have excluded confounding factors in the small subset of anemic dogs. The diagnosis of PTE in 3 dogs with precapillary PH was based on clinical findings in dogs with predisposing disease or echocardiographic evidence of a thrombus inside the pulmonary artery. Necropsy confirmation was available in only 1 case, but advanced imaging techniques (i.e., scintigraphy or computed tomographic angiography) were not used in the diagnostic work. Finally, dogs with cardiopulmonary disease included in the study were either first-opinion or referred cases and almost a quarter of them were receiving drugs for the underlying disease. The effect of these drugs on each measured RDW could not be determined.

Conclusions

A significantly increased RDW was found in dogs with both precapillary PH and postcapillary PH, as well as in dogs with severe PH regardless of the underlying pathophysiological mechanism. Results of the multivariate analyses showed a positive association between RDW and serum urea concentration and WBC count, but not with severity of PH. Although the biologic mechanism underlying the association between raised RDW and PH cannot be determined from this study, it may be associated with dehydration, a proinflammatory or stressful state, or both.

Footnotes

[a] Zone Ultra, Zonare Medical Systems, Mountain View, CA
[b] CX50, Philips, Eindhoven, Netherlands
[c] Advia 120, Hematology system, Siemens, Munich, Germany
[d] AU 400, Mishima Olympus, Shizuoka, Japan
[e] Bio-Rad Laboratories, Segrate, Italy
[f] SAS 9.3, SAS Institute Inc., Cary, NC

Acknowledgments

Conflict of Interest Declaration: Authors declare that they have no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

References

1. Neiger R, Hadley J, Pfeiffer DU. Differentiation of dogs with regenerative and non-regenerative anaemia on the basis of their red cell distribution width and mean corpuscular volume. Vet Rec 2002;150:431–434.

2. Hodges J, Christopher MM. Diagnostic accuracy of using erythrocyte indices and polychromasia to identify regenerative anemia in dogs. J Am Vet Med Assoc 2011;238:1452–1458.

3. Montagnana M, Cervellin G, Meschi T, Lippi G. The role of red blood cell distribution width in cardiovascular and thrombotic disorders. Clin Chem Lab Med 2012;50:635–641.

4. Lippi G, Plebani M. Red blood cell distribution width (RDW) and human pathology. One size fits all. Clin Chem Lab Med 2014;52:1247–1249.

5. Hampole CV, Mehrotra AK, Thenappan T, et al. Usefulness of red cell distribution width as a prognostic marker in pulmonary hypertension. Am J Cardiol 2009;104:868–872.

6. Rhodes CJ, Wharton J, Howard LS, et al. Red cell distribution width outperforms other potential circulating biomarkers in predicting survival in idiopathic pulmonary arterial hypertension. Heart 2011;97:1054–1060.

7. Zorlu A, Bektasoglu G, Kukul Guven FM, et al. Usefulness of admission red cell distribution width as a predictor of early mortality in patients with acute pulmonary embolism. Am J Cardiol 2012;109:128–134.

8. Guglielmini C, Poser H, Dalla Pria A, et al. Red blood cell distribution width in dogs with chronic degenerative valvular disease. J Am Vet Med Assoc 2013;243:858–862.

9. Kellihan HB, Stepien RL. Pulmonary hypertension in canine degenerative mitral valve disease. J Vet Cardiol 2012;14:149–164.

10. Fleming E, Ettinger SJ. Pulmonary hypertension. Compend Contin Educ Pr Vet 2006;28:720–730.

11. Galiè N, Hoeper MM, Humbert M, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension. Eur Heart J 2009;30:2493–2537.

12. Borgarelli M. Pulmonary hypertension. In: Bonagura JD, Twedt DC, eds. Kirk's Current Veterinary Therapy. XV. St. Louis: Elsevier Saunders; 2014: 711–717.

13. Soydan LC, Kellihan HB, Bates ML, et al. Accuracy of Doppler echocardiographic estimates of pulmonary artery pressures in a canine model of pulmonary hypertension. J Vet Cardiol 2015;17:13–24.

14. Atkinson KJ, Fine DM, Thombs LA, et al. Evaluation of pimobendan and N-terminal probrain natriuretic peptide in the treatment of pulmonary hypertension secondary to degenerative mitral valve disease in dogs. J Vet Intern Med 2009;23:1190–1196.

15. Guglielmini C, Civitella C, Diana A, et al. Serum cardiac troponin I concentration in dogs with precapillary and postcapillary pulmonary hypertension. J Vet Intern Med 2010;24:145–152.

16. Kellihan HB, MacKie BA, Stepien RL. NT-proBNP, NT-proANP and cTnI concentrations in dogs with pre-capillary pulmonary hypertension. J Vet Cardiol 2011;13:171–182.

17. Swann JW, Sudunagunta S, Covey HL, et al. Evaluation of red cell distribution width in dogs with pulmonary hypertension. J Vet Cardiol 2014;16:227–235.

18. Thomas WP, Gaber CE, Jacobs GJ, et al. 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;7:247–252.

19. Rishniw M, Erb HN. Evaluation of four 2-dimensional echocardiographic methods of assessing left atrial size in dogs. J Vet Intern Med 2000;14:429–435.

20. Cornell CC, Kittleson MD, Della Torre P, et al. Allometric scaling of M-mode cardiac measurements in normal adult dogs. J Vet Intern Med 2004;18:311–321.

21. Brown DJ, Rush JE, MacGregor J, et al. M-mode echocardiographic ratio indices in normal dogs, cats, and horses: A novel quantitative method. J Vet Intern Med 2003;17:653–662.

22. Welles EG, Hall AS, Carpenter DM. Canine complete blood counts: A comparison of four in-office instruments with the ADVIA 120 and manual differential counts. Vet Clin Pathol 2009;38:20–29.

23. Tvedten H. Laboratory and clinical diagnosis of anemia. In: Weiss DJ, Wardrop KJ, eds. Schalm's Veterinary Hematology, 6th ed. Ames, Iowa: Wiley-Blackwell; 2010:152–161.

24. Johnson L, Boon J, Orton EC. Clinical characteristics of 53 dogs with Doppler-derived evidence of pulmonary hypertension: 1992-1996. J Vet Intern Med 1999;13:440–447.

25. Pyle R, Abbott J, MacLean H. Pulmonary hypertension and cardiovascular sequelae in 54 dogs. J Appl Res Vet Med 2004;2:99–109.

26. Borgarelli M, Savarino P, Crosara S, et al. Survival characteristics and prognostic variables of dogs with mitral regurgitation attributable to myxomatous valve disease. J Vet Intern Med 2008;22:120–128.

27. Borgarelli M, Crosara S, Lamb K, et al. Survival characteristics and prognostic variables of dogs with preclinical chronic degenerative mitral valve disease attributable to myxomatous degeneration. J Vet Intern Med 2012;26:69–75.

28. Hezzell MJ, Boswood A, Moonarmart W, et al. Selected echocardiographic variables change more rapidly in dogs that die from myxomatous mitral valve disease. J Vet Cardiol 2012;14:269–279.

29. Serres FJ, Chetboul V, Tissier R, et al. 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;229:1772–1778.

30. Chiavegato D, Borgarelli M, D'Agnolo G, et al. Pulmonary hypertension in dogs with mitral regurgitation attributable to myxomatous valve disease. Vet Radiol Ultrasound 2009;50:253–258.

31. Baron Toaldo M, Poser H, Menciotti G, et al. Utility of tissue Doppler imaging in the echocardiographic evaluation of left and right ventricular function in dogs with myxomatous mitral valve disease with or without pulmonary hypertension. J Vet Intern Med 2016;30:697–705.

32. Campbell FE. Cardiac effects of pulmonary disease. Vet Clin North Am Small Anim Pract 2007;37:949–962.

33. Haddad F, Doyle R, Murphy DJ, et al. Right ventricular function in cardiovascular disease, part II: Pathophysiology, clinical importance, and management of right ventricular failure. Circulation 2008;117:1717–1731.

34. Sincer I, Zorlu A, Yilmaz MB, et al. Relationship between red cell distribution width and right ventricular dysfunction in patients with chronic obstructive pulmonary disease. Heart Lung 2012;41:238–243.

35. Nathan SD, Reffett T, Brown AW, et al. The red cell distribution width as a prognostic indicator in idiopathic pulmonary fibrosis. Chest 2013;143:1692–1698.

36. Abul Y, Ozsu S, Korkmaz A, et al. Red cell distribution width: A new predictor for chronic thromboembolic pulmonary hypertension after pulmonary embolism. Chron Respir Dis 2014;11:73–81.

37. Ozsu S, Yasin A, Gunaydin S, et al. Prognostic value of red cell distribution width in patients with pulmonary embolism. Clin Appl Thromb 2014;20:365–370.

38. Xi Q, Wang Y, Liu Z, et al. Red cell distribution width predicts chronic thromboembolic pulmonary hypertension in patients with acute pulmonary embolism in a long-term follow-up. Clin Chem Lab Med 2014;52:e191–e195.

39. Wang W, Liu J, Yang Y, et al. Red cell distribution width is increased in chronic thromboembolic pulmonary hypertension. Clin Respir J 2016;10:54–60.

40. Salvagno GL, Sanchis-Gomar F, Picanza A, Lippi G. Red blood cell distribution width: A simple parameter with multiple clinical applications. Crit Rev Clin Lab Sci 2015;52:86–105.

41. Rørtveit R, Sævik BK, Eggertsdottir AV, et al. Age-related changes in hematologic and serum biochemical variables in dogs aged 16-60 days. Vet Clin Pathol 2015;44:47–57.

42. Förhécz Z, Gombos T, Borgulya G, et al. Red cell distribution width in heart failure: Prediction of clinical events and relationship with markers of ineffective erythropoiesis, inflammation, renal function, and nutritional state. Am Heart J 2009;158:659–666.

43. Tvedten H, Raskin RE. Leukocyte Disorders. In: Willard MD, Tvedten H, eds. Small Animal Clinical Diagnosis by Laboratory Methods. Fifth ed. St. Louis, Missouri: Saunders Elsevier; 2012: 66–72.

44. Lippi G, Targher G, Montagnana M, et al. Relationship between red blood cell distribution width and kidney function tests in a large cohort of unselected outpatients. Scand J Clin Lab Invest 2008;68:745–748.

45. Solak Y, Yilmaz MI, Saglam M, et al. Red cell distribution width is independently related to endothelial dysfunction in patients with chronic kidney disease. Am J Med Sci 2014;347:118–124.

46. Schroeder NA. Diuretics. In: Ettinger SJ, Feldman EC, eds. Textbook of Veterinary Internal Medicine, 7th ed. St. Louis, Missouri: Saunders Elsevier; 2010:1212–1214.

47. Bulmer BJ. Angiotensin converting enzyme inhibitors and vasodilators. In: Ettinger SJ, Feldman EC, eds. Textbook of Veterinary Internal Medicine, 7th ed. St. Louis, Missouri: Saunders Elsevier; 2010:1216–1223.

48. Hori Y, Uchide T, Saitoh R, et al. Diagnostic utility of NT-proBNP and ANP in a canine model of chronic embolic pulmonary hypertension. Vet J 2012;194:215–221.

49. Serres F, Chetboul V, Gouni V, et al. Diagnostic value of echo-Doppler and tissue Doppler imaging in dogs with pulmonary arterial hypertension. J Vet Intern Med 2007;21:1280–1289.

50. Tidholm A, Höglund K, Häggström J, Ljungvall I. Diagnostic value of selected echocardiographic variables to identify pulmonary hypertension in dogs with myxomatous mitral valve disease. J Vet Intern Med 2015;29:1510–1517.