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Weekly variability of plasma and serum NT-proBNP measurements in normal dogs

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Abstract *Objectives:* To determine the weekly variability of serum and plasma N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentrations in healthy dogs.
Animals, materials and methods: Fifty-three normal dogs were examined prospectively. Serum ($n = 25$) or plasma ($n = 28$) samples were obtained for NT-proBNP assay at one week interval for 3 consecutive weeks.
Results: Median serum or plasma NT-proBNP concentration did not change over 3 consecutive weeks. Twenty-two of 53 dogs (42%) had at least one NT-proBNP value >500 pmol/L, including 14 dogs with at least one serum NT-proBNP concentration >500 pmol/L and 8 dogs with at least one plasma NT-proBNP concentration >500 pmol/L during the 3-week sampling period. The difference between the maximum and minimum NT-proBNP value obtained over the 3-week sampling period was <100 pmol/L in 40% of dogs, between 100 and 200 pmol/L in 40% of dogs, and >200 pmol/L in 20% of dogs. Of the 19 dogs with a value >500 pmol/L on either week 1 or 2, 11 dogs (58%) had a subsequent NT-proBNP value <500 pmol/L on either week 2 or 3.
Conclusions: There is a high degree of variability in weekly serum and plasma NT-proBNP values in healthy dogs. Individual variability should be considered when interpreting NT-proBNP results in dogs.

Introduction

N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentration has been used to identify

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cardiovascular causes of dyspnea in dogs. Previous studies have reported cut-off values for discrimination between canine patients with cardiac or respiratory disease,[1] and one study reported that a serum NT-proBNP concentration >445 pmol/L discriminated dogs with cardiac disease from normal dogs with a sensitivity of 83.2% and specificity of 90%.[2] The current supplier of the NT-proBNP assay[c] advises that dogs with NT-proBNP <500 pmol/L are very unlikely to possess significant cardiac disease, whereas dogs with NT-proBNP between 500 and 1100 pmol/L have mildly elevated values that may warrant further diagnostics depending on the clinical presentation. NT-proBNP values between 1100 and 1725 pmol/L are moderately elevated, are very likely associated with significant cardiac disease, and further diagnostics are recommended. Finally, dogs with NT-proBNP >1725 pmol/L have severely elevated values and are likely to have congestive heart failure. Previous studies involve sampling for NT-proBNP assay at a single point in time.[1,2] Day-to-day or week-to-week variability in NT-proBNP results could affect the diagnostic utility of the test by creating false-positive or false-negative results; however, the variability of serum and plasma NT-proBNP concentration in either diseased or healthy dogs is unknown. The purpose of this study was to determine the weekly variability of serum and plasma NT-proBNP concentrations in healthy dogs.

Animals, materials and methods

This study was approved by the animal care and use committees of the University of Wisconsin and the University of Pennsylvania. Owner consent was obtained for all dogs enrolled.

Clinically normal dogs were prospectively enrolled from 2 study sites. Inclusion criteria included the absence of a heart murmur and normal electrocardiographic and 2-D, M-mode and Doppler echocardiographic studies. Serum samples from dogs recruited from site A[d] and plasma samples from dogs recruited from site B[e] were obtained for NT-proBNP analysis.[f] Venous blood samples were collected in serum or EDTA plasma tubes and centrifuged at 3200 revolutions per minute for 10 min, separated and frozen at −70 °C within 1 h of phlebotomy. Collection of samples was not standardized with respect to time of day or location of venous access (i.e., jugular vs. cephalic vein). Batched samples were shipped to the assay laboratory on ice according to the guidelines established by the manufacturer.[f] The lower limit of detection, as reported by the manufacturer, was <160 pmol/L.

Descriptive variables were recorded from all patients and the median age, weight and sex distribution between groups were analyzed using Mann–Whitney *U* tests and Chi-square tests. The Spearman method was used to test correlation between median NT-proBNP concentration and age and weight. Median NT-proBNP concentrations at each of 3 time-points were compared within the serum group and within the plasma group using Friedman's test. NT-proBNP values <160 pmol/L were entered as 159 pmol/L for statistical analysis. The number and percentage of dogs with NT-proBNP values greater than 500 pmol/L were tabulated. To evaluate a degree of variability within the population, the difference between each individual dog's maximum and minimum NT-proBNP value over the study period was tabulated and the percentage of dogs with differences <100 pmol/L, 100–200 pmol/L, and >200 pmol/L was calculated. All analyses were performed with standard software.[g] *P*-values <0.05 were considered significant.

Results

Fifty-three dogs were recruited, including 25 dogs from site A and 28 dogs from site B. Median age of dogs from sites A and B was 5 years (range, 0.8–9) and 7 years (range, 2–10), respectively. Median weight of dogs from sites A and B was 26 kg (range, 6.8–50) and 20.4 kg (range, 5.3–46), respectively. There were 12 female dogs and 13 males dogs from site A, and 16 female dogs and 12 male dogs from site B. There was no significant difference in median age ($P = 0.24$), median weight ($P = 0.07$), and gender distribution ($P = 0.51$) between sites A and B. Breed distribution of dogs from site A included 5 Keeshonds, 4 mixed breed, 4 Labrador retrievers, 3 American Staffordshire terriers, and 1 dog each from 8 other breeds. Breed distribution of dogs from site B included, 8 mixed breeds, 3 Labrador retrievers, 2 Golden retrievers,

[c] Cardiopet™ proBNP test, IDEXX Laboratories, Inc., Westbrook, ME, USA.
[d] University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, USA.
[e] University of Wisconsin, School of Veterinary Medicine, Madison, WI, USA.
[f] Canine CardioCare™, Veterinary Diagnostics Institute, Irvine, CA, USA.
[g] GraphPad Prism 5, GraphPad Software Inc, San Diego, CA, USA.