Reference Point

Clinical usefulness of an assay for measurement of circulating N-terminal pro–B-type natriuretic peptide concentration in dogs and cats with heart disease

Mark A. Oyama, DVM, DACVIM; Adrian Boswood, MA, VetMB; David J. Connolly, BVetMed, PhD; Stephen J. Ettinger, DVM, DACVIM; Philip R. Fox, DVM, MS, DACVIM, DACVECC; Sonya G. Gordon, DVM, DACVIM; John E. Rush, DVM, MS, DACVIM, DACVECC; D. David Sisson, DVM, DACVIM; Rebecca L. Stepien, DVM, MS, DACVIM; Gerhard Wess, Dr Med Vet, Dr med vet habil, DACVIM; Faiez Zannad, MD, PhD

The science and clinical usefulness of cardiac biomarker measurement in veterinary patients is a subject of increasing interest. Despite progress in our understanding of the potential clinical applications of NT-proBNP measurement, clear recommendations for use of the NT-proBNP assay are lacking, and for many clinical scenarios, use of this assay requires further study. The purpose of this report is to render the collective opinion of a group of investigators who have been involved in NT-proBNP studies in veterinary patients or in formulating recommendations for use of an NT-proBNP assay in human medicine (FZ). Our objective was to interpret existing investigative data and address potential applications of NT-proBNP testing in veterinary species, with the aim of providing information to help veterinary clinicians apply and interpret assay results. Because proper use of NT-proBNP testing requires an understanding of the basic physiology of the natriuretic peptide system, specific indications and limitations of testing, and appropriate actions to take given the test results, each of these components will be discussed.

Physiologic Basis for BNP and NT-proBNP Testing

B-type natriuretic peptide is a neuroendocrine hormone that is constitutively synthesized in atrial myocytes and stored as proBNP together with the proatrial natriuretic peptide hormone. In response to atrial stretch, proBNP is cleaved into 2 smaller peptides and released into plasma. In human and veterinary patients with chronic cardiac disease, synthesis of proBNP also occurs in the ventricular myocytes and the amount of hormone released is in proportion to disease severity.1-3 Canine proBNP is a 114-amino acid peptide that is proteolytically cleaved into inactive NT-proBNP and a

Abbreviations

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNP</td>
<td>B-type natriuretic peptide</td>
</tr>
<tr>
<td>C-BNP</td>
<td>C-terminal fragment of B-type natriuretic peptide</td>
</tr>
<tr>
<td>CHF</td>
<td>Congestive heart failure</td>
</tr>
<tr>
<td>DCM</td>
<td>Dilated cardiomyopathy</td>
</tr>
<tr>
<td>HCM</td>
<td>Hypertrophic cardiomyopathy</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>LVIDd:Ao</td>
<td>Ratio of end-diastolic left ventricular dimension to aortic root diameter</td>
</tr>
<tr>
<td>MMVD</td>
<td>Myxomatous mitral valve disease</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>N-terminal pro–B-type natriuretic peptide</td>
</tr>
<tr>
<td>ODCM</td>
<td>Occult dilated cardiomyopathy</td>
</tr>
<tr>
<td>proBNP</td>
<td>Pro–B-type natriuretic peptide</td>
</tr>
<tr>
<td>VHS</td>
<td>Vertebral heart size</td>
</tr>
</tbody>
</table>

From the Department of Clinical Studies-Philadelphia, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104 (Oyama); the Department of Clinical Sciences, Royal Veterinary College, University of London, North Mymms, Hertfordshire, AL9 7TA England (Boswood, Connolly); VCA California Animal Hospital Veterinary Specialty Group, 1736 S Sepulveda Blvd, Los Angeles, CA 90025 (Ettinger); Animal Medical Center, 510 E 62nd St, New York, NY 10065 (Fox); the Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843 (Gordon); the Department of Clinical Sciences, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA 01536 (Rush); the Department of Clinical Sciences, College of Veterinary Medicine, Oregon State University, Corvallis, OR 97331 (Sisson); the Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706 (Stepien); Clinic of Small Animal Medicine, Ludwig-Maximilians Universität, Munich, Germany 80539 (Wess); and the Department of Cardiology, Nancy University, Université de Lorraine, Nancy, France 54052 (Zannad).

Over the past 5 years, the following authors served as consultants or on advisory boards for and received support (at least 1 of speaker honoraria, reimbursement of travel expenses, research funding, programmatic support [intern or resident funding or equipment]) from IDEXX Laboratories: Boswood, Fox, Gordon, Oyama, Rush, Stepien, and Wess. Dr. Ettinger received remuneration of travel expenses and programmatic support from IDEXX Laboratories. The remainder (Connolly, Sisson, Zannad) have no disclosures. IDEXX Laboratories did not have knowledge of or participate in formulating the idea to write the manuscript, in writing or review of the manuscript, or in the decision to submit the manuscript for publication.

Address correspondence to Dr. Oyama (maoyama@vet.upenn.edu).
bioactive C-BNP. Bioactive C-BNP binds to A-type natriuretic receptors located in the vasculature and kidney and induces vasodilation and diuresis by inhibiting sodium transport in renal medullary—collecting ducts.

The main mode of C-BNP clearance from circulation is via intracellular degradation following binding to C-type natriuretic peptide receptors, whereas clearance of C-BNP and NT-proBNP occurs via renal excretion. The plasma half-life of C-BNP is reported to be between 12 and 22 minutes in humans and approximately 90 seconds in dogs. The plasma half-life of this peptide in cats is not known. The half-life of NT-proBNP in humans and other animals is between 5 to 15 times as long as that of C-BNP. The C-BNP molecule is also particularly sensitive to collection and storage methods, and for all of these reasons, NT-proBNP generally is regarded as a more reliable analyte for laboratory measurement.

Plasma C-BNP and NT-proBNP concentrations correlate with pulmonary capillary wedge pressure (an indirect measurement of left atrial pressure) and are increased in humans, dogs, and cats with various cardiac disorders, relative to concentrations in those without heart disease. In humans, C-BNP measurement is more accurate than thoracic radiography or ECG for the diagnosis of CHF. The main indication for BNP testing in humans is to distinguish dyspnea of cardiac origin from other noncardiac causes of respiratory compromise. In addition to their usefulness in the diagnostic process, natriuretic peptide concentrations are negatively correlated with survival time, repeated hospitalizations, and other adverse events associated with CHF. On the basis of the physiology of the natriuretic peptide system, the diagnostic and predictive value of NT-proBNP assay in a clinical setting is a subject of considerable interest.

**Important Considerations and Limitations of NT-proBNP Testing**

As with any diagnostic test, certain limitations and considerations must be recognized when using the currently available NT-proBNP assay. Circulating NT-proBNP concentration can be affected by concurrent disease processes such as azotemia, pulmonary hypertension, sepsis, or systemic hypertension as well as incorrect blood sample handling or use of the assay in inappropriate patients. Moreover, because the natriuretic peptide system constitutively operates along a physiologic continuum of activity in a healthy animal, circulating NT-proBNP concentrations in cardiac patients often have a degree of overlap with clinically normal animals, particularly in animals with relatively mild or subclinical heart disease.

The diagnostic sensitivity and specificity of NT-proBNP assays for detection of occult heart disease or heart failure in veterinary patients have been reported. These qualities apply to the performance of the assay in relation to the entire study population, rather than specifically determining the disease status of a sole individual patient. Knowledge of the sensitivity and specificity of a particular diagnostic test can be useful in identifying assay cutoff values that best differentiate the disease condition evaluated. Cutoff values with high sensitivity are important when one intends to screen for a condition that has a low prevalence. This is particularly germane in clinical situations in which measures are taken to avoid false-negative results (eg, designating a cat that actually has cardiomyopathy as cardiologically normal) but in which false-positive results via toleration if these could lead to a relatively benign action (such as performing an echocardiogram). In contrast, cutoff values with high specificity are desirable when the clinician wishes to confirm or reject a particular diagnosis with great certainty and wishes to avoid false results leading to clinical decisions that could potentially cause the patient harm.

The probability of disease in an individual patient depends not only on the test result but also on the patient’s probability of disease before the test is performed (ie, the prevalence of disease within the patient’s population). Performance of NT-proBNP testing in a low-risk population (eg, young healthy cats undergoing neutering) is more likely to result in false-positive results attributable to chance. In contrast, testing a population of cats with a higher likelihood of having heart disease (eg, cats with heart murmurs) is more likely to render true-positive results. For these reasons, selection of an appropriate population for testing is of paramount importance to ensure accurate results.

**Limitations of testing patients with concurrent diseases**—In humans, dogs, and cats, testing patients with concurrent diseases may be extremely low or extremely high. Adapted from Fagan TJ. Letter: Nomogram for Bayes theorem. N Engl J Med 1975;293:257.
nal dysfunction can lead to an increase in circulating NT-proBNP concentration. The precise mechanisms are uncertain, but potential causes include a decrease in renal clearance, renal-related systemic hypertension with associated left ventricular hypertrophy, or mechanisms involved in so-called cardiorenal syndrome.\textsuperscript{2,20,21} One small study\textsuperscript{30} in dogs with renal disease (median serum creatinine concentration, 3.4 mg/dL; IQR, 2.0 to 5.9 mg/dL) but without heart disease found that the mean serum concentration of NT-proBNP was 261 pmol/L in healthy dogs and 617 pmol/L in azotemic dogs. Another study\textsuperscript{31} found that normotensive cats with mild (median serum creatinine concentration, 2.8 mg/dL; IQR, 2.3 to 3.2 mg/dL) or severe (6.0 mg/dL; IQR, 5.5 to 6.9 mg/dL) renal insufficiency had plasma NT-proBNP concentrations greater than those of healthy control cats. In the more severely affected cats, the median NT-proBNP concentration was 79.8 pmol/L, compared with 8.8 pmol/L in control cats. These data suggest that high NT-proBNP concentrations measured in cats with creatinine concentrations > 2.3 mg/dL should be interpreted with caution. It might be possible to adjust NT-proBNP values in a similar method to that used to determine urine protein concentration by indexing NT-proBNP concentration to BUN concentration or serum creatinine concentration,\textsuperscript{30} or glomerular filtration rate,\textsuperscript{35} but these methods have not been thoroughly evaluated in veterinary patients.

Hyperthyroidism in cats is associated with cardiac hypertrophy and potential for heart failure.\textsuperscript{33} In a study\textsuperscript{34} of 85 hyperthyroid cats, serum NT-proBNP was positively correlated with total thyroxine concentration, but unlike in previous studies, NT-proBNP values were not correlated with serum creatinine values. Successful treatment and restoration of a euthyroid state resulted in a significant decrease in NT-proBNP concentration despite a significant increase in serum creatinine concentration, suggesting a direct effect of thyroxine on NT-proBNP, physiologically independent of renal clearance.\textsuperscript{37}

Moderate to severe pulmonary hypertension results in right ventricular hypertrophy, which is another potential stimulus for NT-proBNP production and release. One study\textsuperscript{38} involving dogs revealed that plasma NT-proBNP concentration was significantly correlated to the severity of precapillary pulmonary hypertension secondary to conditions such as chronic interstitial pulmonary disease or heartworm infection. Pulmonary hypertension resulted in NT-proBNP values that overlapped with values associated with CHF caused by left-sided heart disease and could confound the diagnosis of heart failure.\textsuperscript{39}

Limitations associated with patient selection and test interpretation—Measurement of circulating NT-proBNP concentration is not necessary to establish a diagnosis of MMVD in dogs; the presence of an apical left-sided systolic murmur in an adult small-breed dog reliably indicates the presence of this disease. The NT-proBNP assay should also not be used to differentiate between forms of heart disease in an individual dog (eg, MMVD vs DCM). Given the aforementioned potential for false-positive results, NT-proBNP testing is not recommended as an indiscriminate screening test for dogs that have no clinical attributes suggesting the presence of heart disease nor as a routine part of pre-anesthetic testing in healthy dogs.

Use of indiscriminant NT-proBNP testing in healthy cats without auscultatory abnormalities or other clinical findings suggestive of possible heart disease is not recommended. Whether NT-proBNP assays would be useful as part of a geriatric screening profile for otherwise healthy geriatric cats requires additional evaluation. The NT-proBNP assay is best used in cats suspected to have cardiac disease, such as those with a cardiac murmur or gallop rhythm, arrhythmia, radiographic evidence of cardiomegaly, cardiomyopathy in a closely related sibling, or respiratory signs.

Studies conducted to investigate the diagnostic usefulness of blood tests such as NT-proBNP assays attempt to simplify a continuous variable (ie, NT-proBNP concentration) into a dichotomous variable (ie, condition present vs condition absent). Although cutoff values are a useful construct to help segregate groups of individuals, the risk of overinterpretation of results at or near the cutoff value exists. The most useful tests include estimations of pretest probability of disease and consideration of assay results along a continuous scale (Figure 1). Circulating concentrations of NT-proBNP close to the cutoff value impart much less reliable information than values far greater than or less than the cutoff value. In instances where NT-proBNP values fall into this gray area around the cutpoints, the assay might not provide any more reliable information to what was already known prior to testing.

Limitations specific to testing procedures—The NT-proBNP molecule degrades when stored at room temperature,\textsuperscript{30,40} and mishandling of serum or plasma samples can lead to inaccurately low NT-proBNP measurements. At the time of writing this report, collection of plasma samples for the NT-proBNP assay involved collection of whole blood into an EDTA tube, centrifugation to obtain plasma, and subsequent transfer of the plasma into a manufacturer-supplied protease inhibitor tube, which slows the breakdown of NT-proBNP, followed by subsequent storage and sample shipment of the protease tube.\textsuperscript{5} Once collected, same-day shipping of samples to the reference laboratory is recommended. When samples are held overnight, the authors recommend refrigeration at 2° to 8°C, and when the storage period is longer, samples should be stored at −20° or −40°C in a nonself-defrosting freezer because temperature cycling associated with self-defrosting mechanisms could degrade sample quality.

Inter- and intra-assay variability can be high with canine biomarker assays.\textsuperscript{41} The NT-proBNP assay for dogs has gone through several developmental and manufacturing revisions, the latest of which occurred in 2008 coincident with the introduction of protease inhibitor tubes. These changes likely account for some of the variability in circulating NT-proBNP concentrations reported in normal and affected dogs from various studies, but other unidentified factors cannot be excluded. In general, values obtained through studies performed prior to 2008 are lower than values from studies in which the protease inhibitor tubes were used. To the authors’ knowledge and given the consistent findings of multiple studies involving cats, no apparent changes...
have been observed in the performance of the feline NT-proBNP assay.

Week-to-week variability in NT-proBNP concentrations has been reported for dogs and cats. In a study\(^4\) of healthy dogs, serum and plasma NT-proBNP were measured once weekly for 3 weeks and week-to-week variability was < 200 pmol/L in 80% of dogs. Week-to-week plasma variability in a mixed cohort of healthy and cardiomyopathic cats was < 100 pmol/L in 83% of cats.\(^6\) At greatest impact are test results at or near the diagnostic cutoffs given that biological variability can move values to higher or lower than the diagnostic cutoff value, thereby reinforcing the need for cautious interpretation of test results near cutoff values. When test results are equivocal or unexpectedly high or low, repeated or serial measurement can be considered.

The NT-proBNP assay, like many other laboratory tests, is not a definitive diagnostic test specific to any particular etiology. Studies have demonstrated that NT-proBNP testing has its greatest clinical importance when the results are interpreted along with other findings obtained through the physical examination, medical history, echocardiography, thoracic radiography, ECG, arterial blood pressure measurement, and other clinicopathologic testing.\(^1,43\) Such results can provide medical justification to pursue or not pursue additional diagnostic tests. Isolated NT-proBNP measurements recorded in a subclinically affected dog or cat should not be empirically used as the basis to initiate therapy.

### Specific NT-proBNP Testing Recommendations

Differentiation between CHF and noncardiac causes of respiratory signs in cats—Cats with signs of respiratory distress can pose a considerable diagnostic challenge, and differentiation between cardiac and noncardiac causes for that distress is vital to appropriate treatment selection. Historical, physical examination, and thoracic radiographic findings in cats with respiratory signs can be nonspecific. Moreover, the compromised state of cats with severe distress can limit the diagnostic options. Echocardiography greatly facilitates the diagnosis of structural heart disease in cats but might not be available or appropriate, particularly in emergent circumstances. Such challenges have stimulated interest in the use of NT-proBNP assays to facilitate differentiation between CHF and noncardiac causes of respiratory distress.

In humans, NT-proBNP assays can be used to accurately distinguish CHF from noncardiac causes of sudden dyspnea and are superior to clinical evaluation alone for diagnosis.\(^45\) Testing of circulating NT-proBNP concentration is part of the standard diagnostic workup in patients with respiratory distress and is most useful as a test to rule out CHF in patients with respiratory signs.\(^47\) Three studies\(^17,20,4\) have been performed to measure plasma or serum NT-proBNP concentration in cats with respiratory signs, and the results from each study indicate that increased NT-proBNP concentrations distinguish cats with CHF from those with noncardiac causes of dyspnea with a high degree of accuracy (Table 1). In 1 study,\(^17\) an NT-proBNP concentration > 220 pmol/L was 93.9% sensitive and 87.8% specific for detection of CHF. In another study,\(^4\) an NT-proBNP concentration > 277 pmol/L was 95% sensitive and 84.6% specific for the same disease, and in a third study,\(^20\) a value > 265 pmol/L was 90.2% sensitive and 87.9% specific. Collectively, these 3 studies involved 313 cats from the United States and Europe, and each study yielded similar results. Incorporation of an NT-proBNP assay with conventional evaluation such as radiography and physical examination improved the accuracy and confidence of general practitioners to distinguish cats with primary respiratory disease from those with CHF.\(^43\) Addition of an NT-proBNP assay to the clinical workup significantly improved the accuracy of diagnosis from 69.2% to 87.0%,\(^43\) demonstrating that measurement of plasma NT-proBNP concentration was useful when echocardiography was not immediately available. For these reasons, this assay is well-suited to the evaluation of cats with active respiratory signs such as tachypnea, dyspnea, cough, or respiratory distress in which the diagnosis of a cardiac or noncardiac etiology is uncertain and echocardiography is not readily available.

### Recommendations

Cats with respiratory distress should be stabilized on the basis of information obtained through history taking, physical examination, and any other diagnostic testing that can be performed safely (eg, ECG, radiography, thoracocentesis, echocardiography, and NT-proBNP assay). Plasma NT-proBNP concentrations > 270 pmol/L in cats with respiratory signs support CHF as the probable cause of the observed clinical signs with approximately 93% sensitivity and 87% specificity. Results at or near the cutoff values should be interpreted cautiously. Whenever possible, echocardiography should be performed to confirm the presence and identify the type of underlying heart disease. Pursuit of noncardiac causes of respiratory signs should be considered when the NT-proBNP concentration is < 270 pmol/L.

Diagnostic accuracy is improved when an NT-proBNP assay is used in conjunction with other diagnostic modalities, such as physical examination, ECG, and radiography,\(^43\) and as such, this test should not be the sole basis with which to differentiate cardiac from respiratory causes of clinical signs. When echocardiography is readily available and provides sufficient evidence of the underlying disease, NT-proBNP testing is not necessary. At the time this report was written, commercial turnaround time for reporting of plasma NT-proBNP test results limits the use of this assay in emergency situations.

Clinicians who opt for an NT-proBNP assay should consider that the diagnostic threshold for suggestion of a cardiac etiology for respiratory signs (> 270 pmol/L) is greater than the cutoff value for detection of preclinical (occult) cardiomyopathy (≥ 100 pmol/L). This indicates that cats with respiratory signs resulting from noncardiac disease, but with concurrent preclinical heart disease, might have a plasma NT-proBNP concentration between 100 and 270 pmol/L. In such cir-
Table 1—Diagnostic indications and evidence for use of a NT-proBNP assay in dogs and cats.

<table>
<thead>
<tr>
<th>Clinical question</th>
<th>Population</th>
<th>Cutoff assay value (pmol/L)</th>
<th>No. of subjects evaluated</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>Negative predictive value (%)</th>
<th>Actions based on results</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is the cause of this dog’s or cat’s respiratory signs CHF or respiratory disease?</td>
<td>Cats with respiratory signs in which CHF is possible but the diagnosis is unclear given other test results</td>
<td>220 ( ^{27} )</td>
<td>74</td>
<td>93.9</td>
<td>87.3</td>
<td>92.0</td>
<td>85.3</td>
<td>Values greater than the cutoff values indicate CHF is more likely, and values below the cutoff value indicate that CHF is less likely than primary respiratory disease.</td>
<td>Should be interpreted in conjunction with other appropriate tests (radiography or echocardiography) whenever possible. Should not be used when conventional diagnostic tools have already yielded an accurate diagnosis.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>260 ( ^{27} )</td>
<td>167</td>
<td>90.2</td>
<td>87.9</td>
<td>92.0</td>
<td>85.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>277 ( ^{27} )</td>
<td>42</td>
<td>95.0</td>
<td>84.6</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Does this cat or dog have cardiomyopathy?</td>
<td>Cats with a history, physical examination findings, or other preliminary test results suggesting an increased risk of having cardiomyopathy</td>
<td>*1,400 ( ^{48} )</td>
<td>46</td>
<td>92.0</td>
<td>85.5</td>
<td>85.5</td>
<td>81.3</td>
<td>Values greater than the cutoff values indicate CHF is more likely, and values below the cutoff value indicate that CHF is less likely than primary respiratory disease.</td>
<td>Used in human medicine primarily as a rule-out test for CHF (ie, patients with low values are very unlikely to have CHF).</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*1,158 ( ^{48} )</td>
<td>115</td>
<td>92.0</td>
<td>85.5</td>
<td>85.5</td>
<td>81.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doberman Pinschers at increased risk of DCM (&lt; 4 years of age, family history of DCM, PDK4 genetic mutation, gallop rhythm, or heart murmur on auscultation, or presence of ventricular arrhythmia)</td>
<td></td>
<td>*550 ( ^{55} ) or &gt; 50 ventricular premature contractions on Holter recording ( ^{55} )</td>
<td>328</td>
<td>78.6</td>
<td>90.4</td>
<td>87.3</td>
<td>94.7</td>
<td>Values higher than the cutoff value indicate an increased risk of echocardiographic evidence of occult DCM (ie, decreased contractility); echocardiography and Holter recording is recommended. Doberman Pinschers with high values but unremarkable echocardiographic findings might benefit from close monitoring for future development of DCM.</td>
<td>Echocardiography and Holter recording remain the gold standard for diagnosis of occult DCM. The NT-proBNP assay is poor for detection of DCM in dogs with ventricular arrhythmias but without echocardiographic changes. The highest sensitivity and specificity exist when the assay is performed in conjunction with Holter recording.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>457</td>
<td>155</td>
<td>94.5</td>
<td>87.8</td>
<td>87.3</td>
<td>94.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values are derived from studies in which the version of the canine NT-proBNP test used was one available prior to the introduction of protease tubes for sample collection.

— = Not reported.

Sensitivity is the probability of a positive test result in a patient that truly has a given condition. Specificity is the probability of a negative test result in a patient that truly is without a given condition. Positive predictive value (PPV) is the percentage of positive test results that are truly positive. Negative predictive value (NPV) is the percentage of negative test results that are truly negative. Values at or near the cutoff values should be interpreted with caution.
circumstances, a high NT-proBNP concentration can be associated with concurrent ventricular hypertrophy (ie, occult HCM), systemic hypertension, hyperthyroidism, and advanced renal dysfunction.20,31,36

Differentiation between CHF and noncardiac causes of respiratory signs in dogs—Studies32,26,48 have shown that an NT-proBNP assay can be used to differentiate between CHF and noncardiac causes of respiratory signs in dogs. In 1 study,46 21 of 21 (100%) dogs with noncardiac causes of respiratory signs had a serum or plasma NT-proBNP concentration < 800 pmol/L, whereas 23 of 25 (92%) of dogs with CHF had an NT-proBNP concentration > 1,400 pmol/L. In another study,49 an NT-proBNP concentration > 1,158 pmol/L was used to correctly diagnose CHF as the cause of respiratory signs in 83.6% of dogs. The gold standard for diagnosing CHF or respiratory disease in these studies was assimilation of clinical information obtained from the medical history and results of physical examination, thoracic radiography, and echocardiography. These studies involved an early version of the canine NT-proBNP assay that did not include the protease tubes. Their findings suggest measurement of circulating NT-proBNP concentrations can help differentiate between heart failure and noncardiac causes of existing respiratory signs in dogs when routine diagnostic tests are equivocal.

Recommendations

Dogs with respiratory distress should be managed on the basis of the information obtained through history taking, physical examination, and any other diagnostic testing that can be performed safely. Echocardiography is often helpful in this circumstance as is diagnostic thoracocentesis, if applicable, as well as monitoring of the clinical and radiographic response to trial treatment with diuretics and other cardiac drugs. A plasma NT-proBNP concentration < 800 pmol/L in dogs with respiratory signs strongly discounts the possibility of CHF and suggests that noncardiac disease is responsible. In contrast, an NT-proBNP concentration > 1,400 pmol/L increases the likelihood that CHF is the cause of clinical signs.

Plasma NT-proBNP concentration should be evaluated within the context of other diagnostic findings or comorbidities, such as pulmonary hypertension. In dogs in which CHF or respiratory disease is clearly evident from routine diagnostics, performance of an NT-proBNP assay would be of limited value and might even provide erroneous information. Studies in human emergency departments have shown that measurement of NT-proBNP concentration is most useful in patients for whom results of conventional diagnostic tests are ambiguous and that the information from the NT-proBNP assay can help steer the clinician in one direction or another.49,50

Detection of subclinical (occult) cardiomyopathy in cats—Cardiomyopathy is the most prevalent form of heart disease in cats and results in substantial morbidity and mortality rates.35 Detection of cardiomyopathy in subclinically affected cats is often difficult owing to vague physical examination findings and the limited availability and substantial expense of echocardiography.21 Moreover, myocardial diseases have been shown to be exceedingly complex, with some forms having mild structural changes or no apparent structural changes at all.17,20,32,35 Echocardiography is regarded as the gold standard for detection of cardiomyopathy in cats, but its availability might be limited because of the expense or limited expertise. The prevalence of heart murmurs in apparently healthy cats is reportedly between 13.5% and 34%.54,56 Many cats with low-intensity heart murmurs lack clinically important structural or functional heart disease,57 and NT-proBNP testing to help identify this cohort is of interest.36,38 In most situations, determination of whether occult cardiomyopathy is present via cardiac auscultation is not possible, even with the addition of ECG and thoracic radiography, which are both limited by their low diagnostic sensitivity in cats.30,39

High circulating NT-proBNP concentrations have been identified in cats with occult heart disease.16,21 Multiple independent studies have been conducted to investigate the ability of plasma or serum NT-proBNP to distinguish echocardiographically normal cats from those with occult cardiomyopathy. In total, > 560 cats from the United States,21,18 United Kingdom,23 Germany,16 and Japan10 have been evaluated, revealing that NT-proBNP concentration can distinguish healthy cats from those with occult cardiomyopathy with a reasonable degree of accuracy. In 1 report,21 an NT-proBNP concentration > 99 pmol/L was 70.8% sensitive and 100% specific in distinguishing healthy cats from cats with any form of cardiomyopathy. Another study40 revealed an NT-proBNP concentration > 95 pmol/L was 88.1% sensitive and 100% specific in discriminating healthy cats from subclinically affected cats with cardiomegaly and, finally, a third study38 found that an NT-proBNP concentration > 100 pmol/L distinguished clinically normal cats from cats with HCM with 92.4% sensitivity and 93.9% specificity. The NT-proBNP assay performed well in detecting occult cardiomyopathy in cats that had variable degrees of left ventricular hypertrophy as well as in cats with unclassified cardiomyopathy, in which left ventricular wall thickness was within reference limits.51 Findings from these studies are in contrast with those of 2 other studies,23,34 both involving a closed research colony of 40 Maine Coons, in which NT-proBNP measurements failed to reliably identify subclinical HCM. The reason for this discrepancy is not clear. The NT-proBNP assay appears best suited to distinguish cats with healthy hearts from those with moderate or severe heart disease,18,60 and this diseased group is the population most likely to benefit from additional confirmatory diagnostic testing or therapy. The most recent studies showed that a high degree of repeatability exists regarding specific cutoff values and the resultant clinical performance of the assay.18,21,40 The reliability of NT-proBNP assays as a general screening test to detect occult cardiomyopathy (ie, when used without regard to history or physical examination findings) has not been demonstrated and requires further investigation.

Recommendations

The NT-proBNP assay is most reliable when used to assess cats with a medical history or physical ex-
amination findings suggestive of heart disease, such as when siblings are known to have been affected with cardiomyopathy; physical examination includes a heart murmur, gallop rhythm, or arrhythmia; or radiographic heart enlargement is detected.

A plasma NT-proBNP concentration > 100 pmol/L suggests that morphologic cardiac changes are present, and further evaluation via echocardiography, thoracic radiography, and other diagnostic tests is needed to provide a definitive diagnosis and to determine the severity of disease. Given the reported sensitivity and specificity of the feline NT-proBNP assay, the test will occasionally yield false-positive or false-negative results. Therefore, owners should be made aware of the fact that some cats with a high NT-proBNP concentration will have not have evidence of heart disease on echocardiography.

The dependability of plasma NT-proBNP measurement to screen for cardiomyopathy in the general feline population has not been documented. The prevalence of heart disease is likely lower in this population than in the populations typically used for sample selection for studies involving cats with a suspicion of heart disease. For this reason, indiscriminate testing of NT-proBNP concentrations in a general population with a low prevalence of disease will lead to a greater possibility of a false-positive result (Figure 1). Testing does not specifically identify cats with a particular form of cardiomyopathy but rather identifies cats with morphologic cardiac changes. Echocardiography, blood pressure measurement, and thyroid hormone testing is often needed to clarify the diagnosis. Therefore, NT-proBNP testing is not a substitute for traditional cardiac diagnostics but alerts the clinician of a high likelihood of detecting clinically important structural or functional changes on echocardiography. The NT-proBNP assay is not suited as a test of breeding soundness in young healthy cats because it cannot be used to predict the potential for development of cardiomyopathy as the cat ages. In instances when a genetic mutation has been identified as associated with disease (ie, in Maine Coons with a mutation in myosin binding protein-C), genetic testing is the method of choice for evaluation of breeding soundness in young apparently healthy cats.

Screening for ODCM in dogs—Idiopathic DCM is particularly common in Doberman Pinschers, with a lifetime incidence ranging from 4% to 62% in the United States and Europe. Various other breeds are at risk for DCM, including the Irish Wolfhound, Great Dane, Newfoundland, Portuguese Water Dog, Saint Bernard, and Airedale Terrier. Boxers are predisposed to a particular form of cardiomyopathy that has been termed arrhythmogenic right ventricular cardiomyopathy. Diagnosis of dogs with ODCM (in which structural heart disease is present, but the patient has never had clinical signs) relies on the detection of ventricular arrhythmias via Holter monitoring or 3- to 5-minute ECG rhythm strips, with or without consistent morphologic abnormalities detected via echocardiographic evaluation of left ventricular dimensions, volume, and function. In high-risk breeds, yearly screening over the life of the dog is recommended but can be cost prohibitive and requires a devoted owner. Therefore, use of the NT-proBNP assay as a means to complement screening for ODCM is of interest.

The usefulness of NT-proBNP testing for the detection of DCM in Doberman Pinschers has been evaluated. In 1 study of 328 Doberman Pinschers, plasma NT-proBNP concentration was significantly higher in Doberman Pinschers with ODCM, including those with ODCM diagnosed through left ventricular systolic dysfunction or both left ventricular systolic dysfunction and arrhythmias, than in healthy dogs. The NT-proBNP assay was not clinically useful to detect disease in those dogs solely with ventricular arrhythmias. At a cutoff value of > 400 pmol/L, the sensitivity of the NT-proBNP assay for detection of left ventricular systolic dysfunction was 90.0% and specificity was 75.0%. This cutoff value is less than half the cutoff value advised by the manufacturer to distinguish cardiologically normal dogs from dogs with overt heart disease, creating somewhat of a dilemma for implementation of NT-proBNP testing for this application. Twenty-six Doberman Pinschers originally assessed as healthy by echocardiography and Holter recording developed DCM within 1.5 years after the original examination. At time of original assessment, NT-proBNP concentrations in this group were significantly greater, compared with those of dogs that did not develop DCM.

In a second study, the combined use of an NT-proBNP cutoff value > 457 pmol/L and a Holter recording led to detection of DCM with a sensitivity of 94.5%, specificity of 87.8%, and overall accuracy of 91.0%. Similar to the aforementioned study, NT-proBNP concentration was most accurate for detection of DCM when Doberman Pinschers had echocardiographic changes but had poor accuracy for identification of dogs that only had ventricular arrhythmias. Both of the aforementioned studies were performed without use of the protease inhibitor tubes for sample collection, and ideally, these studies should be repeated with the most current recommended collection and handling methods. Despite its reported usefulness, the NT-proBNP assay does not replace recommended diagnostic procedures such as echocardiographic examination wherein the sensitivity and specificity of detecting left ventricular dysfunction can be as high as 97%.

Doberman Pinschers > 4 years of age, particularly those that are known to carry mutations associated with DCM, are reasonable candidates for NT-proBNP screening. Testing could be considered when the owner cannot afford the expense of echocardiography and Holter recording. Use of the NT-proBNP assay is not indicated for evaluating Doberman Pinschers when these other established modalities are available and affordable. In Boxers, no significant difference exists in plasma BNP concentration between those with arrhythmogenic right ventricular cardiomyopathy and those that are clinically normal, which makes NT-proBNP testing in breeds other than Doberman Pinscher of unestablished value.

Recommendations

Determination of plasma NT-proBNP concentration does not replace echocardiography or Holter recording for ODCM screening. Rather, the assay could help owners and veterinarians identify dogs with a high probability of having ODCM and that would most likely benefit from further and more costly diagnostic test-
ing. In circumstances in which Holter recording is not possible, a 3- to 5-minute in-hospital ECG recording is typically performed. In circumstances when echocardiography is not readily accessible, NT-proBNP concentrations appear to have good sensitivity and specificity for predicting the presence of systolic dysfunction and would support pursuit of echocardiographic examination. Concentrations of NT-proBNP lower than the cut-off values would make ODCM less likely but would not guarantee against future development of disease.

Whether the NT-proBNP assay can be used to identify ODCM in breeds other than the Doberman Pinscher requires further investigation. It is important to note that NT-proBNP concentrations overlap in groups of dogs with and without ODCM, and false-negative and false-positive results can occur in any individual dog. For these reasons, owners should be made aware of the fact that dogs with a high NT-proBNP concentration occasionally will have no evidence of disease when echocardiography is performed.

Prediction of first-onset CHF in dogs with preclinical MMVD—Myxomatous mitral valve disease is the most common acquired heart disease of dogs. Mitral valve murmurs are detected in 11%, 24%, and 27% of dogs 5 to 8 years, 9 to 12 years, and > 13 years of age, respectively. At necropsy, virtually all dogs > 9 years of age have gross evidence of MMVD, with approximately 40% of these dogs judged as having clinically important MMVD. Many dogs with MMVD develop chronic, progressive blood volume overload that results in CHF and ultimately death, whereas in other dogs, disease progression is relatively slow.

The ability to predict which dogs with preclinical MMVD are at highest risk for developing CHF would assist clinicians in formulating monitoring, surveillance, and, potentially, treatment recommendations. No standard clinical method exists for predicting the risk of CHF development in dogs with MMVD. Clinicians mainly rely on radiographic heart size and echocardiographic measurements of left ventricular and atrial dimensions to estimate the likelihood of future CHF. Given the relationship between production of NT-proBNP and atrial and ventricular size and mechanical stress, NT-proBNP could potentially help predict risk of first-onset CHF with preclinical MMVD. In a study of 269 humans with preclinical MMVD, plasma C-BNP concentration, along with estimates of heart size and mitral regurgitation severity, were predictive of future CHF. Patients with a high C-BNP value were 4.1 times as likely to develop CHF myocardial failure, or death, compared with those with a lower value.

The usefulness of NT-proBNP concentrations to predict CHF in dogs with preclinical MMVD has been reported. In a study, 55 dogs with preclinical MMVD were followed for 12 months, and plasma NT-proBNP concentration at baseline was significantly higher in dogs that eventually developed CHF (n = 10) versus dogs that remained free of clinical signs (45). A baseline NT-proBNP concentration > 466 pmol/L was 80% sensitive and 76% specific for predicting CHF development within the next year. In a second study, 65 dogs with preclinical MMVD were monitored for a median of 335 days, during which time serial measurements of plasma NT-proBNP concentration were performed. During the study period, 30 dogs developed CHF and 35 dogs remained CHF free. At any given examination, an NT-proBNP concentration > 1,500 pmol/L and echocardiographic LVIdd:Ao > 3.0 were significantly associated with an increased risk of developing CHF over the following 3 to 6 months. If echocardiographic variables were removed from the analysis, radiographic VHS > 12 and NT-proBNP concentration > 1,500 pmol/L were significantly predictive of CHF development over the following 3 to 6 months. Overall, dogs with an NT-proBNP value > 1,500 pmol/L were between 5.8 and 9.4 times as likely to develop CHF as were dogs with lower values. For each additional increase in NT-proBNP of 730 pmol/L, the risk of future CHF increased by another 1.4 times. With regard to heart size, dogs with an LVIdd:Ao > 3.0 or VHS > 12 were 6.11 and 15.8 times as likely, respectively, to develop CHF as were dogs with lower values. Thus, echocardiographic and radiographic measurements of heart size in combination with NT-proBNP concentration identified dogs that were likely to go on to develop CHF.

Although the 2 aforementioned studies led to similar conclusions, the NT-proBNP values that indicated increased risk were quite different (> 466 pmol/L vs > 1,500 pmol/L). Reasons for this discrepancy might include a difference in the version of the NT-proBNP assay used or the difference in time between NT-proBNP measurement and outcome determination (12 months79 vs 3 to 6 months80). The rate of change in radiographic heart enlargement as well as NT-proBNP concentration appears to accelerate during the approximately 6 months immediately prior to CHF development. Thus, the greatest change in NT-proBNP concentration is expected to occur in the interval immediately preceding CHF; however, the exact extent of change and the period during which the change develops requires additional evaluation.

Recommendations

Use of the NT-proBNP assay is recommended for dogs with subclinical MMVD and radiographic or echocardiographic evidence of heart enlargement. A plasma NT-proBNP concentration > 1,500 pmol/L, VHS > 12, or LVIdd:Ao > 3.0 indicates an increased risk for developing CHF within the subsequent 3 to 6 months. Meeting a combination of these factors signifies greater risk than any 1 factor alone. Increased owner vigilance for subtle changes that would signal development of CHF, such as changes in respiratory rate and effort or activity, is recommended. For dogs identified as at high risk, the authors routinely counsel owners to monitor their dog's respiratory rate while it is resting or sleeping and to contact their veterinarian if the rate increases to greater than a prespecified value. In dogs with MMVD, rates > 40 breaths/min are suggestive of CHF. In dogs at high risk of CHF, the clinician might also shorten the period between routine reexaminations with the intent of catching early clinical or radiographic signs of CHF. Whether medical treatment decisions should be made solely on the basis of NT-proBNP concentration, however, requires further evaluation and is not recommended at this time.
A high circulating NT-proBNP concentration is a surrogate marker for an increase in left atrial and ventricular size and wall stress. Radiographic detection of pulmonary edema remains the gold standard for clinical diagnosis of CHF and indicates the need to initiate medical treatment effective in reducing clinical signs and improving outcome. Neither NT-proBNP testing nor echocardiography should be considered a substitute for thoracic radiography when attempting to diagnose CHF. In dogs with a systolic heart murmur over the mitral valve region, absence of radiographic or echocardiographic evidence of left heart enlargement greatly reduces the clinical usefulness of the NT-proBNP assay because in the absence of heart enlargement, the risk for developing CHF in the next 6 months is relatively low.

Prognostication in dogs with acquired cardiovascular disease—Many studies have shown that natriuretic peptide concentrations increase with advancing MMVD severity and that blood natriuretic peptide concentrations correlate with measurements of heart size and heart failure stage. Dogs with clinical signs of heart failure have higher NT-proBNP concentrations than those without those clinical signs. High concentrations of these peptides are associated with more advanced cardiac disease and a worse prognosis. Six studies have demonstrated associations between NT-proBNP or atrial natriuretic peptide and outcomes in dogs with MMVD. In 1 study, a plasma NT-proBNP concentration > 1,500 pmol/L was a predictor of death in the subsequent 6 months; however, this study was limited by the lack of time-to-event analysis. In 2 studies involving overlapping cohorts of dogs, serum NT-proBNP values predicted outcome when considered as a continuous and an ordinal (low, medium, and high) variable. Another study found the median all-cause survival time for dogs with an NT-proBNP concentration > 739 pmol/L (318 days) was significantly shorter than that for dogs with an NT-proBNP concentration > 391 but ≤ 739 pmol/L (786 days). Fewer than 50% of dogs with concentrations ≤ 391 pmol/L died; therefore, a median survival time could not be estimated for that group.

The combination of serum NT-proBNP concentration > 524 pmol/L and serum cardiac troponin-I concentration > 0.025 ng/mL was identified as indicating a higher risk of death than in dogs with lower values in a different study. For each 100 pmol/L increase in NT-proBNP concentration, the risk of death due to cardiac problems increased by 7%. In a study of dogs with newly diagnosed CHF caused by MMVD, NT-proBNP concentration was measured at the time of CHF detection as well as 7 to 30 days after successful treatment. Dogs in which the NT-proBNP concentration was < 965 pmol/L at the time of reexamination survived longer than did dogs with greater concentrations, and the NT-proBNP concentration at reexamination was a better predictor of cardiac survival than was the NT-proBNP concentration at the time of CHF detection.

The concentration of circulating NT-proBNP is high in dogs with DCM and highest in dogs in the clinical stage of the disease, compared with dogs with ODCM. A study in which conventional survival analysis was not performed found that NT-proBNP concentrations were higher in dogs with DCM that died in the 60 days after NT-proBNP testing, compared with those in dogs that survived during that period. In another study, the median survival time of Doberman Pinschers with DCM and an NT-proBNP concentration > 900 pmol/L (284 days) was significantly shorter than for dogs with an NT-proBNP concentration < 900 pmol/L (1,743 days). The amount of data supporting prognostic value in dogs with DCM is less than that in dogs with MMVD.

Recommendations

Testing of NT-proBNP concentration in dogs with MMVD provides additional insight relative to prognosis and risk of death. Dogs with a high NT-proBNP concentration might benefit from close monitoring including respiratory rate, repeated physical examination, or other testing. Concurrent measurement of serum cardiac troponin-I concentration with a high-sensitivity assay and radiographic and echocardiographic determination of heart size in addition to NT-proBNP testing provides more information than any test alone. The usefulness of NT-proBNP testing to estimate prognosis in dogs with DCM requires additional study.

Caution should be taken in applying a predictive test that has been validated in a defined population to a different population or to an individual animal. Plasma NT-proBNP results should be considered within the context of information gleaned from medical history taking, physical examination, and clinical tests. Rather than concluding that the test result provides evidence of certain impending death and poor prognosis, veterinary clinicians should consider that dogs with higher NT-proBNP concentrations are members of a population at greater risk and therefore warrant closer scrutiny than those with lower concentrations. No evidence exists to suggest that treatment can be effectively initiated or modified on the basis of any given NT-proBNP concentration or that high NT-proBNP concentrations should be used as a basis for decisions regarding euthanasia.

Future Directions

In this report, the existing literature on use of NT-proBNP testing in veterinary patients has been reviewed and recommendations made. Despite the large amount of research into the clinical usefulness of this testing in dogs and cats, several opportunities remain for additional investigation.

One of the most intriguing uses of the NT-proBNP assay involves the concept of NT-proBNP–guided interventions. In veterinary medicine, it has yet to be established whether medical strategies to decrease patients’ NT-proBNP concentrations to lower than a prespecified value would lead to a decrease in morbidity and mortality rate and improvement in quality of life. Studies in humans have found that strategies to manage CHF that achieved an approximately 30% decrease in pre-treatment NT-proBNP concentrations resulted in a decreased hospital readmission rate and longer survival. Two large meta-analyses revealed that natriuretic peptide–guided treatment of human heart failure leads
to better outcomes, although these results are controversial. Little comparative data are available for dogs, although a small pilot study showed that serial NT-proBNP concentrations predicted the clinician's decision to modify a patient's medications on the basis of their clinical assessment.

Whether testing of multiple biomarkers of heart disease is better than testing of only 1 is unknown. The NT-proBNP assay should be used in concert with a complete clinical assessment, including medical history collection, physical examination, thoracic radiography, echocardiography, or other relevant testing. In humans, combined use of the NT-proBNP assay with assays of other cardiac biomarkers improves predictive ability and potentially widens the applicability of these tests. In dogs, 1 study revealed that the combined use of serum NT-proBNP and high-sensitivity cardiac troponin-I assays improved the accuracy of risk assessment, compared with the use of either biomarker assay alone.

The question arises as to whether point-of-care or cage-side NT-proBNP testing is feasible. This method of testing would reduce or eliminate the need for special sample collection, handling, and shipping; make rapid biomarker analysis readily available; and assist diagnostic and management strategies in unstable patients. A pilot study revealed that a feline-specific point-of-care NT-proBNP ELISA test could be used to help detect occult cardiomyopathy in cats. Further development of commercial assays and related study is required.

Biomarker testing is an emerging and promising field for veterinary medicine. Optimal use of the NT-proBNP assay in clinical practice requires careful patient selection and test interpretation. As a diagnostic test, NT-proBNP assays should be considered as an adjunct to existing diagnostic tools. The concept of biomarker assay-guided treatment is intriguing but requires validation through clinical trials. Optimal patient diagnosis and management remains reliant on the synthesis of data from the medical history, physical examination, and selected diagnostic tests to promote timely and cost-effective diagnosis and prognosis and favorable outcomes.

References


