Studies on the clinical significance of N-terminal pro-atrial natriuretic peptide in dogs

Summary of Doctoral Thesis

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Myxomatous degenerative mitral valve disease (MMVD) is the most commonly acquired cardiac disease in dogs. Studies have reported that the concentration of plasma N-terminal pro-atrial natriuretic peptide (NT-proANP) concentration increased with the progression in MMVD. However, studies on plasma NT-proANP concentration are lacking compared with studies conducted on other cardiac biomarkers (CBs), such as the concentration of N-terminal pro-brain natriuretic peptide (NT-proBNP). Besides, factors influencing this concentration remain unclear. Additionally, the clinical usefulness of plasma NT-proANP concentration in dogs has not been compared with that of other CBs.

Therefore, in Chapter 2 of this study, we investigated the effect of diet on plasma NT-proANP concentration, in addition to the effect of diurnal variations on plasma NT-proANP concentration in clinically healthy dogs. Chapter 3 assessed the effect of dehydration on plasma NT-proANP concentration in healthy dogs. In Chapter 4, we evaluated the effect of the glomerular filtration rate (GFR) on plasma NT-proANP concentration in dogs, where plasma iohexol clearance test was used to measure the GFR. Moreover, in Chapter 5, we examined the usefulness of plasma NT-proANP concentration as a CB in dogs diagnosed with MMVD. Finally, in chapter six, we investigated the clinical significance of plasma NT-proANP concentrations in dogs with coughing by investigating the ability of plasma NT-proANP concentrations to differentiate between causes of MMVD and respiratory diseases. In this study, we also investigated plasma NT-proANP and NT-proBNP concentrations in chapters three, five, and six, to clarify differences in factors increasing plasma NT-proANP and NT-proBNP concentrations, to compare their characteristics as CB, and to examine their use in clinical practice.

1. Effects of diet and diurnal variations on plasma N-terminal pro-atrial natriuretic peptide concentration in dogs (Chapter 2)

Studies have shown that plasma NT-proANP concentration is influenced by diet and show diurnal variations in rats. However, this fact is unknown in dogs. Additionally, although diurnal variations in heart rate and heart rate variability (HRV), an index of autonomic function, have been reported in dogs, they have also been associated with

plasma NT-proANP concentration in humans. Therefore, in this chapter, we evaluated the effect of diet and diurnal variation on plasma NT-proANP concentration in clinically healthy dogs. Also, we investigated whether autonomic function was involved in the diurnal variation of plasma NT-proANP concentration when it exists.

For this study, five clinically healthy dogs were used. To investigate the effect of food intake, we compared plasma NT-proANP concentration at 5, 15, and 30 min, then 1 and 2 h after feeding the dogs, using 5 min before feeding as the baseline (BL). However, to evaluate diurnal variations, plasma NT-proANP concentration, heart rate, and HRV parameters were compared and evaluated at 10:30, 13:30, 16:30, 19:30, and 22:30, then at 1:30, 4:30, and 7:30 the following day.

Results showed that plasma NT-proANP concentration was unaffected by diet. In contrast, although no statistically significant difference was observed, plasma NT-proANP concentration increased transiently at 19:30 and showed a decreasing trend at night (from 22:30 to 7:30 the next day). Furthermore, plasma NT-proANP concentration was inversely correlated with the square root of the mean-squared differences between adjacent RR intervals (RMSSD), and transiently increased when the sympathetic activity was temporarily dominant.

These findings therefore propose that the influence of food should not be taken into account when drawing blood for measuring plasma NT-proANP concentration. However, although no statistically significant difference in plasma NT-proANP concentration at different periods was observed, plasma NT-proANP concentration can increase when the sympathetic nerve activity is increased, suggesting that caution should be exercised in interpreting plasma NT-proANP concentration when dogs are excited or nervous during blood sampling.

2. Studies on the effect of hydration status on plasma N-terminal pro-atrial natriuretic peptide and N-terminal pro-B-type natriuretic peptide concentrations in dogs (Chapter 3)

Studies have reported that plasma ANP concentrations, which are released into the blood in equimolar quantities to plasma NT-proANP concentration, show a decreasing trend during dehydration in dogs. It has also been reported that the plasma NT-proBNP

concentration decreases during dehydration in humans. Therefore, in this chapter, we evaluated the effect of dehydration on plasma NT-proANP and NT-proBNP concentrations in dogs.

Furosemide was administered intravenously to five clinically healthy dogs at a dose of 2–4 mg/kg every 1–2 h, to create a dehydration model. The dehydration model was completed when the dogs lost at least 5% of their body weight and showed signs of dehydration on physical examination. Afterward, plasma NT-proANP and NT-proBNP concentrations were compared at three time points: before the dehydration model (BL), after completing the dehydration model, and when the dehydration was judged to have improved.

Results showed that plasma NT-proANP concentration decreased significantly after completing the dehydration model compared with BL. In contrast, the plasma NT-proBNP concentration showed a decreasing trend but did not significantly differ before and after dehydration. Also, although the plasma NT-proANP concentration was significantly correlated with body weight and plasma NT-proBNP concentration, plasma NT-proBNP concentration was significantly correlated with electrolytes and echocardiographic parameters (diastolic left ventricular internal diameter [LVIDd], including the LVIDd parameter normalized for body weight [LVIDdN]).

These findings therefore propose that plasma NT-proANP concentration can underestimate the severity of cardiac diseases in dehydrated dogs. As observed, although plasma NT-proBNP concentration was not statistically significant before and after dehydration, it was significantly correlated with the plasma NT-proANP concentration, plasma sodium concentration, and left ventricular internal diameter parameters, suggesting that it can decrease significantly with a reduction in ventricular internal diameter during more severe dehydration conditions.

3. The effect of glomerular filtration rate on plasma N-terminal pro-atrial natriuretic peptide concentration in dogs (Chapter 4)

In humans, 17% of NT-proANP released into circulation is excreted through the kidneys. If a portion of NT-proANP is also excreted through the kidneys in dogs, a decrease in the glomerular filtration rate (GFR) is proposed to be responsible for the increase in

plasma NT-proANP concentration. Therefore, in this chapter, we investigated the relationship between plasma NT-proANP concentration and GFR in dogs.

We included dogs whose GFR was measured using the plasma iohexol clearance (PCio) test at the Department of Nephrology in Nippon Veterinary and Life Science University or at an external veterinary hospital. Dogs with a PCio greater than 1.8 ml/min/kg were classified as normal (control), those with a PCio between 0.9 and 1.8 ml/min/kg as having mildly depressed GFR (M-GFR), and those with a PCio less than 0.9 ml/min/kg were classified as having severely depressed GFR (S-GFR).

Thus, 59 dogs were included in this chapter (control, 19; M-GFR, 26; S-GFR, 14). Results showed that plasma NT-proANP concentration was significantly higher in the S-GFR group than in the control and M-GFR groups.

These findings therefore indicate that GFR did not affect plasma NT-proANP concentration, except only when GFR was severely reduced. Moreover, mildly reduced GFR had little effect on plasma NT-proANP concentration.

4. Comparing discriminatory abilities of plasma N-terminal pro-atrial natriuretic peptide concentration and three cardiac biomarkers against clinical stages in dogs diagnosed with myxomatous mitral valve disease (Chapter 5)

In dogs diagnosed with MMVD, no studies comparing the diagnostic usefulness of plasma NT-proANP concentration and the three CBs currently used in dogs (plasma NT-proBNP, ANP, and cardiac troponin I [cTnI] concentrations) exist. Therefore, this chapter evaluated characteristics of plasma NT-proANP, NT-proBNP, ANP, and cTnI concentrations, in addition to their discriminatory abilities during diagnosis of cardiac enlargement and congestive heart failure (CHF) in dogs with MMVD.

Data were collected from the Department of Cardiology at the Nippon Veterinary and Life Science University and from seven external institutions. The dogs were then classified as either normal (having a normal heart, following the American College of Veterinary Internal Medicine [ACVIM] stage A classification) or MMVD (having differing heart disease severities, following the ACVIM classification stages B1, B2, C, and D).

As a result, 105 dogs were included in this chapter (normal group, 36 dogs;

MMVD group, 69 dogs, comprising 34 dogs at stage B1; 14 dogs at stage B2; 14 dogs at stage C; and 7 dogs at stage D). It was also observed that CBs other than plasma cTnI concentration were significantly elevated in stages B1, B2, C, and D relative to the normal group. During the multivariate analysis, the left atrium/aorta ratio (LA/Ao) was selected as a variable, which was independently related to plasma NT-proANP, NT-proBNP, and ANP concentrations. From the receiver operating characteristic curve analysis, plasma NT-proANP, and NT-proBNP concentrations showed a higher sensitivity and negative predictive value for cardiac enlargement and CHF than the other CBs. However, no difference in discriminatory abilities between the two CBs existed.

These findings therefore proposed that plasma NT-proANP, NT-proBNP, and ANP concentrations increased with left atrial enlargement, regardless of the presence or absence of clinical signs. In this chapter, we also confirmed that both plasma NT-proANP and NT-proBNP concentrations were associated with left atrial enlargements and were equally discriminatory for cardiac enlargement and CHF.

5. The comparative evaluation of the usefulness of cardiac biomarkers as a tool for differentiating between the cause of coughing in dogs with myxomatous mitral valve disease without signs of congestive heart failure and dogs with respiratory diseases (Chapter 6)

The clinical sign of coughing is frequently present in dogs with MMVD, even in those with less severe cardiac enlargements. Therefore, during clinical practice, it is difficult to distinguish the cause of coughing in dogs with MMVD from those with respiratory diseases, such as a tracheal collapse. Hence, in this chapter, we investigated the usefulness of plasma NT-proANP and NT-proBNP concentrations in differentiating the cause of coughing in dogs with MMVD without signs of CHF and dogs with respiratory diseases.

We included dogs that visited the Department of Cardiology or the Department of Respiratory Medicine of the Nippon Veterinary and Life Science University who had a cough at the time of the visit. The dogs whose coughing was judged to be due to MMVD were included in the MMVD group, whereas those whose coughing was due to

respiratory diseases were included in the respiratory disease group (RD group). In this chapter, dogs with both MMVD and respiratory disease were excluded.

Thus, 57 dogs were included (MMVD group, 31 dogs; RD group, 26 dogs). Results showed that plasma NT-proANP and NT-proBNP concentrations were significantly higher in the MMVD group than in the RD group. As observed, the sensitivity and specificity of plasma NT-proANP and NT-proBNP concentrations were 93.9% and 97.0%, respectively, then 82.1%, respectively.

These findings therefore indicated that plasma NT-proANP and NT-proBNP concentrations were excellent in differentiating causes of coughing in dogs with MMVD and dogs with respiratory diseases. It was also observed that the discriminatory ability of these two CBs was comparable. In dogs, it is occasionally difficult to distinguish the cause of coughing from chest radiography alone. In such cases, the plasma NT-proANP concentration is as useful as the plasma NT-proBNP concentration.

This study revealed that the plasma NT-proANP concentration showed a decreasing trend at night, which is proposed to increase with increasing sympathetic nerve activity. Findings also revealed that this concentration was affected by dehydration and GFR. Moreover, the clinical usefulness of plasma NT-proANP concentration for MMVD was considered comparable to that of plasma NT-proBNP concentration, which is currently the most widely used in clinical practice. Additionally, CBs differentiated between causes of coughing in dogs with MMVD without signs of CHF and causes of coughing in dogs with respiratory disease. Therefore, the effect of these CBs should be taken into account when choosing between plasma NT-proANP and NT-proBNP concentrations for CB testing in dogs with MMVD.