Evaluation of Plasma N-Terminal Pro-Brain Natriuretic Peptide (NT-proBNP) Concentrations in Dogs with Mitral Valve Insufficiency

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ABSTRACT. The diagnostic significance of the plasma concentration of N-terminal pro-brain natriuretic peptide (NT-proBNP) was evaluated in 72 dogs with mitral valve insufficiency and 36 control dogs. In the controls, the plasma NT-proBNP concentration was 163.9 ± 114.7 (SD) pmol/l. The values in those with International Small Animal Cardiac Health Council (ISACHC) functional classification of heart failure class Ia, Ib, II and IIIa mitral valve insufficiency were 302.8 ± 257.1 (n=21), 634.2 ± 642.5 (n=23), 1,277.9 ± 756.2 (n=18) and 1,908.9 ± 538.8 (n=10) pmol/l, respectively; those in the class Ib or severer groups were significantly higher than that in the controls. In dogs in which the intensity of cardiac murmurs was Levine 3, 4, 5 and 6, plasma NT-proBNP concentrations were 647.6 ± 577.3 (n=27), 1,184.7 ± 841.0 (n=18), 1,532.4 ± 784.2 (n=10) and 1,461.8 ± 932.2 (n=4) pmol/l, respectively, and were significantly higher than that in the controls. The plasma NT-proBNP concentration was significantly correlated with the cardiac size (VHS) and LA/Ao (r=0.611, n=89, p<0.01; and r=0.705, n=91, p<0.01, respectively). When dogs with ISACHC class II or IIIa were regarded as heart failure, the cut-off value was 713.5 pmol/l, and the sensitivity and specificity were 0.913 and 0.857, respectively. These findings could indicate that plasma NT-proBNP concentration was significantly associated with the severity of heart failure due to mitral valve insufficiency in dogs. Further investigation is required to determine factors other than heart failure affecting plasma NT-proBNP concentration.

KEY WORDS: canine, mitral valve insufficiency, NT-pro BNP.

FULL PAPER

Mittal valve insufficiency is the most common cardiac disease in dogs. To manage this disease, it is essential to evaluate the severity of heart failure. For the classification of the severity of this disorder in small animals, the criteria proposed by the International Small Animal Cardiac Health Council (ISACHC) functional classification of heart failure are routinely employed [9]. In animals with chronic heart failure, thoracic radiography and echocardiography are also performed to evaluate the severity.

Brain natriuretic peptide (BNP) is a hormone synthesized in the heart. Signal peptides are removed from peptides synthesized as proBNP in the heart, especially the ventricle, forming pro-BNP. This is cleaved into BNP and N-terminal pro-BNP (NT-proBNP), and then released into the circulation. BNP exhibits various physiological actions including natriuresis and inhibition of the renin-angiotensin system, whereas NT-proBNP does not show any physiological actions [14, 17]. In humans, the plasma concentration of BNP is emphasized as a parameter of the severity of heart failure, a prognostic factor, and an item for evaluating the treatment response [15, 16, 18]. In addition, some studies have indicated that NT-proBNP is diagnostically more significant than BNP [3, 5, 6, 10, 11].

In dogs, several studies have investigated the plasma concentration of BNP [1, 2, 7, 12], but studies on NT-proBNP are limited. In the present study, the authors evaluated the clinical significance of the plasma NT-proBNP concentration in dogs with mitral valve insufficiency with respect to the severity of heart failure, the intensity of cardiac murmurs, cardiac size and left atrial enlargement.

MATERIALS AND METHODS

The subjects were 108 dogs presented to 6 small animal hospitals (4 private clinics and 2 university hospitals) in Japan. Of these dogs, 72 (37 males, 5 castrated males, 14 females and 16 spayed females, age: 10.6 ± 2.45 [SD] years) were diagnosed with mitral valve insufficiency based on the medical history, physical examination, thoracic radiography and echocardiography. Seventeen dogs were Maltese; 13 were Shih Tzu; 12 were mongrels; 6 were Cavalier King Charles Spaniels; 5 were Pomeranians; 4 were Beagles; 3 were American Cocker Spaniels; 2 each were Miniature Schnauzers, Toy Poodles, Chihuahuas and Shetland Sheepdogs; with 1 each of Miniature Pinschers, Miniature Dachshunds, and Maltipoo.
tetraacetic acid, and then placed at 4°C saphenous veins, immediately mixed with ethylenediamine-}

diethanolamine 

described [8].

enlargement was classified according to the Vertebra Heart

failure was assessed according to the ISACHC criteria [9] by 3 Board Certified Members of Japanese Society.

The intensity of cardiac murmurs was evaluated according to Levine’s classification. The grade of cardiac

Pyrenean Mountain Dog.

Newfoundland, Chihuahua, Standard Poodle, Shiba and Shitzu Terrier, Flat-coated Retriever, Bichon Frise, Papillon, Pomeranians and Shetland Sheepdogs; with 1 each of Yorkshires, Toy Poodles; 2 each were Miniature Schnauzers, Miniature Dachshunds and mongrels; 4 were Shih Tzu; 3

were assigned as the controls. Seven dogs each were

were Toy Poodles; 2 each were Miniature Schnauzers, Pomeranians and Shetland Sheepdogs; with 1 each of Yorkshire Terrier, Flat-coated Retriever, Bichon Frise, Papillon, Newfoundland, Chihuahua, Standard Poodle, Shiba and Pyrenean Mountain Dog.

In this study, the authors evaluated the severity of heart failure, the intensity of cardiac murmurs, grade of cardiac enlargement on radiography, and the left atrial diameter/aortic diameter ratio (LA/Ao) on echocardiography. The severity of heart failure was assessed according to the ISACHC criteria [9] by 3 Board Certified Members of Japanese Society of Veterinary Cardiology and 3 well-experienced veterinarians. The intensity of cardiac murmurs was evaluated according to Levine’s classification. The grade of cardiac enlargement was classified according to the Vertebra Heart Scale (VHS) method [4]. LA/Ao was evaluated as previously described [8].

Blood was collected through the jugular, cephalic or saphenous veins, immediately mixed with ethylenediaminetetraacetic acid, and then placed at 4°C for 30 min. Subsequently, plasma was separated and stored at –20°C. The plasma concentration of NT-proBNP was measured at a commercial laboratory center (ADTEC Co., Ltd., Ohita, Japan) was used. P<0.05 was regarded as significant.

RESULTS

In the control dogs, the plasma concentration of NT-proBNP was 163.9 ± 114.7 pmol/l. Among the dogs with mitral valve insufficiency, the values in those with ISACHC class Ia, Ib, II and IIIa were 302.8 ± 257.1 (n=21), 634.2 ± 642.5 (n=23), 1,277.9 ± 756.2 (n=18) and 1,908.9 ± 538.8 (n=10) pmol/l, respectively; the plasma concentrations of NT-proBNP in the class Ib or severer heart failure groups were significantly higher than that in the controls (Fig. 1). In addition, the plasma concentration of NT-proBNP increased with the severity of heart failure.

In dogs in which Levine 1 or 2 murmurs were heard, the plasma concentrations of NT-proBNP were 149.5 ± 87.5 (n=4) and 177.8 ± 82.8 (n=8) pmol/l, respectively, without significant differences from the value of controls. However, in dogs in which Levine 3, 4, 5 or 6 murmurs were heard, the plasma concentrations of NT-proBNP were 647.6 ± 577.3 (n=27), 1,184.7 ± 841.0 (n=18), 1,532.4 ± 784.2 (n=10) and 1,461.8 ± 932.2 (n=4) pmol/l, respectively. These values were significantly higher than that in controls (Fig. 2). In addition, the plasma concentration of NT-proBNP increased with the intensity of the cardiac murmurs.

As shown in Figs. 3 and 4, the plasma concentration of NT-proBNP was correlated with VHS and LA/Ao (r=0.611, n=89, p=0.01; and r=0.705, n=91, p=0.01, respectively).

Figure 5 shows the ROC curve when ISACHC class II or IIIa were regarded as heart failure (heart failure: 28 dogs, normal heart: 80 dogs). The area under the curve (AUC) using the kit was according to manufacturer’s recommendation. Briefly, freeze-dried standards were dissolved in 0.3 ml of distilled water and left to stand at room temperature for 30 min. Twenty µl of each standard concentration and samples was added to respective wells, and 200 µl of tracer was added to all wells. Then, plates were incubated in the dark overnight (16–24 hr) at room temperature. The contents of the wells were discarded and washed adequately with diluted wash buffer. Any remaining washing buffer was removed, and 200 µl of substrate was added to all wells and mixed well. The plates were incubated in the dark for 30 min at room temperature. Fifty µl of stop solution was added to all wells, and absorbance was determined immediately with a reader at 450 nm.

Receiver operator characteristic (ROC) curve was analyzed to evaluate the diagnostic features of the plasma NT-proBNP concentration and establish the cut-off value.

The data are expressed as the mean ± standard deviation (SD). Multiple comparisons of the plasma NT-proBNP concentrations were performed using Tukey’s HSD test with respect to the severity of heart failure and the intensity of cardiac murmurs. To examine correlations, linear regression analysis was performed. In performing these tests and preparing the ROC curve, commercially available statistical software (Dr. SPSS II for Windows, Version 11.0, 1J, SPSS Japan, Inc., Tokyo, Japan) was used. P<0.05 was regarded as significant.

<table>
<thead>
<tr>
<th>ISACHC class</th>
<th>Medication</th>
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<tr>
<td>Ia</td>
<td>ACEI</td>
<td>21</td>
</tr>
<tr>
<td>Ib</td>
<td>ACEI+ISDN</td>
<td>2</td>
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<tr>
<td>II</td>
<td>ACEI+β-blocker</td>
<td>0</td>
</tr>
<tr>
<td>IIIa</td>
<td>ACEI+ISDN+β-blocker</td>
<td>0</td>
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<tr>
<td></td>
<td>ACEI+ISDN+digoxin</td>
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<tr>
<td></td>
<td>ACEI+ISDN+digoxin+furosemide</td>
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<td>ACEI+furosemide+pimobendan</td>
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<td>ACEI+furosemide+pimobendan+ISDN</td>
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<td>% dogs on medication</td>
<td>9.5</td>
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ACEI: Angiotensin converting enzyme inhibitor, ISDN: Isosorbide dinitrate.

Table 1. Heart failure medication administered in each ISACHC class

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<thead>
<tr>
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<tr>
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<td>% dogs on medication</td>
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was 0.930. The cut-off value was 713.5 pmol/l, and the sensitivity and specificity were 0.913 and 0.857, respectively.

DISCUSSION

In general, the severity of chronic heart failure in small animals is staged using ISACHC functional classification of heart failure. This classification is mainly based on the interview and history of the illness, and simple and rapid staging is possible. However, for more accurate staging of the severity, additional examinations including physical examination, thoracic radiography and echocardiography...
are essential.

BNP is a hormone that exhibits antagonistic actions against the renin-angiotensin-aldosterone system, as reported for atrial natriuretic peptide (ANP) [14, 17]. Several studies have documented the significance of BNP in small animals with chronic heart failure [1, 2, 7, 12]. NT-proBNP is a peptide released from the heart, especially the ventricle, in response to cardiac overload. The diagnostic significance of NT-proBNP has been well-established in human medicine. However, no study has examined the significance of the NT-proBNP level in dogs, because an assay specific for canine NT-proBNP has not been developed to date. In this study, the authors evaluated the diagnostic significance of the plasma NT-proBNP concentration in dogs with mitral valve insufficiency using the EIA kit currently available to measure canine NT-proBNP.

In mitral valve insufficiency, the intensity of cardiac murmur is considered to roughly reflect the severity of mitral regurgitation. In the present study, the plasma NT-proBNP level was significantly higher in the group with grade 3 or louder murmur than in the control group, showing that mitral valve insufficiency with grade 1 or 2 murmur can not be detected based on the NT-proBNP level alone. Measurement of the plasma NT-proBNP level may not be suitable for detecting mitral valve insufficiency with a low-grade cardiac murmur.

When the left atrium and ventricle expand in response to volume overload associated with mitral valve insufficiency, the airway is compressed. This is the cause of coughing, a typical symptom of left heart failure. The left atrial size is more accurately evaluated as LA/Ao by echocardiography. An increase in the LA/Ao is associated with the cause of the airway compression-induced coughing described above. In addition, an increased LA/Ao indirectly reflects the left atrial pressure and subsequently the pulmonary venous pressure, and thus is associated with the risk of pulmonary edema.

The correlation between the diagnostic imaging findings and plasma NT-proBNP level attracts attention because these are directly associated with the severity of chronic heart failure. Increases in VHS and LA/Ao result from volume overload associated with mitral valve insufficiency. This volume overload may have stimulated ventricular synthesis and the secretion of prepro-BNP in the ventricle, elevating the plasma NT-proBNP level.

The plasma NT-proBNP concentration was associated with the ISACHC cardiac function classification in the present study. The plasma NT-proBNP level in class Ia was not significantly different from that in the control group. However, those in class Ib, II and IIIa were significantly higher when compared to that in control group. These results would indicate that the plasma NT-proBNP concentration might have a diagnostic importance to detect cardiac enlargement rather than heart failure in dogs with mitral valve insufficiency.

The representative clinical symptoms of mitral valve insufficiency include exercise intolerance and chronic cough. The latter is also caused by various disorders of the upper and lower airway such as tracheal collapse. Therefore, it is sometimes difficult to evaluate the main etiology of coughing in dogs with mitral valve insufficiency and chronic respiratory disorder. To determine the diagnostic importance of the plasma NT-proBNP concentration to differentiate cardiac and non-cardiac coughing, the cut-off value was established by analyzing ROC curve in the present study. As the result, the sensitivity and specificity could be acceptable as the one of the clinical cardiac examinations.

Large variation in the plasma NT-proBNP concentration was found in the present study, especially in ISACHC class II and IIIa. ISACHC class II is defined as mild to moderate heart failure [9]. Therefore, such a variation in the plasma NT-proBNP concentration might result from the vagueness of the definition. In addition, there were limited cases with ISACHC IIIa in the present study. This might also have contributed to the marked variation in the present study.

Based on the above findings, it was concluded that dogs with class Ia can not be detected by measuring the plasma NT-proBNP concentration, although it is significantly associated with the severity of heart failure in dogs with mitral valve insufficiency.

There are some limitations in the present study. The authors did not investigate changes in the plasma concentration of NT-proBNP related to the deterioration and improvement of the ISACHC class in the same dogs in this study. In addition, we did not examine diurnal changes in the plasma concentration of NT-proBNP. Moreover, the concentration might also be influenced by breed, age or sex. Thus, the present results might have been partially affected by such factors. Finally, it was shown that the effects of body mass...
index and age on the plasma NT-proBNP concentration were associated with the glomerular filtration rate in humans with chronic heart failure [13]. These factors were also not considered in our study. These limitations will be overcome in a further investigation.

REFERENCES


