

Evaluation of ST2 and NT-proBNP as Cardiac Biomarkers in Dogs With Chronic Mitral Valve Disease

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Abstract : Recently assessment of suppression of tumorigenicity 2 (ST2) level has become a useful cardiac biomarker in human medicine. This study compared serum ST2 levels and N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels between healthy dogs and dogs with chronic mitral valve disease. Twenty client-owned dogs were investigated. Dogs were divided into normal, asymptomatic, and symptomatic groups. Serum samples were used to measure levels of NT-proBNP and ST2. Samples for NT-proBNP were sent to IDEXX laboratory for analysis while ST2 levels were measured by using a canine interleukin 33 receptor ELISA kit. There was a significant difference in NT-proBNP levels between asymptomatic and symptomatic groups ($P < 0.01$), and between normal and symptomatic groups ($P < 0.01$). In contrast, ST2 levels were not relatively different between asymptomatic and symptomatic groups ($P > 0.05$). There was no significant difference was observed among all groups in ST2 study. The usefulness of measuring NT-proBNP level as a cardiac biomarker in dogs with chronic mitral valve disease was confirmed, but usefulness of the ST2 level was not observed. Further investigations are needed to evaluate the potential usefulness of ST2 level as a cardiac biomarker in canines.

Key words : ST2, Cardiac Biomarker, CMVD, NT-proBNP, level.

Introduction

Chronic mitral valve disease (CMVD) is one of the most common diseases in old dogs. In addition, it has a high rate of occurrence in small and medium-sized dogs. CMVD is characterized by reflux from the left ventricle to the left atrium, and the prominent enlargement of the left atrium, which leads to clinical symptoms such as cardiogenic pulmonary edema as the disease progresses.

In managing CMVD, patients are typically divided into stages based on the results of physical examination and radiologic criteria. The recommended diets and medications for CMVD patients are dependent on disease stage (1,12,14). However, there are not many objective indicators that can be obtained quickly and easily for evaluating the prognosis of patients with CMVD.

Measuring N-terminal pro-B-type natriuretic peptide (NT-proBNP) level is reported to be useful in the prognosis process which is considered to be the gold standard in evaluating heart conditions, along with Troponin T (cTn T and cTn I) (4,7,15,19).

According to previous studies, biomarker is a characteristic that can be objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to therapeutic intervention (3). Two or more biomarkers should be used, because the prediction of risk or mortality cannot be exact when only one biomarker is

used. Recently, a new biomarker, suppression of tumorigenicity 2 (ST2), has been used in conjunction with the NT-proBNP. In addition, three biomarkers have a higher predictive value compared to using two biomarkers (2,7,11).

NT-proBNP levels have been shown to be positively correlated with the severity of heart disease in human and veterinary medicines and have been used not only for the management of patients with heart failure, but also for excluding patients with non-cardiogenic respiratory symptoms and for predicting the expression of congestive heart failure (CHF) (3,9,16). However, there is a limitation that, it could be increased by kidney disease or inflammation, so use of NT-proBNP level as a sole biomarker is not recommended (4,8).

ST2 level, when used as a biomarker, can indicate cardiac volume overload, and its assessment can be divided into two types: sST2 and ST2L. In normal status subjects, ST2L type binds to interleukin 33 (IL-33) to produce a cardioprotective effect. However, in CMVD patients, it is more likely that IL-33 binds to sST2 than to ST2L. As a result, the associated cardioprotective effect is reduced, and cardiac remodeling as well as myocardial fibrosis proceeds (15).

In human medicine, ST2 measurement is obtained via a test kit, which allows the measurement to be performed quickly in approximately 20 minutes in a hospital. Thus, ST2 levels of hospitalized patients with heart failure can be measured quickly and repeatedly to predict not only improvement or deterioration, but also the probability of sudden death in those with non-proliferative heart failure (6,13,15).

In contrast to NT-proBNP levels, the ST2 level is unaffected by renal function. This lack of renal effect on ST2 is advantageous because the occurrence of cardiorenal syndrome

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in heart failure patients is not uncommon (4).

Considering these potential advantages, there is a requirement to provide objective and realistic data via biomarkers for veterinary patients admitted to hospital due to heart condition. This study compared serum ST2 and NT-proBNP levels between healthy dogs and dogs with CMVD.

Materials and Methods

Study Design and Animals

In this study, patients' NT-proBNP and ST2 levels were measured, which is currently being used as a new biomarker in human medicine. Twenty dogs that visited Chungnam National University Animal Hospital were enrolled. Of those dogs, 4 were placed in the normal group, 8 in the asymptomatic CMVD groups, and 8 in the symptomatic CMVD groups (Table 1). With the exception of one dog, all dogs participating in the experiment were small breeds. Dogs were classified into groups based on the basic physical examination, complete blood count, serum biochemistry, urinalysis, thoracic and abdominal radiology, and echocardiography results.

Normal group dogs showed no specific abnormality in any of these test results. The subjects placed in the asymptomatic and symptomatic CMVD groups were classified based on any abnormalities in kidney and urinalysis. The mean age of total subjects was 12.5 ± 5.0 years old and the mean body weight was 5.61 ± 5.05 kg.

Sample Collection

All blood samples were collected from external jugular or cephalic veins after 12 h of fasting. Samples were contained in plain tubes using a 26G catheter and the obtained sera were separated within 30 minutes after coagulation. A por-

tion of the serum was used to measure the ST2 level and the remainder was refrigerated before sending to an external laboratory (IDEXX Laboratories, Maine, USA) for determination of NT-proBNP levels via the SNAP test. The ST2 levels were measured by using a canine IL-33 test (ST2 ELISA kit, Mybiosource, USA). The quantitative sandwich ELISA method was used for ST2 assessment of 4 standards and 16 serum samples with duplicates. The optical density of each ELISA well was determined by using an ELISA reader (Apollo LB913, BERTHOLD Technologies) set to 450 nm.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS version 24.0, IBM, USA) program was used for the statistical analysis. The values were expressed as mean values with using standard deviations. One-way ANOVA was used to compare NT-proBNP and ST2 values among the three groups. The Scheffe test was used to confirm the association between asymptomatic and symptomatic groups. A P-value less than 0.05 in all analyses was considered statistically significant.

Results

There was a significant difference in the NT-proBNP levels between the asymptomatic and symptomatic CMVD groups ($P < 0.01$). In addition, there was a significant difference in NT-proBNP levels between the normal and symptomatic groups ($P < 0.01$). The mean NT-proBNP value of the normal group, 302 pmol/L (95% CI, 221-382 pmol/L).

In the asymptomatic group, the mean NT-proBNP value was 517 pmol/L (95% CI, 345-668 pmol/L), whereas the symptomatic group had a markedly higher mean NT-proBNP value of 2,599 pmol/L (95% CI, 1,640-3,558 pmol/L) (Fig 1).

In contrast, ST2 levels were not significantly different among the normal, symptomatic, and asymptomatic groups

Table 1. Characteristics of the study population in this study

	Groups		
	Normal	Asymptomatic	Symptomatic
N(20)	4	8	8
Age	3.75 ± 2.21	14.25 ± 2.65	15.12 ± 1.88
Sex	M(4)	M(3), F(5)	M(5), F(3)
BW	4.75 ± 1.65	7.03 ± 7.5	4.61 ± 2.76
Maltese	1	3	4
Shih-Tzu		2	3
Dachshund		1	
Chihuahua	1		
Yorkshire Terrier		1	1
Poodle	1		
Italian Greyhound	1		
Poongsan		1	

BW: body weight, M: male, F: female, N: number
All data used mean value (\pm standard deviation)

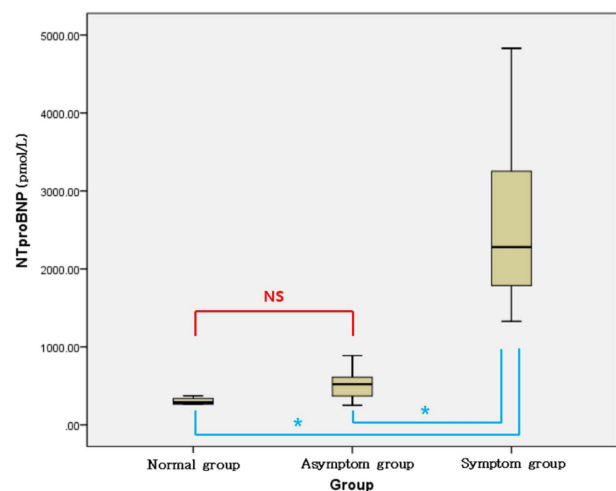


Fig 1. Comparison of NT-proBNP levels among normal, asymptomatic and symptomatic groups. Significant difference was observed between normal and symptomatic groups ($P < 0.01$). Significant difference was observed between asymptomatic and symptomatic groups ($P < 0.01$). *Significant ($P < 0.01$), NS = Not significant.

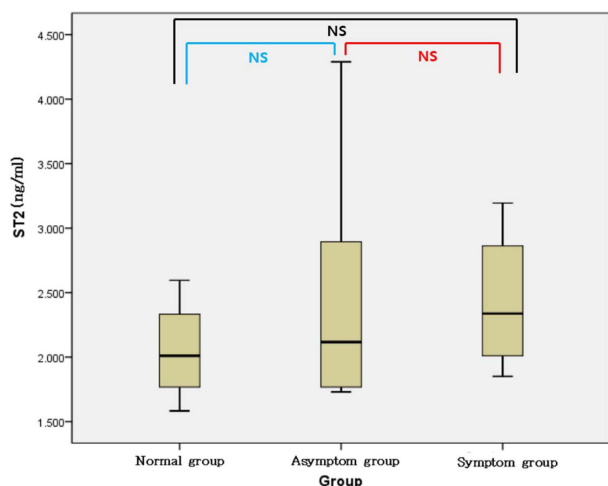


Fig 2. Comparison of ST2 levels between three groups. No significant difference was observed among all groups. NS = Not significant.

($p > 0.05$). The mean ST2 value of the normal group, 2.050 ng/mL (95% CI, 1.38-2.71 ng/mL), whereas the mean ST2 value of the asymptomatic group was 2.447 ng/mL (95% CI, 1.68-3.21 ng/mL), and the mean ST2 value of the symptomatic group was 2.433 ng/mL (95% CI, 2.01-2.85 ng/mL) (Fig 2).

As expected, the NT-proBNP level was elevated when symptoms of CMVD were present, however the ST2 results failed to indicate any relationships between CMVD status and ST2 levels.

Discussion

Currently, the tools used to assess cardiac remodeling in patients with heart failure in veterinary medicine include the identification of clinical symptoms by veterinarians, derivation of vertebral heart score (VHS) through thoracic radiography, and measurements obtained from echocardiography. These assessments are not completely objective because the result can be different depending on the equipment operator or data interpreter. Therefore, in order to obtain more objective data for use in the management of heart disease patients, the assessment of more than one biomarker is necessary (2,5,7).

In human medicine, predict the progress of the heart disease, the patient's ST2 level along with NT-proBNP level should be measured sequentially in patients with CHF (2). In veterinary medicine, NT-proBNP is typically measured by using an external referral process; thus, it is often measured for patients for whom the focus is for long-term management. The ST2 level has lower variability than other biomarkers, and it is relatively easy to monitor and use to evaluate disease prognosis (19). To date, research on ST2 as a biomarker has not been widely reported in veterinary medicine. According to a report by Oyama *et al.* (14), ST2 level was not significantly elevated in an experimental group suffering from heart disease compared to the level in the control group (13). Most recently, in the 2017 American College of Veterinary Internal Medicine Forum, Gimenes *et al.* (6) announced that dogs with CHF had elevated ST2 values compared to

those of non-CHF dogs. However, it was not possible to distinguish the measured value of ST2 at each CMVD stage because the ST2 level showed no clear difference between groups. Since ST2 is a newly studied biomarker in veterinary medicine, conventionally accepted significant values have not been reported, and there is no accepted value that would differentiate patients into different CMVD stages. In this study, comparison of ST2 levels among normal, asymptomatic, and symptomatic groups showed no significant difference among those groups.

CMVD is characterized primarily by volume overload of the left ventricle and left atrium. It is believed that evaluation of CMVD status would benefit a second cardiac biomarker, in addition to NT-proBNP which is reported to be effective for short-term and long-term prognosis and evaluation (18). When NT-proBNP level is over 900 pmol/L, the risk of heart failure is a concern (4). In this study, the mean NT-proBNP level of the normal group was 302 pmol/L, that of asymptomatic group was 517 pmol/L; thus, both had limited risk of heart failure. However, the symptomatic group had a mean NT-proBNP level of 2,599 pmol/L. In the present study, NT-pro BNP level was significantly higher in the symptomatic group than in the asymptomatic and normal groups. Thus, the results of this study indicate that NT-proBNP level is an effective cardiac biomarker of CMVD in canines.

This study was our first attempt at using ST2 level as a biomarker in canine CMVD patients in South Korea. One limitation of this study was that ST2 levels were not measured sequentially in a patient as has been done in previous experiments. In addition, the number of dogs included in the study was small.

In conclusion, the usefulness of measuring NT-proBNP level as a cardiac biomarker in dogs with chronic mitral valve disease was reconfirmed but ST2 level's was not validated by the results of this study. Therefore, further longitudinal study with a larger study population is required to examine changes in ST2 levels and estimate its changing bioavailability during CMVD progress.

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