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Evaluation of Serum NT-proBNP and Cardiac Troponin I Concentrations in Dogs with Heartworm Disease

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Abstract : Biomarkers used in dogs with heartworm disease include N-terminal pro B-type natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI), which are associated with damage to the myocardium. Pulmonary hypertension is one of the clinical signs of canine heartworm disease. The purpose of this study is to investigate the change in the concentration of each biomarker, severity of pulmonary hypertension and the correlation between biomarkers according to the severity of clinical signs. Five healthy dogs and 10 heartworm-infected dogs were recruited for the study. The heartworm-infected group was classified based on the history, clinical signs, and blood assay, thoracic radiography, and echocardiography after confirming the infection according to the results of the commercial ELISA kit (SNAP test, IDEXX Laboratories, Maine, USA). NT-proBNP was higher in the severely infected group than the control group ($p < 0.05$); cTnI was also higher in the severely infected group than the control group ($p < 0.05$). The pressure gradient of pulmonary hypertension was higher in the severe group than the mild group ($p < 0.05$). The severity of pulmonary hypertension was correlated with NT-proBNP ($r = 0.818$, $p < 0.01$), cTnI ($r = 0.894$, $p < 0.01$). When the correlation of the two serum values for each group was examined, a correlation was not found in the mild group ($r = 0.707$, $p = 0.182$), but a correlation was found in the severe group ($r = 0.9$, $p < 0.05$). NT-proBNP and cTnI were significantly increased and correlated with severe clinical signs. Pulmonary hypertension was significant higher in the severe group than in the mild group ($p < 0.05$). Evaluation of blood biomarker concentration and severity of pulmonary hypertension and referring to each correlation between these indicators may be helpful to assess the severity of the heartworm disease.

Key words **:** NT-proBNP, cTnI, pulmonary hypertension, dirofilaliasis, dog.

Introduction

Canine heartworm disease is an infectious disease caused by *Dirofilaria (D.) immitits* as a causative middle agent of mosquitoes and cause cardiopulmonary system diseases in dogs (1,13). *D. immititis* is widely distributed in tropical and subtropical climate regions, including South Korea (2,18). Clinical signs can range from asymptomatic to fatal chronic lung parenchyma and symptoms related to cardiopulmonary diseases including the blood vessels and heart. Severe infections can cause fatal symptoms such as caval syndrome and disseminated intravascular coagulation (DIC), in which multiple organs are damaged (1,2,13). Diagnosis of dirofilariasis is mainly based on medical history, clinical signs, Heartworm antigen test, microfilaria test, radiography, and echocardiography. Heartworm disease is divided into 4 stages according to clinical signs, and the higher the stage, the poorer the prognosis (1,2). In this disease, biomarkers can be used as screening tests to evaluate prognosis or treatment progress (5). Significant biomarkers in dirofilariasis include N-terminal pro B-type natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI) (6).

NT-proBNP is produced in cardiac ventricular myocardial cells and secreted from myocardial cells due to stimulation and stress caused by hypoxia, ventricular overload and myocardial hypertrophy (7). This biomarker is useful for severe heart disease in small animals, and it helps to evaluate the treatment effect and the severity of the disease (9,14).

Cardiac Troponin is a biomarker that provides information about the specific damage to the heart and is commonly used to assess myocardial integrity (9,20). In dirofilariasis, Troponin I is significantly increased compared to the normal dog in infected individuals. So, Troponin I is a useful biomarker in the diagnosis of heartworms (4,9,19).

The proliferation of arterial vessels and pulmonary embolism cause the pulmonary hypertension. Echocardiography is a non-invasive diagnostic tool for diagnosing pulmonary hypertension in dogs (2,10,16-17). The pressure gradient (PG) can be calculated by measuring peak regurgitant flow velocity of tricuspid regurgitation or pulmonic insufficiency (2,15).

According to existing studies, only the tendency of each blood level to increase with the severity of clinical signs has been studied (5,6), however, the correlation between each biomarker has not been researched.

Therefore, the purpose of this study is to identify the change in the concentration of biomarkers and PG of pulmonary hypertension according to the severity of canine heartworm disease and the correlation between pulmonary hypertension and each biomarker.

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Materials and Methods

Study animals

Client-owned dogs participated at the Veterinary Medical Teaching Hospital of Chungnam National University. A total of 10 dogs infected with heartworm and 5 healthy dogs were used in the study. Before entering the experiment, the client's informed consent was obtained. Diagnosis of dirofilariasis consists of history, clinical signs, thoracic radiography, echocardiography and enzyme-linked immunosorbent assay (ELISA) kit results. A commercial ELISA kit (SNAP test, IDEXX Laboratories, Maine, USA) was used for diagnosis, according to the manufacturer's instructions. After diagnosis, dogs infected with heartworms were divided into two groups; mild (n = 5), severe (n = 5). According to the classification system described in Lee et al. (11), dogs of class 1 and 2 were included in the mild group, and dogs of class 3 and 4 were included in the severe group. Dogs in the mild group showed no clinical signs, only mild coughing, no or mild regurgitation on the echocardiography, whereas the dogs in the severe group showed clinical signs such as ascites, syncope, dyspnea, and hemoglobinemia and hemoglobinuria. Additionally, cardiac remodeling was also observed in thoracic radiation in severe group and tricuspid and pulmonary regurgitation was observed on the echocardiography.

Blood sample collection

A blood sample was taken from all dogs after fasting for more than 12 hours and it was collected through jugular vein. The 3 ml of whole blood was centrifuged at 1,500 g for 5 minutes, and the centrifuged serum was divided into 2 Eppendorf tubes and stored frozen at −80°C until analysis.

NT-proBNP assays

To measure NT-proBNP, a serum tube containing 1ml of serum was packed in an ice pack and sent to the IDEXX reference laboratory. The reference range set by the laboratory is indicated as < 900 pmol/L in the normal dog.

Cardiac troponin I assays

Serum cTnI concentrations were measured using an ELISA (Dog Cardiac Troponin I ELISA Kit, MYBIOSOURCE, USA).

In a quantitative sandwich ELISA method, 7 standards and 15 serum samples 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.

Echocardiography

Echocardiography iU22® (Philips, Bothell, WA, USA) was used to check tricuspid regurgitation (TR) in the left parasternal four-chamber view and MPA expansion and heartworm in the right parasternal short axis view. The PG was measured according to the Bermoulli equation (pressure gradient = 4 × Velocity^2) from its backflow velocity.

Statistical analysis

Statistical analysis was performed with a commercially

Table 1. Demographic distribution and biomarker concentration data for dogs in this study

Group	Control (n = 5)	Mild (n = 5)	Severe (n = 5)
Age (yr)	6.2 ± 1.7	5 ± 1.3	4 ± 0.6
Weight (kg)	12.7 ± 5.8	15.8 ± 6.4	13 ± 3
NT-proBNP (pmol/L)	250	265.4 ± 15.4	1272.8 ± 513.4
cTnI (ng/ml)	0.05 ± 0.01	0.06 ± 0.01	0.15 ± 0.03
PG (mmHg)	-	15.2 ± 9.4	85.2 ± 12.7

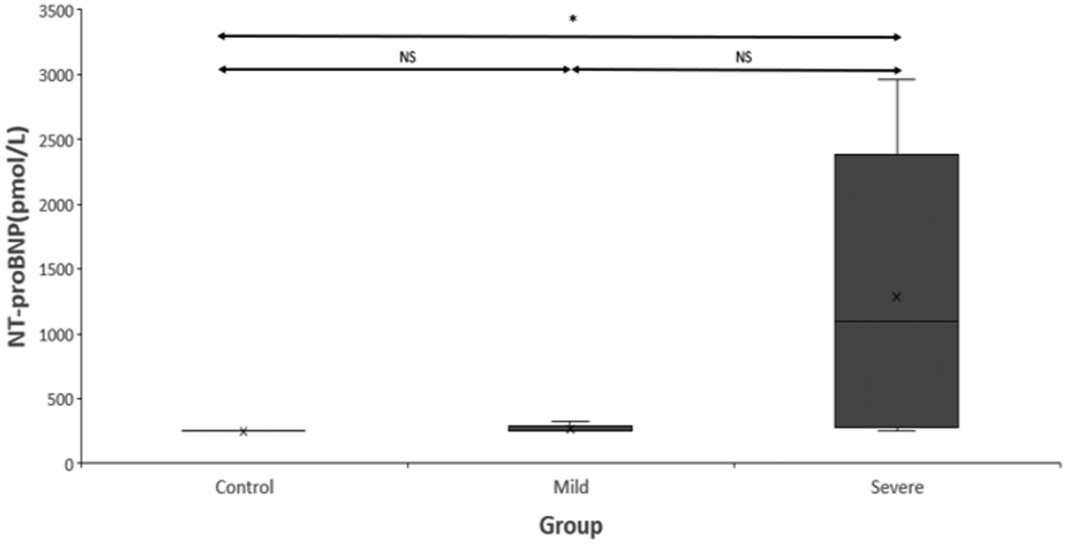

Fig 1. NT-proBNP concentration in control, mild and severe group. NS; no significant difference *p < 0.05; significant difference was observed between two groups.

available computer-based software program (IBM SPSS statistics 24.0.0, SPSS Inc., USA). The concentrations of NT-proBNP and cTnI and PG were expressed as the mean ± standard error of each group.

The Mann-Whitney method (nonparametric test) was used to compare and analyze the concentrations and PG of each group, and independent-2sample nonparametric tests were performed.

Finally, Pearson correlation coefficients(r) were used to confirm the correlation between the PG and serum NT-proBNP and cTnI concentration for each individual and the correlation between serum NT-proBNP and cTnI concentration in each group. The value of $p < 0.05$ is significant.

Results

The NT-proBNP concentration was 250 pmol/L in the healthy population, 265.4 ± 15.4 pmol/L in the mild group, 1272.8 ± 513.4 pmol/L in the severe group. The NT-proBNP concentration was higher in severe group than control group ($p < 0.05$) (Table 1 and Fig 1).

The cTnI concentration was 0.05 ± 0.01 ng/ml in the con-

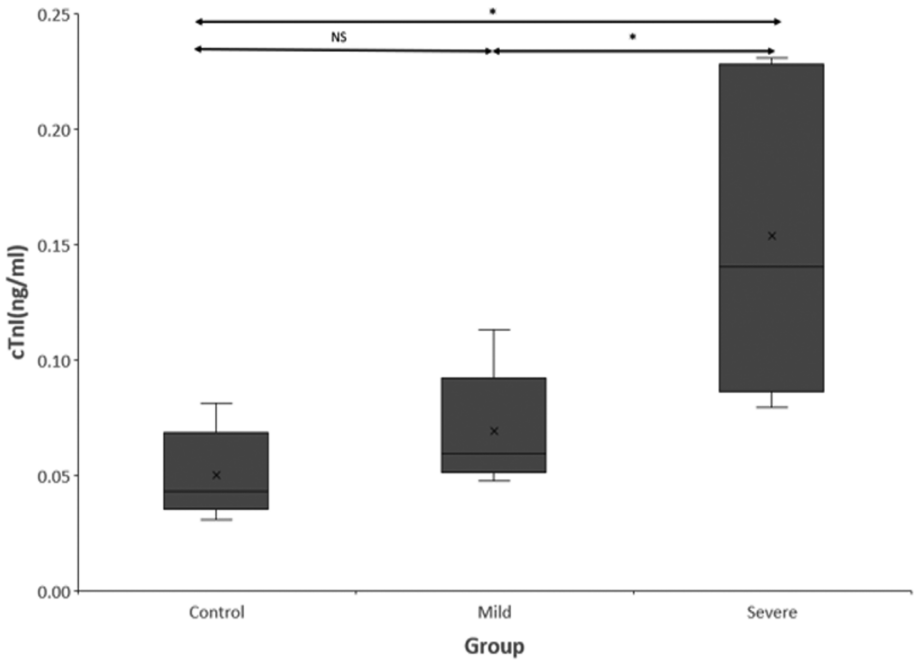

Fig 2. cTnI concentration in control, mild and severe group. NS; no significant difference *$p < 0.05$; significant difference was observed between two groups.

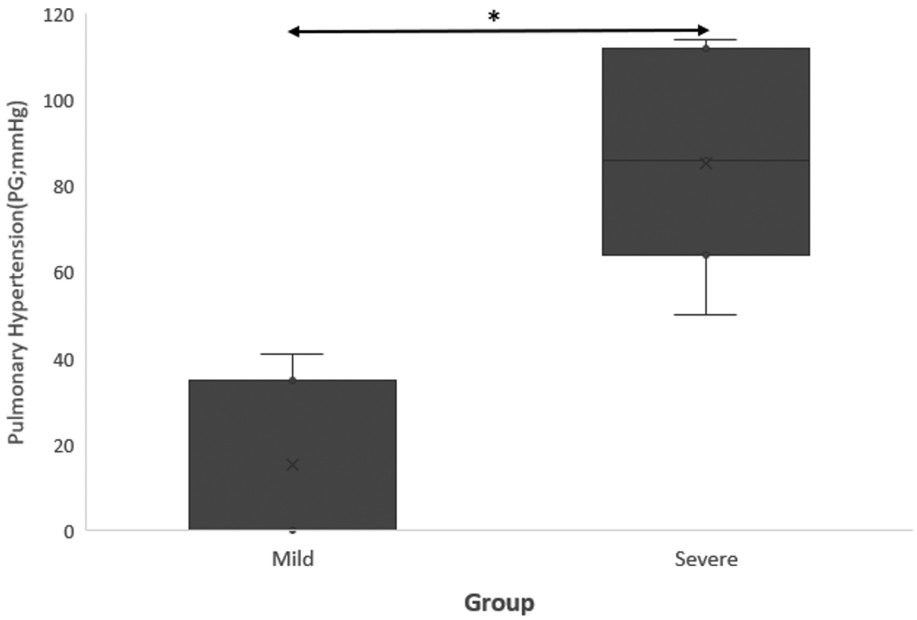

Fig 3. Pressure gradient of pulmonary hypertension in mild and severe group. *$p < 0.05$; significant difference was observed between two groups.

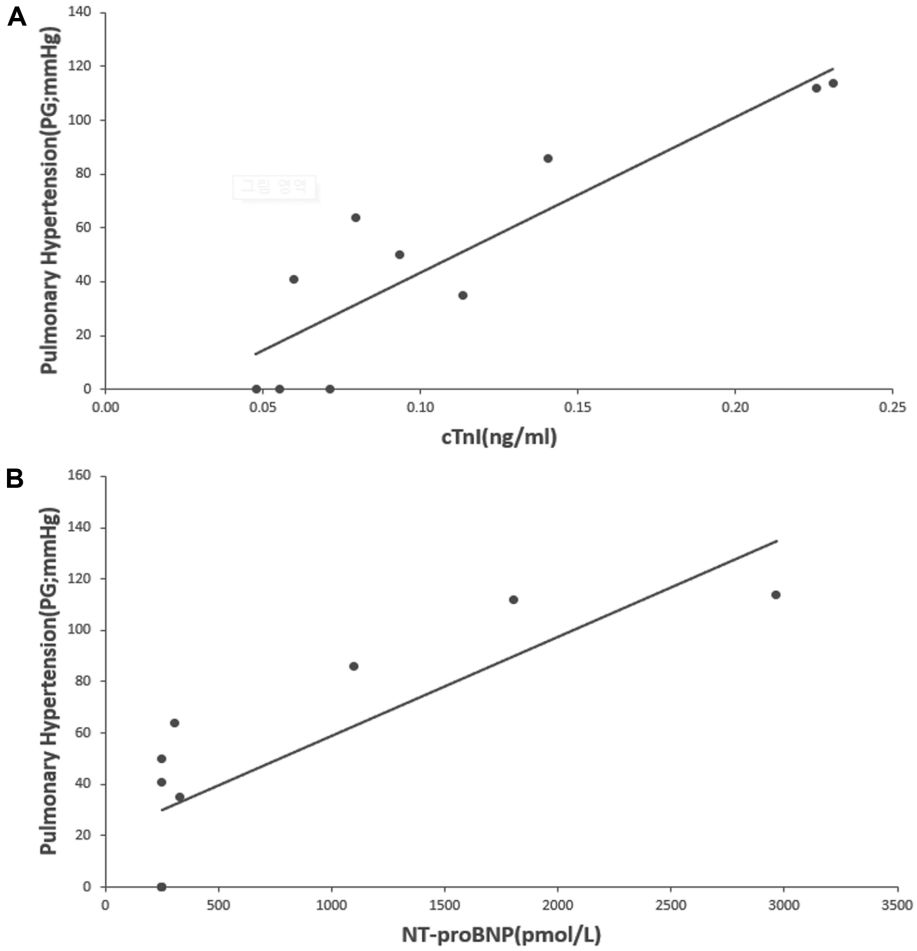

Fig 4. The correlation between the pulmonary hypertension and concentration of biomarkers in this study. A, Pulmonary hypertension vs cTnI ($r = 0.894$, $p < 0.01$). B, Pulmonary hypertension vs NT-proBNP ($r = 0.818$, $p < 0.01$).

trol group, 0.06 ± 0.01 ng/ml in the mild group, and 0.15 ± 0.03 ng/ml in the severe group. The cTnI concentration was higher in the severe group than in the control group ($p < 0.05$) (Table 1 and Fig 2).

The PG of pulmonary hypertension was 15.2 ± 9.4 mmHg in the mild group and 85.2 ± 12.7 mmHg in the severe group. When the PG values were compared between the two groups, it was significant higher in the severe group than the mild group ($p < 0.05$) (Table 1 and Fig 3).

When comparing the pulmonary arterial pressure of the individual according to the biomarker concentration, there was a significant positive correlation between the PG and NT-proBNP. ($r = 0.818$, $p < 0.01$) (Fig 4). When comparing the pulmonary arterial pressure of the individual according to the biomarker concentration, cTnI also had a significant positive correlation with PG ($r = 0.894$, $p < 0.01$) (Fig 4).

In addition, the concentrations of NT-proBNP and cTnI in the each group were compared. In the severe group, there was a significant correlation between the two values ($r = 0.9$, $p < 0.05$), but it was not in the mild group ($r = 0.707$, $p = 0.182$) (Fig 5).

Discussion

Canine heartworm disease is a threatening and chronic disease that causes inflammatory reactions, pulmonary hypertension, and even right-sided heart failure in the pulmonary system due to the presence of *D. immitis* in the pulmonary artery (8).

In one study, when NT-proBNP was applied to canine heartworm disease, normal values were shown in the mild cases, but pathologic values were shown in the severe cases (11). In this study, there was no significant difference of the numerical value between the control group and the mild group, but the severe group showed a significant higher levels than the control group.

In cTnI levels, there was no significant difference between the control and mild groups, but there was a marked increase in severe groups than in control group. In this study, the heartworm infection progresses chronically, the myocardial damage becomes serious.

In canine heartworm disease, embolism of dead worms can cause clinical signs of pulmonary hypertension, and the proliferation of vascular lumen causes pulmonary hypertension making irreparable structural damage of blood vessels (13). Objective measurement is important because pulmonary hypertension provides important information in measuring the severity of clinical signs (8,12). In one study, the severity of pulmonary hypertension was higher in the high parasite burden than in the low parasite burden in heart-

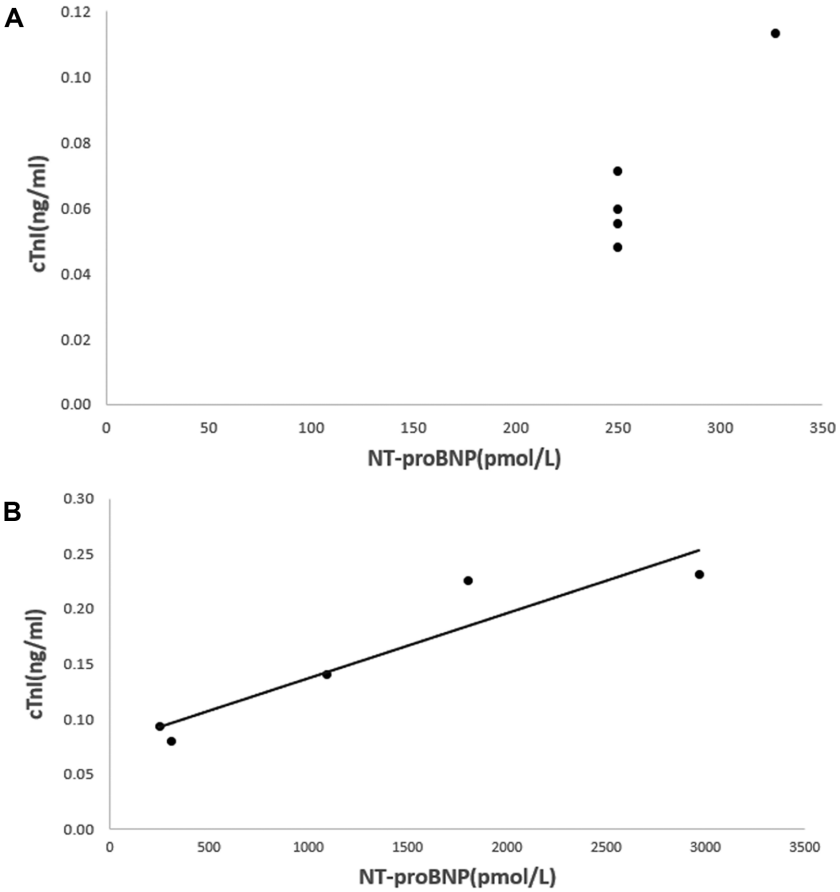

Fig 5. The correlation between NT-proBNP and cTnI in severe group and mild group. A, Mild group ($r = 0.717$, $p = 0.182$). B, Severe group ($r = 0.9$, $p < 0.05$).

worm disease (17). In this study, the pulmonary hypertension was higher levels in the severe group than the mild group.

In addition, when evaluating the association between each biomarker and PG, there was a positive correlation in pulmonary hypertension and NT-proBNP, and a positive correlation in cTnI. When comparing correlations among concentrations, there was no significant correlation between NT-proBNP and cTnI in the mild group. However, there was a significant positive correlation between NT-proBNP and cTnI in the severe group.

There are several limitations to this study. First, the number of dogs participating in the study was small. Second, the golden standard for pulmonary hypertension measurement is a direct measurement by attaching a catheter to the right ventricle, but in this study, echocardiography was used to calculate the pulmonary hypertension.

In conclusion, for diagnosis of canine heartworm disease, evaluation of blood biomarker concentration and severity of pulmonary hypertension may be helpful to assess the severity of this disease. Also, it is helpful to refer to each correlation between these indicators.

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