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*Original article*

# NT-pro-BNP and troponin I as predictors of mortality in dogs with heart failure

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## Abstract

The purpose of this study was to develop prognostic models for heart failure in dogs with dilated cardiomyopathy (DCM). The prospective study included 26 dogs with DCM and 58 healthy dogs. The survival time median was 250 days (1-600 days). All the dogs were clinically examined, had echocardiography, electrocardiography, and morphological and biochemical blood sampling. Twenty four deaths were found in the group of dogs with DCM and 1 demise in the healthy dog's group. There was a significant increase in the level of NT-pro-BNP and cTnI ( $p < 0.0005$ ) in the group of dogs with DCM and a significant higher level of NT-pro-BNP and cTnI ( $p < 0.0005$ ) in the dead dogs from group with DCM that died or were euthanized up to the 60<sup>th</sup> day of observation, compared to the animals that outlasted over 60 days of observation. The median level of NT-pro-BNP in the dogs which had short survival period (no more than 60 days) was 4865 pmol/L and the median level of cTnI in the same group of dogs was 0.63 ng/ml. The median level of NT-pro-BNP in the group of dogs with DCM, which lived longer than 60 days of observation was 978 pmol/l and the median level of cTnI in this group was 0.1 ng/ml. The level of NT-pro-BNP ( $r = 0.79$ ) and cTnI ( $r = 0.4$ ) correlated with the dogs' death. NT-pro-BNP and cTnI measurements could be useful to evaluate the survival the dogs with DCM. Increased level of NT-pro-BNP and cTnI is a bad prognosis. In the performed analysis of the Cox hazard regression it was found that cTnI level has a significant impact of the survival of the dogs (HR=8.54; CI 1.1-46.6;  $p = 0.02$ ).

**Key words:** brain natriuretic peptide, dogs, heart, troponin I

## Introduction

Dilated cardiomyopathy (DCM) is one of the most common organic heart failures observed in large and giant dog breeds. It is characterized by dilatation of heart ventricle and the systolic and diastolic dysfunction of the myocardium. The etiology is still not recognized, but from among reasons given are gen-

etic, nutritional (deficiency of aminoacids), metabolic, inflammatory, infectious and toxicological factors (Tidholm et al. 2001). In humans, mice and hamsters, the genetic background was confirmed and multiple mutations responsible for the development of DCM were found (Wiersma et al. 2008). In many breeds of dogs, the hereditary aspect of DCM was described, but still the gene mutations responsible for the devel-

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opment of this sickness are unknown, even though studies are continued for many years and many gene loci were checked (Meurs et al. 2008, Werner et al. 2008, Wiersma et al. 2008). In Dobermans, Dalmatians, Bull Mastiffs and Irish Wolfhounds irregularities in the proteins of cytoskeleton: dystrophin, desmin, h-sarcoglycan and  $\beta$ -dystroglycan were described, but it was not confirmed that the irregularities of proteins in cytoskeleton were responsible for DCM (Spier et al. 2001, Noszczyk-Nowak et al. 2009). It might be that the irregularities are the effect of the disorganization of cardiomyocytes during DCM (Noszczyk-Nowak et al. 2009). It is described that in Great Danes, DCM is a monogenetic recessive disease linked with chromosome X (Meurs et al. 2001). In Newfoundlands, it is a dominant autosomal with incomplete gene penetration (Dukes-McEwan and Jakson 2002). In male Irish Wolfhounds with DCM, a different genotypic layout is described compared to females with DCM (Distl et al. 2007). Males seem to have DCM more often than females, with the appearance of symptoms in 4 to 10 year old dogs (Tidholm et al. 2001). DCM can be confirmed by doing the echocardiography. The enlargement of the left ventricle, the increase of sphericity of left ventricle and the reduction of the systolic function was observed in the echocardiography. Additionally the enlargement of left atria, increase of the relation between the size of left atria towards the aorta diameter (LA:Ao ratio) and the insufficiency of the tricuspid and mitral valve can be found (Dukes-McEwan et al. 2003). In the echocardiography supraventricular and ventricular heart beat disorders were described (Dukes-McEwan et al. 2003). From the moment of diagnosing DCM the dogs were still alive from 2 to 24 months, but this period of time depends on many variables, that is why it is difficult to evaluate it (Monnet 1995, Tidholm et al. 1997, Tidholm 2006). The time of survival is related to the development of the heart failure symptoms, enlargement of the heart cavities, appearance of the rhythm disorders and the prescribed treatment (Tidholm 2006). Worse prognosis is when lung swelling appears, together with pleura exudation and ascites (Monnet 1995, Tidholm et al. 1997). Among different parameters used for the evaluation of the clinical status of the dog with DCM, shortening fraction (FS), left ventricle ejection fraction (LVEF), relation between the size of left atria towards aorta diameter (LA:Ao ratio) are described (Tidholm et al. 1997). There are no relevant biochemical indicators that can be used to predict the time of survival of dogs with DCM, which would be easy to assay, would not demand expensive equipment and which result of this tests would correlate with the time of survival and the

mortality of dogs with DCM. Among so called cardiac indicators, troponin I and T (cTnI and cTnT) and creatinine kinase (CK) together with its isoenzyme (CK-MB) are mentioned as the myocardium breakdown (necrosis) indicators, brain natriuretic peptide and atrial natriuretic peptide (BNP and ANP) as cardiomyocytes overload indicators, C-reactive protein (CRP) as an inflammatory indicator, metalperoxidase (MPO) as a neutrophil's activation indicator or endothelin (ET) as an endothelium activation sign (O'Brien 1997, Oyama and Sisson 2004, Oyama et al. 2007, Oyama and Singletary 2010, O'Sullivan et al. 2007). Many of the above mentioned cardiac indicators were determined in dogs with DCM, although most of all, the focus was put on evaluating their usefulness for early detection of DCM (Oyama and Sisson 2004, Oyama et al. 2007, O'Sullivan et al. 2007, Vazquez et al. 2009). The usefulness of ET and BNP as bad prognosis indicators in dogs with DCM was confirmed (Oyama et al. 2007, O'Sullivan et al. 2007).

The aim of the present study was to determine the usefulness of NT-pro-BNP and cTnI as prognosis indicators of dogs with DCM.

## Materials and Methods

### Reference population

Prospective studies were performed on 84 dogs, Boxers, Great Danes and Dobermans breeds, aged from 1 year up to 11 years, various sex (47 male dogs and 37 female dogs). Among the study group, 26 dogs had DCM (Boxers-8, Great Danes-9 and Dobermans-9) and clinical signs of heart failure (Ib, II and IIIa, ISACHC score) and 58 dogs (Boxers -20, Great Danes-19 and Dobermans-19) were healthy (Freeman et al. 2005). The observation time was between 1 and 600 days (end of the study). None of the dogs were treated earlier with cardiac drugs. All of the owners were interviewed with special attention put on defining symptoms that could lead to heart failure.

### Methods

All of the dogs were clinically examined, had echocardiography, which was the basis of diagnosing DCM, electrocardiography, morphological and biochemistry blood tests. During echocardiography, the size of the left ventricle at systole and diastole was evaluated together with the thickness of the ventricular septum and the left ventricle open wall during systole and diastole, left ventricle ejection fraction

(LVEF) and left ventricle shortening fraction (FS), size of the left atria and the relation of the atria size towards aorta diameter (LA:Ao ratio). For further analysis LVEF, FS and LA:Ao ratio as independent parameters from the body mass (range between 30-70 kg) were used. During electrocardiography the heart beat frequency and the presence of any rhythm disorders were evaluated.

### Blood analyses

Blood samples were collected after clinical examination. Using minimum stasis, cephalic venous blood was obtained with a 21 G disposable butterfly needle and Vacutainer system into serum (6 mL), EDTA (2 mL) and special tubes to Nt-proBNP (IDEXX), in the order. Morphological blood tests were based on evaluating the total level of red blood cells, white blood cells, thrombocytes (PLT), haematocrit (Ht) and haemoglobine (Hb). Morphological blood tests were performed on Animal Blood Center abc VET analyzer. Biochemistry tests were based on evaluating the activity of alanine aminotransferase (ALT), asparate aminotransferase (AST), level of urea, creatinine, bilirubin, total protein and albumins, level of ions: Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, total Ca and inorganic phosphorus. Biochemistry tests were performed on MaxMat PI analyzer. The concentration of NT-pro-BNP and cTnI as cardiac indicators were evaluated. The plasma concentration of NT-proBNP and cTnI were measured at a commercial laboratory center IDEXX. ELISA test was used to NT-proBNP evaluation. Automated immunoluminescence test was used to cTnI evaluation.

Healthy dogs were controlled every 6 months, dogs with DCM were examined depending on their clinical status. The date of dog's death and observation time were registered. Statistical analysis was performed based on the data collected.

### Statistical analysis

The differences between groups based on parametric data with standard distribution were analyzed by using t-Student test. In case of non-parametric data or lack of standard distribution, U Mann-Whitney test was used. The correlation was analyzed by using R Spearman test. A single factor analyze ANOVA was performed together with the evaluation of Cox hazard regression. The testing was done based on the significance level  $p < 0.05$ .

The study was approved by the ethics committee of the University of Environmental and Life Sciences, Wroclaw, Poland No. 06/2008.

### Results

The group of dogs did not differ based upon the sex and body weight, but the average age of dogs with diagnosed DCM was higher than that of the healthy dogs (Table 1). During 600 days of observation, 24 deaths of dogs with DCM were noticed, including 9 dogs (37.5%) which died before 60 days of observation. One of the dogs that was found to be healthy was also noticed dead (1.7%). In the group of dogs with DCM, the correlation of LA:Ao ratio ( $p < 0.001$ ) was higher, the FS and LVEF ( $p < 0.001$ ) were lower. Factors FS and LVEF were correlated with the dog's mortality ( $r = -0.7$ ;  $p < 0.05$ ). In the group of dogs with DCM, the resting heart rhythm ( $148.5 \pm 43.7$  bpm) was higher compared to that observed in the healthy dogs ( $131.6 \pm 27.9$  bpm) ( $p = 0.039$ ). The frequency of heart rhythm was correlated with the mortality of dogs with DCM ( $r = 0.4$ ;  $p < 0.05$ ). Significantly lower was RBC in DCM dogs ( $p < 0.01$ ). The rest of the morphological blood parameters did not differ between the groups. Dogs with DCM showed a higher level of ALT, but the value median in any group did not exceed the reference values and there was no correlation between ALT and the dogs' mortality. Dogs with DCM had also a higher concentration of NT-pro-BNP and cTnI ( $p < 0.001$ ). The concentration of both indicators was significantly higher in dogs which died in less than 60 days counting from the day of sampling compared to those that lived more than 60 days of observation (Table 2). Correlation between NT-pro-BNP and the mortality of dogs with DCM ( $r = 0.79$ ;  $p < 0.05$ ) was observed as well as the correlation between the concentration of cTnI and the dogs mortality ( $r = 0.4$ ;  $p < 0.05$ ). The concentration of NT-pro-BNP was correlated with the relation of LA:Ao ratio ( $r = 0.71$ ;  $p < 0.05$ ) as well as LVEF and SF ( $r = -0.7$ ,  $p < 0.05$ ). The concentration of cTnI was correlated with the relation of LA:Ao ratio, showing an increase in LA ( $r = 0.54$ ;  $p < 0.05$ ) as well as in LVEF and SF ( $r = -0.27$ ;  $p < 0.05$ ). The Cox regression hazard analyze showed that cTnI has a significant influence on the dogs' survival (HR=8.54; CI 1.1-46.6;  $p = 0.02$ ).

### Discussion

The presented results show the prognosis value of NT-pro-BNP and cTnI in dogs with DCM. Dogs with DCM were older than the animals from the control group, which is correlated with the screening type of the study. The research included dogs more than 1.5 year old, after the growing chase. DCM usually appears in dogs aged between 4 and 10 years, therefore the age of dogs with DCM in the study was signifi-

Table 1. Result of group characterization, echocardiography examination, morphological and biochemical blood tests in healthy dogs and dogs with DCM.

Parameter	Healthy n=58 Median (range)	DCM n=26 Median (range)	Differences between groups
Age [years]	4 (1.5-13)	8 (1.5-11)	p=0.003
Sex	34 ♂/24 ♀	18 ♂/8 ♀	ns
Body mass [kg]	43 (24-81)	42 (26-78)	ns
HR [bpm]	131 (81-206)	146 (98-260)	p=0.04
LVEF [%]	61 (38-79)	32 (6.6-38)	p<0.001
FS [%]	32 (21-48)	18 (2.9-19)	p<0.001
LA:Ao ratio	1.1 (0.9-1.2)	2.1 (1.6-2.6)	p<0.001
ALT [U/l]	36 (10-124)	68 (33-394)	p=0.05
AST [U/l]	36.5 (20-61)	41 (13-168)	ns
Urea [mmol/L]	5.1 (3-12)	6.7 (3.2-14)	ns
Creatinine [mmol/L]	94 (64-135)	104 (71-149)	ns
Na <sup>+</sup> [mmol/L]	143.5 (124.4-148)	146 (137-152)	ns
K <sup>+</sup> [mmol/L]	4.5 (3.4-5.1)	4.4 (3.8-5.1)	ns
Mg <sup>2+</sup> [mmol/L]	0.7 (0.55-0.87)	0.77 (0.44-0.86)	ns
Cl <sup>-</sup> [mmol/L]	119 (108-122)	112 (102-124)	ns
Ca [mmol/L]	2.6 (1.9-2.8)	2.5 (2.0-2.8)	ns
P inorganic [mmol/L]	1.45 (1.05-2.4)	1.4 (1.16-2.07)	ns
Total protein [g/L]	65 (59-77)	63 (54-73)	ns
Albumin [g/L]	33 (29-36)	33 (27-35)	ns
Bilirubin [μmol/L]	4.1 (1.8-11)	3.8 (1.6-9)	ns
RBC [10 <sup>12</sup> /L]	7.5 (5.7-8.7)	7.0 (4.4-8.8)	p<0.01
WBC [10 <sup>9</sup> /L]	11.9 (5.3-18.0)	11.5 (6.4-26.0)	ns
PLT [10 <sup>3</sup> /L]	244 (98-449)	228 (73-500)	ns
Hb [mmol/L]	10.5 (7.9-12.2)	10.25 (6.2-15.9)	ns
Ht [L/L]	0.51 (0.38-0.59)	0.48 (0.29-0.58)	ns
cTnI [ng/ml]	0.03 (0.01-0.05)	0.2 (0.06-10.8)	p<0.001
NT-pro-BNP [pmol/l]	136 (10-879)	2180 (780-5065)	p<0.001
Deaths	1	24	p<0.001
Days of observation	250 (120-600)	145 (1-220)	p<0.001

Table 2. Concentration of cTnI and NT-pro-BNP in healthy dogs, dogs with DCM, which did not survive over a period of 60 days of observation and dogs with DCM which died after the 60<sup>th</sup> day of observation.

Parameter	Healthy N=58 Median (range)	DCM with observation time less than 60 N=9 Median (range)	DCM with observation time over 60 days N=15 Median (range)
NT-pro-BNP	136 (10-879)	4865 (2180-5065)	978 (167-3026)
cTnI	0.03 (0.01-0.05)	0.63 (0.1-10.84)	0.1 (0.06-0.27)

cantly higher (Tidholm et al. 2001). DCM is characterized by cardiectasia and the decrease in the systole and diastole function of the left ventricle (Tidholm et al. 2001, Dukes-McEwan and Jakson 2002). The late diastole value of the left ventricle is dependent from the body weight and is significantly different in Dobermans, Boxers and Great Danes, that is why for further statistical analysis LA:Ao ratio, LVEF and FS

were chosen, which in the segment between 30-80 kg have similar reference values. The identification of dilated cardiomyopathy in dogs was done based upon echocardiography. In the resting ECG, a higher heart rhythm frequency in dogs with DCM was observed and its correlation with the mortality in this group of animals. Tachycardia is one of the compensating mechanism, activated in heart failure, but on the

other hand the increase in heart rhythm frequency leads to higher demand for oxygen by cardiomyocytes. During an observation of a large group of humans with heart failure, it has been confirmed that a higher heart rhythm is correlated with a higher mortality in this group of patients (Vazquez et al. 2009). Additionally, tachycardia may lead to a development of tachycardiomyopathy, which will worsen the functionality of the left ventricle.

In the group of dogs with DCM, a low level of RBC was observed, although the RBC median did not exceed the lowest reference values. Observed low value of RBC in the group of dogs with RBC might be correlated with cardio-renal anemia syndrome (CRA). Anemia developing due to chronic disease is the major type appearing in dogs. In many cases, anemia is related to disturbances during the synthesis of erythropoietin and renal failure which develop very often in dogs with DCM. An important factor in the anemia development is the blood cell hemolysis due to the damage of red blood cells by urea toxins. In many cases the anemia is intensified by insufficient bone marrow reaction towards erythropoietin, due to iron, folic acid, vitamin B<sub>12</sub> deficiency. Anemia appearing in patients with heart failure is one of the factors modulating mortality in this group of patients (Palterieri et al. 1998, Iana et al. 2005). The prognosis for dogs with DCM is bad and the time of survival is between 2 and 24 months, depending on many factors (Monnet et al. 1995, Tidholm et al. 1997, Tidholm 2006). That is why there are efforts made to find indicators which would help to evaluate the survival time for dogs with DCM. NT-pro-BNP is a N-final part of pro-BNP. Its advantage is that it has a longer half-life, higher stability in the serum compared to BNP which means it can be easier determined (Oyama and Singletary 2010). In healthy dogs, the level of NT-pro-BNP should not exceed 500 pmol/l, but sometimes higher values can be found, which is correlated with individual variation (Kellihan et al. 2009). In these cases, the test needs to be repeated after 2-3 weeks. In the study done by Oyama et al. (2008) the level of NT-pro-BNP over 445 pmol/l did differ the dogs with cardiac disease from the healthy ones (83% of sensitivity, 90% of specificity). According to the same authors, the level of NT-pro-BNP was highly correlated with the heart size and frequency of heart rhythm in the dogs with cardiac disease. In the present study, the concentration of NT-pro-BNP highly correlated with LA:Ao ratio and the systole parameters of the left ventricle and dog's mortality. The concentration of NT-pro-BNP was significantly higher in dogs with DCM, which died shorter than 60 days calculating from the first observation day (median 4865 pmol/l), what leads to a conclusion that a high

level of NT-pro-BNP is a negative prognosis factor.

Troponin I is a protein belonging to the troponin-myosin complex in cardiomyocytes. Its concentration is very low or hardly detectable in healthy dogs. The level of cTnI increases during cardiomyocytes' damage due to hypoxia, toxemia or myocarditis. Dogs with cardiac diseases have a higher level of cTnI, and the values <0.2 ng/ml are correlated with shorter time of survival (Oyama et al. 2007). In the present study, the level of cTnI was significantly higher in dogs with DCM, that died in shorter than 60 days of observation and in those animals which had a longer period of survival. According to the Cox hazard regression analysis, cTnI is an independent factor having an impact on the survival of dogs with DCM and the 0.1 ng/ml increase in cTnI elevates the risk of death by as much as 8.5 times.

NT-pro-BNP and cTnI are good indicators of the time of death of dogs with DCM. They allow to evaluate the probability of death and specify the prognosis.

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