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Plasma atrial natriuretic peptide is an early diagnosis and disease severity marker of myxomatous mitral valve disease in dogs

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ABSTRACT

The aim of this study was to retrospectively assess the clinical usefulness of plasma atrial natriuretic peptide (ANP) concentrations for determining the severity of myxomatous mitral valve disease (MMVD) in dogs. Plasma ANP levels were found to be significantly higher in dogs with MMVD compared to healthy dogs, and plasma ANP levels increased significantly in dogs with progressive heart failure. In dogs with MMVD, stepwise regression analysis revealed that the left atrium/aorta ratio and fractional shortening could be used to predict the plasma ANP concentration. These results indicated that plasma ANP rose with an increase in the volume overload of the left side of the heart. Plasma ANP discriminated cardiomegaly from non-cardiomegaly caused by asymptomatic MMVD. We conclude, therefore, that plasma ANP concentrations may be a clinically useful tool for early diagnosis of asymptomatic MMVD in dogs.

1. Introduction

Myxomatous mitral valve disease (MMVD) in dogs is the most commonly acquired heart disease (Serfass et al., 2006). MMVD causes mitral regurgitation due to rupture of chordae tendineae and poor coaptation of the mitral valve leaflets during systole. Mitral regurgitation leads to the left atrial and the left ventricular dilatation, and causes pulmonary congestion (Gouni et al., 2007). This mechanical stress imparted on the cardiomyocytes stimulates the synthesis and secretion of atrial natriuretic peptide (ANP) (Edwards et al., 1988). Normally, ANP is secreted mainly by atrial cardiomyocytes, ventricular contribution corresponding to less than 3% (Nakayama, 2005). However, heart failure stimulates ANP release from ventricular cardiomyocytes to a level equivalent to the atrial cardiomyocytes. This small peptide plays an important role in intravascular volume homeostasis, such as natriuresis, vasodilation, and inhibition of the renin–angiotensin–aldosterone system (Ruskoaho, 2003). Precisely, release of ANP is a protective mechanism against ventricular volume overload (de Almeida et al., 2012).

Heart diseases in dogs are associated with high concentration of ANP in the bloodstream. For instance, plasma ANP level increases with pulmonary capillary wedge pressure, a measure of left atrial pressure in dogs (Asano et al., 1999; Hori et al., 2010). Häggström et al. reported that left atrial enlargement causes an increase in plasma ANP concentration, and ANP level was a good indicator of decompensation in Cavalier King Charles Spaniels with mitral regurgitation (Häggström et al., 1994, 2000). In addition, Greco et al. reported that plasma ANP level reflects survival in dogs with heart failure including dilated cardiomyopathy and MMVD (Greco et al., 2003). These studies suggest plasma ANP may be a marker of severity of heart disease in dogs.

The aim of this study was to test the potential of plasma ANP levels as diagnostic marker of MMVD severity in dogs. First, we determined the relationship between plasma ANP levels and MMVD severity. Second, the accuracy as a diagnostic indicator of MMVD severity was determined by the sensitivity and specificity of plasma ANP levels.

2. Materials and methods

2.1. Study population

The study population consisted of client-owned dogs that presented with systolic murmur in the mitral area (grade > 2) and owner consent was obtained for all dogs included in this study. Five veterinary cardiology practices prospectively recruited dogs between October 2009 and May 2010. Both healthy dogs (healthy group) and dogs with MMVD (MMVD group) were recruited for

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this study. The healthy dogs were considered healthy on the basis of results of physical examination, including cardiac auscultation, and blood test; total protein, albumin, complete blood count, urea nitrogen, creatinine, aspartate amino transferase, and alanine aminotransferase. Exclusion criteria included congenital heart disease and acquired heart disease other than MMVD. For the MMVD group, dogs with liver disease, kidney disease, or systemic disease were excluded from the study. Dogs with prerenal azotemia associated with MMVD were included in the study.

2.2. Assessment of cardiac disease severity

All dogs with MMVD underwent thoracic radiography and 2-D, M-mode, and color-flow Doppler echocardiography. The left ventricular end-diastolic diameter (LVEDD), the left ventricular end-systolic diameter (LVESD), the aortic diameter (Ao), and the left atrial diameter (LA) were measured by standard echocardiographic techniques (Hansson et al., 2002; Thomas et al., 1993). LA to Ao ratio was calculated (LA/Ao). The left ventricular fractional shortening (FS) was calculated with LVEDD and LVESD. LVEDD and LVESD values were divided by the Ao to normalize them to the size of the dog (LVEDD/Ao and LVESD/Ao, respectively). In dogs with MMVD, the severity of heart failure was classified according to the International Small Animal Cardiac Health Council (ISACHC) recommendations based on clinical symptoms and thoracic radiographs (International Small Animal Cardiac Health Council, 1999).

2.3. Blood sampling and measurement of ANP

Blood samples were obtained from the jugular or cephalic vein and were immediately collected in a tube containing aprotinin and EDTA and centrifuged at 3000 rpm for 10 min. The supernatant (plasma) was transferred to a plastic tube and stored at −80 °C. Plasma ANP concentrations were determined with a chemiluminescence enzyme immunoassay for human α-ANP (Shionoria-ANP, Shionogi Co., Osaka, Japan) (Hori et al., 2011). The detection limit of plasma ANP concentrations assay was 5 pg/mL.

2.4. Statistical analysis

Values are presented as the median and the interquartile range (IQR, 25th–75th percentile). Mann–Whitney's *U* test was used to compare plasma ANP concentrations between healthy dogs and dogs in each ISACHC Class of MMVD. The Kruskal–Wallis test, followed by the Dunn multiple comparison test, was used to compare physical examination results, echocardiography variables, and plasma ANP concentrations among dogs in each ISACHC Class of MMVD. Differences in the numbers of dogs receiving medical treatment in each ISACHC Class were determined by use of the chi-square test.

Pearson's correlation coefficient test was used to examine correlations between plasma ANP concentration and heart rate, LA/Ao, LVEDD/Ao, LVESD/Ao, and FS. The Spearman rank correlation was calculated to assess the correlation between plasma ANP concentration and heart murmur grade. Stepwise multiple regression analysis was performed to identify continuous variables associated with plasma ANP concentration.

Receiver operating characteristic (ROC) analyses were performed to determine the optimal cut-off values for plasma ANP concentration in discriminating between dogs with MMVD and healthy dogs. Furthermore, ROC analyses were performed to assess the predictive accuracy of the plasma ANP concentration for detecting left atrium enlargement and pulmonary edema. ROC curves were drawn by plotting all the sensitivity values against their corresponding 1 − specificity values. The area under the ROC curve and the 95% confidence interval (CI) of the prediction of the area were calculated. All analyses were performed with standard software (Prism version 5.0c, GraphPad Software Inc., CA, USA) and values of $P < 0.05$ were considered significantly different.

3. Results

A total of 36 healthy dogs and 127 dogs diagnosed with MMVD were included in this study. Both groups were comparable in terms of mean age, gender ratio and body weight, as well as breed variety. The healthy group comprised of male ($n = 16$, 44.4%) and female ($n = 20$, 55.6%), adult aged (median, 56 months; IQR, 42–87 months), and small-breed dogs (median, 9.0 kg; 5.4–11.9 kg). Breeds consisted of 16 Beagles, seven Miniature Dachshunds, three Mixed breeds, two Yorkshire Terriers, and one French Bulldog, Labrador Retriever, Maltese, Papillon, Shetland Sheepdog, Shiba, Toy Poodle, and Welsh Corgi. The MMVD group was mostly composed of male ($n = 81$, 63.8%), adult aged (median, 145 months; IQR, 115–161 months), small-breed dogs (median, 5.5 kg; 3.8–7.7 kg). Breeds consisted of 17 Mixed breeds, 16 each of Maltese and Cavalier King Charles Spaniels, 14 Shih Tzus, 13 Miniature Dachshunds, 12 Chihuahuas, eight each of Yorkshire Terriers and Pomeranians, six Shibas, five Toy Poodles, three each of Beagles and Miniature Schnauzers, two Papillons, and one Akita, American Cocker Spaniel, Japanese Chin and West Highland White Terrier.

The MMVD dogs were classified according to disease severity based on the ISACHC classification. A total of 57 dogs (44.9%) had asymptomatic disease (Class I), 47 dogs (37.0%) were in ISACHC Class II, and 23 dogs (18.1%) were in ISACHC Class III. The heart murmur rate gradually doubled with disease severity, from a median value of 2 for Class Ia to 4 for Class IIIb. Heart rate also increased significantly with disease severity, but the most dramatic increase was detected for Class IIIa, and not Class IIIb. The healthy dogs were not taking any medication. In contrast, the MMVD dogs at baseline, 96 (75.6%) dogs were receiving medical treatment for their cardiac disease. Twenty-nine dogs were receiving monotherapy with angiotensin converting enzyme (ACE) inhibitor. Sixty-seven dogs were receiving combination therapy with more than two of the following drugs: ACE inhibitor, carvedilol, digoxin, diuretics (furosemide or torsemide), spironolactone, and/or pimobendan. A higher proportion of dogs in ISACHC Class II (91.5%) were receiving medical treatment compared with dogs in ISACHC Class I (63.2%, $P < 0.01$) and Class III (73.9%, $P < 0.05$) MMVD. Ventricular overload increased linearly starting with Class Ib as detected in terms of LA/Ao, LVEDD/Ao, and LVESD/Ao. The most remarkable finding is the drastic age difference between Class Ia and Class Ib, which marks a change in the rate of disease progression. Seventeen dogs presented with pulmonary edema in ISACHC Class III. Baseline values of the continuous variables for the 127 dogs at first examination are summarized in Table 1.

The relationship between MMVD and plasma ANP concentration was demonstrated by comparing the median values of the healthy dogs to those of each Class of MMVD dogs. Table 2 shows that all MMVD Classes of dogs exhibited a median plasma ANP level significantly higher than healthy dogs. Also, plasma ANP levels were significantly higher in Classes Ib, II, IIIa and IIIb, compared to Class Ia, showing a gradual progression with disease severity.

Regression analyses were conducted to determine whether an increase in plasma ANP level correlates with the increase of ventricular overload in MMVD dogs. Table 3 shows that plasma ANP level was correlated significantly with heart rate, LA/Ao, LVEDD/Ao, and FS, but not LVESD/Ao that is reflected by pressure overload and systolic function. Stepwise multiple regression analysis revealed that the LA/Ao ratio and FS could be used to predict the plasma ANP concentration.