Assessment of serum N-terminal pro-B-type natriuretic peptide concentration for differentiation of congestive heart failure from primary respiratory tract disease as the cause of respiratory signs in dogs

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Objective—To determine whether serum N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentration is useful in discriminating between cardiac and noncardiac (ie, primary respiratory tract disease) causes of respiratory signs (ie, coughing, stertor, stridor, excessive panting, increased respiratory effort, tachypnea, or overt respiratory distress) in dogs.

Design—Multicenter cross-sectional study.

Animals—115 dogs with respiratory signs.

Procedures—Dogs with respiratory signs were solicited for study. Physical examination, thoracic radiography, and echocardiography were used to determine whether respiratory signs were the result of cardiac (ie, congestive heart failure) or noncardiac (ie, primary respiratory tract disease) causes. Serum samples for NT-proBNP assay were obtained at time of admission for each dog. Receiver-operating characteristic curves were constructed to determine the ability of serum NT-proBNP concentration to discriminate between cardiac and noncardiac causes of respiratory signs.

Results—Serum NT-proBNP concentration was significantly higher in dogs with cardiac versus noncardiac causes of respiratory signs. In dogs with primary respiratory tract disease, serum NT-proBNP concentration was significantly higher in those with concurrent pulmonary hypertension than in those without. A serum NT-proBNP cutoff concentration > 1,158 pmol/L discriminated between dogs with congestive heart failure and dogs with primary respiratory tract disease with a sensitivity of 85.5% and a specificity of 81.3%.

Conclusions and Clinical Relevance—Measuring serum NT-proBNP concentration in dogs with respiratory signs helps to differentiate between congestive heart failure and primary respiratory tract disease as an underlying cause. (*J Am Vet Med Assoc* 2009;235:1319–1325)

Differentiating between causes of respiratory distress in animals that have acute signs is challenging. Clinical signs are generally nonspecific and can result from congestive heart failure as well as primary respiratory tract disease. Data gathered through medical history, physical examination, and thoracic radiography can be ambiguous or nonconfirmatory. Differentiation of the common causes of respiratory signs (ie, coughing, stertor, stridor, excessive panting, increased respiratory effort, tachypnea, or overt respiratory distress) is essential to optimize management and outcome. Thus, a blood-based assay to differentiate between cardiac versus noncardiac (ie, primary respiratory tract disease) causes of respiratory signs would be useful.

Abbreviations

AUC_{ROC}	Area under the receiver-operating characteristic curve
C-BNP	C-terminal B-type natriuretic peptide
CI	Confidence interval
IQR	Interquartile range
LA:Ao	Left atrial-to-aortic root dimension ratio
LVIDd	Left ventricular internal dimension at end-diastole
LVIDs	Left ventricular internal dimension at end-systole
NT-proBNP	N-terminal pro-B-type natriuretic peptide
ROC	Receiver-operating characteristic
VHS	Vertebral heart size

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N-terminal pro-B-type natriuretic peptide is a cardiac biomarker that is increased in the blood of dogs with heart disease.[1,2] N-terminal pro-B-type natriuretic peptide is formed when its parent pro-hormone, pro-B-type natriuretic peptide, is cleaved into 2 molecules, NT-proBNP and C-BNP. The pro-B-type natriuretic peptide is produced in response to an increase in intracardiac hydrostatic pressure, increased cardiac wall stress, angiotensin II, myocardial hypoxia, and heightened sympathetic tone.[3] As such, both serum NT-proBNP and C-BNP concentrations represent potential diagnostic tools to help diagnose congestive heart failure.[4,5] In humans, C-BNP[6–8] and NT-proBNP[9–11] assays possess high sensitivity and specificity in differentiating between cardiac and pulmonary causes of respiratory distress. In circulation, C-BNP is rapidly degraded,[12] making laboratory testing difficult. Commercially available tests in dogs and cats exclusively detect NT-proBNP, which is thought to possess a longer half-life.

Results of a previous study[1] indicate that dogs with respiratory signs resulting from congestive heart failure have significantly higher serum NT-proBNP concentrations than dogs with primary respiratory tract disease. The magnitude of difference between the 2 populations was such that the serum NT-proBNP concentration provided a clinically useful test with an adequate sensitivity and specificity for differentiating between the 2 populations. However, all dogs did not undergo the same set of diagnostic tests, and a substantial proportion of the study population lacked both evaluation of thoracic radiographs and an echocardiographic examination. Thus, the usefulness of this peptide as an adjunct diagnostic test merits closer evaluation. We hypothesized that serum NT-proBNP concentration would be higher in dogs with congestive heart failure than in dogs with primary respiratory tract disease and that serum NT-proBNP concentration would help to differentiate between cardiac and noncardiac causes of respiratory signs in dogs. The purpose of the multicenter study reported here was to assess the clinical sensitivity and specificity of a serum NT-proBNP assay to differentiate between cardiac versus noncardiac (ie, primary respiratory tract disease) causes of respiratory signs, and to determine an optimal cutoff value that would facilitate this assessment.

Materials and Methods

Animals—Study procedures were approved by the institutional animal use and care committees of each qualifying participating institution. Owner consent was obtained for all dogs included in the study. Fourteen veterinary cardiology practices prospectively recruited dogs between September 2006 and November 2007. Dogs were eligible for inclusion if the owner reported a complaint of respiratory signs that were severe enough to affect the dog's quality of life. Qualifying signs included coughing, stertor, stridor, excessive panting, increased respiratory effort, tachypnea, or overt respiratory distress. Dogs were excluded if respiratory signs were caused by obvious trauma (eg, vehicular trauma).

Assessment of disease—All dogs underwent thoracic radiography and M-mode, 2-D, and Doppler echocardiography. Vertebral heart size was measured on the left or right lateral radiographic projection of the thorax by the attending cardiologist as previously described.[13,14] Additional diagnostic testing to help determine the underlying cause of the respiratory signs (eg, fluoroscopy, tracheal wash, and bronchoscopy) was performed at the discretion of the attending clinician. The LVIDd, LVIDs, and LA:Ao were measured by means of standard echocardiographic techniques.[15,16] Detection of tricuspid regurgitation by use of echocardiography was recorded. Pulmonary hypertension was arbitrarily defined as a velocity of tricuspid regurgitation > 3.25 m/s (pressure gradient > 42.3 mm Hg). In the absence of tricuspid regurgitation, a presumptive echocardiographic diagnosis of pulmonary hypertension was made in dogs with a combination of severe right ventricular hypertrophy or dilation, flattened interventricular septum, and an enlarged main pulmonary artery, in the absence of increased right ventricular to pulmonary artery outflow velocity.

A standardized patient datasheet was used to record patient signalment, clinical signs, and diagnostic findings. On the basis of this workup, and without knowledge of the NT-proBNP assay results, a board-certified veterinary cardiologist assigned the dog to 1 of 4 groups as follows: group 1 included dogs with congestive heart failure (findings of severe cardiac disease, perihilar or caudodorsal pulmonary interstitial pattern, cardiomegaly, enlarged pulmonary veins, pleural effusion, and ascites) and without primary respiratory tract disease; group 2 included dogs with primary respiratory tract disease (parenchymal lung disease [findings of cranioventral or lobar alveolar pattern, lung lobe torsion, and bullae] or upper airway disease [findings of laryngeal paralysis, brachycephalic syndrome, collapsing trachea, and moderate or severe bronchiolar pulmonary pattern]) and without underlying cardiac disease (no heart murmur and no evidence of clinically relevant left-sided cardiac disease on echocardiography); group 3 included dogs with primary respiratory tract disease (as described for group 2 dogs) that also had heart disease that was not congestive heart failure (detection of heart murmur or cardiac disease on echocardiography without evidence of congestive heart failure on thoracic radiography, physical examination, or other diagnostic tests); and group 4 included dogs for which the etiology of respiratory signs could not be reliably ascribed to either respiratory tract disease or congestive heart failure.

Measurement of serum NT-proBNP concentration—Venous blood samples were collected at the time of admission. Blood was drawn into plain evacuated glass tubes that did not contain any additives. Samples were centrifuged within 60 minutes after collection, and serum was stored at –20°C prior to batched overnight shipment for analysis. Samples were shipped with cold packs and packing materials provided by the assay manufacturer. Serum NT-proBNP concentration was determined with a commercially available canine-specific NT-proBNP assay[a] as previously described.[2]

Statistical analysis—Data were summarized as median (IQR [ie, 25th to 75th percentile]) or mean ±

SD. Multiple group comparisons of radiographic, echocardiographic, and NT-proBNP data were performed via Kruskal-Wallis analysis and the Dunn multiple comparison tests. The Spearman method was used to test for correlations between serum NT-proBNP concentration and body weight, age, and radiographic and echocardiographic heart size. Multivariate linear regression was performed to identify whether these variables were associated with serum NT-proBNP concentration; cutoff *P* values for entry into and removal from the multivariate model were < 0.05 and > 0.10, respectively. Receiver-operating characteristic curves were constructed to determine the sensitivity, specificity, positive and negative predictive values, accuracy (defined as the sum of the concordant results divided by the sum of all results in a 2 × 2 table), and AUC_{ROC} of NT-proBNP results, compared with the cardiologist's diagnosis. All analyses were performed with standard software.[b,c] Values of $P < 0.05$ were considered significant.

Results

Animals—One hundred ninety-six dogs from 14 sites were entered into the study (University of Pennsylvania, n = 59 dogs; Tufts University, 59; The Animal Medical Center, 11; Oregon State University, 10; Texas A&M University, 9; Chesapeake Veterinary Cardiology Associates, 8; Veterinary Cardiology Consultants, 8; Advanced Veterinary Care Center, 6; Angell Animal Medical Center, 6; MedVet Medical & Cancer Center for Pets, 6; University of Florida, 5; Cornell University, 4; Fifth Avenue Veterinary Specialists, 3; Heartsound Consultants, 2). Complications involving shipping of serum samples resulted in the loss of the first 81 samples obtained. These samples were shipped according to original manufacturer recommendations but arrived at the laboratory at room temperature (approx 23°C). Following this incident, the manufacturer changed its shipping recommendations to ensure transport and overnight arrival at 4°C. This involved use of extra cold packs and heavily insulated shipping containers, and the following 115 samples shipped in this fashion were used in the study analysis. Fifteen of the 115 (13%) dogs were of mixed breeding, 9 (8%) were Cavalier King Charles Spaniels, 6 (5%) were Shih Tzus, 5 (4%) were Cocker Spaniels, and 5 (4%) were Chihuahuas; the remaining dogs represented 41 other breeds. Forty (35%) dogs were spayed females, 4 (3%) were sexually intact females, 61 (53%) were neutered males, and 10 (9%) were sexually intact males. Clinical signs of respiratory problems were recorded for the 196 originally enrolled dogs as follows: 76 instances of coughing, 46 instances of moderate tachypnea or dyspnea, 27 instances of mild tachypnea or dyspnea, 24 instances of excessive panting, 19 instances of severe tachypnea or dyspnea, and 4 instances of stertor or stridor. Thirty-seven of the 115 (32%) dogs had only 1 clinical sign, 69 (60%) had 2 clinical signs, and 9 (8%) had ≥ 3 clinical signs.

Etiology of respiratory signs—On the basis of findings on physical examination, thoracic radiography, and echocardiography, 62 of the 115 (54%) dogs were placed into group 1, 21 (18%) into group 2, 27 (23%) into group 3, and 5 (4%) into group 4. For the 62 group 1 dogs, underlying causes of congestive heart failure included chronic degenerative mitral valve disease (44/62 [71%]), dilated cardiomyopathy (13/62 [21%]), congenital heart disease (2/62 [3%]), endocarditis (1/62 [2%]), heart block (1/62 [2%]), and arrhythmogenic right ventricular cardiomyopathy (1/62 [2%]). For the 21 group 2 dogs, primary respiratory tract diseases included small airway disease (8/21 [38%]), upper airway disease including collapsing trachea (5/21 [24%]), thoracic or pulmonary neoplasia (3/21 [14%]), pneumonia (3/21 [14%]), lung lobe torsion (1/21 [5%]), and noncardiogenic pulmonary edema (1/21 [5%]). For the 27 group 3 dogs, primary respiratory tract diseases included small airway disease (12/27 [44%]), upper airway disease including collapsing trachea (8/27 [30%]), pneumonia (4/27 [15%]), pulmonary or thoracic neoplasia (2/27 [7%]), and suspected pulmonary thromboembolism (1/27 [4%]). For the 27 group 3 dogs, concurrent underlying heart diseases included degenerative mitral valve disease (25/27 [93%]), severe tricuspid valve disease (1/27 [4%]), and left ventricular hypertrophy resulting from systemic hypertension (1/27 [4%]). Group 4 included 5 dogs with various combinations of severe pulmonary hypertension, parenchymal pulmonary disease, severe degenerative valvular disease, pericardial effusion, myocardial failure, and ventricular arrhythmias. Because of the inability to definitively diagnose the etiology of respiratory signs in group 4 dogs, they were excluded from further analysis.

Vertebral heart size, LVIDd, and LVIDs indexed to body weight were significantly different among groups. Median (IQR) VHS was 12.3 (11.5 to 13.0), 10.5 (10.0 to 11.0), and 11.0 (10.0 to 11.5) for group 1, 2, and 3 dogs ($P < 0.001$), respectively. Median VHS was significantly different between group 1 and 2 dogs ($P < 0.001$) as well as between group 1 and 3 dogs ($P < 0.001$). Median (IQR) LVIDd indexed to body weight was 0.355 (0.216 to 0.534), 0.214 (0.136 to 0.317), and 0.303 (0.231 to 0.451) for group 1, 2, and 3 dogs ($P = 0.005$), respectively. Median LVIDd was significantly different between group 1 and 2 dogs ($P = 0.001$) as well as between group 2 and 3 dogs ($P = 0.015$). Median LVIDs indexed to body weight was 0.196 (0.151 to 0.283), 0.095 (0.078 to 0.168), and 0.183 (0.135 to 0.235) for group 1, 2, and 3 dogs ($P < 0.001$), respectively. Median LVIDs indexed to body weight was significantly different between group 1 and 2 dogs ($P < 0.001$) as well as between group 2 and 3 dogs ($P = 0.003$). Median (IQR) LA:Ao was 2.24 (1.90 to 2.79), 1.29 (1.15 to 1.49), and 1.40 (1.19 to 1.70) for group 1, 2, and 3 dogs ($P < 0.001$), respectively. Median LA:Ao was significantly different between group 1 and 2 dogs ($P < 0.001$) as well as between group 1 and 3 dogs ($P < 0.001$).

Serum NT-proBNP concentrations—Median serum NT-proBNP concentration was significantly ($P < 0.001$) different among groups. Median (IQR) serum NT-proBNP concentration of group 1 dogs (2,445 pmol/L [1,499 to 3,134 pmol/L]) was significantly higher than in group 2 (413 pmol/L [245 to 857 pmol/L]; $P < 0.001$) and group 3 (478 pmol/L [323 to 1,158 pmol/L]; $P < 0.001$) dogs. Median serum NT-proBNP concentration between group 2 and 3 dogs was not significantly different (**Figure 1**).

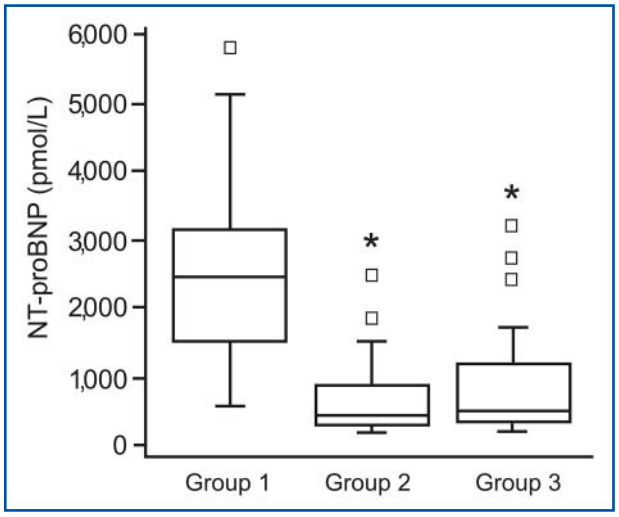

Figure 1—Box and whisker plot of serum NT-proBNP concentration in dogs in which the etiology of respiratory signs is congestive heart failure (group 1; n = 62), primary respiratory tract disease (group 2; 21), and respiratory tract disease with concurrent heart disease (group 3; 27). For each plot, the box represents the IQR, the horizontal line in the middle of the box represents the median, and the whiskers denote the range extending to 1.5 times the IQR from the upper and lower quartiles. Outlier values between 1.5 to 3.0 times the IQR are denoted as squares. *Significantly ($P < 0.005$) different from value for group 1 dogs.

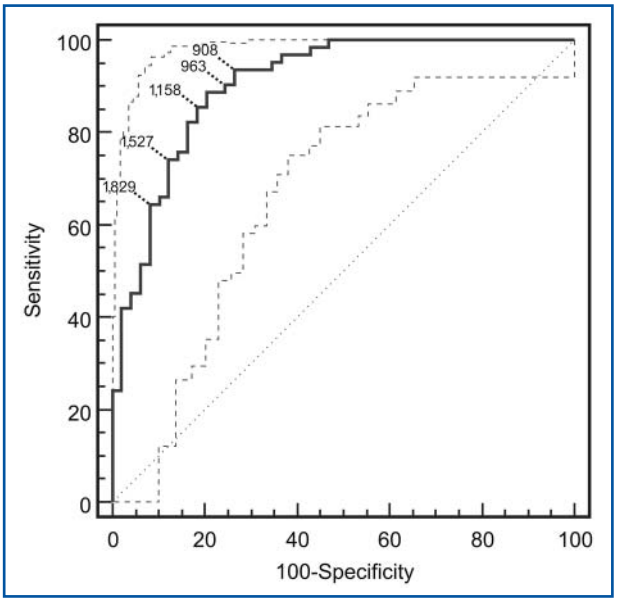

Figure 2—The ROC curve displaying the sensitivity and specificity of serum NT-proBNP concentration to distinguish between cardiac and noncardiac (ie, primary respiratory tract disease) as the cause of respiratory signs in 110 dogs (solid line). The 95% CIs are displayed as the dashed lines. The diagonal dotted line represents the line of no discrimination. Various potential diagnostic cutoff values (pmol/L) are indicated along the curve. $AUC_{ROC} = 0.905$.

Tricuspid regurgitation was detected in 34 (34/62 [55%]), 5 (5/21 [24%]), and 18 (18/27 [67%]) group 1, 2, and 3 dogs, respectively. A diagnosis of pulmonary hypertension was made for 16 (16/62 [26%]) group 1 dogs (mean ± SD tricuspid regurgitation velocity, 3.78 ± 0.49 m/s; n = 16), 7 (7/21 [33%]) group 2 dogs (4.69 ± 0.89 m/s; 5), and 5 (5/27 [19%]) group 3 dogs (4.27 ± 0.60 m/s; 5). Median (IQR) serum NT-proBNP concentration in group 2 dogs with pulmonary hypertension was significantly ($P = 0.002$) higher than in group 2 dogs without pulmonary hypertension (1,028 pmol/L [559 to 1,829 pmol/L] vs 309 pmol/L [196 to 461 pmol/L], respectively). In contrast, median (IQR) serum NT-proBNP concentration in group 1 dogs with pulmonary hypertension was not significantly ($P = 0.41$) higher than in group 1 dogs without pulmonary hypertension (2,078 pmol/L [1,484 to 3,071 pmol/L] vs 2,541 pmol/L [1,539 to 3,141 pmol/L], respectively). Also, median (IQR) serum NT-proBNP concentration in group 3 dogs with pulmonary hypertension was not significantly ($P = 0.81$) higher than in group 3 dogs without pulmonary hypertension (478 pmol/L [348 to 1,616 pmol/L] vs 504 pmol/L [301 to 1,107 pmol/L], respectively).

Univariate regression analysis revealed that serum NT-proBNP concentration was significantly but poorly correlated to body weight ($r = 0.21$; $P = 0.029$), VHS ($r = 0.55$; $P < 0.001$), LVIDs indexed to body weight ($r = 0.27$; $P = 0.021$), and LA:Ao ($r = 0.61$; $P < 0.001$). Multiple regression analysis revealed that only LA:Ao (β coefficient = 757.9; $P < 0.001$) and VHS (β coefficient = 218.3; $P = 0.043$) were correlated with serum NT-proBNP concentration.

Receiver-operating characteristic curve analysis—Serum NT-proBNP concentrations > 1,158 pmol/L differentiated group 1 dogs from group 2 and 3 dogs with a sensitivity of 85.5%, specificity of 81.3%, positive predictive value of 85.5%, negative predictive value of 81.3%, and accuracy of 83.6%. The AUC_{ROC} was 90.5% (95% CI, 83.4% to 95.2%; **Figure 2**). Additional cutoff values for serum NT-proBNP that yielded either a sensitivity > 90% or a specificity > 90% were identified (NT-proBNP > 963 pmol/L [sensitivity, 90.3%; specificity, 73.5%]; NT-proBNP > 1,829 pmol/L [sensitivity, 64.5%; specificity, 91.7%]). The AUC_{ROC} was 93.0% (95% CI, 85.2% to 97.4%) when differentiating between group 1 dogs and group 2 dogs and was 88.5% (95% CI, 79.9% to 94.3%) when differentiating between group 1 dogs and group 3 dogs.

Discussion

Our results indicate that serum NT-proBNP concentration in dogs with respiratory signs helps to differentiate between congestive heart failure and primary respiratory tract disease as an underlying cause. Our results are consistent with those of other studies[1,17–19,d] that have evaluated circulating C-BNP and NT-proBNP concentrations in dogs with respiratory tract disease. DeFrancesco et al[17] reported that plasma C-BNP concentration had a sensitivity of 90% and a specificity of 78% in differentiating between 101 dogs with congestive heart failure and 78 dogs with respiratory tract disease. Prosek et al[18] reported that plasma C-BNP concentration had a sensitivity of 86.4% and a specificity of 80.8% in differentiating between 22 dogs with congestive heart failure and 26 dogs with respiratory tract disease. Wess et al[d] reported that a plasma NT-proBNP concentration > 520 pmol/L had a sensitivity of 94.7% and a specificity of 96.2% in differentiating between 19 dogs with cardiac disease and 57 dogs with respiratory tract disease. In a study of 46 dogs, Fine et al[19] reported that a serum or plasma NT-proBNP concentration > 1,400 pmol/L detected 92% of dogs with heart fail-

ure. Boswood et al[1] reported that a plasma NT-proBNP concentration > 210 pmol/L had a sensitivity of 85.5% and a specificity of 82.4% in differentiating between dogs with cardiac disease and those with respiratory tract disease. Thus, our results are in general agreement with those of other studies. Our study was unique in its large multicentered design and the requirement that all dogs undergo both thoracic radiography and echocardiography as part of their diagnostic workup. In our study as well as in others in humans[20–22] and dogs,[2,23] serum NT-proBNP concentration was correlated with echocardiographic and radiographic measures of cardiac enlargement.

Our results as well as those of Fine et al[19] indicate a higher diagnostic cutoff value for serum NT-proBNP concentration than those of studies by Boswood et al[1] and Wess et al[d]; the reason for the discrepancy is unclear. Results of previous studies have revealed ranges of circulating NT-proBNP concentrations in both healthy dogs[2,24] and dogs with heart disease or respiratory tract disease[2,24,25] that are higher than those initially reported by Boswood et al.[1] It is interesting to note that the lower circulating NT-proBNP concentrations were reported from studies[1,d] in Europe, and differences in the study population or sample handling may be responsible for the variation in values. In humans, NT-proBNP, while relatively stable, compared with C-BNP, degrades if samples are left at room temperature,[26] and careful attention to sample collection, handling, and shipping is required. To the authors' knowledge, the in vitro stability of canine NT-proBNP has not been reported; however, our experience with shipping protocols indicates that NT-proBNP degrades during overnight shipping if not maintained at 4°C. Future studies are needed to further evaluate different handling and shipping protocols before wide-ranging clinical recommendations regarding NT-proBNP assay results can be made.

The clinical usefulness of diagnostic assays can be compared among various studies[27] by examining results of ROC curve analysis. Inspection of the ROC curve indicates assay sensitivity and specificity at any given cutoff value. The AUC_{ROC} reflects the discriminatory ability of the test with 50% indicating no ability and 100% indicating perfect ability to discriminate between conditions. To be clinically useful, diagnostic tests should possess an AUC_{ROC} of at least 75% to 85%.[27] Our study yielded an AUC_{ROC} of 90.5%, which is comparable to results from 3 of the largest studies[9,10,28] in humans. Maisel et al[28] reported that plasma or whole blood C-BNP concentration had a 90% sensitivity, a 76% specificity, and an AUC_{ROC} of 91% for predicting the presence or absence of congestive heart failure in a cohort of 1,586 patients with shortness of breath. Januzzi et al[9] reported that a circulating NT-proBNP concentration > 900 pg/mL had an 87% sensitivity, an 86% specificity, and an AUC_{ROC} of 94% for predicting acute congestive heart failure in 600 patients with dyspnea, while Moe et al[10] reported that, compared with traditional evaluation without the use of biomarker assays, use of a NT-proBNP assay increased the AUC_{ROC} from 83% to 90% for predicting acute heart failure in 500 patients presenting to the emergency room with dyspnea. In humans, natriuretic peptide testing is currently recommended as one of the first clinical steps in evaluating patients suspected of having heart failure.[29] Use of NT-proBNP assay in these patients is strongly associated with shortened hospital stay, lower rate of rehospitalization, and reduced financial cost.[8,10]

In our study as well as in others,[9,11,28,30] the diagnostic gold standard was the clinical evaluation by a board-certified cardiologist of a uniform set of diagnostic tests, including physical examination, thoracic radiography, and echocardiography. Our study included a diagnostic category for instances where a definitive clinical diagnosis could not be confidently made (group 4), and it is unlikely that large numbers of misdiagnoses were made in groups 1 through 3. Despite this protocol, differentiation of cardiac versus respiratory tract disease in dogs can be challenging, and it is possible that misdiagnoses were made in our study, especially in dogs with concurrent heart and respiratory tract disease. If so, this may have affected the sensitivity and specificity that were determined for the NT-proBNP assay. One potential limitation of our study involves the use of secondary and tertiary referral centers from which patients were recruited. This may introduce bias into the study's patient population; however, workup by cardiology specialists performing echocardiography was an integral part of the study and necessitated use of this particular patient population.

A diagnostic assay has potential clinical value if the current means of diagnosis possesses accuracy lower than that afforded by the assay. In our study, the accuracy of NT-proBNP assay was 83.6%. In humans, the accuracy of correctly diagnosing congestive heart failure by emergency room physicians is surprisingly low, ranging from 60% to 75%, and missed diagnoses contribute to the in-hospital mortality rate.[27,31] In one of the largest studies,[7] involving 1,538 patients admitted to the emergency room with congestive heart failure, accuracy of diagnosis by the primary physician was only 74%. This accuracy increased to 81.5% if circulating NT-proBNP concentrations were used in conjunction with the primary physician's assessment. To our knowledge, the accuracy of diagnosing congestive heart failure in dogs by veterinarians in private practice has not been reported. This variable should be explored against NT-proBNP–assisted workup in future studies.

The clinical performance of a diagnostic test is a competing balance between sensitivity and specificity. Gains in sensitivity are made at the expense of specificity and vice versa. The ROC curve analysis allows identification of various cutoff values, which maximize either parameter. In our study, lowering the cutoff value to 963 pmol/L increased sensitivity to > 90%, while increasing the cutoff value to 1,829 pmol/L increased specificity to > 90%. In previous studies in dogs[2] and humans,[27,27,32] an intermediate gray zone, in which assay results are less reliable, has been proposed. In a population of dogs with mitral valve disease and dilated cardiomyopathy,[2] a serum NT-proBNP concentration > 1,725 pmol/L or < 820 pmol/L yielded good sensitivity and specificity for discriminating between dogs with and without congestive heart failure; however, the clinical usefulness of serum NT-proBNP concentrations between those 2 values was limited. In humans, NT-proBNP clinical algorithms

use subjective diagnostic terminology, such as unlikely, possible, and very likely, to convey assay results.[28,29,33] Results that fall into the possible category automatically trigger additional diagnostic investigation to increase (or decrease) the confidence of diagnosis. These algorithms underscore the diagnostic limitations of natriuretic peptide assay in both humans and dogs, and NT-proBNP assay results should complement and not replace the medical history, physical examination, and conventional diagnostic testing.

In our study, most of the patient population comprised geriatric small-breed dogs, and as such, many dogs had concurrent cardiac and respiratory tract disease or pulmonary hypertension. Results of previous studies[2,24] indicate that, although circulating NT-proBNP concentration is generally low in dogs with asymptomatic heart disease, it can be markedly high in individual dogs. These high concentrations would decrease the discriminatory ability of NT-proBNP in dogs with concurrent cardiac and respiratory tract disease. Indeed, in our study, the discriminatory power of NT-proBNP was lower in distinguishing between group 1 and 3 dogs versus between group 1 and 2 dogs. Our results indicate that serum NT-proBNP concentration is high in dogs with respiratory tract disease and pulmonary hypertension. In humans, NT-proBNP is produced by the right ventricle in response to myocyte stress,[34,35] and although the high plasma NT-proBNP concentrations are generally lower than for diseases that primarily affect the left ventricle,[36] serum NT-proBNP concentration is correlated with severity of right ventricular dysfunction and pressure overload.[33–36] In our study, dogs with primary respiratory tract disease and pulmonary hypertension had a 3-fold increase in the median serum NT-proBNP concentration, compared with dogs with respiratory tract disease without pulmonary hypertension. This phenomenon may contribute to a likelihood of false-positive result for congestive heart failure (ie, the diagnosis of congestive heart failure in dogs with primary respiratory tract disease as the cause of respiratory signs). This concept of false-positive result for congestive heart failure on the basis of NT-proBNP assay results in patients with respiratory and cardiac disease is intriguing. On one hand, the production of NT-proBNP in response to pulmonary hypertension confounds the determination of the cause of respiratory signs. On the other hand, assay results in this population should not be summarily discounted, as NT-proBNP is strongly correlated to mortality and morbidity rates. In human patients with pulmonary hypertension, a serum NT-proBNP concentration > 1,400 pmol/L had a sensitivity of 100% for predicting the 3-year mortality rate and, in this respect, was superior to other parameters, such as exercise test results, pulmonary vascular resistance, functional clinical class, and cardiac index.[37] Thus, NT-proBNP assay results may have important prognostic value in dyspneic patients despite their potential to confound diagnosis of etiology.

In conclusion, serum NT-proBNP concentration in dogs with respiratory signs in the present study helped to differentiate between congestive heart failure and primary respiratory tract disease as the underlying cause. The presence of concurrent cardiac and respiratory tract disease or pulmonary hypertension could confound diagnosis. The NT-proBNP assay should be used in conjunction with medical history, physical examination findings, and other diagnostic test results to help achieve a diagnosis for the cause of respiratory signs in dogs.

a. Canine CardioCare NT-proBNP, Veterinary Diagnostics Institute, Irvine, Calif.
b. GraphPad Prism, version 4.0, Graph Pad Software Inc, San Diego, Calif.
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