



Serum NT-pro BNP estimation for diagnosis of cardiac diseases in dogs with and without Holter Electrocardiographic arrhythmias

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Abstract

The study was conducted with an objective to rule out the dogs presented with a complaint of clinical signs suggestive of cardiac disease. A total of 14 animals were chosen for the assessment of serum NT-pro BNP level. The animals were grouped into two groups: group 1 with Holter electrocardiographic arrhythmias and group 2 without Holter electrocardiographic arrhythmias. Group 1 showed a significant increase in the NT-pro BNP levels in contrast to the control and group 2 animals. NT-pro BNP estimation in screening of dogs suspected for cardiac sickness was found to be a reliable step when done concurrently with other diagnostic tests.

Keywords: Biomarker, NT-pro BNP, cardiac diseases, dogs

The biomarker N-terminal-pro brain natriuretic peptide (NT-pro BNP) is a peptide in nature, synthesized and released by the myocardium in the form of prohormones into circulation in response to various stimuli such as myocardial wall stress (volume overload and pressure overload).

On release into the system, specific proteases cleave the prohormones into pro hormones: C-terminal and N-terminal pro BNP (Goetze *et al.*, 2005; Hogenhuis *et al.*, 2007; Oyama and Singletary, 2010). Physiologically it performs various antagonistic functions to that of the renin-angiotensin system viz., diuresis, increased renal blood flow, vasodilation, improves cardiac

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diastolic function and counters cardiac muscle fibrosis (Chen *et al.*, 2000; Lainchbury *et al.*, 2000; Oyama and Singletary, 2010). NT-pro BNP also possess various clinical diagnostic important usessuch as the identification of occult heart disease, recognition of etiology of cough, improvement of diagnostic accuracy and forecasting risk of morbidity and mortality in affected dogs (Oyama and Singletary, 2010).

A total of 24 dogs were brought to the Teaching Veterinary Clinical Complex (TVCC) of Wayanad district, with a complaint of various clinical signs pointing towards cardiac illness. Animals showing signs of exercise intolerance, cough, respiratory distress, weakness, inappetence and ascites were selected. A total number of 14 dogs were preferred out of the presented 24 dogs (58.33 per cent) for performing NT-pro BNP assay. The animals were categorized into two groups based on the Holter electrocardiographic arrhythmia findings viz., group 1, dogs with Holter arrhythmias and group-2, those without Holter arrhythmias. Six apparently healthy dogs were selected as for the control. Blood sample was collected from either the cephalic or saphenous veins. Serum was separated with the help of centrifugation and stored at -20° C. The NT-pro BNP was

estimated using Cusabio ELISA Kit specific for canine NT-pro BNP following sandwich ELISA test as per the procedure provided with the assay. Optical density was determined using a microplate reader at 450 nm (Fig.1).

The serum NT-pro BNP findings were statistically analysed between the control, group 1 and group 2. The mean \pm SE values for serum NT-pro BNP were 345.71 ± 25.6 , 1212.81 ± 192.22 and 442.31 ± 64.11 pmol/L (picomoles per litre) for the control, group 1 and group 2 animals. Significant increase in the mean \pm SE values for NT-pro BNP levels was found in group 1 when compared to the control and group 2 animals. Group 2 animals showed no significant difference from that of the control group. The individual serum NT-pro BNP findings are given in Table 1. Mean Serum NT-pro BNP observations are presented in Table 2.

The study was carried out in the dogs showing clinical signs suggestive of cardiac illness irrespective of the severity of disease. Diagnosis was made based on the combined Holter ECG, echocardiogram, radiography and NT-pro BNP estimation (Fig.2). Myxomatous mitral valve disease (MMVD) was diagnosed in two dogs (T2 and T6). Dilated cardiomyopathy (DCM) was observed in two dogs (T3 and T5), occult dilated cardiomyopathy (ODCM) in two

Table 1. Serum NT-pro BNP finding

Group	Animals	NT-pro BNP (pmol/L)
Group-1	T1	1354.6
	T2	844.33
	T3	881.04
	T4	660.78
	T5	2386.15
	T6	1163.71
	T7	1450.04
	T8	961.80

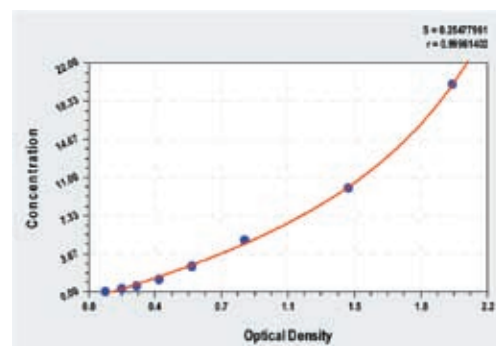


Fig 1. Standard ELISA curve

Table 2. Serum NT-pro BNP

Parameter	Control	Group 1	Group 2	F value	P value
NT-pro BNP (pmol/L)	345.71 ± 25.6^b	1212.81 ± 192.22^a	442.31 ± 64.11^b	12.473**	<0.001

** Significant at 0.01 level; ns Non-significant ($P > 0.05$)

Means having different letter as superscript differ significantly

Echocardiogram

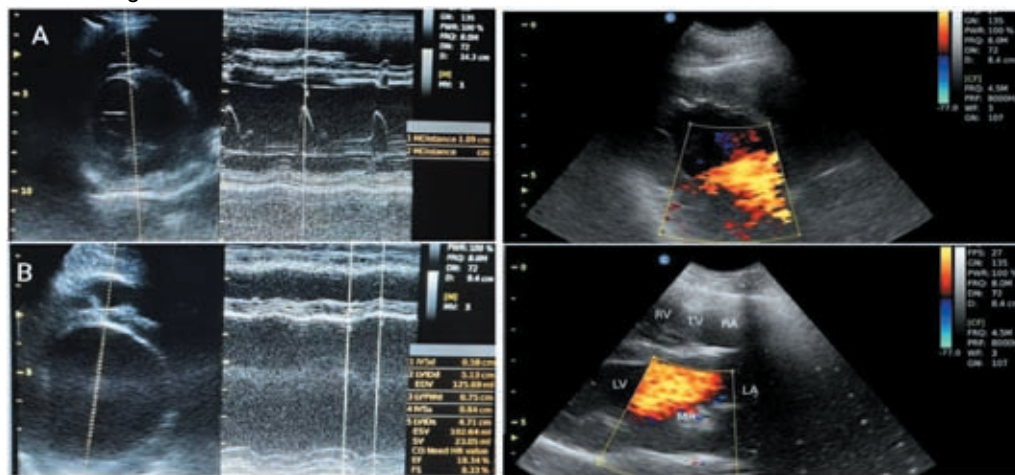


Fig2. Echocardiogram in Dogs- A. Increased EPSS and B. Dilated Ventricle seen in a dog with severe signs Congestive heart failure (CHF). Mitral regurgitation (MR) seen by mosaic pattern of colour (picture on the right side) in a dog with the complaint of cough and exercise intolerance.

dogs (T4 and T7), Hypertrophic cardiomyopathy (HCM) in one dog (T8) and one dog (T1) was not categorised into any disease condition due to non-specific findings. Only one dog had a severe form of congestive heart failure in the current study. Animals diagnosed with ODCM had a value of 660.78 pmol/L and 1450.04 pmol/L. Both the animals did not show any clinical symptoms except aslight exercise intolerance in one. Dogs with MMVD had a value of 844.33 pmol/L and 1163.71 pmol/L. It had a complaint of cough and weakness. Dog with DCM had a value of 881.04 pmol/L and 2386.15 pmol/L. One of the dogs was on long-term premedication with pimobendan which might be the reason for lower serum NT-pro BNP concentration. Dog diagnosed with HCM had a value of 961.80 pmol/L with a complaint of exercise intolerance. A concentration of 1354.6 pmol/L was found for dog (T1) with no specific diagnosis. The dogs diagnosed with several cardiac disorders had a serum NT-pro BNP concentration of 660.78 pmol/L to 2386.15 pmol/L in the present study. The present study found that dog (T5) with signs of congestive heart failure had a concentration of > 1800 pmol/L which is similar to the findings (Oyama *et al.*, 2008a). In addition, it had developed ascites, echocardiogram revealed severely dilated left atrium with a LA/Ao ratio of 2.4, revealed atrial fibrillation in both routine electrocardiogram

(ECG) and Holter ECG. Other members of the group had a complaint of cough, dyspnea and exercise intolerance without showing any other overt clinical symptoms. According to Oyama *et al.* (2008b), NT-pro BNP concentration between 820 pmol/L to 1725 pmol/L was the undefined zone for diagnosis of congestive heart failure which was similar to the observations of the present study. Animals from group 2 were mainly had complaints of exercise intolerance, inappetence and weakness. There was no Holter arrhythmia and echocardiographic, radiographic findings were within the normal range. Importance of Serum NT-pro BNP concentration is determining the level of stress attended by the ventricular wall in response to the degree of compensation going on. It aids in assessing the treatment efficacy which is attended by T3. It helps in tracking the disease progression and its severity.

Summary

Higher levels of NT-pro BNP concentration were found in the dogs having Holter arrhythmias when compared to those without Holter arrhythmias. Combined application of electrocardiogram, echocardiogram, radiography and NT-pro BNP assay will help in better understanding of the disease progression.

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Conflict of interest

There is no conflict of interest

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