

Biomarkers for differentiation of causes of respiratory distress in dogs and cats: Part 1 – Cardiac diseases and pulmonary hypertension

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Abstract

Objective – To review the current veterinary and relevant human literature regarding biomarkers of cardiac disease leading to respiratory compromise.

Data Sources – Veterinary and human medical literature: original research articles, scientific reviews, consensus statements, and recent textbooks.

Human Data Synthesis – Cardiac troponins (cTn) and natriuretic peptides are routinely used in human medicine.

Veterinary Data Synthesis – Although biomarkers should not be accepted in lieu of gold standard diagnostics, they may be useful in directing care in the stabilization process. Biomarkers of congestive heart failure (CHF) include natriuretic peptides, cTn, and endothelin. cTnI is useful in differentiating causes of pericardial effusion, but is unlikely to be useful in differentiating CHF from other causes of respiratory distress. The most extensively studied and promising cardiac biomarker is amino-terminal probrain natriuretic peptide, although a bedside test is not currently available. Other natriuretic peptides have also proven useful, but have lower availability. Endothelin is unlikely to be clinically useful. Although critically evaluated for their use in cardiac diseases, many of the biomarkers are affected by more than one type of respiratory or systemic disease. Several cardiac biomarkers are increased in cases of pulmonary hypertension (PH), but discerning CHF alone from PH or a combination of heart disease and PH is challenging when evaluating biomarkers alone.

Conclusion – At this time, there are no point-of-care tests for biomarkers that can reliably differentiate among causes of dyspnea of cardiac origin in dogs and cats, although there are reference laboratory tests that show promise and future development of point-of-care tests that may be useful in certain situations.

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Abbreviations

ANP	atrial natriuretic peptide
ARDS	acute respiratory distress syndrome
AUC	area under the curve
BNP	brain natriuretic peptide
C-ANP	C-terminal atrial natriuretic peptide
C-BNP	C-terminal brain natriuretic peptide

CHF	congestive heart failure
CNP	C-type natriuretic peptide
cTn	cardiac troponin
cTnI	cardiac troponin I
DCM	dilated cardiomyopathy
DNP	dendroaspis natriuretic peptide
ET-1	endothelin 1
HCM	hypertrophic cardiomyopathy
MVD	mitral valvular disease
NT-proANP	amino-terminal pro-atrial natriuretic peptide
NT-proBNP	amino-terminal probrain natriuretic peptide
PH	pulmonary hypertension
ROC	receiver operating curve
Tn	troponin
VNP	ventricular natriuretic peptide

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Introduction

Respiratory distress is a common presenting complaint to the emergency practitioner.¹⁻⁴ If left untreated, respiratory distress may lead to exhaustion, respiratory failure, and death.¹ Therefore, early recognition of respiratory compromise, diagnosis of the underlying cause, and treatment are imperative.¹ Dyspneic patients are fragile and any intervention, such as handling and routine diagnostics, will increase cardiovascular and respiratory demands and may result in rapid decompensation.² The purpose of this review is to present and appraise the current literature exploring the utility of biomarkers in disease processes that result in respiratory distress.

In 2001, the Biomarkers Definition Working Group defined a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.”⁵ Traditionally, in veterinary and human medicine, a biomarker is a protein or molecule that is easily obtained, quantitatively assessed, and helps to guide diagnostic decisions and patient therapy. For the purposes of this review, the definition of a biomarker will be limited to a protein or molecule that is objectively measured from an available sampling medium such as exhaled breath or blood components.

Several characteristics of a biomarker must be considered when evaluating it for clinical utility in practice. Ideally, a biomarker should be measurable in an in-house laboratory utilizing accessible equipment and minimal technical procedures.⁴ The results should have a rapid turnaround time.⁴ Additionally, a clinically useful biomarker should have a high-positive and/or negative predictive value.⁴ This enables the results to succinctly rule a disease process in or out.⁴ The test results should also be relatively easy to interpret, allowing for minimal clinical error when evaluating findings.⁴ The biomarkers discussed in this review will be evaluated in light of these characteristics

Respiratory distress caused by upper respiratory diseases, pleural space diseases, and other extrapulmonary conditions have not been thoroughly investigated for associated changes in biomarkers or are beyond the scope of this discussion. There is evidence that many biomarkers can be affected by multiple disease states. The grouping throughout this review, therefore, is reflective of the majority of the research for each biomarker, and these generalizations should not be interpreted as indication that the biomarker is exclusive to that particular disease category. This review has been divided into 2 sections: this first section will focus on biomarkers of cardiac diseases and pulmonary hypertension (PH) and part 2 will focus on biomarkers of lower airway, inflammatory and pulmonary thromboembolic diseases.⁶

Cardiac diseases

The most common cause of respiratory distress from cardiac disease is congestive heart failure (CHF). CHF describes a clinical syndrome that is characterized by cardiac, hemodynamic, renal, neurohormonal, and cytokine abnormalities.⁷ In dogs, there are several causes of heart disease, but 2 of the most common are dilated cardiomyopathy (DCM) and mitral valvular disease (MVD).^{7,8} Although other types of cardiomyopathies occur, hypertrophic cardiomyopathy (HCM) is the most common cause of heart disease in cats.⁹ In dogs, thoracic radiographs often give valuable information about the underlying disease process leading to CHF, but in cats, radiographs alone may be insufficient to diagnose underlying heart disease.¹⁰ Furthermore, the presence of a heart murmur does not necessarily indicate the presence of heart disease.¹¹ Conversely, the absence of a murmur does preclude the presence of cardiac disease.⁹ Although echocardiography is the gold standard for the antemortem diagnosis of heart disease, this diagnostic test may not be readily available to emergency practitioners and the distressed patient may not tolerate the procedure. Ultimately, the use of a biomarker to identify heart disease may aid the clinician in the immediate, as well as long-term management of patients.

Cardiac troponin

Troponins (Tns) are proteins that are part of the contractile apparatus in cardiac and skeletal muscle.¹⁰ There are 3 individual proteins, troponin C (TnC), troponin I (TnI), and troponin T (TnT), that interact to assist in the regulation of muscle contraction.¹² Cardiac TnC is not clinically useful as it has high homology with noncardiac TnC and cannot be readily distinguished.¹³ The majority of troponins (Tn) are bound to the contractile apparatus of the cell, but a very small proportion remain free within the cytosol (2–8%).^{14,15} With the onset of myocardial injury and resulting damage to cardiomyocyte cell membrane, the release of the cytosolic component causes an early but small rise in Tn concentrations.^{14,15} This initial mild increase is followed by a greater and more sustained rise from the bound component.^{14,15}

Cardiac Tn (cTn) has been used for many years as biomarkers of cardiac cellular injury and are currently part of the criteria to diagnose an acute myocardial infarction in people.¹⁶ Cardiac Tn are sensitive markers of myocardial injury, even an ischemic episode as short as 3 minutes lead to increases in cTnI.¹⁷ They do not, however, identify the cause of the injury and ultimately if the increase arises from primary cardiac disease or cardiac damage secondary to systemic disease.¹⁷⁻²⁴

It has been documented that people with CHF have increases of cTn, and concentrations positively correlate with the risk of mortality.^{25,26} It is believed that

cTn is released secondary to severe left ventricular wall strain encountered in CHF.²⁷ Even in the absence of ischemia, cTn has been documented to increase in people with aortic valve disease,²⁸ precapillary PH,²⁹ tachyarrhythmias,^{30,31} pericarditis,³² and those with left ventricular hypertrophy.³³ Pulmonary diseases that have resulted in increases in cTn include acute respiratory distress syndrome (ARDS),³⁴ chronic obstructive pulmonary disease,³⁵ and pneumonia.³⁶ Other disease processes that can result in increases in cTn in people include ultra-endurance exercise,^{18,19} scorpion envenomation,²⁰ trauma,²¹ and kidney disease.²² In critically ill human patients with sepsis, cTnI increases in 31%–85% of patients and this appears to be correlated with increased morbidity.^{37–42}

cTn has been evaluated in several disease processes in large animals including horses and cows. Similar to people, these processes include both cardiac-specific disease processes and those which are more pulmonary or systemic in nature.^{43–57}

In small animal veterinary medicine, the use of cTn has been evaluated in a number of pathologic conditions as an aid for diagnosis and has shown applicability in numerous conditions (see Table 1).^{58–89} Although each analyzer has its own reported reference interval, typically these values are very low (<0.2 ng/mL).^{58–89} In dogs, concentrations of cTn have been found to rise within hours after the cardiac insult and reach a peak value 10–16 hours later.⁵⁸ Concentrations keep on increasing for 7–10 (cTnI) and 10–14 days (cTnT).^{14,15} The reported half-life of cTnI in dogs is approximately 2 hours.⁵⁹ Although both cTnI and cTnT have been evaluated in veterinary medicine, cTnT has shown to be less sensitive as a marker than cTnI and is therefore much less studied.^{58–89}

There have been 2 studies in dogs evaluating the use of cTnI as a diagnostic tool to differentiate respiratory distress caused by cardiac or noncardiac etiologies (see Table 1).^{60,61} The first study did not find a statistical difference in cTnI concentrations in dogs with cardiac compared to noncardiac etiologies of respiratory distress.⁶⁰ In this study the investigators evaluated dogs with CHF secondary to DCM, MVD, patent ductus arteriosus, and subaortic stenosis and compared them to dogs with interstitial pneumonia, pulmonary neoplasia, neoplastic pleural effusion, laryngeal paralysis, and chronic bronchitis.⁶⁰ A second study by Payne et al⁶¹ did identify a statistical difference in cTnI concentration in dogs with cardiogenic respiratory distress compared to noncardiogenic causes of respiratory distress.⁶¹ In this study, the investigators expanded the cardiac disease group to include pericardial effusion, PH, and ventricular tachycardia.⁶¹ The discrepancy in the results between the studies likely relates to differences in the populations

evaluated; noncardiac causes were fairly similar between the two studies, but the underlying heart disease in the cardiac groups were different. Both investigators determined, however, that patients with respiratory distress of any etiology had a higher cTnI concentration than healthy controls.^{60,61}

Occult MVD,^{62–65} DCM,^{65–67} arrhythmogenic right ventricular cardiomyopathy,⁶⁸ bradyarrhythmias,⁶⁹ doxorubicin-induced DCM,⁷⁰ and subaortic stenosis⁶⁵ all cause increases in cTnI concentration in dogs. Via the use of multiple regression analysis, cTnI concentrations positively correlated with age, C-reactive protein concentration, and heart rate in dogs with MVD.⁶³ Furthermore, increase in cTnI concentration was negatively correlated with survival in dogs with MVD with a shorter median survival time (67.5 days, range 1–390 days) compared to those with a normal cTnI concentration (390 days, range 20–912 days).^{62,63}

cTnI has been evaluated in dogs with pericardial effusion due to various causes. Studies demonstrate that peripheral cTnI concentrations are significantly higher in dogs with pericardial effusion compared to healthy controls.^{71–73} Moreover, those that were diagnosed with cardiac hemangiosarcoma had significantly higher concentrations of cTnI than those with other causes of pericardial effusion.^{71,72} Measurement of the cTnI concentrations in the pericardial effusion fluid did not improve the sensitivity of the test in distinguishing hemangiosarcoma from other neoplastic or nonneoplastic causes.⁷³ Splenic and dermal hemangiosarcoma were not associated with increased cTnI concentrations.⁷²

Noncardiac causes of increased cTnI concentrations have been studied in dogs. Normal Greyhounds and Boxers have higher baseline cTnI concentrations than other breeds.^{68,74} Blunt trauma,^{75,76} gastric dilatation volvulus (GDV),^{76,77} and high intensity exercise⁷⁸ have all been shown to cause increases in cTnI (see Table 1). Dogs with kidney failure were found to have a slightly, but statistically significant, higher cTnI concentrations than controls.⁷⁹ Inflammatory processes may lead to increases in cTn concentrations and specific populations in which this has been shown include pyometra,⁸⁰ ehrlichiosis,⁸¹ and babesiosis.⁸² A recent study found that dogs with critical illness requiring admission into ICU and evidence of systemic inflammation (characterized by an increased C reactive protein concentration) had a significantly higher cTnI and cTnT concentration. Furthermore, an increased cTnI and cTnT were significantly correlated with higher risk of mortality.⁸³

The use of cTnI to distinguish cardiac from noncardiac causes of dyspnea in cats that present for respiratory distress has been evaluated.^{84,85} Separate studies found that cats with CHF had a higher median cTnI concentration

Table 1: Reportable cardiac troponin I by analyzer and disease evaluated

Analyzer	Disease condition	Results	How reported
Access AccuTnI ^h	Respiratory distress in dogs CHF versus pulmonary disease ⁵⁹	CHF: 0.42 (0.02–10.90) Pulmonary disease: 0.29 (0.02–8.13)	Mean, range (ng/mL)
	DCM in dogs ⁶⁵	Normal: 0.06, 0.01* Asymptomatic DCM: 0.21, 0.1*	Mean, SEM (ng/mL)
	Heart disease in dogs ⁶⁴	Normal: 0.03 (0.01–0.15)*,§,† DCM: 0.14 (0.03–1.88)* MVD: 0.11 (0.01–9.53) § SAS 0.08 (0.01–0.94)†	Median, range (ng/mL)
	Mitral valve disease in dogs ⁶²	Normal: 0.001 (0.001–0.004)*,§ Mild MVD: 0.003 (0.001–0.024)†,¶ Moderate MVD: 0.014 (0.008–0.029)*,‡,¶ Severe MVD: 0.043 (0.031–0.087)§,†,‡	Median, IQR (ng/mL)
OPUS Troponin I ⁱ	Blunt trauma in dogs ⁷⁴	Normal: 0 (0–1.37)* Trauma: 0.97 (0–31.84)*	Mean, range (ng/mL)
	GDV in dogs ⁷⁶	GDV: median not reported (0.5–381) Mild ECG changes: 0.53 (<0.5–104)*,§ Moderate ECG changes: 3.29 (<0.5–63.4)*,† Severe ECG changes: 35 (1.2–81)§,†	Median, range (ng/mL)
Stratus CS Stat [†]	Pericardial effusion in dogs ⁷²	Normal: 0.02 (0–0.03)*,§,† Pericardial effusion: 0.64 (0.03–47.18)* PE, hemangiosarcoma: 2.77 (0.09–47.18) §,‡ PE, nonhemangiosarcoma: 0.05 (0.03–0.09) †,‡	Median, range (ng/mL)
	Pericardial effusion in dogs ⁷⁰	Normal: 0.03 (0.03–0.08)* Pericardial effusion: 0.19 (0.04–69.89)*	Median, range (ng/mL)
	Pericardial effusion in dogs ⁷¹	PE, hemangiosarcoma: 10.7 (0–101)*,§ PE, nonhemangiosarcoma: 0.1 (0–0.2)* Noncardiac hemangiosarcoma: 0.6 (0–2.4)§	Mean, range (ng/mL)
	Bradycardia in dogs ⁶⁸	Prepacing: 1.99, 2.86* Postpacing: 0.24, 0.29*	Mean, SD (mg/dL)
	HCM in cats ⁶⁵	Healthy: <0.03 (<0.03–0.16)* HCM: 0.66 (0.05–10.93)*	Median, range (ng/mL)
	Respiratory distress in cats CHF versus pulmonary disease ⁶⁴	CHF: 1.59 (0.2–30.24)* Pulmonary disease: 0.165 (0.01–1.42)*	Median, range (ng/mL)
Abbott AxSYM ^k	GDV and blunt trauma in dogs ⁷⁵	Healthy: 0 (0.1–1.1)*,§ GDV: median not reported (0.3–369)* Trauma: median not reported (0–82.4)§	Median, range (µg/L)
Immolute 1000 ^l	Renal failure in dogs ⁷⁸	Healthy: 0.2 (0.2–0.4)* Renal failure: 0.35 (0.2–25.95)*	Median, range (ng/mL)
	DCM in Doberman Pinschers ⁶⁶	Healthy: 0.07 (0.05–0.08)*,§,†,‡ Occult with VPC: 0.36 (0.29–0.43)*,¶ Occult, echo changes: 0.33 (0.22–0.44) §,¶ Occult, VPC, and echo changes: 0.45 (0.39–0.51) †,‡ CHF: 1.04 (0.82–1.27) †,¶,*,‡	Mean, 95% CI (ng/mL)
	Pulmonary hypertension in dogs ²⁶⁵	Healthy: 0.1 (0.1–0.17)§,†,‡,¶ MVD: 0.12 (0.10–1.00)*,*,§§ PH-MVD: 0.21 (0.1–2.1) §,**,†† Precapillary PH: 0.25 (0.1–1.9) *,†,‡,¶,‡‡ CHF, PH-MVD: 0.38 (0.1–2.1) †,*,**,‡ CHF, MVD: 0.45 (0.1–1.00) ¶,§§,††,‡‡	Median, range (ng/mL)
	Pulmonary hypertension in dogs ²⁶⁴	Pulmonary disease: 0.2 (0.19–0.82) Precapillary PH: 0.285 (0.19–1.13)	Median, range (ng/mL)

(Continued)

Table 1: Continued

Analyzer	Disease condition	Results	How reported
	HCM in cats ⁸⁶	Healthy: <0.2 (<0.2–0.25)*,§ HCM: 0.91 (0.24–4.1)* Hypertrophic obstructive CM: 1.3 (<0.2–1.7) §	Median, range (ng/mL)
	Respiratory distress in cats CHF versus pulmonary disease ⁸³	CHF: 0.94 (0.54–4.0) Pulmonary disease: <0.2 (<0.2–0.33)	Median, IQR (ng/mL)
Unicel DXI ^m	Arrhythmogenic right ventricular cardiomyopathy in Boxer dogs ⁶⁷	Healthy non-Boxer: 0.023, 0.01*,§ Healthy Boxer: 0.079, 0.03*,† ARVC: 0.142, 0.05 §,†	Mean, SD (ng/mL)
	Healthy Greyhound dogs ⁷³	Non-Greyhound: 0.02 (0.01–0.05)* Greyhound: 0.1 (0.03–0.57)*	Median, range (ng/mL)
Reagent Flex cTnI ⁿ	<i>Ehrlichia canis</i> in dogs ⁸⁰	Healthy: 0.04 (0.04–0.1)* <i>E. canis</i> : 0.4 (0.04–9.12)*	Median, range (ng/dL)
i-STAT cTnI ^o	Respiratory distress in dogs	Healthy: 0.03 (0.00–0.11)*	Median, range (ng/mL)
	CHF versus pulmonary disease ⁶⁰	CHF: 1.74 (0.05–17.11)*,§ Pulmonary disease: 0.14 (0.01–4.31) §	
	High intensity exercise in sled dogs ⁷⁷	Resting: 0.02 (0.0–0.12)* 48 hours: 0.07 (0.02–0.21)*	Median, range (ng/mL)

*, §, †, ‡, ¶, **, §§, ††, ††† Paired values are statistically different from one another.

CHF, congestive heart failure; DCM, dilated cardiomyopathy; MVD, mitral valve disease; SAS, subaortic stenosis; IQR, interquartile range; GDV, gastric dilatation and volvulus; PE, pericardial effusion; HCM, hypertrophic cardiomyopathy; CI, confidence interval; VPC, ventricular premature contractions; Echo, echocardiographic changes; PH, pulmonary hypertension; PH-MVD, postcapillary pulmonary hypertension secondary to mitral valve disease; ARVC, arrhythmogenic right ventricular cardiomyopathy.

than cats with pulmonary disease with an area under the curve (AUC) of the receiver operator curve (ROC) of approximately 0.84.^{84,85} However, both of those studies found considerable overlap between the groups and were unable to identify a cut-off point at which a clinician could relatively reliably diagnose cardiac-related dyspnea.^{84,85}

There is a discrepancy in the literature as to whether cTnI can be used to differentiate cats with nonclinical HCM from cats with CHF secondary to HCM. Herndon et al⁸⁶ found that cats with HCM and associated CHF had significantly higher cTnI concentrations compared to cats with asymptomatic HCM.⁸⁶ This study also found that cats with HCM and untreated CHF had higher cTnI concentrations than those cats with HCM and medically well-controlled CHF.⁸⁶ However, a study by Connolly et al⁸⁷ did not find a significant difference between HCM cats with CHF and asymptomatic HCM cats.⁸⁷ These groups, however, were utilizing different assays. Herndon et al⁸⁶ used a more sensitive assay with a lower level of detection of 0.03 ng/mL while Connolly et al⁸⁷ used an assay with a lower level of detection of 0.2 ng/mL.⁸⁷ These differences in assays and their lower levels of detection likely created a discrepancy in results when the 2 studies are compared. Cats with untreated hyperthyroidism had increased cTnI

concentrations that decreased after treatment with radioactive iodine.⁸⁸

There are multiple veterinary cTnI analyzers available (see Table 1), some of which are cost effective point-of-care assays.⁸⁹ One of the major drawbacks of the available cTnI assays is the lack of standardization; each company uses a different target on the cTnI protein for their assay.⁸⁹ Values obtained on one analyzer cannot be directly compared to values obtained on a different analyzer, making it impossible to accurately compare cTnI concentrations.^{89,90} Until assays are standardized, each individual assay must have a specific reference interval determined.^{89,90} A cTnI value that is used as a cut-off point in a certain study will only have meaning if that same assay is used in clinical practice.⁸⁹

cTnI has shown promise as a prognostic indicator in several disease processes and in its ability to identify neoplastic pericardial effusions in dogs. Unfortunately, while increased concentration of cTnI may indicate myocardial damage, the damage can be due to a variety of underlying causes. Due to the discrepancies in the literature and considerable overlap in values, it seems unlikely that cTnI will be useful in determining the origin of dyspnea on presentation. Furthermore, the inability to compare values from different analyzers limits the utility of using cTnI in clinical situations.

Natriuretic peptides

Natriuretic peptides are a group of structurally related proteins, which include atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), dendroaspis natriuretic peptide (DNP), and ventricular natriuretic peptide (VNP).^{91–95} All natriuretic peptides are synthesized as prohormones and are rapidly processed to form prohormones.^{96–98} Prohormones are further cleaved into 2 parts creating the biologically inert amino-terminal prohormone (ie, amino-terminal probrain natriuretic peptide [NT-proBNP]) and the biologically active C-terminal hormone (ie, [C-terminal brain natriuretic peptide], C-BNP).⁹⁸ Once in circulation, the active hormone is degraded into fragments.^{99,100}

ANP and BNP function in plasma volume control.^{101,102} Volume overload increases and hypovolemia decreases the concentration of these proteins in the blood.^{101–103} Additionally, catecholamines and other hormones can modify natriuretic peptide release and in the face of hypovolemia can cause a paradoxical rise in their concentrations.^{104,105} In the kidney, these hormones induce diuresis and natriuresis to modulate blood volume and blood pressure, and to antagonize the renin-angiotensin-aldosterone system.^{101,102} CNP is produced by the vascular endothelium.¹⁰⁶ CNP has diminished diuretic and natriuretic effects compared to ANP and BNP.^{107,108} In general, CNP has autocrine and paracrine effects and is thought to have a role in vascular tone.¹⁰⁹ While a DNP-like peptide, originally discovered in the venom of *Dendroaspis angusticeps*,⁹⁴ has been identified in the human and canine heart and has natriuretic effects, the exact role of DNP in mammals has yet to be determined.^{110–112} VNP is important in primitive fishes, but is not present in mammals.⁹⁵ As potential physiologic markers of diseases involving the cardiovascular system, many of these peptides have been evaluated in human medicine, but research has been limited to ANP, BNP, and CNP and the respective amino-terminal prohormones in veterinary medicine.

Natriuretic peptides are partially cleared by the kidneys;^{113,114} cats with severe CKD have higher concentrations of amino-terminal pro-atrial natriuretic peptide (NT-proANP) and NT-proBNP,¹¹⁵ and dogs with CKD and acute kidney injury have increases in NT-proBNP concentrations.^{116–118} The median NT-proBNP of severely affected animals was up to 5× higher than the median of control animals in dogs and up to 35× higher in cats with hypertensive azotemia.^{115–118} Thus, natriuretic peptides should be interpreted with caution in patients with advanced kidney disease.

C-terminal atrial natriuretic peptide

ANP was first isolated in the atrium,⁹¹ but has also been found in other tissues.^{113,119} PreproANP is released primarily by the cardiac atria in response to atrial stretch and is cleaved into the amino-terminal proANP and the active form C-terminal atrial natriuretic peptide (C-ANP).^{120,121}

In people, C-ANP is increased in CHF¹²² and tachyarrhythmias.¹²³ Although mostly considered a cardiac biomarker, C-ANP has also been evaluated in other conditions in people and has been shown to be increased in female patients,¹²⁴ pregnancy,¹²⁵ advanced liver disease,^{122,126,127} kidney failure,¹²⁸ PH,¹²⁹ systemic hypertension,^{122,130} pneumonia,¹³¹ lung cancer,¹³² sepsis,¹³³ and pulmonary embolism.¹³⁴

Concentrations of C-ANP have been shown to be increased in cows and horses with cardiac stresses from various causes.^{135–140} Concentrations of ANP decreased significantly after fluid resuscitation in hypovolemic adult horses, which was thought to be due to secondary catecholamine induced ANP production.¹³⁷

There is sufficient homology between canine and human C-ANP to use a human assay for measuring canine C-ANP.¹⁴¹ ANP has been shown to be increased in experimentally induced compensated aortic stenosis¹⁴¹ and experimentally induced volume overload¹⁰³ in dogs. C-ANP was found to have a low positive predictive value in diagnosing occult cardiomyopathy in Doberman Pinschers.⁶⁶ Dogs with CHF secondary to MVD and other forms of spontaneous heart disease have been found to have higher concentrations of C-ANP compared with healthy controls.^{142,143} Dogs with CHF had significantly higher C-ANP concentrations than dogs with MVD that were not yet clinical for their disease.¹⁴² Furthermore, increases in C-ANP concentration were significantly correlated with vertebral heart score, fractional shortening, and the left atrial to aortic root diameter ratio (LA:Ao ratio).¹⁴³ While this study found too much overlap in C-ANP concentrations between dogs with and without CHF to determine a clinically useful cut-off value,¹⁴³ another study found that the mean concentration of C-ANP was 7× higher in dogs with CHF secondary to MVD than normal dogs.¹⁴²

Similar to dogs, the physiologically active C terminal of ANP in cats is nearly identical to that of people, and human assays for C-ANP can be used to measure this protein in cats.¹⁴⁴ Experimental fluid overload in cats showed that C-ANP concentrations positively correlated with directly measured left atrial pressures.¹⁴⁵ Cats with various cardiomyopathies have increased C-ANP concentrations compared to healthy controls.¹⁴⁵ Furthermore, cats with systemic thromboembolism or with symptoms of CHF had higher

Table 2: Natriuretic peptides evaluated in disease that can lead to respiratory distress in cats

Peptide	Disease condition	Results	How reported
ANP	Cardiomyopathy ± CHF ¹⁴⁴	Healthy: 18.5 (10–57)*,§ CM – CHF: 111 (78–300)* CM + CHF: 228 (95–585)§	Mean, range (pg/mL)
NT-proANP	Respiratory distress CHF versus pulmonary disease ¹⁵¹	CHF: 1,690 (556–4,987)* Pulmonary disease: 614 (208–3,109)*	Median, range (fmol/mL)
	Cardiomyopathy ± CHF ¹⁵²	Healthy: 682 (530–834)*,§ CM – CHF: 1,176 (810–1,543)*,† CM + CHF: 1,865 (1,499–2,231)§,†	Median, 95% CI (fmol/mL)
	Cardiomyopathy ± CHF ¹⁵³	Healthy: 381 (52–450)*,§ CM – CHF: 763 (167–2,386)*,† CM + CHF: 2,443 (1,189–15,462)§,†	Median, range (fmol/mL)
	Cardiomyopathy ± CHF ¹⁵⁴	Healthy: 413 (52–940)*,§ CM – CHF: 1,254 (167–2,818)*,† CM + CHF: 3,208 (1,189–15,462)§,†	Median, range (fmol/mL)
NT-proBNP	Respiratory distress CHF versus pulmonary disease ²⁰¹	CHF: 754 (437–1,035)* Pulmonary disease: 76.5 (24–180)*	Mean, IQR (pmol/L)
	Respiratory distress CHF versus pulmonary disease ¹⁵¹	CHF: 523 (95–982)* Pulmonary disease: 45 (6–394)*	Median, range (fmol/mL)
	Cardiomyopathy ± CHF ¹⁵²	Healthy: 33.6 (11.2–56.1)*,§ CM – CHF: 184 (111–257)*,† CM + CHF: 525 (437–612)§,†	Median, 95% CI (fmol/mL)

*, §, † Paired values are statistically different from one another.

ANP, atrial natriuretic peptide; NT-proANP, amino-terminal pro-atrial natriuretic peptide; NT-proBNP, amino-terminal pro-brain natriuretic peptide; CM, cardiomyopathy; CHF, congestive heart failure; CI, confidence interval; IQR, interquartile range.

C-ANP concentrations than normal healthy cats.^a A comparison of 8 cats with cardiomyopathy and concurrent CHF to 6 cats with asymptomatic cardiomyopathy failed to find significant differences in C-ANP (see Table 2); however, the median concentration of C-ANP in the CHF group was higher than the non-CHF group.¹⁴⁵

The limitation of using C-ANP to aid in the diagnosis of heart disease is that it is less stable than other natriuretic peptides.¹⁴² The half-life in dogs is approximately 10 minutes.^{146,147} Measurement of C-ANP requires radioimmunoassay, which is not readily available in most veterinary practices.^{116–118,141–145} Due to the lack of studies evaluating C-ANP to differentiate cause of dyspnea in animals that present with respiratory distress and inconsistent findings for animals with CHF, use of C-ANP as a biomarker for respiratory distress cannot be advised at this time. Theoretically, if measurement of C-ANP did become more readily available, an increase in the concentration of this protein, as seen in experimental volume overload of dogs and cats, could help to monitor for increased left atrial pressures and risk of volume overload in patients on aggressive fluid support.

Amino-terminal proANP

Amino-terminal proANP is the nonactive portion of the ANP prohormone that is formed in equimolar concentrations to C-ANP.¹⁴⁸ The half-life is considerably longer than that of C-ANP, and therefore theoretically easier to accurately measure at peak concentrations.^{148,149} It has been shown to be increased in people with CHF,¹⁵⁰ myocardial infarction,¹⁵¹ tachyarrhythmias,¹²³ sepsis,¹³³ hypertension,¹²⁷ and during pregnancy.¹²⁵

Amino-terminal proANP has been evaluated as a tool to differentiate dyspnea of cardiac origin from noncardiac origin in dogs.^{60,118} Both of the studies looking at NT-proANP in dyspneic dogs were able to demonstrate an increase in NT-proANP in cases with CHF over those with other causes of dyspnea.^{60,118} The groups found variable accuracy, however, with Prosek et al⁶⁰ demonstrating an excellent sensitivity and specificity with an AUC of the ROC of 0.946 while Boswood et al¹¹⁸ found an AUC of 0.793.^{60,118} If serum was evaluated rather than plasma, Boswood et al found that it increased sensitivity and specificity with an AUC of 0.852.¹¹⁸

Studies have demonstrated that cats with respiratory distress secondary to CHF had a higher concentration of NT-proANP than those that had respiratory

Table 3: Natriuretic peptides evaluated in disease that can lead to respiratory distress in dogs

Peptide	Disease condition	Results	How reported
NT- proANP	Respiratory distress CHF versus pulmonary disease ⁵⁹	Pulmonary disease: 0.26 (0.01–2.11)* CHF: 1.38 (0.33–4.41)*	Mean, range (nmol/L)
	Respiratory distress CHF versus pulmonary disease ¹¹⁷	Pulmonary disease: 1,110 (520–3,792)*,§ Heart disease – CHF: 1,645 (731–3,320)*,† Heart disease + CHF: 2,785 (760–4,100)§,†	Median, range (pmol/L)
	Pulmonary disease ± precapillary PH ²⁶⁴	Pulmonary disease: 1,209 (976–1,389) Precapillary PH: 1,747 (894–2,884)	Median, range (pmol/L)
BNP	Respiratory distress CHF versus pulmonary disease ⁵⁹	Pulmonary disease: 12.18 (5.18–28.1)* CHF: 34.97 (4.45–245.9)*	Mean, range (pg/mL)
	Respiratory distress CHF versus pulmonary disease ¹⁷⁵	Pulmonary disease: 1.9 (0.5–3.9)* Pulmonary + heart disease: 3.3 (1.2–5.4)§ CHF: 24.6 (12.3–40.0)*,§	Median, IQR (pg/mL)
NT-proBNP	Respiratory distress CHF versus pulmonary disease ¹⁹⁰	Pulmonary disease: 357 (192–565)* CHF: 2,544 (1,652–3,476)*	Median, IQR (pmol/L)
	Respiratory distress CHF versus pulmonary disease ¹⁹¹	Pulmonary disease: 413 (245–857)* Pulmonary + heart disease: 478 (323–1,158)§ CHF: 2,445 (1,499–3,134)*,§	Median, IQR (pmol/L)
	Respiratory distress CHF versus pulmonary disease ¹¹⁷	Pulmonary disease: 113 (<42–362)*,§ Heart disease – CHF: 468 (<42–3,980)§,† Heart disease + CHF: 1,700 (186–9,280)§,†	Median, range (pmol/L)
	Pulmonary disease ± precapillary PH ²⁶⁴	Pulmonary disease: 744 (531–2,710)* Precapillary PH: 2,011 (274–7,713)*	Median, range (pmol/L)
	Doberman DCM ¹⁹³	Healthy: 303 (22–1,325)* DCM + CHF: 2,960 (386–3,000)*	Median, range (pmol/L)

*, §, † Paired values are statistically different from one another.

NT-proANP, amino-terminal pro-atrial natriuretic peptide; BNP, brain natriuretic peptide; NT-proBNP, amino-terminal probrain natriuretic peptide; CHF, congestive heart failure; HD, heart disease; PH, pulmonary hypertension; IQR, interquartile range; DCM, dilated cardiomyopathy.

distress of noncardiac origin (see Table 2).^{152–155,b} Although the AUC of the ROC was relatively high at 0.88, it was less precise than NT-proBNP, which was simultaneously evaluated in this study and had an AUC of 0.96.¹⁵² Other studies have shown that cats with occult cardiomyopathies have higher median NT-proANP concentrations than normal cats.^{152–154,c} Furthermore, cats with CHF have been found to have higher NT-proANP concentrations compared to normal cats and cats with occult cardiomyopathy.^{153–155,d} However, there is overlap in NT-proANP values between groups and although sometimes the overlap was minimal, a distinct line between healthy cats, those with occult cardiomyopathy and those with CHF has not been convincingly demonstrated.^{152–156} This is unfortunate as a clear cutoff would more easily categorize cats with respiratory distress due to noncardiac disease with underlying heart disease versus those with CHF.

The NT-proANP assay utilized in recent veterinary studies was a commercially available human sandwich ELISA.^e Similar to C-ANP evaluation, the assays require equipment not readily available in most

veterinary hospitals.^{152–156} The half-life of canine NT-proANP is approximately 45 minutes.¹⁴⁷ The longer half-life compared with that of C-ANP gives researchers and clinicians a longer window to document abnormalities. If NT-proANP were available as a bedside test, it appears that it would be a valuable tool to rule in or out CHF in both dogs and cats.

C-terminal BNP

BNP was first identified in the brain,⁹² but it is actually produced in larger amounts in the heart.¹⁵⁷ BNP is rapidly produced by the ventricular myocytes in response to stretch and hypoxia.^{158–160} In people, CHF is associated with significant increase in C-BNP concentration.¹⁶¹ Noncardiac factors that have been found to be associated with increases in C-BNP concentration in people include advanced age,¹⁶² female gender,¹⁶² presence of pneumonia,¹⁶³ systemic inflammatory response syndrome,¹⁶⁴ sepsis,^{164–166} pulmonary embolism,¹⁶⁷ PH,¹⁶⁸ systemic hypertension,¹³⁰ hyperthyroidism,¹⁶⁹ lung tumors,¹⁷⁰ stroke,^{171,172} and

Table 4: Cardiac biomarkers currently available

Biomarker	Availability
cTnI ^{h-o}	Numerous point of care, see Table 1
C-ANP	No practical point-of-care or commercial send-out test available
NT-proANP ^b	No practical point-of-care or commercial send-out test available
C-BNP ^c	No practical point-of-care or commercial send-out test available
NT-proBNP ^d	Requires special media for outside lab, 1–2 days turnaround time, point-of-care test currently in development
Endothelin ^g	No practical point-of-care or commercial send-out test available

liver disease.¹⁷³ Obesity is linked to lower concentrations of C-BNP.¹⁷⁴ A close inverse relationship between increased plasma C-BNP concentrations and left ventricular function has been documented in people with sepsis-induced impaired left ventricular function.¹⁷⁵

There are 2 studies in the veterinary literature evaluating C-BNP as a biomarker to differentiate dogs with CHF from those with noncardiac causes of dyspnea.^{60,176} Dogs with CHF had a significantly higher median C-BNP concentrations with an AUC of the ROC between 0.89 and 0.91, and the magnitude of elevation increased as the severity of the heart disease increased.^{60,176} Although it appeared as though dogs with concurrent heart disease and a noncardiac etiology of cough or dyspnea had higher C-BNP concentrations than those with dyspnea without evidence of concurrent heart disease, this was not statistically significant ($P = 0.20$).¹⁷⁶ A cutoff of 6 pg/mL had a sensitivity of 90% and specificity of 78% for differentiating CHF from other causes of cough or dyspnea, while a cutoff of 25 pg/mL had a sensitivity of only 50%, but a specificity of 96%.¹⁷⁶ In the same study, when dogs were divided into groups based on underlying condition, median C-BNP concentrations were as follows: DCM 36.9 pg/mL, pre- and postcapillary PH 14.8 pg/mL, chronic degenerative valvular disease 5.0 pg/mL, and pericardial effusion 1.24 pg/mL (see Table 3).¹⁷⁶ Despite these findings, both studies found a large amount of overlap between dogs with dyspnea from cardiac origin and dogs with dyspnea from noncardiac origin.^{60,176}

An additional study evaluated C-BNP in dogs with heart disease with or without CHF and compared them to other diseases that did not result in dyspnea (eg, trauma, toxicity, neurological, gastrointestinal, renal, neoplastic, or metabolic diseases).¹⁷⁷ The median C-BNP was significantly increased in dogs with CHF compared to all other groups, and a cut-off value of >6.0 pg/mL

was found to have a sensitivity of 93% and a specificity of 87% for diagnosis of dyspnea secondary to cardiac disease.¹⁷⁷ However, there were several individuals with various systemic diseases that had C-BNP concentrations that were as increased or even higher than dogs with CHF, underscoring the problem of comorbidities confounding the diagnosis of CHF using a single C-BNP measurement.¹⁷⁷

Numerous investigators have looked at C-BNP in dogs with naturally occurring and experimentally induced heart disease.^{66,142,178,179} C-terminal BNP is increased in Doberman Pinschers with occult cardiomyopathy and may be a valuable screening tool in this breed.⁶⁶ Concentrations are increased in experimentally induced myocardial failure from long term pacing, but were not shown to be increased in Boxer dogs that are clinically affected by arrhythmogenic right ventricular cardiomyopathy.¹⁷⁸ Dogs with naturally acquired mitral valve disease have also been found to have higher concentrations of C-BNP, but elevations occur only when clinical signs of CHF appear.¹⁴² This study found that increases in NT-proANP (AUC of 0.99) and C-ANP (AUC of 0.97) concentrations more accurately correlated with decompensated heart failure as compared to C-BNP (AUC of 0.80).¹⁴² In a retrospective study evaluating canine patients with chronic degenerative valvular disease, C-BNP concentrations increased with increasing severity of heart disease, and correlated with the International Small Animal Cardiac Health Council Heart Failure Classification groups.¹⁷⁹ Additionally, this group also found that every 10 pg/mL rise in plasma C-BNP concentration was associated with a 44% increase in mortality.¹⁷⁹

C-terminal BNP concentrations have been found to be increased in cats with asymptomatic cardiomyopathy and concentrations increase further with the onset of CHF or arterial thromboembolism.^f However, overlap between groups exists.⁸ Use of angiotensin converting enzyme inhibitor did not change C-BNP concentrations in Maine Coon cats with asymptomatic familial HCM.¹⁸⁰

Point-of-care C-BNP analyzers are available for people, but currently are not available or validated for veterinary species.¹⁸⁰ All assays from the studies mentioned above either required equipment that is not readily available^{60,142,179,180} or were sent as batched samples to a reference laboratory,^{h,176,177} limiting their current use in the emergency setting. The half-life of C-BNP in dogs is very short at approximately 90 seconds, making it difficult to capture the peak level.¹⁸¹ However, this biomarker could ultimately prove useful in determining the presence of CHF in dogs. In cats, there appears to be insufficient data to recommend routine use of C-BNP as a biomarker.

Amino-terminal proBNP

As discussed previously, NT-proBNP is the nonactive amino-terminal portion of the BNP preprohormone⁹⁸ and is increased in people with CHF,¹⁸² tachyarrhythmias,¹⁸³ and bradyarrhythmias.¹⁸⁴ Other disease processes, which have been shown to cause NT-proBNP increases in people, include pneumonia,¹⁶⁸ sepsis,¹⁶⁵ pulmonary embolism,¹⁸⁵ PH,¹⁸⁶ systemic hypertension,¹⁸⁷ stroke,¹⁸⁸ ARDS,¹⁸⁹ and liver disease.¹⁷³ Obesity and insulin resistance is linked with lower concentrations of NT-proBNP.¹⁹⁰

Amino-terminal proBNP is the most widely studied biomarker of cardiac disease in veterinary medicine.^{118,191–211} When NT-proBNP was evaluated as a biomarker to differentiate CHF from other causes of respiratory distress, it was found to be significantly higher in dogs with respiratory distress from CHF compared to dogs with respiratory distress from noncardiac diseases including pneumonia, pulmonary neoplasia, neoplastic pleural effusion, laryngeal paralysis, chronic bronchitis, collapsing trachea, lung lobe torsion, noncardiogenic pulmonary edema, and eosinophilic bronchopneumopathy.^{118,191,192} In addition, NT-proBNP was significantly higher in dogs with CHF compared to those with primary pulmonary disease and concurrent asymptomatic heart disease.¹⁹² The AUC of the ROC ranged from 0.89 to 0.91 when using NT-proBNP to differentiate dyspnea of cardiac origin from noncardiac origin.^{118,192} Oyama *et al*¹⁹² found that a value >158 pmol/L was 85.5% sensitive and 81.3% specific for the diagnosis of CHF.¹⁹²

There are a multitude of studies evaluating the use of NT-proBNP in dogs with different heart diseases at different stages of disease.^{141,193–201} In an experimental model of compensated aortic stenosis, NT-proBNP not only significantly rose compared to baseline, but was also positively correlated with left ventricular end-diastolic pressure and end-diastolic intraventricular septal wall thickness.¹⁴¹ The use of pimobendan has been shown to significantly reduce concentrations of NT-proBNP in dogs with postcapillary PH and CHF from MVD.¹⁹³ However, despite continued improvement in quantitative and qualitative parameters (tricuspid regurgitant flow velocity, quality of life scores, heart rate, and echocardiographic parameters of systolic function) NT-proBNP did not persistently improve.¹⁹³ This differs from what is seen in human studies, and the authors attributed this species discrepancy to day-to-day variation, small sample size, or progression of disease.¹⁹³ In Doberman Pinschers, median NT-proBNP concentration were increased in occult DCM, but significantly increased when patients developed CHF or clinically significant arrhythmias.¹⁹⁴ Furthermore, dogs with CHF secondary to DCM had lower NT-proBNP concentrations after

therapy was initiated and the CHF had resolved.¹⁹⁵ In dogs with mitral valve disease, animals with increased NT-proBNP concentrations have more severe disease,^{196–198} develop CHF sooner,^{199,200} and have a shorter overall life span.^{62,196,201}

When using NT-proBNP to determine if the cause of feline respiratory distress is secondary to CHF versus a noncardiac cause, NT-proBNP was significantly higher in cats with CHF with an AUC of 0.94–0.96.^{152,202} Connolly *et al*¹⁵² found that a value >220 pmol/L diagnosed CHF in cats with a sensitivity of 93.9% and a specificity of 87.8%.¹⁵² Similarly, Fox *et al*²⁰² found that a value >265 pmol/L had a 90.2% sensitivity and 87.9% specificity for the diagnosis of CHF.²⁰² Use of NT-proBNP in combination with thoracic radiographs has been shown to improve diagnostic accuracy of CHF in cats that present for respiratory distress over thoracic radiographs alone.²⁰³ Furthermore, clinician confidence in diagnosing CHF significantly improved when NT-proBNP was increased.²⁰³ While this study demonstrated significance, one cat with pulmonary disease had an NT-proBNP that fell into the intermediate range and clinician interpretation of radiographic findings did not improve with the NT-proBNP information.²⁰³ This stresses the importance that interpretation of NT-proBNP should still be in light of clinical signs and other diagnostic tests.

Amino-terminal proBNP has been found to be increased in any degree of severity of occult feline cardiomyopathy,^{153,204} but other studies have found that NT-proBNP was only significantly increased if there were severe echocardiographic changes.^{205,206} Given the discrepancy in the data reported, it is concerning that there may not be accurate or consistent correlations between the NT-proBNP concentration and the echocardiographic findings in feline patients with occult heart disease. However, concentrations of NT-proBNP are significantly higher in cats with CHF when compared to healthy controls (AUC = 0.99) or cats with compensated heart disease (AUC = 0.90).¹⁵³ Finally, NT-proBNP was found to be higher in azotemic cats with systemic hypertension compared to azotemic cats with normal systemic blood pressure.¹¹⁵

The half-life of NT-proBNP in dogs is not known, but the molecule is much more stable than C-BNP and in some animal models the half-life is 6- to 15-fold longer than C-BNP.^{208,212–214} Amino-terminal proBNP is not maintained at a static concentration in the body and a normal dog may have more than a 200 pmol/L change from 1 week to the next.²⁰⁹ Due to normal variation, some normal dogs may actually have values that are worrisome for heart disease, and NT-proBNP values must be interpreted in light of physical exam findings and other biochemical and radiographic abnormalities.

Additionally, echocardiograms are recommended in cases in which heart disease cannot be completely ruled out and in patients with increased NT-proBNP concentrations.

Amino-terminal proBNP is a commercially available diagnostic testⁱ with a turnaround time of 1–2 business days. This test has been validated in dogs and cats and no spurious changes were seen with the introduction of lipids, hemoglobin, or bilirubin to the plasma.^{j,118} Sample type and handling are vital for accurate measurement of NT-proBNP.^{k,210} Special sampling tubes that contain a protease inhibitor to minimize NT-proBNP degradation in plasma are recommended and should be maintained at room temperature.^{211,1} A recently completed pilot study by the commercial laboratory evaluated a point-of-care test for NT-proBNP in cats and found that the test had a sensitivity of 87% and a specificity of 78% to identify cats with moderate to severe occult heart disease.^m The authors concluded that this test is useful in ruling out heart disease,ⁿ but has not been evaluated specifically in cats that present with respiratory distress. The sensitivity, specificity, and the availability of this test to most individuals (especially if and when the point-of care test becomes available) makes NT-proBNP probably one of the most promising biomarkers that will be useful in the emergency setting. Although NT-proBNP appears to be the most useful biomarker in determining if cardiac failure is the underlying cause of respiratory distress, this test still has various limitations.

Endothelin – 1

Endothelin-1 (ET-1) is a protein that is produced by numerous cells including vascular endothelial cells, vascular smooth muscles cells, airway epithelial cells, macrophages, fibrocytes, cardiac myocytes, and others.^{215–221} It is produced as the large preproendothelin, then is cleaved into an inactive intermediate termed big ET-1.²²² Big ET-1 is then cleaved into the biologically active ET-1.²²² In the vasculature, this protein is made in response to shear stress,²¹⁹ hypoxia,²²³ angiotensin II,²²⁴ and vasopressin.²²⁵ ET-1 affects the cardiovascular system in a complex manner through a variety of receptors, inducing intense and sustained vasoconstriction, vasodilation, and increased inotropy in various situations.^{222,226–229} It is most commonly measured in circulation, but also has been measured in exhaled breath condensate in people.^{230,231}

ET-1 has been shown to be increased and may play a role in the progression of a variety of human diseases including pulmonary fibrosis,²³² systemic hypertension,²³³ PH,²³⁴ atherosclerosis,²³⁵ myocardial remodeling following CHF,²³⁶ and disseminated

intravascular coagulation.²³⁷ ET-1 has been shown to be increased in multiple causes of dyspnea in people including lung cancer,²³⁸ pneumonia,²³⁹ ARDS,²⁴⁰ and asthma.²⁴¹ It is unclear if increases in endothelin concentrations are involved in the pathogenesis of certain diseases or are a sequelae of them.²³⁴

ET-1 appears to play an important role in the pathophysiology of equine laminitis^{242,243} and lower airway inflammation,^{244,245} and is increased in horses after exercise²⁴⁶ and those with obstructive pulmonary disease.^{247,248} Investigations on ET-1 concentrations in cows have been limited to reproduction.^{249,250}

ET-1 has been evaluated as a marker of heart disease in dogs that present for dyspnea, and it was found that dogs with CHF had significantly higher ET-1 concentrations compared to dogs with other causes of dyspnea with an AUC of the ROC of 0.85.⁶⁰ A cut-off value of 0.478 fmol/mL yielded a sensitivity of 85.7% and a specificity of 80.8%.⁶⁰

There has been some research on dogs evaluating the use of ET-1 and big ET-1 as markers for CHF.^{251–254} Experimentally induced heart failure secondary to over-pacing resulted in ET-1 overexpression in dogs.^{251,254} Doberman Pinschers with DCM and CHF had significantly increased big ET-1 concentration.²⁵² Furthermore, increased big ET-1 concentrations were associated with shorter survival times.²⁵² An additional study evaluating dogs with CHF caused by acquired heart disease found that the dogs with CHF had increased ET-1 concentration compared to healthy controls and dogs with compensated heart disease.²⁵³

ET-1 is increased in cats with asymptomatic cardiomyopathy compared to healthy controls.²⁵⁵ There was an even greater difference when comparing cats with CHF or thromboembolic sequelae from their heart disease compared to healthy controls.²⁵⁵ However, there was not a significant difference between cats with CHF and those with compensated heart disease.²⁵⁵

Although ET-1 and big ET-1 have shown some promise as indicators of heart disease, there are other pathological conditions that have been shown to cause ET-1 and big ET-1 elevations, decreasing the specificity of ET-1 as a marker of heart disease in critically ill animals. Experimental canine studies performed in vitro and in vivo have shown that ET-1 may have a role in the pathogenesis and disease progression in arrhythmogenesis,^{256,257} ischemia-reperfusion injury,^{258,259} systemic hypertension,²⁶⁰ PH,²⁶¹ and myxomatous mitral valve disease.²⁶² A study evaluating dogs with CHF, kidney failure, diabetes mellitus, and hyperadrenocorticism found that big ET-1 was significantly increased in the dogs with illness compared to healthy

controls, but there was no significant difference between groups.²⁶³ Serum ET-1 concentrations were significantly increased in dogs with idiopathic pulmonary fibrosis compared to healthy Beagles, healthy age-matched West Highland White Terriers, dogs with chronic bronchitis, and dogs with eosinophilic bronchopneumopathy.²⁶⁴

Although ET-1 has some promise as a biomarker for cardiogenic dyspnea in dogs, it was confirmed to be less accurate than either NT-proANP or C-BNP.⁶⁰ Endothelin measurements are not routinely performed and there is not a point-of-care test available for use that has been validated in dogs or cats. Commercial tests are available,⁹ but these require equipment that is typically not available in most practices and may be time prohibitive as a diagnostic tool as it requires overnight incubation.²⁵³ Due to clinical, canine experimental, and human studies showing that ET-1 may play a role in disease progression and the fact that it is increased in a variety of disease processes, future studies are warranted to determine its role in clinical situation.

Pulmonary hypertension

Precapillary PH can be caused by idiopathic primary arterial hypertension, heart worm disease, cardiovascular shunts, vascular occlusive disease, or chronic hypoxemia from various pulmonary diseases.²⁶⁵ Postcapillary PH is often the result of left-sided heart disease, and most commonly occurs in dogs secondary to MVD.²⁶⁶ However, without other diagnostic tools such as echocardiography and cardiac catheterization, it is often difficult to determine if PH is the cause of respiratory distress. Many of the biomarkers evaluated in dogs with cardiac disease have also been evaluated as tools in diagnosing PH.^{176,193,265,266}

In people, PH has been associated with increased concentrations of exhaled nitric oxide,²⁶⁷ and blood cTn,²⁹ C-ANP,¹²⁹ C-BNP,¹⁶⁸ NT-proBNP,¹⁸⁶ and D-dimers.²⁶⁸ Dogs with precapillary PH have been shown to have increased cTnI compared to healthy control dogs and dogs with varying stages of MVD (see Table 1).²⁶⁶ Decompensation of postcapillary PH was found to result in increased cTnI concentration compared to dogs with compensated MVD with or without concurrent PH.²⁶⁶ However, there was a good deal of overlap in all groups.^{265,266} Dogs with CHF and concomitant PH have increases in C-BNP concentration.¹⁷⁶ Dogs with both precapillary and postcapillary PH have been shown to have increased NT-proBNP concentration.^{193,265} Amino-terminal proANP concentrations were not different between healthy dogs and those with precapillary PH.²⁶⁵ Although several biomarkers have been shown to be increased in dogs with PH, there does not appear to be a single test that can differentiate PH from CHF at this time.

Conclusion

Numerous cardiac biomarkers have been evaluated and some may prove useful in clarifying the cause of respiratory distress. cTnI can be measured with point-of-care analyzers and has been shown to be increased in patients with CHF. One of the biggest drawbacks to its use, however, is the lack of standardization that would allow a clinician to interpret values obtained from different analyzers than used in published reports. Furthermore, most of the conditions evaluated for cTnI levels had significant amounts of overlap, which complicate clinical decision making. To date, NT-proBNP is regarded as the most useful cardiac biomarker, and several studies have provided some evidence that NT-proBNP concentrations are different in healthy veterinary patients compared to those with respiratory disease, occult heart disease, and CHF. An area requiring further investigation is the development and validation of a point-of-care test for NT-proBNP. It should be stressed that this test should be interpreted in light of other diagnostic findings. Other natriuretic peptide tests may also be of benefit, but due to availability of testing and limited clinical data on these biomarkers, their use is at this time is limited to research settings. ET-1 may be useful in suspected cases of idiopathic pulmonary fibrosis, but its role in other disease processes is unclear and unlikely to be clinically appropriate. Unfortunately, there is currently no biomarker that can reliably distinguish CHF from PH as the cause of dyspnea. Further research in this area is warranted.

Footnotes

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- ^b Vetsign Canine CardioSCREEN, Guildhay Limited, Guilford, Surrey, UK.
- ^c Biosite Incorporated, San Diego, CA.
- ^d Cardiopet proBNP, IDEXX Laboratories, Westbrook, ME.
- ^e Carrier A, Beardow A, Farace G, et al. Analytical validation of a commercially-available feline N-terminal prohormone brain natriuretic peptide ELISA. *J Vet Intern Med* 2009; 23: 747. Abstract 212 presented at 2009 ACVIM forum and CVMA convention June 3-6, 2009. Montreal, Quebec, Canada.
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- ^g Big endothelin-1 EIA kit, Immuno-Biological Laboratories Co, Ltd, Gunma, Japan, Endothelin EIA, ALPCO Diagnostics, Windham, NH.
- ^h Access AccuTnI, Beckman Coulter, Inc., Fullerton, CA.
- ⁱ OPUS Immunoassay System, OPUS Troponin I, Behring Diagnostics Inc., Westwood, MA.
- ^j Stratus CS stat flurometric analyzer, Dade Behring Inc., Newark, DE.
- ^k Abbott AxSYM System, Abbott AG, Diagnostics Division, Baar, Switzerland.
- ^l Immulite 1000 Troponin I kit, Siemens, Los Angeles, CA.
- ^m Unicel DXI, Beckman Coulter.
- ⁿ Reagent Flex CTNI (RF421C), Dade Behring Inc., Deerfield IL.
- ^o I-STAT cTnI test, Heska Corporation, Loveland, CO.

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