

Cardiac Biomarkers for Monitoring Canine Heartworm Disease: A Comparative Study of N-Terminal Pro B Type Natriuretic Peptide and Cardiac Troponin I Levels

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Abstract

Canine heartworm disease is a global mosquito-borne parasitic illness that affects dogs. The main species of filarids responsible for this disease is *Dirofilaria immitis*. This article specifically explores the use of cardiac biomarkers, such as N-terminal pro B type natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI), for diagnosing and monitoring canine heartworm disease. These biomarkers can indicate myocardial injury and heart failure, particularly in chronic infections. The study compares the levels of NT-proBNP and cTnI in dogs with heartworm disease, both clinically diagnosed cases and those without symptoms. The results reveal that cTnI is elevated in both cases, while NT-proBNP is increased only in dogs with clinical signs. These findings suggest that cTnI may be more accurate in detecting heartworm disease than NT-proBNP. The article concludes by emphasizing the significance of cardiac biomarkers in monitoring the different stages of the disease and evaluating the severity of heartworm disease in dogs.

Keywords: Cardiac Biomarkers, Canine Heartworm, NT-proBNP, cTnI

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Introduction

Canine filariasis disease is important and has a worldwide distribution, including in Thailand. Filariasis is a mosquito-borne parasitic disease which is a significant public health concern in tropical and subtropical areas. Filariasis in dogs is caused by several species of filarids such as *Dirofilaria immitis*, *Dirofilaria repens*, *Brugia malayi*, *Brugia pahangi* and *Acanthocheilonema reconditum*. In Thailand, canine filariasis disease is caused by *D. immitis* and *B. pahangi* (Jitsamai et al., 2021). The most pathogenic canine filarid is *D. immitis*, which causes heartworm disease and canine dirofilariasis. The clinical signs of canine heartworm diseases can range from asymptomatic to symptoms related to cardiopulmonary diseases including blood vessels and heart. A severe infection can cause fatal symptoms. Adult *D. immitis* heartworms lead to vascular disease, potentially compromising blood flow and impacting the vascular and pulmonary systems, with severe cases even affecting the right heart chambers. The damage to pulmonary endothelium and vascular occlusion from worm death will reduce cardiac output. The resulting pulmonary hypertension may lead to compensatory right-side heart enlargement and progress to right heart failure (Bowman and Atkins, 2009; Ames and Atkins, 2020).

The diagnosis tool for dirofilariasis is mainly based on history, clinical signs in heartworm antigen test, microfilaria test, radiography and echocardiography. The biomarkers can be used in screening tests to monitor or evaluate prognosis, disease stages and monitor treatment (Carreton et al., 2017). The cardiac biomarkers that are important in canine heartworms are N-terminal pro B type natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI). The cardiac biomarkers demonstrated the presence of myocardial injury and heart failure, especially in chronic infection. Ventricular myocytes primarily produce NT proBNP, although the atria also contribute a small amount to their synthesis. The study of NT pro-BNP has many reported in heart and cardiopulmonary diseases in dogs (Carreton et al., 2017; Costa-Rodríguez et al., 2023; Sastravaha et al., 2010; Yasue et al., 1994). Troponin is a structural protein and part of the contractile apparatus of skeletal and cardiac muscle. Troponin regulates the interaction of actin and myosin to control muscle cell contraction. Troponin consists of three subunits: troponin C, troponin I and troponin T. Troponin I is one of the subunits that is specific to the myocardial muscle (Panteghini et al., 2008). Cardiac troponin has many reports in heart diseases such as myocardial infarction, cardiac trauma, primary cardiac disease, secondary myocardial injury and heartworm disease (Langhorn and Willson, 2016; Lee et al., 2020). The cTnI was also reported in the study of monitoring during treatment of heartworm diseases (Carreton et al., 2013). There is little information about the cardiac biomarkers: NT-proBNP and cTnI in non-clinical heartworm diseases. Therefore, the aim of this study was to compare cardiac biomarkers NT-proBNP and cTnI concentrations in non-clinical and clinical canine heartworm diseases.

Materials and Methods

Sample collection: A total of 15 canine samples were collected from animal hospitals and clinics in Bangkok and the vicinity. The research protocol was approved by the Chulalongkorn University Animal Committee (approved no.2031065). Samples of canine blood were gathered and sorted into three categories: a control or healthy group (n=5), a non-clinical sign group (n=5), and a clinical group exhibiting signs of heart abnormalities (n=5). The clinical signs were confirmed through echocardiography, along with the presence of adult heartworms in the pulmonary artery and right ventricle. The non-clinical group showed positive findings for *D. immitis* microfilaria using the buffy coat thin blood smear and Giemsa staining techniques. All samples were determined microfilaria by Modified Knott's test and specified species by acid phosphatase staining technique (Chungpivat and Taweethavonsawat, 2008). All canine blood samples were gathered using EDTA and serum collection tubes. Subsequently, all serum samples were stored at -20°C until analysis.

Laboratory measurements of NT-proBNP: The NT-proBNP concentration was measured using a fluorescent immunoassay (Vcheck Canine CRP 2.0 test kit, Bionote, South Korea). The serum samples from control (n=5), non-clinical sign (n=5) and clinical sign (n=5) were determined NT-proBNP concentration. The 100 µl of each sample was diluted with diluent buffer in the bottle. The 100 µl diluted sample was mixed and added to the test device. The V200 analyzer showed NT-proBNP results on the screen after 15 minutes. The NT-proBNP concentration <900 pmol/L means normal value.

Laboratory measurements of cTnI: The concentration of cTnI was measured using the Vcheck Canine CRP 2.0 test kit, a fluorescent immunoassay manufactured by Bionote, South Korea. Serum samples from the control group (n=5), non-clinical sign group (n=5), and clinical sign group (n=5) were analyzed to determine their cTnI concentration. Each sample, consisting of 100 µl, was diluted with diluent buffer in a bottle. After mixing, the diluted sample (100 µl) was added to the test device. The V200 analyzer displayed the cTnI result on the screen after 10 minutes. A cTnI concentration below 0.03 ng/ml indicates a normal value.

Results and Discussion

NT-pro BNP concentration: In both the normal and non-clinical sign groups, the concentration of NT-pro BNP was found to be less than 500 pmol/L. In the clinical sign group displayed higher concentrations of NT-proBNP. The average concentration in the clinical sign group was 1,131.04 pmol/L (Table 1). The highest recorded concentration of NT-proBNP in the clinical sign group was 2,017.70 pmol/L, while the lowest concentration observed was 610.10 pmol/L.

Table 1 The results of NT-proBNP and cTnI in clinical, non-clinical sign and control group

Variable	Control (Mean ± SD) (n=5)	Clinical sign (Mean ± SD) (n=5)	Non-clinical sign (Mean ± SD) (n=5)
NT-proBNP (pmol/L)	<500	1131.04 ± 597.98	<500
cTnI (ng/ml)	0.03 ± 0.02	0.23 ± 0.43	0.16 ± 0.17

cTnI concentration: In the normal group, the concentration of cTnI was found to be 0.03 ± 0.02 ng/ml, while in the non-clinical sign group it was 0.16 ± 0.17 ng/ml, and in the clinical sign group it was 0.23 ± 0.43 ng/ml (Table 1). Out of the clinical sign group, three samples displayed significantly abnormal cTnI concentrations, measuring 1.40, 0.4, and 0.12 ng/ml, respectively.

The findings of this study revealed that levels of cTnI were elevated in both the non-clinical and clinical sign groups. Additionally, the concentration of NT-proBNP was within the normal range in the non-clinical group but increased in the clinical sign group. These results align with previous studies that demonstrated an increase in NT-proBNP and cTnI levels, which were also correlated with severe clinical signs. The severity of pulmonary hypertension was found to be higher in the severe group compared to the mild group (Lee *et al.*, 2020). According to this study, cTnI appears to be more sensitive than NT-proBNP as a biomarker. This is because cTnI levels increased in both non-clinical and clinical sign groups, whereas NT-proBNP levels only increased in the clinical sign groups. Multiple studies have reported elevated serum cTnI levels in dogs with heartworm (Carretón *et al.*, 2013; Puente *et al.*, 2018; Yoon *et al.*, 2017). Canine heartworm disease produces an elevation of cTnI due to right heart myocardial damage (Carretón *et al.*, 2012). Cardiac troponins are biomarkers specific to cardiomyocyte injury (Liquori *et al.*, 2014). When the cardiomyocyte experiences destruction and membrane rupture, there is a significant release of free cardiac troponins into the bloodstream. This is accompanied by a subsequent slow and continuous release of structurally bound troponins. As a result, there is a sustained increase in the concentration of cardiac troponins in the serum (Wells and Sleeper, 2008). Serum cTnI can be detected within 4-6 hours after induced trauma, reaches its peak concentration at 10-16 hours, and remains elevated for up to 200 hours (Cummins and Cummins, 1987).

The study revealed that an increase in NT-proBNP was observed only in groups exhibiting clinical signs, which correlated with findings from other studies. Several studies determined NT-proBNP levels in canine heartworm disease, demonstrating that mild cases exhibited normal values while severe cases displayed significantly higher levels than the control group (Lee *et al.*, 2018; Lee *et al.*, 2020). NT-proBNP, which is produced in the ventricular myocardial cells, is secreted in response to ventricular overload and myocardial hypertrophy-induced stress. These biomarkers are utilized to assess severe heart disease in small animals and to evaluate the effectiveness of treatment and the severity of cardiac diseases (Kim *et al.*, 2013; Oyama *et al.*, 2013).

In conclusion, to compare cTnI and NT-proBNP, the cTnI can be useful for non-clinical and clinical

signs. NT-proBNP is probably elevated in severe cases of canine heartworm disease. In the case of non-clinical symptoms, the cardiac biomarker was important to monitor the stage of diseases and check health status. In the clinical group, the cardiac biomarker is one of the tools for dividing the staging of the diseases and helping veterinarians to monitor the treatment and progress of the disease.

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