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NT-proBNP, NT-proANP and cTnI concentrations in dogs with pre-capillary pulmonary hypertension

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KEYWORDS
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Abstract *Objectives:* To compare [NT-proBNP], [NT-proANP] and [cTnI] between control dogs with respiratory disease without pulmonary hypertension (PH) and dogs with pre-capillary PH, and to assess the accuracy of [NT-proBNP], [NT-proANP], [cTnI] to predict Doppler-derived peak tricuspid regurgitation (TR) gradient.
Animals: 20 dogs. 8 control dogs with respiratory disease with no PH and 12 with pre-capillary PH.
Methods: [NT-proBNP], [NT-proANP] and [cTnI] were compared between the 2 groups and simple linear regression analysis was used to predict peak TR gradients from various blood biomarkers.
Results: Median [NT-proBNP] was higher in the dogs with PH (2011 pmol/L, 274—7713 pmol/L) compared to control dogs (744 pmol/L; 531—2710 pmol/L) ($p = 0.0339$). [NT-proBNP] was associated with peak TR gradient ($R^2 = 0.7851$, $p = 0.0001$). Median [NT-proANP] did not differ between dogs with PH (1747 fmol/L; 894—2884 fmol/L) and control dogs (1209 fmol/L; 976—1389 fmol/L ($p = 0.058$). [NT-proANP] was not associated with peak TR gradient ($R^2 = 0.2780$, $p = 0.0781$). Median [cTnI] did not differ between dogs with PH (0.2850 ng/mL; 0.19—1.13 ng/mL) and control dogs (0.2 ng/mL; 0.19—0.82 ng/mL, $p = 0.3051$). Median [TnI] was not associated with peak TR gradient ($R^2 = 0.024$, $p = 0.6307$).
Conclusions: [NT-proBNP] concentration is significantly higher in dogs with pre-capillary PH when compared to dogs with respiratory disease without PH, and [NT-

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proBNP] may be useful to predict the severity of estimated PH. Elevations in [NT-proBNP] due to pre-capillary PH may complicate the interpretation of [NT-proBNP] elevations in patients presenting with cardiorespiratory abnormalities. [NT-proANP] and [cTnI] were not elevated in dogs with pre-capillary PH.

Abbreviations

BNP	Brain natriuretic peptide
CT-ANP	C-terminal atrial natriuretic peptide
cTnI	Cardiac troponin I
NT-proANP	N-terminal proatrial natriuretic peptide
NT-proBNP	N-terminal proBNP
PCV	Packed cell volume
PH	Pulmonary hypertension
proANP	Pro-atrial natriuretic peptide
RA	Right atrial
SPAP	Systolic pulmonary arterial pressure
TR	Tricuspid regurgitation
VHS	Vertebral heart score

Introduction

In veterinary medicine, pulmonary hypertension (PH) has been reported in dogs when echocardiographic estimates of systolic pulmonary arterial pressure (SPAP) exceed ~30 mm Hg.[1–5] Pre-capillary PH is defined as PH which results from abnormalities on the arterial side of the pulmonary vascular system. In dogs, this type of PH occurs secondary to idiopathic primary arterial PH, heartworm disease, left-to-right cardiovascular shunts, vascular occlusive disease, hypoxemia and various pulmonary diseases. In contrast, post-capillary PH is typically associated with left-sided heart disease and is defined as PH that occurs as a result of pulmonary venous hypertension, which leads to pulmonary capillary hypertension and subsequent pulmonary arterial hypertension.

The gold standard of PH diagnosis is a right-heart catheterization directly measuring the SPAP, but in the clinical setting of veterinary medicine, tricuspid regurgitation (TR) interrogation by Doppler echocardiography is more frequently used to estimate right ventricular systolic pressure, which reflects peak SPAP. There are several limitations associated with this measurement in people and in dogs. Firstly, in people, there is conflicting data regarding the correlation of peak systolic TR gradients to invasively measured SPAP.[6,7] Secondly, although the diagnosis of TR is usually straightforward the actual measurement of the peak velocity TR jet may be affected by patient compliance during the exam, the degree of TR present, the position of the TR jet in relation to the Doppler cursor and experience of the echocardiographer. Thirdly, in some instances of PH, TR may not be present, precluding the clinician from definitively diagnosing and quantifying PH.[4]

Brain natriuretic peptide (BNP) exists as a prohormone that is cleaved into the inactive N-terminal fragment (N-terminal proBNP [NT-proBNP]) and biologically active hormone BNP prior to release into the blood circulation primarily in response to ventricular myocyte stretch. NT-proBNP is more stable in vivo and is typically used as a surrogate marker for the biologically active form.[8] Historically, the left ventricle has been thought to be the major source of BNP, with the right ventricle having a smaller contribution. In human and veterinary medicine, BNP and NT-proBNP concentration has been used to discriminate between cardiac and respiratory disease in patients with abnormal respiratory signs.[9–14] Elevated NT-proBNP concentration has been described in dogs with post-capillary PH due to left heart failure secondary to severe mitral regurgitation.[15] In people, NT-proBNP concentration is elevated in the presence of pre-capillary PH, and has been used to stratify disease severity, monitor response to treatment, and serve as a prognostic parameter.[16–18]

Pro-atrial natriuretic peptide (proANP), another blood-based cardiac biomarker, is released primarily from the atrial myocytes in response to stretch. ProANP is then cleaved into N-terminal proANP (NT-proANP) and C-terminal ANP (CT-ANP). ANP, NT-proANP and CT-ANP concentration has been used in veterinary medicine primarily to evaluate left-sided cardiac disease in dogs.[19–29] A single study evaluated ANP concentration in dogs with right-sided heart disease due to dirofilariasis.[30]

Cardiac troponin I (cTnI) plasma concentration elevation indicates the presence of myocardial damage and reflects the severity of myocardial injury. In veterinary medicine, cardiac troponin concentration has been used to evaluate myocardial injury in

cardiac and non-cardiac disease processes.[31–42] Use of cTnI concentration to specifically assess the right heart in PH and pulmonic stenosis has been described.[43,44] To our knowledge, there have been no veterinary studies systematically evaluating cardiac biomarkers in dogs solely with pre-capillary PH.

The aims of this prospective study were to compare cardiac biomarker concentrations (NT-proBNP, NT-proANP, cTnI) and estimated systolic pulmonary artery pressures based on peak systolic TR gradients between control dogs with respiratory disease without PH to those of dogs with pre-capillary PH, and to assess the accuracy of cardiac biomarker (NT-proBNP, NT-proANP, cTnI) concentrations to predict Doppler-derived peak systolic TR gradient.

Animals, materials and methods

Study population

This study was approved by the animal care and use committee of the University of Wisconsin. Owner consent was obtained for all dogs enrolled. All dogs were recruited for the study between November 2008 and March 2009.

Control group

The control group included 8 dogs with respiratory disease examined prospectively. Animals were considered for inclusion if the dogs had current clinical signs of cough or dyspnea, apparent pulmonary or airway disease diagnosed by thoracic radiography (e.g. alveolar infiltrates consistent with pneumonia, nodular infiltrates consistent with metastatic neoplasia or fungal pneumonia, bronchial thickening consistent with bronchitis or collapsing trachea) or other clinical testing (e.g. tracheal wash), and normal findings for the following clinical tests: 2-D, M-mode and Doppler echocardiographic findings, systolic blood pressure, serum creatinine concentration and packed cell volume (PCV) based on University of Wisconsin normal ranges for each test. The presence of PH was echocardiographically excluded if: there was a normal peak systolic TR gradient ($\leq$30 mm Hg) in dogs with TR in addition to subjectively normal right ventricular wall thicknesses, subjectively normal right ventricular and right atrial dimensions, absence of septal flattening and a normal main pulmonary artery diameter (main pulmonary artery diameter less than aortic root diameter). All control patients were negative for heartworm by serum antigen test. Animals were excluded from this group if idiopathic chronic interstitial pulmonary disease was suspected (e.g. pulmonary crackles present on auscultation in the absence of radiographic evidence of pneumonia, neoplasia or atelectasis). The exclusion of heartworm serum antigen positive dogs and idiopathic chronic interstitial pulmonary disease suspect dogs was to increase the likelihood that echocardiographically undiagnosed PH was not present.

Study group

Twelve consecutive clinical patients with echocardiographically diagnosed pre-capillary PH were studied prospectively. Each patient underwent echocardiographic examination based on the suspicion of PH in the presence of abnormal respiratory signs/findings (i.e. cough, dyspnea, pulmonary crackles), syncope, a diagnosis of previous or active heartworm infection or suspect cyanotic congenital heart disease. All enrolled patients had a diagnosis of pre-capillary PH based on Doppler assessment of peak systolic T gradient greater than 30 mmHg in the absence of mitral insufficiency, left atrial enlargement,[45] abnormal left ventricular dimensions/function or right ventricular outflow tract obstruction. Since azotemia, systemic hypertension and anemia are known to affect NT-proBNP concentrations,[46–48] all patients enrolled had normal serum creatinine concentration (0.5–2.0 mg/dL or 44.2–176.8 μmol/L), normal systolic oscillometric or Doppler-derived blood pressure values ($<$160 mm Hg) and a normal PCV (37–55%) based on University of Wisconsin normal ranges for each test. Dogs were categorized as having mild (31–50 mmHg), moderate (51–75 mmHg) or severe PH ($>$75 mmHg) based on the Doppler-derived peak systolic TR gradient.[1,2,4,49] Additional echocardiographic indices suggestive of PH (i.e. right ventricular hypertrophy, septal flattening, increased peak diastolic pulmonic insufficiency, pulmonary artery dilation, abnormal pulmonary artery systolic flow profiles, right ventricular systolic time intervals) were assessed but not reported in this study.

Echocardiography

Full echocardiographic studies, including standard 2-D views[50] and Doppler studies, were performed on all dogs by using a cardiac ultrasound unit[c] with the patient in right and left lateral recumbency. Images were obtained from the dependent side of the patient. Doppler evaluations were performed using a 3 or 5-MHz transducer. The right- or left-sided view that allowed for the optimal alignment of the continuous wave Doppler interrogation beam

[c] Vivid 7, General Electric Medical System, Waukesha,WI, USA.

through the regurgitant flow across the tricuspid valve was used to measure instantaneous peak systolic TR velocities. Pulmonic stenosis was ruled out by evaluating for normal valvular anatomy and mobility on 2-D echocardiography and identification of laminar pulmonic flow profile via pulsed-wave Doppler echocardiography with peak pulmonary artery flow velocities less than 1.5 m/s. The modified Bernoulli equation was applied to the peak systolic TR flow velocity to calculate the instantaneous right ventricular to right atrial pressure gradient. Doppler flow interrogations of TR jets provided estimates of SPAP, allowing diagnosis of PH and estimation of severity. A peak systolic TR flow velocity $\geq$ 2.8 m/s (peak systolic TR flow gradient $\geq$ 31 mm Hg) was considered to be indicative of PH in this setting.[1,2,4] Right atrial (RA) pressures were not measured or estimated based on recent human data suggesting that the addition of estimated RA pressure may lead to overestimation of PH severity.[7]

Thoracic radiography

All thoracic radiographs were reviewed by a single person (HBK) blinded to patient identity. Vertebral heart scale (VHS) was measured and the cardiac structure was evaluated for specific chamber enlargement. The presence and distribution of any airway or pulmonary parenchymal abnormalities were recorded. A clinical diagnosis of probable idiopathic chronic interstitial pulmonary disease was made if the following composite clinical findings were present: chronic exercise intolerance, shortness of breath, exercise-induced cyanosis, cough, auscultable pulmonary crackles, negative heartworm antigen test, normal pulmonary pattern or interstitial infiltrates on thoracic radiographs and absence of left-sided heart disease and left-sided congestive heart failure on the basis of echocardiography and thoracic radiography.[4,51]

Cardiac biomarkers

In order to measure the concentrations of the variables assessed, venous blood samples were collected in EDTA plasma tubes and centrifuged at 3200 revolutions per minute for 10 min, separated and frozen at −80 °C within 1 h of phlebotomy. Batched samples were shipped to the assay laboratory on ice according to the guidelines established by the manufacturer.[d]

Statistical analysis

Data analysis was performed using statistical calculation software.[e] Normal distribution could not be assumed with the small sample size and all variables were analyzed with nonparametric methods. A Mann−Whitney *U* test was used to compare median age, body weight, NT-proBNP, NT-proANP, and cTnI concentration between the control ($n = 8$) and PH ($n = 12$) groups. A Fisher's exact test was used to evaluate for sex proportion differences between the control and PH groups. A Kruskal−Wallis test was used to analyze the variance among severity subgroups of PH and control dogs and if a significant difference was found a Dunn's post-test was used to compare all pairs of medians. Simple linear regression analysis was used to predict peak systolic TR gradients from various blood cardiac biomarkers. The R^2 value was reported for linear regression models and p values <0.05 and R^2 values >0.75 were considered statistically and clinically significant, respectively, for all tests. To evaluate the ability of the biological variables (i.e. age and sex) to predict peak systolic TR, a stepwise regression procedure was used. In each step, one variable was added or removed from the model and a variable was required to have a significance level of 0.05 to stay in the model by a type III F test. The final predictive model using stepwise selection contained only the significant NT-proBNP concentration. Sensitivity and specificity with 95% confidence intervals were calculated for blood cardiac biomarkers. The specific NT-proBNP, NT-proANP and cTnI concentration cutoff values for determining sensitivity and specificity were based on values suggestive of cardiac disease reported by the analyzing laboratory.[d]

Results

Study population

The PH group ($n = 12$), included 2 Yorkshire terriers, 2 Labrador retrievers, 2 mixed-breed dogs, and 1 each of the following breeds: miniature Pinscher, Cocker spaniel, German shorthaired pointer, French bulldog, American water spaniel and miniature Poodle. The control group ($n = 8$) consisted of 1 dog each of the following breeds: great Dane, German shepherd dog, Rottweiler, Dachshund, Boxer dog, Maltese, bearded Collie and Cocker spaniel. The proportion of females was

[d] Cardiopet NT-proBNP, NT-proANP and cTnI tests, IDEXX Laboratories, Inc., Westbrook, ME, USA.

[e] GraphPad Prism 5.0b, GraphPad Software Inc, San Diego, CA, USA.

Table 1 Comparison of selected physical and cardiac biomarker results in 20 dogs.

	Control dogs $n = 8$[a]	PH dogs $n = 12$[a]	p value
Age (years)	6 (1–9)	12 (2–14)	**0.0102**
Weight (kilograms)	28.5 (3.4–55)	14 (2.5–43.3)	0.2167
Females (percent)	1/8 (12.5%)	8/12 (67%)	**0.0281**
[NT-proBNP] (pmol/L)	744 (531–2710)	2011 (274–7713)	**0.0339**
[NT-proANP] (fmol/L)	1209 (976–1389)	1747 (894–2884)	0.0587
[cTnI] (ng/ml)	0.2 (0.19–0.82)	0.285 (0.19–1.13)	0.3051

PH, pulmonary hypertension.
Bold p values indicate significance ($p < 0.05$).
[a] Values are presented as median (range).

significantly higher in the PH group (8/12, 67%) compared to the control group ($n = 1/8$, 12.5%, $p = 0.0281$) (Table 1). The median age of dogs with PH (12 years; 2–14 years) was significantly higher than that of control dogs (6 years; 1–9 years) ($p = 0.0102$) (Table 1). Median body weight was not significantly different between the dogs with PH (14 kg; 2.5–43.3 kg) and control dogs (28.5 kg; 3.4–55 kg, $p = 0.2167$) (Table 1). Sex and age did not show significant association with peak systolic TR gradient. The underlying etiology of PH in the affected group consisted of presumed idiopathic chronic interstitial pulmonary disease ($n = 7$), pulmonary neoplasia ($n = 1$), historical heartworm infection ($n = 1$), presumed chronic obstructive pulmonary disease ($n = 1$) and Eisenmenger's syndrome secondary to a patent ductus arteriosus ($n = 2$). The underlying respiratory disease in the control group consisted of pneumonia ($n = 4$) and 1 each of the following: eosinophilic pneumonopathy, collapsing trachea, carcinoma and pulmonary alveolar infiltrates of unknown cause.

Cardiac biomarkers

NT-proBNP

Median NT-proBNP concentration was significantly higher in the dogs with PH (2011 pmol/L, 274–7713 pmol/L, $n = 12$) compared to control dogs (744 pmol/L; 531–2710 pmol/L, $n = 8$) ($p = 0.0339$) (Table 1, Fig. 1a). When the dogs with

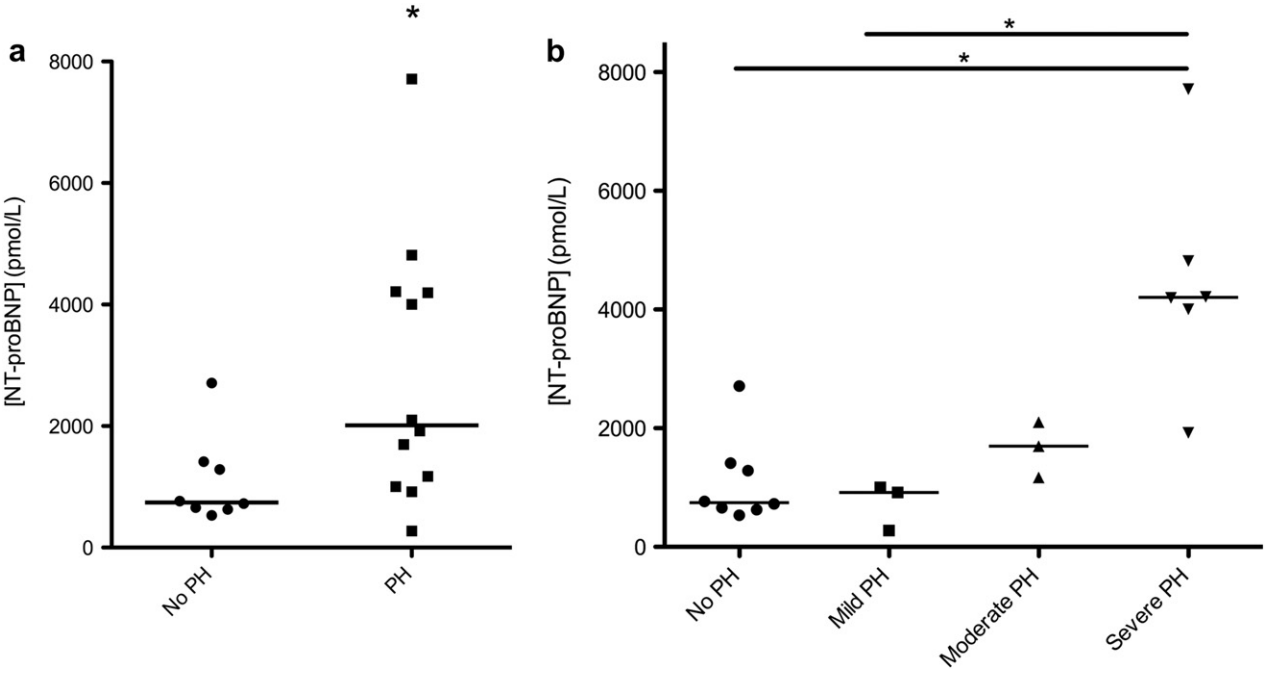

Figure 1 NT-proBNP concentration in control dogs with respiratory disease in the absence of PH and dogs with precapillary PH. Bold line represents the median. Points represent individual patients. * represents significant difference. (a) Comparison between control ($n = 8$) and dogs with PH ($n = 12$). (b) NT-proBNP concentration and severity of PH based on (peak systolic TR gradient). No PH ($n = 8$), mild PH ($n = 3$) (31–50 mm Hg), moderate PH ($n = 3$) (51–75 mm Hg), severe PH ($n = 6$) (>75 mm Hg).

Table 2 Results of simple linear regression analysis in the prediction of peak systolic TR gradient in dogs with pre-capillary pulmonary hypertension.

Independent variable	R^2	95% CI	*p* value
[NT-proBNP]	0.7851	(0.008, 0.018)	**0.0001**
[NT-proANP]	0.278	(−0.004, 0.064)	0.0781
[cTnI]	0.024	(−94.8, 60.3)	0.6307

TR, tricuspid regurgitation; R^2, coefficient of determination; CI, confidence interval; NT-proBNP, N-terminal-pro-brain natriuretic peptide; NT-proANP, N-terminal-pro-atrial natriuretic peptide; cTnI, cardiac troponin I.
Bold *p* values indicate significance ($p < 0.05$).

PH were separated into categories of PH severity, median NT-proBNP concentration was significantly higher in severe PH (4204 pmol/L; 1921—7713, $n = 6$) compared to control dogs (744 pmol/L; 531—2710, $n = 8$) and dogs with mild PH (919 pmol/L; 274—1004, $n = 3$) ($p < 0.05$) (Fig. 1b). All other comparisons did not reveal significant differences in median NT-proBNP concentration. ($p > 0.05$) (Fig. 1b). NT-proBNP concentration was significantly associated with peak systolic TR gradient ($R^2 = 0.7851$, $p = 0.0001$) (Table 2, Fig. 4a). The sensitivity and specificity of NT-proBNP concentration in this population of dogs with and without PH was 91.7% and 62.5%, respectively, for an NT-proBNP concentration cutoff of ≥900 pmol/L.

NT-proANP

There was no significant difference between median NT-proANP concentration in the dogs with PH (1747 fmol/L; 894—2884 fmol/L, $n = 12$) and control dogs (1209 fmol/L; 976—1389 fmol/L, $n = 8$)($p = 0.058$) (Table 1, Fig. 2a). When the dogs with PH were separated into categories of PH severity, NT-proANP concentration did not differ among the control and PH severity groups ($p = 0.0924$) (Fig. 2b). NT-proANP concentration was not significantly associated with peak systolic TR gradient ($R^2 = 0.2780$, $p = 0.0781$) (Table 2, Fig. 4b). The sensitivity and specificity of NT-proANP concentration in this population of dogs with and without PH was 58.3% and 100%, respectively, for an NT-proANP concentration cutoff of >1700 fmol/L.

cTnI

Median cTnI concentration did not differ between dogs with PH (0.2850 ng/mlL; 0.19—1.13 ng/mL, $n = 12$) and control dogs (0.2 ng/mL; 0.19—0.82 ng/mL, $n = 8$) ($p = 0.3051$) (Table 1, Fig. 3a). When the dogs with PH were separated into categories of PH severity, median cTnI concentration did not differ among the control and PH severity groups ($p = 0.3572$) (Fig. 3b). Cardiac TnI concentration was not significantly associated with peak systolic TR gradient ($R^2 = 0.024$, $p = 0.6307$) (Table 2, Fig. 4c). The sensitivity and specificity of cTnI concentration in this population of dogs with and

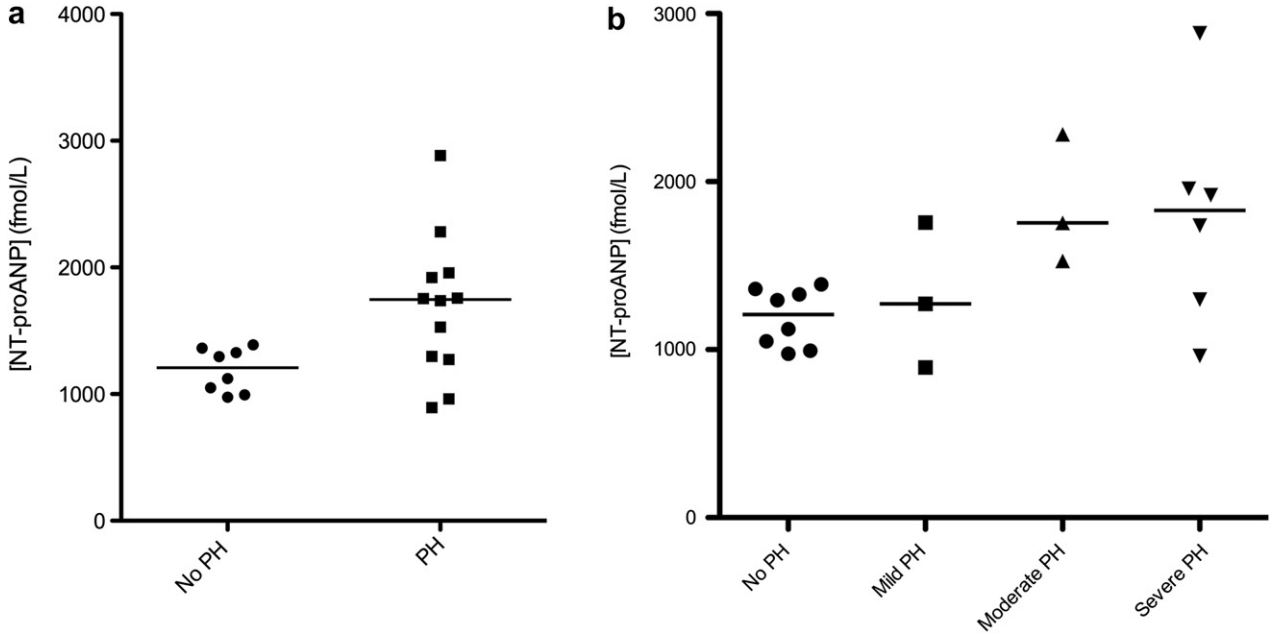

Figure 2 NT-proANP concentration in control dogs with respiratory disease in the absence of PH and dogs with pre-capillary PH. Bold line represents the median. Points represent individual patients. (a) Comparison between control ($n = 8$) and dogs with PH ($n = 12$). (b) NT-proANP concentration and severity of PH based on (peak systolic TR gradient). No PH ($n = 8$), mild PH ($n = 3$) (31—50 mm Hg), moderate PH ($n = 3$) (51—75 mm Hg), severe PH ($n = 6$) (>75 mm Hg).

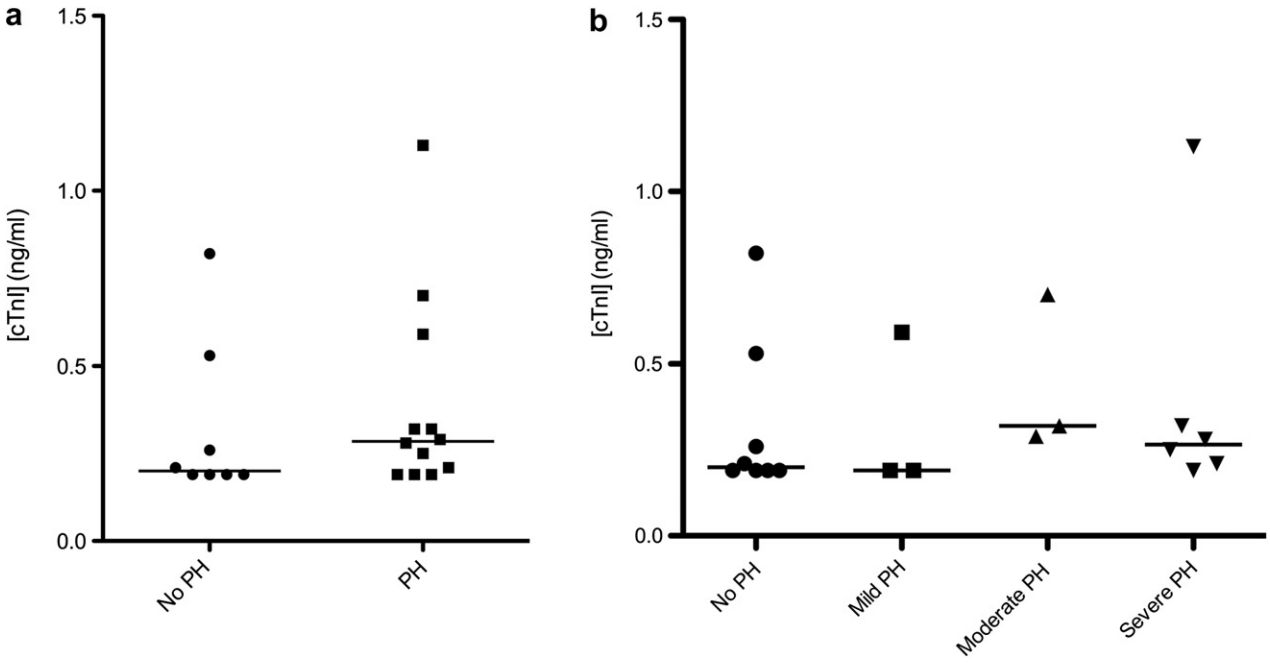

Figure 3 cTnI concentration in control dogs with respiratory disease in the absence of PH and dogs with pre-capillary PH. Bold line represents the median. Points represent individual patients. (a) Comparison between control ($n = 8$) and dogs with PH ($n = 12$). (b) cTnI concentration and severity of PH based on (peak systolic TR gradient). No PH ($n = 8$), mild PH ($n = 3$) (31−50mmHg), moderate PH ($n = 3$) (51−75mmHg), severe PH ($n = 6$) (>75 mm Hg).

without PH was 75% and 50%, respectively, for a cTnI concentration cutoff of >0.2 ng/mL.

Discussion

Fifty-eight percent of the dogs with PH had PH secondary to presumed idiopathic chronic interstitial pulmonary disease, which appears to be more common in smaller terrier breeds, most notably the West Highland white terrier.[4,51,52] A study by Schober and Baade demonstrated that PH occurred frequently in West Highland white terriers secondary to chronic interstitial pulmonary disease.[4] In that study, 45 West Highland white terriers with chronic interstitial pulmonary disease were examined and 16% ($n = 7$) had normal estimated SPAPs based on peak systolic TR gradients, 40% ($n = 18$) had no detectable TR to positively or negatively diagnose PH and 44% ($n = 20$) had evidence of PH based on the elevated peak systolic TR gradient. The predominance of female dogs with PH has only been reported previously by Kellum and Stepien in a study evaluating both pre- and post-capillary PH in dogs.[1]

NT-proBNP concentration was significantly elevated in dogs with pre-capillary PH in the present study. In contrast to the many of studies exploring NT-proBNP concentrations in dogs with left ventricular (LV) dysfunction,[12,15,21,53−55] there is only a single study in veterinary medicine briefly describing an elevation in NT-proBNP concentration in dogs with pre-capillary PH.[12] In people, NT-proBNP concentration is significantly elevated in the presence of pre-capillary PH causing right ventricular stress and the release of BNP from the right ventricular myocardium in response to pressure overload.[16,17,56−63]

In dogs with abnormal respiratory signs, NT-proBNP concentration has been a proposed way to differentiate left-sided heart disease or heart failure from pulmonary disease. This study suggests elevated NT-proBNP concentration is also seen in pre-capillary PH and may obscure the diagnosis of left-sided heart disease in dogs with clinical signs of cough or dyspnea. In the present study, 11/12 of dogs with pre-capillary PH would have been misdiagnosed as having left-sided heart disease or heart failure based on the laboratory reference ranges reported at the time of evaluation.[d] Only one of 12 dogs with PH had a NT-proBNP concentration that fell within the reference range and this dog had the mildest degree of estimated PH based on the peak systolic TR gradient (40 mm Hg). Despite the common recommendation that a clinical decision to treat a dog in heart failure should never be based solely on cardiac biomarker evaluation alone, a misdiagnosis of dogs with pre-capillary PH as having heart disease and congestive heart failure

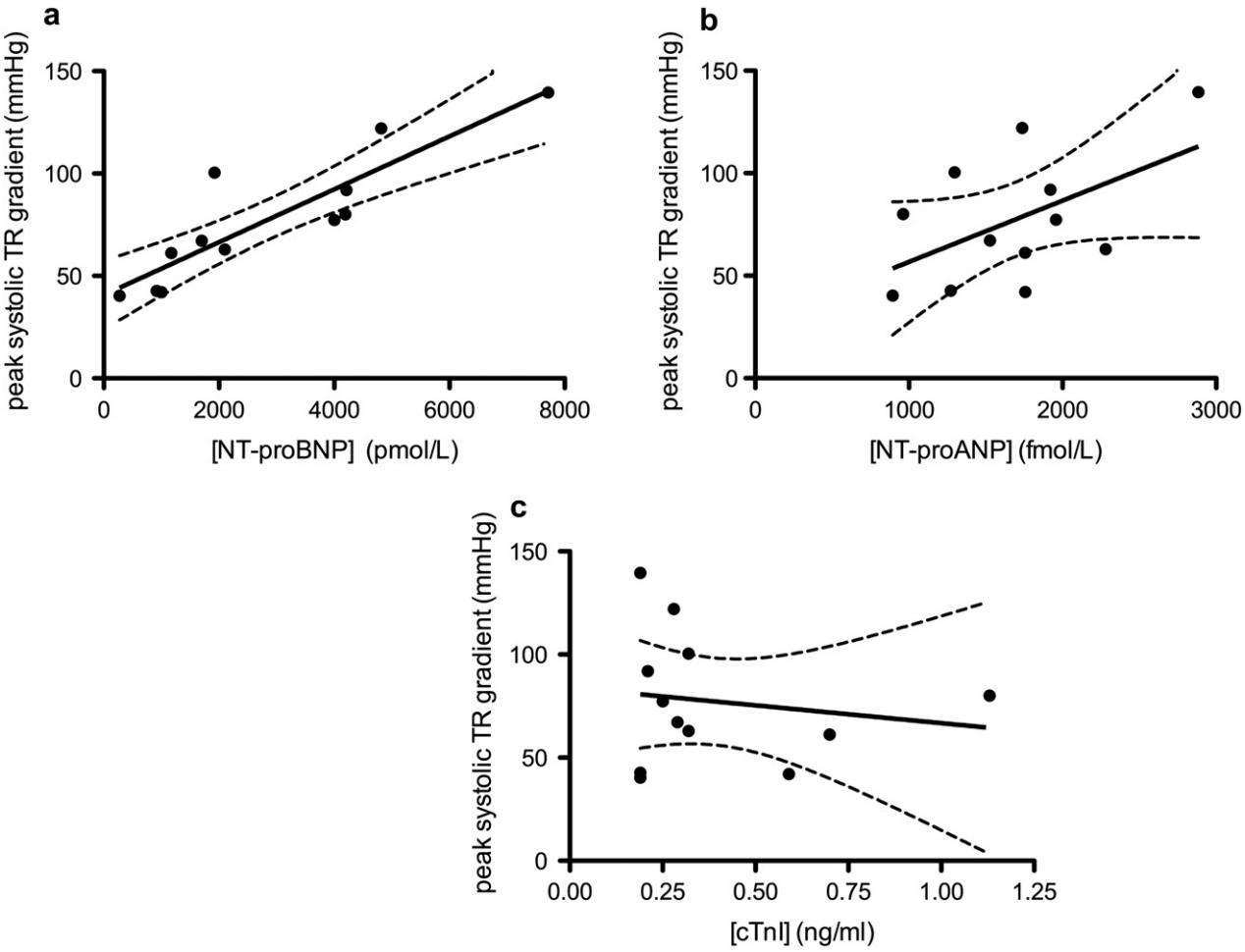

Figure 4 Simple linear regression analysis in the prediction of peak systolic TR gradient in dogs with pre-capillary pulmonary hypertension. (a) NT-proBNP ($R^2 = 0.79$, $p = 0.0001$). (b) NT-proANP ($R^2 = 0.28$, $p = 0.0781$). (c) cTnI ($R^2 = 0.024$, $p = 0.6307$).

may lead to inappropriate therapy with cardiac medications, resulting in delay of appropriate therapy and potentially, worsening of clinical signs and prognosis.

Extra-cardiac factors that have been reported to affect NT-proBNP concentration in people and dogs include variations in renal function, blood pressure and hemoglobin concentration.[46–48,54,64–66] In the evaluation of NT-proBNP concentration in dogs with heart disease, exclusion criteria often include azotemia and systemic hypertension since these concurrent abnormalities may falsely elevate NT-proBNP concentration and result in inaccurate interpretations. The results of the present study suggest PH may be added to this list of extra-cardiac factors affecting NT-proBNP concentration.

In the present study, there was a reasonable association between NT-proBNP and peak systolic TR gradients in dogs with PH, suggesting NT-proBNP may be a good predictor of estimated SPAP and offer a surrogate estimation of PH severity in dogs with pre-capillary PH. This population of dogs often have clinically significant respiratory distress limiting the quality of echocardiographic images. This, in addition to the standard difficulties associated with TR jet interrogation, may make accurate Doppler assessment of PH severity challenging. In people, NT-proBNP concentration has been correlated to PH severity and NT-proBNP concentration serves a diagnostic aide, as a tool in monitoring treatment response and as a prognostic predictor of mortality.[16–18]

NT-proANP concentrations were not significantly elevated in dogs with pre-capillary PH when compared to dogs with respiratory disease without PH. A possible reason for the historical unpredictability of NT-proANP concentration in dogs may be the mechanism of ANP release. ANP is stored in secretory granules in atrial myocytes and released acutely in response to atrial stretch.[67] BNP is

continuously released from ventricular myocytes due to continued synthesis by mRNA in response to ventricular stretch.[68] It has been suggested that an elevated ANP concentration may occur in the more acute phase of disease since ANP granular stores may become depleted with continued stimulation whereas BNP release may represent a more chronic phenomenon.[53,68–72] Depending on the degree and acuity of the cardiac dysfunction at the time of ANP concentration analysis, the ANP concentration may or may not be representative of the current underlying pathology. Another possible hypothesis for the lack of significant elevation in NT-proANP concentration in the dogs with PH in this study may be due to the population of dogs in the control group. Our control group consisted of patients with current pulmonary disease rather than a normal or disease-free cohort. Studies have shown that hypoxia induces the release of ANP as a compensatory mechanism to result in endogenous pulmonary artery vasodilation (i.e. a mechanism to prevent the development of PH).[73–75] In the present study, NT-proANP concentration in the respiratory control group may have been higher than normal dogs due to hypoxia associated with pulmonary disease, ultimately yielding in a lack of significance between the control and PH groups.

No association was found between NT-proANP concentration and peak systolic TR gradients in dogs with pre-capillary PH. Although, based on sensitivity and specificity of NT-proANP concentration in this group of dogs, one could suspect that a normal NT-proANP measurement suggests no PH is present. In people, ANP concentration correlates with the severity of PH,[76] yet in the present study no correlation was found. In people, Wiedemann and colleagues found that ANP was significantly correlated with parameters of RV function, but not with calculated PAPs.[76] Based on the limited and conflicting veterinary and human literature, ANP and NT-proANP concentration results appear to have variable and unpredictable outcomes when assessing pulmonary hypertension.

Cardiac TnI, a biomarker of acute myocyte injury, was not significantly elevated in dogs with pre-capillary PH compared to dogs with respiratory disease in the absence of PH. In a single veterinary study by Guglielmini and colleagues, that evaluated cTnI in dogs with pre- and post-capillary PH, there was a significantly higher cTnI concentration in dogs with pre-capillary PH when compared to normal dogs.[43] There was also a modest positive correlation between dogs with pre-capillary PH and estimated SPAP based on peak systolic TR gradients ($r = 0.56$, $p < 0.05$). In people, the use of cTnI concentration in patients with PH is primarily reserved for evaluation of PH in the setting of acute pulmonary thromboembolism, which results in elevated cTnI concentration that correlates with the severity of calculated PAP.[77] The disparity between the present study and Guglielmini's findings may be a result of patients presented to the hospital in different stages of the disease. Twenty-four percent of the dogs with pre-capillary PH in Guglielmini's study had a diagnosis of pulmonary thromboembolism vs. none of the dogs in the present study having a diagnosis of PH secondary acute disease processes.[43]

Our study has several limitations. The small study population limits statistical analysis and may affect applicability of findings to a larger population. The diagnosis of PH in this study was based on estimated SPAPs derived from calculated peak systolic TR gradients and not from the gold standard of direct PAP measurements. Doppler interrogation of TR can be difficult and even when velocities are carefully measured, they may not accurately represent the true SPAP. The respiratory control group was defined as having the absence of PH, but PH can be present in the absence of TR and could not be completely ruled out in these patients. In our linear regression model only dogs with PH were represented, suggesting NT-proBNP concentrations may be good at predicting PH in dogs with the disease present, but due to the absence of the control dogs in the analysis the outcome may have been less significant. Unfortunately, the control dogs were not added into the linear regression analysis due to the lack of consistent TR jets in the majority of patients. Even though statistical significance was shown with NT-proBNP concentration in dogs with PH, there is evidence of a substantial degree of individual variability in NT-proBNP concentration in normal dogs.[78] The individual variability of NT-proANP and cTnI concentrations in normal and abnormal dogs is unknown at this time. The dogs in the two groups were not matched for age, sex and breed. Lastly, due to diagnostic limitations, the true underlying pulmonary diagnosis of many dogs remained presumed rather than proven (i.e. idiopathic chronic interstitial pulmonary disease requires histopathologic confirmation to secure a diagnosis).

Conclusions

In conclusion, NT-proBNP concentration is significantly higher in dogs with pre-capillary PH when compared to dogs with respiratory disease without PH. NT-proBNP concentration is reasonably predictive of the severity of SPAP elevation. Elevations in NT-proBNP concentration due to pre-

capillary PH may complicate the interpretation of NT-proBNP concentration elevations in canine patients presenting with cardiorespiratory abnormalities, but may serve as an indicator of the presence of PH in patients with respiratory signs not due to heart disease. NT-proANP and cTnI concentration was not elevated in dogs with precapillary PH and do not appear to be good predictors of estimated SPAP.

Conflict of interest

None.

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