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Plasma atrial natriuretic peptide and N-terminal pro B-type natriuretic peptide concentrations in dogs with right-sided congestive heart failure

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ABSTRACT. The clinical utility of plasma natriuretic peptide concentrations in dogs with right-sided congestive heart failure (CHF) remains unclear. We investigated whether plasma levels of atrial natriuretic peptide (ANP) and N-terminal pro B-type natriuretic peptide (NT-proBNP) are useful for assessing the congestive signs of right-sided heart failure in dogs. This retrospective study enrolled 16 healthy dogs and 51 untreated dogs with presence (n=28) or absence (n=23) of right-sided CHF. Medical records of physical examinations, thoracic radiography and echocardiography were reviewed. The plasma concentration of canine ANP was measured with a chemiluminescent enzyme immunoassay. Plasma NT-proBNP concentrations were determined using an enzyme immunoassay. Plasma ANP and NT-proBNP concentrations in dogs with right-sided CHF were significantly higher than in healthy controls and those without right-sided CHF. The plasma NT-proBNP concentration >3,003 pmol/l used to identify right-sided CHF had a sensitivity of 88.5% and specificity of 90.3%. An area under the ROC curve (AUC) was 0.93. The AUC for NT-proBNP was significantly higher than the AUCs for the cardiothoracic ratio, vertebral heart score, ratio of right ventricular end-diastolic internal diameter to body surface area, tricuspid late diastolic flow and ratio of the velocities of tricuspid early to late diastolic flow. These results suggest that plasma ANP and NT-proBNP concentrations increase markedly in dogs with right-sided CHF. Particularly, NT-proBNP is simple and helpful biomarkers to assess the right-sided CHF.
KEY WORDS: canine, congestive heart failure, natriuretic peptide, NT-proBNP, right heart disease

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Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are neurohumoral peptides involved in the regulation of body fluid homeostasis and blood pressure. These peptides are synthesized as a prohormone, which is proteolytically cleaved into a biologically active fragment (ANP and BNP) and an inactive fragment N-terminal prohormone (NT-proANP and NT-proBNP) [28]. Both peptides are mainly produced and released from the myocardium in response to wall stretching, as during volume overloading [28].

Many studies have investigated the clinical utility of natriuretic peptides in dogs with left-sided heart failure [1, 6, 11, 21, 25, 26, 31, 32]. Plasma ANP concentration concomitantly increases with cardiac enlargement and left atrial dilation in dogs with this condition [1, 9, 11]. The serum or plasma NT-proBNP concentration was correlated with the vertebral heart score and ratio of left atrial to aortic dimension in dogs with cardiac disease [25, 31]. An increased level of natriuretic peptide can indicate disease severity and prognosis in dogs with left-sided heart failure [4, 9, 11, 31]. This finding is expected to be useful in assessing the severity of heart failure in clinical practice.

Plasma ANP and BNP and/or NT-proBNP levels have been used as a non-invasive screening tool in human patients with right-sided heart failure [19, 20, 22, 27]. Plasma BNP levels were significantly elevated in patients with right ventricular systolic dysfunction and were negatively correlated with right ventricular ejection fractions [22]. In addition, children with left-sided congenital heart defects (e.g., ventricular septal defects and a patent ductus arteriosus) had significantly higher plasma ANP and BNP concentrations than those with right-sided congenital heart defects (e.g., primum atrial septal defect, secundum atrial septal defect and partial anomalous pulmonary venous return) [10]. Therefore, it is possible that the clinical implications of natriuretic peptide levels are different between dogs with left-sided and right-sided heart failure.

Few reports have investigated changes in plasma ANP and BNP levels in dogs with right-sided congestive heart failure (CHF) [2, 16]. One study found that the plasma ANP concentration was significantly increased in dogs with vena cava syndrome, concomitant with elevated right atrial and ventricular pressure [16]. However, the diagnostic utility of plasma ANP and NT-proBNP concentrations in dogs with right-sided CHF remains unclear. In the present study, we investigated changes in plasma natriuretic peptide concentrations in dogs with right-sided CHF. In addition, the sensitivity and specificity of plasma ANP and NT-proBNP con-

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centrations used to diagnose this disease were determined.

MATERIALS AND METHODS

Animals: This retrospective study population consisted of 67 dogs. All of the dogs were examined between February 2008 and October 2012 at the Kitasato University Veterinary Teaching Hospital and Miyazaki University Veterinary Teaching Hospital, Japan. The data collected included breed, sex, age, weight, clinical signs and the presence or absence of symptoms of right-sided CHF. Results of echocardiographic examination, plasma chemical examinations and plasma measurements of ANP and NT-proBNP were recorded. All of the examinations were performed without sedation in a quiet examination room.

Clinically healthy dogs (*n*=16) were recruited from healthy laboratory beagles. Clinically healthy dogs of both sexes (3 males and 13 females), aged 2–8 years and weighing 7.8–13.0 kg, were enrolled as study subjects. Regarding examinations, the procedures followed the guideline for Institutional Laboratory Animal Care and Use of the School of Veterinary Medicine at Kitasato University.

Medical records of 51 dogs with right-sided heart disease were reviewed. Owners provided informed consent to obtain information about the current status of each animal. The dogs were divided into two groups based on the presence of clinical right-sided CHF. Dogs with mild to moderate heart failure (exercise intolerance, prolonged recovery and tachypnea with exercise) without fluid retention were classified as the negative for CHF [non-CHF] group (*n*=23). Dogs with congestive signs, such as ascites, pleural effusion, subcutaneous edema and pericardial effusion, in combination with radiographic and echocardiographic evidence were classified in the right-sided CHF group (*n*=28).

Diseased dogs with heartworm disease, tricuspid valve insufficiency and pulmonary valve insufficiency were included. Heartworm disease was diagnosed by the presence of circulating microfilariae and/or a positive blood antigen test (SNAP Heartworm, IDEXX Laboratories, Westbrook, ME, U.S.A.). Dogs with suspected precapillary pulmonary hypertension (PH) were included [13–15]. Tricuspid valve regurgitation (TR) velocity >2.8 m/sec or pulmonary valve regurgitation velocity >2.2 m/sec concomitant with main pulmonary artery dilation without left-sided heart failure were considered precapillary PH [13–15]. The medical records of untreated dogs with non-CHF and right-sided CHF were reviewed at the first medical examination. After all of the examinations was completed, if necessary, dogs received immediate medical treatment for heart disease.

Dogs with pulmonary stenosis, double-chambered right ventricle, pulmonary arterial embolization, suspected post-capillary PH [15]; i.e., left-sided heart failure or concurrent systemic illness other than cardiovascular disease, were excluded from the study.

Radiography and echocardiography: The cardiothoracic ratio and vertebral heart score were evaluated using thoracic radiography. Conventional echocardiographic and Doppler examinations were performed using an ultrasonographic unit (iE33, Philips Electronics Japan, Tokyo, Japan and EUB7500, Hitachi Medical, Utsunomiya, Japan) with a 7.5 to 12 MHz probe. These examinations were performed by 2 experienced veterinarians (YH and NK). The diagnosis of tricuspid and pulmonary valve insufficiency was made based on color flow Doppler echocardiographic evidence of regurgitated flow. Diagnosis of vena cava syndrome was made based on echocardiographic evidence of heartworm (small, bright parallel echoes) in the pulmonary arterial, right atrial or right ventricular cavity.

Left ventricular end-diastolic internal diameter (LVID) and right ventricular internal diameter (RVID) were measured by M-mode echocardiography in the right parasternal short-axis view. The ratio of RVID to LVID (RVID/LVID ratio) and the ratio of RVID to body surface area (RVID/BSA ratio) were calculated. The ratio of main pulmonary artery to aorta (PA/Ao ratio) was measured in the right parasternal short-axis view [12].

Pulsed Doppler echocardiography was used to measure right ventricular inflow velocities with the sample volume positioned at the tip of the tricuspid valve leaflets in the left apical four-chamber view. The early diastolic (E wave) and atrial systolic (A wave) velocities of transtricuspid flow and the ratio of E wave to A wave velocities (E/A ratio) were measured. Similarly, TR velocities and pulmonary valve regurgitation velocities were measured using continuous wave Doppler echocardiography.

Plasma ANP and NT-proBNP measurements: To determine the concentrations of ANP and NT-proBNP, blood samples were collected from the jugular vein in tubes containing aprotinin (for ANP) and ethylene diamine tetraacetic acid (for NT-proBNP), and were centrifuged immediately for 15 min at 4°C and 3,500 rpm. The plasma was separated and stored at −80°C. The ANP and NT-proBNP assay was performed in duplicates. The plasma concentration of canine ANP was measured with a chemiluminescent enzyme immunoassay with a human ANP assay kit (Shionoria-ANP, Shionogi Co., Osaka, Japan) [11, 12]. Plasma NT-proBNP concentrations were determined using an enzyme immunoassay for canine NT-proBNP (Cardiopet proBNP, IDEXX Laboratories) [12].

Statistical analysis: Normality of data was assessed with the Kolmogorov–Smirnov test. All values are expressed as medians [min-max]. The Kruskal–Wallis test was used to analyze the distributed data among all groups. A post hoc analysis was performed with the Dunn test for multiple comparisons. Spearman's nonparametric correlation analysis was applied to compare plasma ANP and NT-proBNP concentrations to the thoracic radiographic or echocardiographic measurements. Forward stepwise regression analysis was used to determine the natriuretic peptides that correlated best with the radiographic and echocardiographic variables. A value of $F>2.0$ was considered statistically significant. Receiver-operating characteristic (ROC) analyses were used to assess the predictive accuracy of plasma natriuretic peptide concentrations for identification of dogs with or without right-sided CHF. Comparisons of ROC curves were analyzed using a previously described method (MedCalc version 12.2.1.0;

Table 1. Comparison of physical examination and thoracic radiographic variables among clinically healthy dogs and 2 groups of dogs with heart disease

	Healthy control	Non-CHF	Right-sided CHF
n	16	23	28
Sex (Males/ Females)	3/13	10/13	15/13
Age (years)	7.0 [2.0–8.0]	10.0 [0.6–15.0][a)]	10.0 [3.2–19.0][a)]
Body weight (kg)	10.1 [7.8–13.0]	13.6 [3.4–23.1]	10.8 [2.5–30.8]
Body temperature (°C)	38.3 [37.7–40.1]	38.6 [37.7–39.4]	38.1 [36.2–39.9]
Respiratory rate (/min)	29 [20–40]	36.0 [24.0–42.0]	48.0 [16.0–180.0][b)]
Heart rate (beat/min)	110 [88–144]	118.0 [72.0–180.0][a)]	140.0 [36.0–200.0][a)]
Cardiothoracic ratio (%)	48.1 [42.7–53.3]	61.3 [45.7–81.8][c)]	63.3 [47.1–78.5][c)]
Vertebral heart score	10.1 [8.8–10.3]	11.5 [9.1–13.5][c)]	11.7 [10.2–15.6][c)]

Data are described as median values [min-max]. a) $P<0.05$ v.s. healthy controls, b) $P<0.01$ v.s. healthy controls, c) $P<0.001$ v.s. healthy controls.

MedCalc Software, Ostend, Belgium) [7]. A *P*-value <0.05 was considered to indicate significance.

RESULTS

The diseased dogs included 26 Mixed breeds, 4 Golden retrievers, 3 Miniature Dachshunds, 3 Welsh Corgis, 2 Beagles, 2 Pomeranians, 2 Toy poodles and 9 other breeds. The causes of heart diseases included 24 tricuspid valve insufficiency, 23 heartworm disease, 3 pulmonary valve insufficiency and 1 Ebstein anomaly. Of these, 37 dogs (72.5%) were concomitant with suspected precapillary PH, which included 22 tricuspid valve insufficiency, 13 heartworm disease and 2 pulmonary valve insufficiency.

In dogs with the right-sided CHF, 21 dogs had ascites, 4 dogs had pleural effusion, 3 dogs had pulmonary edema, 2 dogs had subcutaneous edema, and 1 dog had pericardial effusion. The results of the physical examinations and thoracic radiographic variables of the groups are shown in Table 1. Respiratory rate and heart rate were significantly higher in dogs with right-sided CHF compared to those in healthy control dogs. The cardiothoracic ratio and vertebral heart score in both diseased groups were significantly higher than those in the healthy control group.

Echocardiographic data are shown in Table 2. The RVID/BSA and RVID/LVID ratios were significantly higher in dogs with right-sided CHF compared to those in the healthy controls and non-CHF group. The PA/Ao ratio was significantly higher in dogs with both diseased groups compared to that in the healthy controls. The TR velocity was not different between the right-sided CHF and non-CHF groups. Additionally, pulmonary valve regurgitation velocity was not different between the right-sided CHF and non-CHF groups.

Plasma ANP concentrations in dogs with right-sided CHF (116.0 *p*g/m*l* [28.5–260.0]) were significantly higher than those in healthy controls (24.8 [15.7–39.9]) and those in the non-CHF group (41.7 [8.1–210.0], Fig. 1A). Plasma ANP levels were higher in non-CHF dogs than those in the healthy controls, but the differences were not significant. In addition, plasma NT-proBNP concentrations in dogs with right-sided CHF (6,554 pmol/*l* [1,583–9,568]) were significantly higher than those in the healthy controls (510 [55–1,252]) and the non-CHF dogs (1,643 [107–8,411], Fig. 1B). Plasma NT-proBNP levels were higher in non-CHF dogs than those in healthy controls, but the difference was not significant.

Plasma ANP level was significantly correlated with the vertebral heart score (r=0.65), cardiothoracic ratio (r=0.56), RVID/BSA ratio (r=0.43), RVID/LVID ratio (r=0.40) and PA/Ao ratio (r=0.46) (Table 3). Stepwise regression analysis showed that vertebral heart score (F=8.5), cardiothoracic ratio (F=4.8), RVID/LVID ratio (F=3.5) and PA/Ao ratio (F=2.7) could predict plasma ANP (r=0.74, r^2=0.55; $P<0.001$). Plasma NT-proBNP level was significantly correlated with vertebral heart score (r=0.67), E wave velocity (r=0.55), A wave velocity (r=0.50), RVID/LVID ratio (r=0.48), cardiothoracic ratio (r=0.46), RVID/BSA ratio (r=0.45), PA/Ao ratio (r=0.39) and TR velocity (r=-0.38) (Table 3). Stepwise regression analysis showed that vertebral heart score (F=36.3) and heart rate (F=5.8) could predict plasma NT-proBNP (r=0.77, r^2=0.60; $P<0.001$).

Sensitivity and specificity for detecting dogs with presence or absence of right-sided CHF at relevant cutoff values were determined. Use of a plasma ANP concentration >35.3 *p*g/m*l* to detect non-CHF had a sensitivity of 61.9% and a specificity of 81.3%. The area under the ROC curve (AUC) was 0.67 (95% CI, 0.49 to 0.86; P=0.075, Fig. 2A). Use of a plasma NT-proBNP concentration >595.5 pmol/*l* to identify non-CHF had a sensitivity of 73.3% and specificity of 56.3%. The AUC was 0.75 (95% CI, 0.57 to 0.94; $P<0.017$, Fig. 2B). Diagnostic accuracies for detection of dogs with right-sided CHF are shown in Table 4. Use of a plasma ANP concentration >47.6 *p*g/m*l* to detect right-sided CHF had sensitivity of 81.3% and specificity of 75.7%. The AUC was 0.86 ($P<0.0001$, Fig. 3A). Use of a plasma NT-proBNP concentration >3,003 pmol/*l* to identify right-sided CHF had a sensitivity of 88.5% and specificity of 90.3%. The AUC was 0.93 ($P<0.0001$, Fig. 3B).

The ROC curves of ANP or NT-proBNP levels used to diagnose right-sided CHF were compared to those of thoracic radiographic and echocardiographic parameters (Table 5). The AUC for ANP was insignificant than that of the other parameters. In contrast, the AUC for NT-proBNP was significantly higher than the AUCs for the cardiothoracic ratio, the vertebral heart score, the RVID/BSA ratio, the A wave and the E/A ratio.

Table 2. Comparison of echocardiographic variables among groups

	Healthy Control (*n*)	Non-CHF (*n*)	Right-sided CHF (*n*)
RVID/BSA ratio	2.1 [1.2–2.8] (16)	2.4 [1.0–9.0] (20)	4.8 [2.2–8.4] (28)[c, e)]
RVID/LVID ratio	0.3 [0.2–0.4] (16)	0.5 [0.2–2.2] (20)	1.1 [0.5–2.6] (28)[c, e)]
PA/Ao ratio	1.0 [0.9–1.2] (15)	1.2 [0.9–2.1] (21)[b)]	1.4 [1.0–2.2] (28)[c)]
TR velocity (m/sec)	–	3.9 [2.5–4.9] (12)	4.6 [2.3–5.9] (24)
PR velocity (m/sec)	–	3.2 [0.8–3.3] (9)	3.4 [1.4–3.8] (19)
E wave (cm/sec)	48.5 [25.0–67.0] (13)	42.7 [24.9–132.3] (16)	78.2 [44.6–123.3] (12)[b, f)]
A wave (cm/sec)	49.1 [36.9–65.3] (13)	66.4 [17.5–99.6] (15)	64.4 [48.0–120.0] (8)
E/A ratio	0.9 [0.6–1.4] (13)	0.6 [0.4–1.9] (15)	1.0 [0.6–2.0] (9)[d)]

All data are described as median values [min-max]. A wave; tricuspid late diastolic flow, E/A ratio; the ratio of E wave to A wave velocities, E wave; tricuspid early diastolic flow, PA/Ao ratio; ratio of main pulmonary artery to aorta, PR; pulmonary valve regurgitation, RVID/BSA ratio; ratio of right ventricular end-diastolic internal diameter to body surface area, RVID/LVID ratio; ratio of right ventricular end-diastolic internal diameter to left ventricular end-diastolic internal diameter, TR; tricuspid valve regurgitation. a) $P<0.05$ v.s. healthy controls, b) $P<0.01$ v.s. healthy controls, c) $P<0.001$ v.s. healthy controls, d) $P<0.05$ v.s. non-CHF, e) $P<0.01$ v.s. non-CHF, f) $P<0.001$ v.s. non-CHF.

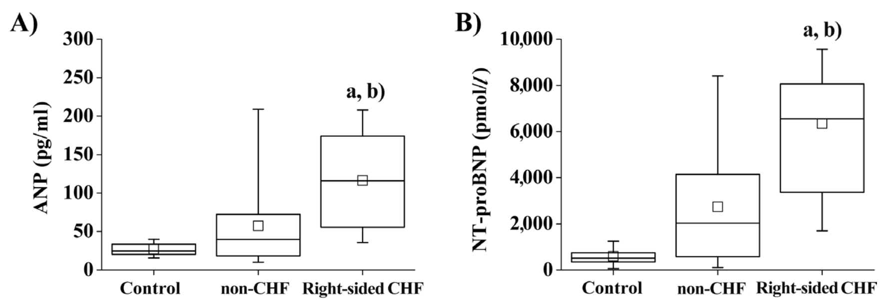

Fig. 1. Plasma ANP (A) and NT-proBNP (B) concentrations in dogs with or without right-sided CHF. a) $P<0.001$ v.s. healthy controls, b) $P<0.01$ v.s. non-CHF.

Table 3. Results of single regression analyses of natriuretic peptides and radiographic or echocardiographic measurements

	ANP			NT-proBNP		
	r	r^2	*P*	*r*	r^2	*P*
Cardiothoracic ratio	0.56	0.31	<0.001	0.46	0.21	<0.001
Vertebral heart score	0.65	0.42	<0.001	0.67	0.45	<0.001
RVID/BSA ratio	0.43	0.19	<0.001	0.45	0.21	<0.001
RVID/LVID ratio	0.40	0.16	<0.01	0.48	0.23	<0.001
PA/Ao ratio	0.46	0.21	<0.001	0.39	0.16	<0.01
E wave	0.28	0.08	0.08	0.55	0.30	<0.001
A wave	0.19	0.04	0.27	0.50	0.25	<0.01
E/A ratio	−0.16	0.03	0.36	0.01	<0.01	0.96
TR velocity	−0.16	0.03	0.39	−0.38	0.15	0.03
PR velocity	−0.06	<0.01	0.78	0.31	0.10	0.14

A wave; tricuspid late diastolic flow, E/A ratio; the ratio of E wave to A wave velocities, E wave; tricuspid early diastolic flow, PA/Ao ratio; ratio of main pulmonary artery to aorta, *r*; regression coefficient, PR; pulmonary valve regurgitation, RVID/BSA ratio; ratio of right ventricular end-diastolic internal diameter to body surface area, RVID/LVID ratio; ratio of right ventricular end-diastolic internal diameter to left ventricular end-diastolic internal diameter, TR; tricuspid valve regurgitation.

DISCUSSION

Right-sided CHF in dogs is the clinical outcome of various congenital or acquired cardiac and pulmonary conditions, such as tricuspid valve insufficiency, heartworm disease, pulmonary artery stenosis, pulmonary disease and PH [8, 15, 16, 18]. Ascites is a frequent clinical symptom in right-sided CHF and is concomitant with disease severity [5, 16]. In addition, dogs with severe right-sided heart failure showed right ventricular dilation and main pulmonary artery enlargement

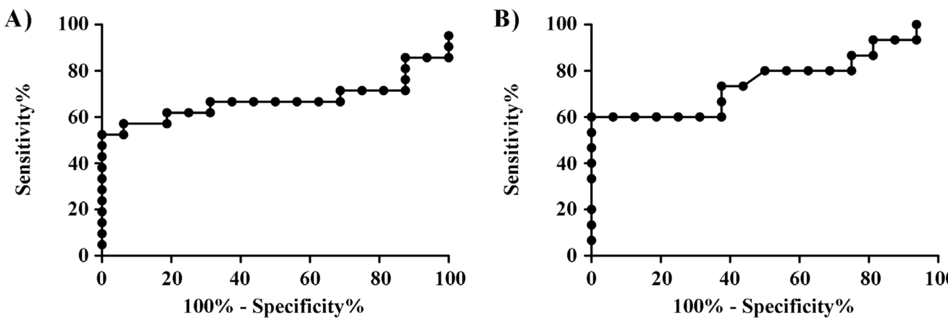

Fig. 2. Receiver operating characteristic curves displaying sensitivity and specificity using plasma ANP (A) and NT-proBNP (B) concentrations for detecting dogs without right-sided CHF.

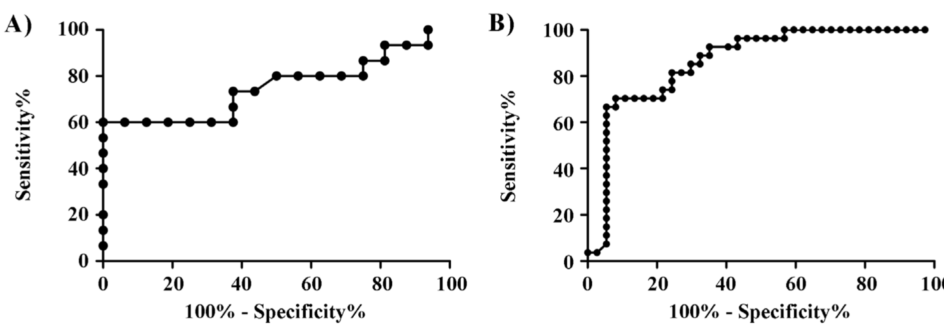

Fig. 3. Receiver operating characteristic curves displaying sensitivities and specificities when plasma ANP (A) and NT-proBNP (B) concentrations were used to identify dogs with right-sided CHF.

Table 4. Diagnostic accuracy of natriuretic peptides to detect dogs with right-sided CHF

Parameter	ANP	NT-proBNP
Cutoff value	47.6 *pg*/m*l*	3,003 pmol/*l*
AUC	0.86	0.93
95% CI	0.77–0.96	0.86–0.99
Sensitivity (%)	81.3	88.5
Specificity (%)	75.7	90.3
FPR (%)	24.3	9.7
FNR (%)	18.7	11.5
PPV (%)	74	88
NPV (%)	88	89

ANP; atrial natriuretic peptide, AUC; area under the ROC curve, CI; confidence intervals, FNR; False negative ratio, FPR; False positive ratio, NPV; Negative predictive value, NT-proBNP; N-terminal pro B-type natriuretic peptide, PPV; Positive predictive value.

[3, 16]. In the present study, dogs with right-sided CHF had congestive signs, which were concomitant with echocardiographic evidence of an increased RVID/BSA ratio, RVID/LVID ratio and PA/Ao ratio, as well as the cardiothoracic ratio and vertebral heart score. These results were consistent with those of previous reports and were observed as typical echocardiographic and radiographic features of right-sided CHF.

Measurement of plasma ANP levels is mainly used to diagnose the severity of disease and to evaluate the prognosis, of dogs with left-sided heart failure [1, 9, 11, 24], whereas recent human clinical studies reported that the measurement of plasma ANP is useful in assessing the severity of right-sided heart disease [10, 33]. Plasma ANP levels were significantly elevated in human patients with right ventricular pressure overload, and the level showed a significant inverse correlation with the right ventricular ejection fraction [33]. Similarly, plasma ANP concentrations are significantly higher in dogs with vena cava syndrome than those in normal dogs, which is concomitant with increased right atrial and ventricular pressure [16]. These results have led to the speculation that circulating ANP levels are regulated by the severity of right ventricular dysfunction in right-sided heart failure. However, whether plasma ANP concentrations reflect the presence or absence of right-sided CHF or disease severity, in dogs remains unclear. Our results demonstrate that plasma ANP levels were significantly elevated in dogs with right-sided CHF, but were unchanged in dogs with non-CHF. These results suggest that plasma ANP levels increase with right-sided CHF in dogs.

Most studies have reported that circulating NT-proBNP levels are elevated in dogs with left-sided heart failure [4, 25, 26, 31, 32]. In dogs with mitral valve disease, the plasma NT-proBNP concentration was significantly higher in dogs with CHF than in asymptomatic dogs [25, 31]. In addition, the plasma NT-proBNP concentration was significantly elevated in dogs with symptomatic pulmonary stenosis

Table 5. Comparison of ROC curve analysis to detect dogs with right-sided CHF

	v.s. ANP	v.s. NT-proBNP
Cardiothoracic ratio		
Difference between areas	0.109	0.204
95% CI	−0.002–0.220	0.087–0.321
z statistic	1.93	3.42
P value	0.054	0.0006
Vertebral heart score		
Difference between areas	0.033	0.133
95% CI	−0.064–0.129	0.037–0.229
z statistic	0.665	2.716
P value	0.506	0.007
RVID/BSA ratio		
Difference between areas	0.022	0.097
95% CI	−0.109–0.152	0.008–0.185
z statistic	0.324	2.144
P value	0.746	0.032
RVID/LVID ratio		
Difference between areas	0.032	0.066
95% CI	−0.086–0.150	−0.008–0.139
z statistic	0.529	1.758
P value	0.597	0.079
PA/Ao ratio		
Difference between areas	0.022	0.071
95% CI	−0.099–0.143	−0.009–0.151
z statistic	0.36	1.749
P value	0.719	0.080
E wave		
Difference between areas	0.146	0.007
95% CI	−0.028–0.319	−0.129–0.143
z statistic	1.646	0.104
P value	0.100	0.917
A wave		
Difference between areas	0.070	0.192
95% CI	−0.165–0.305	0.016–0.367
z statistic	0.853	2.144
P value	0.560	0.032
E/A ratio		
Difference between areas	0.068	0.270
95% CI	−0.199–0.335	0.030–0.511
z statistic	0.498	2.204
P value	0.619	0.028

ANP; atrial natriuretic peptide, A wave; tricuspid late diastolic flow, E/A ratio; the ratio of E wave to A wave velocities, E wave; tricuspid early diastolic flow, CI; confidence intervals, NT-proBNP; N-terminal pro B-type natriuretic peptide, PA/Ao ratio; ratio of main pulmonary artery to aorta, RVID/BSA ratio; ratio of right ventricular end-diastolic internal diameter to body surface area, RVID/LVID ratio; ratio of right ventricular end-diastolic internal diameter to left ventricular end-diastolic internal diameter.

compared to asymptomatic dogs, and the Doppler-derived pulmonic pressure gradient was significantly correlated with the plasma NT-proBNP concentration [17]. These results led to the speculation that plasma NT-proBNP concentrations can be useful for diagnosing and estimating the severity of right-sided CHF in dogs. However, the clinical implications of plasma NT-proBNP levels in dogs with or without right-sided CHF have never been studied. In the present study, dogs with increased plasma NT-proBNP concentration tended to have increased disease severity, and plasma NT-proBNP levels were significantly elevated in dogs with right-sided CHF. This is the first study to demonstrate that plasma NT-proBNP measurements reflect the severity of right-sided CHF in dogs.

There are a few reports on plasma concentrations of cardiac biomarkers in dogs with right-sided heart failure [2, 9, 16], but the diagnostic utility of plasma ANP and NT-proBNP concentrations in dogs with right-sided CHF remains unclear. In the present study, a plasma ANP level >47.6 *p*g/m*l* and a plasma NT-proBNP concentration >3,003 pmol/*l* to identify dogs with right-sided CHF had AUCs of 0.86 and 0.93, respectively. The best accuracy was afforded by NT-proBNP. Furthermore, the AUC for NT-proBNP, but not ANP, was significantly higher than the AUCs for the cardiothoracic ratio, vertebral heart score, RVID/BSA ratio, A wave and E/A ratio. These results indicate that the plasma NT-proBNP concentration can be used to diagnose right-sided CHF in dogs.

Although direct pulmonary arterial pressure measurement is considered the gold standard for diagnosing PH, dogs require general anesthesia to obtain an accurate pulmonary arterial pressure. In contrast, when TR >2.8 m/sec is present, Doppler echocardiography provides an estimate of PH in dogs [13, 14, 29]. Thus, indirect pulmonary arterial pressure measurement using echocardiography has been proposed to diagnose PH in a clinical setting. Previous studies have shown that plasma ANP and NT-proBNP concentrations were significantly elevated in dogs with severe PH (systolic pulmonary arterial pressure >75 mm Hg), but were unchanged in mild PH (<50 mmHg) [12, 15]. In addition, the TR gradient in dogs with precapillary PH strongly correlates with plasma NT-proBNP concentrations [12, 15]. Thus, the NT-proBNP level is expected to be useful in assessing the severity of PH. In the present study, 37 dogs (72.5%) with suspected precapillary PH were diagnosed; however, the TR velocity was weakly correlated with the plasma NT-proBNP level (r=−0.38 and r^2=0.15), but not the ANP. Reasons for this difference of correlation coefficient may reflect the differences of underlying disease and population; our study enrolled dogs with several etiologies of right-sided heart disease, but the previous study enrolled dogs with only PH [12, 15]. Because our study focused on investigating the relationship between right-sided CHF and cardiac biomarkers, the association between PH and cardiac biomarkers should be interpreted with caution.

Limitations: Subjects with several forms of heart disease or PH were enrolled. Additional large-scale, disease matched prospective studies are necessary to explore the clinical implications of plasma natriuretic peptide levels in dogs with right-sided CHF. Several factors may affect plasma natriuretic peptide concentrations in dogs; these include renal function, systemic blood pressure and gender [4, 23, 30].

Conclusions: Plasma ANP and NT-proBNP levels were significantly elevated in dogs with right-sided CHF. An ANP cutoff value >47.6 *p*g/m*l* and NT-proBNP >3,003 pmol/*l* had high sensitivity and specificity for detection of right-sided CHF. Our results suggest that measuring these peptides, particularly NT-proBNP, is simple and can be used to assess the presence of right-sided CHF in dogs.

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