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Evaluation of ST2 and NT-proBNP as cardiac biomarkers in dogs with heartworm disease

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Abstract

This study compared serum concentrations of suppression of tumorigenicity 2 (ST2) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) between healthy and heartworm- infected dogs. Eighteen heartworm-infected dogs and five healthy dogs were included in the study. Dogs were diagnosed and categorized by history, clinical signs, and blood assay, thoracic radiography, echocardiography, and commercial ELISA kit results. Serum samples were sent to the IDEXX reference laboratory for NT-proBNP measurement. ST2 was examined by using a canine interleukin 33 receptor ELISA kit with the quantitative sandwich ELISA method. The severely infected group showed significant elevation of NT-proBNP concentration over those of the control (P=0.03) and mildly infected (P=0.04) group. There were no significant difference in ST2 concentrations among the three groups. The usefulness of NT-proBNP as a cardiac biomarker in dogs with severe heartworm disease was confirmed by the results of this study. Further investigations to assess ST2 as a cardiac biomarker are warranted.

Key words : ST2, NT-proBNP, Heartworm disease, Biomarker, Dog

INTRODUCTION

Dirofilariasis, commonly called heartworm disease, is widely distributed in the tropics and subtropics, including South Korea (Song et al, 2010). Domestic dogs are definitive hosts for *Dirofilaria*, while cats and ferrets may also be infected. For infection, a susceptible mosquito ingests the *Dirofilaria* larvae by taking a blood meal from an infected host and, after further larval development, transmits the larvae to another host. Mildly infected dogs may be asymptomatic or show cough, while severe cases may exhibit life-threatening clinical signs such as dyspnea, ascites and syncope. Diagnosis is mainly based on history, clinical signs, antigen and/or microfilaria tests, radiography, and echocardiography. Heartworm disease can be classified into class 1 through class 4 (caval syndrome), and both treatment and prognosis varies with severity (American Heartworm Society, 2014).

In addition to the screening tests mentioned above, several biomarkers are being used for diagnostic and prognostic purposes (Boswood, 2009). A biomarker is defined as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (Atkinson et al, 2001). On that basis, new cardiac biomarkers are being investigated in both human and veterinary medicine.

The level of N-terminal pro-B-type natriuretic peptide (NT-proBNP) in blood is one of the most commonly used cardiac biomarkers in veterinary medicine. A number of researchers have shown its usefulness in evaluating dogs with heart disease (Ebisawa et al, 2012; Ettinger et al, 2012). Furthermore, severity of heartworm disease in patients has been assessed by using NT-proBNP and other biomarkers such as c-reactive protein (CRP), troponin I (cTnI), myoglobin, and D-dimer levels (Carretón

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et al, 2011; Carretón et al, 2014). However, due to limitations of those biomarkers, more accurate and comprehensive biomarkers are being researched. The suppression of tumorigenicity 2 (ST2) protein, which is thought to be a biomarker of remodeling and myocardial stress, has been discussed as a risk predictor in heart failure patients in human medicine (Braunwald, 2008). Other human study has shown that simultaneous measurement of ST2 and NT-proBNP levels improves determination of the risk for death in heart failure patients (Bayes-Genis et al, 2012).

As dogs with severe heartworm disease show right heart remodeling and myocardial stress, the authors hypothesized that the ST2 may be useful as prognostic biomarker as well as diagnostic biomarker in dogs with severe heartworm disease. The aim of this study is to apply and assess ST2 as a cardiac biomarker candidate in veterinary medicine. This study includes the measurement of ST2 and NT-proBNP levels in dogs with dirofilariasis of different severity levels.

MATERIALS AND METHODS

Animals

Eighteen heartworm-infected dogs and five control healthy dogs were used in this study. Dog breed, sex, and age were randomized. The dirofilariasis diagnosis was made by examining subject history, clinical signs, and blood assay, thoracic radiography, echocardiography, and enzyme-linked immunosorbent assay (ELISA) kit results. A commercial ELISA kit (SNAP test, IDEXX Laboratories, Maine, USA) was used, and the manufacturer's instructions were followed. As renal disease can elevate NT-proBNP level in dogs without heart disease, blood urea nitrogen (BUN) and creatinine assessments were included in the blood assay (Schmidt et al, 2009). Dogs showing azotemia were excluded from the study.

After the diagnosis, heartworm-infected dogs were divided into two groups; mild (n=9) and severe (n=9). Following the classification system described by Di Sacco and Vezzoni (1992), dogs of classes 1 and 2 were included in the mild group, while dogs of classes 3 and 4 were included in the severe group. Dogs in the mild group showed either no clinical signs or a mild cough while those in the severe group showed critical clinical signs including ascites, syncope, hemoglobinemia, and hemoglobinuria. Cardiac remodeling was observed on thoracic radiography and echocardiography in the severe group. All heartworm-infected dogs were diagnosed based on the results of the commercial ELISA kit.

Laboratory evaluations

Jugular and cephalic veins were used for the collection of blood in all 23 cases. Whole blood (3 mL) was collected in a serum tube, which then underwent centrifugation at 3000 r/min for 20 min. Immediately after centrifugation, a portion of the serum was used in the commercial ELISA kit and for blood assays such as BUN and creatinine. The remaining portion of the serum samples was divided into two conical tubes and stored at −80°C within 30 min of centrifugation to maintain sample stability until other tests. As repetitive freezing and thawing may lead to false results, serum samples for NT-proBNP assessment were sent on the same day as that for the ST2 measurement. One of the serum tubes containing 1 mL of serum was packed with ice and sent to the IDEXX reference laboratory for NT-proBNP measurement. A reference range of <900 pmol/L for a normal healthy dog was set by the IDEXX laboratory. The other serum tube was used in the measurement of ST2, which was performed by using a ELISA kit (Canine interleukin 33 receptor, MyBioSource, San Diego, USA). A quantitative sandwich ELISA method was used to examine 6 standards and 23 serum samples, which were assessed in duplicate. The optical density of each ELISA kit well was determined by using an ELISA reader (Apollo LB913, BERTHOLD Technologies) set to 450 nm. As the reference range for ST2 in dogs is not yet established, the results of 5 healthy control dogs were compared with those of the infected dogs.

Statistical analysis

GraphPad Prism software version 6 was used to per-

form the statistical analysis of all cases. Data are summarized as mean ± SE values. For significance determination, the Mann-Whitney method (a non-parametric test) was selected in accordance with the results of the D'Agostino-Pearson omnibus normality test. Identification of outliers was performed by using Grubb's test, also called the extreme studentized deviate method. Values of $P<0.05$ were considered significant.

RESULTS

The mean NT-proBNP concentration of dogs in the severe group (2681 pmol/L [95% CI, 1610 to 3752 pmol/L; n=9]) was significantly ($P<0.05$) higher than those of the control (347.8 pmol/L [95% CI, 303.34 to 392.26; n=5]) and mild (564.2 pmol/L [95% CI, 390.8 to 737.6; n=9]) group (Fig. 1). However, mean ST2 concentration among the control (2.831 ng/mL [95% CI, 2.4768 to 3.1852; n=5]), mild (3.479 ng/mL [95% CI, 2.6170 to 4.3410; n=8]), and severe (2.713 ng/mL [95% CI, 2.2263 to 3.1997; n=8]) group did not show significant differences (Fig. 2). In accordance with Grubb's test of outliers, one dog in the mild group and one in the severe group were excluded.

DISCUSSION

NT-proBNP is a neuroendocrine hormone that is produced by ventricles in accord with chronic pressure and volume overload levels (Carretón et al, 2014). Previous studies have demonstrated that serum NT-proBNP concentration is a useful cardiac biomarker for assessing the severity of various heart diseases including mitral valve disease, dilated cardiomyopathy, and heartworm disease (Oyama et al, 2008; Carretón et al, 2014). The results of the present study confirmed that elevation of NT-proBNP level depends on the severity of the heart disease. The NT-proBNP concentrations of the control and mild group were within the normal reference range and were not significantly different from each other. On the contrary, the NT-proBNP level of the severe group dogs were significantly higher than those of the other groups. These results indicate that NT-proBNP can be used for the evaluation of canine patients with heartworm disease. However, as the NT-proBNP has a high degree of variability and can be falsely elevated in renal patients, a combination of NT-proBNP with other cardiac biomarkers is recommended (Kelihan et al, 2009; Schmidt et al, 2009).

On the basis, this study evaluated a new candidate biomarker, ST2, a member of the interleukin 1 receptor family. ST2 has two isoforms, a transmembrane form of

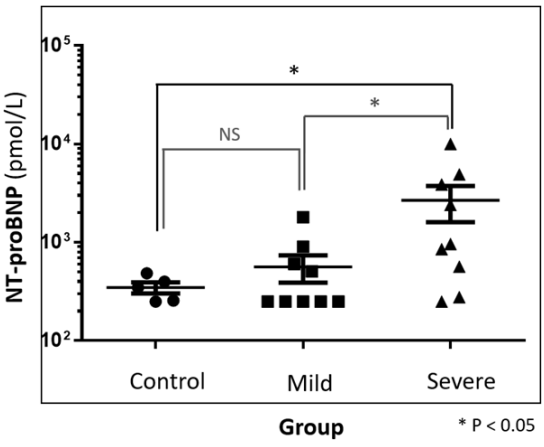

Fig. 1. NT-proBNP concentrations in control, mild, and severe group. Horizontal line represents mean value and upper/lower lines represent SE. NT-proBNP concentrations of the severe group were significantly higher than those of the control and mild group. *Significantly ($P<0.05$) different from value for control dogs. NS = Not significant.

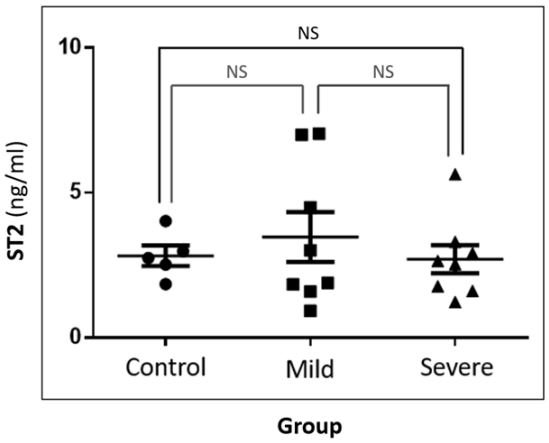

Fig. 2. ST2 concentrations in control, mild, and severe group. Horizontal line represents mean value and upper/lower lines represent SE. There were no differences between any groups. One dog in the mild group and one dog in the severe group were excluded as an outlier in accordance with the Grubb's test. NS = Not significant.

ST2 ligand (ST2L) and a circulating form of soluble ST2 (sST2) (Shah and Januzzi, 2010). ST2L has cardioprotective effects through its interaction with interleukin-33 (IL-33), whereas sST2 is thought to be a 'decoy receptor' competing with ST2L and inhibiting its positive effects (Villacorta and Maisel, 2016). Therefore, sST2 has been used as a predictor of mortality, as well as a marker of cardiac structure and function, in human medicine (Shah, 2010).

However, in this study, there were no significant difference in ST2 levels among the three study groups. Thus, the results show that ST2 does not elevate in accordance with the severity of canine heartworm disease. It is not known whether ST2-related physiological mechanisms differ between human and other animals. Regardless, there are some weaknesses in the evaluation of ST2 in this study. First, some of the samples were stored at −80℃ for approximately 1 year prior to being assessed. Based on research in humans, ST2 results did not show significant variation until 6 months of −30℃ storage; however, further assessment over a longer period was not described (Piper et al, 2016). Unfortunately, in veterinary medicine, only variation in NT-proBNP and troponin-I levels in dogs have been evaluated, thus making the ST2 results difficult to assess (Ruaux et al, 2015). Another weakness of the study is that only a small number of dogs were included in the study. Further study that includes more dogs should be undertaken. Additionally, as linear variability of ST2 levels was not assessed, serial testing is recommended.

CONCLUSION

The usefulness of NT-proBNP as a cardiac biomarker in dogs with severe heartworm disease was reconfirmed by this study. Mild heartworm infection does not elevate serum NT-proBNP level. In contrast, ST2 did not show significant differences among the study groups. Further investigations assessing ST2 as a cardiac biomarker in dogs are warranted.

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