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Short communication

BLOOD VARIABLES ASSOCIATED WITH SURVIVAL IN CANINE CONGESTIVE HEART FAILURE PATIENTS

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Summary

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Our study aimed to investigate the blood variables: coenzyme Q_{10}, N-terminal pro-B-type natriuretic peptide (NT-proBNP), electrolytes, cholesterol, triglyceride, glutathione peroxidase, white blood cell, neutrophil, lymphocyte and monocyte counts and relative numbers of neutrophils, lymphocytes and monocytes that may be associated with survival of dogs in congestive heart failure (CHF). Twenty-one client-owned dogs in CHF were included in the study. Cox regression analysis showed significant association only between NT-proBNP blood concentration and survival.

Key words: blood variables, cardiovascular diseases, coenzyme Q_{10}, dogs, N-terminal pro-B-type natriuretic peptide, survival

In human patients with decompensated heart failure, some basic laboratory parameters, such as increased white blood cell (WBC) and neutrophil counts, higher serum C-reactive protein, glucose, blood urea nitrogen and creatinine concentrations, and higher aspartate aminotransferase activity are significantly associated with in-hospital deaths (Ostrowska *et al*., 2017). Furthermore, plasma coenzyme Q_{10} (CoQ_{10}) concentration was found as an independent predictor of mortality in human patients with chronic heart failure (Molyneux *et al.,* 2008). Coenzyme Q_{10} is an essential cofactor in oxidative phosphorylation in mitochondria and a powerful antioxidant (Littarru & Tiano, 2007).

Various factors associated with survival in dogs with mitral valve disease (MVD; Serres *et al.*, 2009; Moonarmart *et al*., 2010; de Madron *et al*., 2011; Hezzell *et al.*, 2012; Sargent *et al.*, 2015) or di-

lated cardiomyopathy (DCM; Slupe *et al.*, 2008; Martin *et al.*, 2010; Noszczyk-Nowak, 2011) were investigated. Evaluated factors consisted mostly of echocardiographic and electrocardiographic variables, clinical findings and N-terminal pro-B-type natriuretic peptide (NT-proBNP). However, the data on association of blood variables with survival in dogs with congestive heart failure (CHF) are sparse, with the exception of NT-proBNP. Moreover, antioxidant parameters, such as plasma CoQ_{10} and intracellular antioxidant enzyme, glutathione peroxidase (GPX), and some basic laboratory parameters, such as total and differential white blood cell counts, have not been evaluated as factors associated with survival, yet.

The aim of this study was to find out which of the patients' blood variables measured at admission: plasma CoQ_{10} (lipid-standardised CoQ_{10}), serum NT-proBNP, sodium (Na), potassium (K), chloride (Cl), total cholesterol and triglyceride concentrations, whole blood GPX activity, WBC, neutrophil, lymphocyte and monocyte counts and relative numbers of neutrophils, lymphocytes and monocytes were associated with survival of canine CHF patients (ISACHC II and III classes; International Small Animal Cardiac Health Council) due to MVD and DCM.

Twenty-one client-owned dogs (Table 1) with MVD (n=17) or DCM (n=4), were included in the study and classified into ISACHC II or III groups. Cardiovascular patients included in our study were of the following breeds: Mixed breed dogs (n=5), Cavalier King Charles Spaniel (n=3) and one dog of each breed: Doberman Pinscher, Great Dane, German Boxer, German Shepherd, Golden Retriever, Tibetan Terrier, Dogue de Bordeaux, Jack Russell Terrier, English Cocker Spaniel, Leonberger, Dalmatian, Miniature Schnauzer and Wire-Haired Dachshund. Cardiovascular disease was confirmed on the basis of history and re-

Table 1. Baseline characteristics of canine congestive heart failure patients and laboratory results of five variables used in Cox proportional-hazards models. Results are presented as median and interquartile range (IQR) in the case of non-normal distribution of the data or as mean ± standard deviation (SD) in the case of normal distribution of the data

	Patients (n=21)
Sex (female/male)	4/17
ISACHC II/ ISACHC III	13/8
Cardiac diseases (MVD/DCM)	17/4
Age (years); mean ± SD	9.9 ± 2.6
Weight (kg); median, IQR	16.8, 10.5–35.2
NT-proBNP (nmol/L), mean ± SD	5.540 ± 2.763
WBC (x 10^9/L), mean ± SD	11.6 ± 3.2
Neutrophil count (x 10^9/L), mean ± SD	8.48 ± 2.5
K (mmol/L), mean ± SD	4.06 ± 0.52
CoQ_{10} (mg/L), median, IQR	1.07, 0.51–1.39

ISACHC – International Small Animal Cardiac Health Council classification for cardiac disease; MVD – mitral valve disease; DCM – dilated cardiomyopathy; NT-proBNP – N-terminal pro-B-type natriuretic peptide; WBC – white blood cell count; K – potassium; CoQ_{10} – coenzyme Q_{10}.

Table 2. Hazard ratios (HR) and corresponding 95% confidence intervals (CI) and P values.

Variable	HR, (95% CI for HR)	P value
CoQ_{10}, (mg/L)	0.74 (0.39, 1.38)	0.34
WBC (x 10^9/L)	1.09 (0.93, 1.27)	0.29
Neutrophil count (x 10^9/L)	1.13 (0.95, 1.36)	0.18
K (mmol/L)	2.72 (0.78, 9.44)	0.12
NT-proBNP (nmol/L)	1.27 (1.07, 1.52)	< 0.01

CoQ_{10} – coenzyme Q_{10}; WBC – white blood cell count; K – potassium; NT-proBNP – N-terminal pro-B-type natriuretic peptide.

sults of a clinical examination, radiographic examination of the thorax, standard ECG examination, and echocardiography with 2-dimensional, M-mode, colour, and spectral Doppler modes (Vingmed System Five, General Electric Healthcare, USA). Blood samples for determination of selected blood variables were collected at admission. Haematological variables were measured with automated haematology analyser ADVIA 120 (Siemens, Germany), total cholesterol and triglyceride with automated biochemistry analyser RX-Daytona (Randox, Great Britain) and electrolytes (Na, K, Cl) with Ilyte analyser (Instrumentation Laboratory, USA). Whole blood GPX activity and plasma CoQ_{10} (lipid standardised CoQ_{10}) and serum NT-proBNP concentrations were measured as reported previously (Svete *et al.*, 2017). All procedures complied with applicable governmental regulations (Animal Protection Act University of Ljubljana Republic of Slovenia, 43/2007).

Data were analysed using R programming language. Significance level was set to 5%. Survival time was counted from the day of admission to the time of death or euthanasia. A broader exploratory analysis was made from which the variables of interests were identified. Considering there was no censoring present in the data, the initial correlation between time-to-death and a set of 16 blood variables was evaluated using Spearman's rank correlation coefficient. Five variables of interest: K (r= –0.37, P=0.09), CoQ_{10} (r=0.12, P=0.61), WBC (r= –0.15, P=0.51), neutrophil count (r= –0.21, P=0.36) and NT-proBNP (r= –0.562, P=0.008) were further analysed using Cox proportional-hazards models. Hazard ratios (HR), 95% confidence intervals (CI) and corresponding P values were calculated. Obtained P values were not adjusted as they do not refer to an inference and will only serve as a basis for future research. During the study period, all dogs died or were euthanised due to their cardiac disease. Median survival time was 11.6 months (range: 0.1–73.7 months).

In canine CHF patients, low plasma CoQ_{10} concentration was associated with the greater severity of the disease (Svete *et al*., 2017). Furthermore, we demonstrated leukocytosis and neutrophilia in dogs with severe, i.e. decompensated CHF (Domanjko Petrič *et al.,* 2018). Contrary to our expectations, CoQ_{10}, as well as WBC, neutrophil count and K, were not significantly associated with the survival of our patients (Table 2). The lack of significant effect of these variables on survival might be due to low number of patients included in the present study and

inclusion of two diseases or may be inherent to dogs with CHF. In the study of Molyneux *et al*. (2008), where low plasma CoQ_{10} concentration was an independent predictor of mortality, high number (236) of severely symptomatic human heart failure patients with median ejection fraction of 37% were included. We assume that the phase of decompensation in particular played a key role in the process of decreasing plasma CoQ_{10} concentration.

A significant association was found only between NT-proBNP and survival (Table 2), which has already been reported in canine cardiovascular patients (Serres *et al.*, 2009; Moonarmart *et al.*, 2010, Noszczyk-Nowak, 2011; Hezzell *et al.*, 2012). According to the Cox model in our study, an increase of NT-proBNP for 1 unit (1 nmol/L), increased the risk for death by 27.5 % ($p = 0.0063$), which is in general agreement with results of Serres *et al.* (2009). The increase in risk for death, obtained in our study, was lower when compared with reported values in dogs with MVD (Moonarmart *et al.*, 2010; Hezzell *et al.*, 2012). According to our opinion, the difference might be attributed to difference in NT-proBNP assay methods used, as well as to mixed severity of studied dogs in those two studies, while in our study, only CHF dogs were included. Additionally, lower HR value obtained in the current study might be attributed to the lack of survival outcomes in our study.

According to the results of our study, we may conclude that the investigated blood variables, with the exception of NT-proBNP, were not associated with survival of CHF patients. Our results warrant further studies on association of blood variables with survival in a larger group of severely affected canine CHF patients with a single disease in order to confirm the results of the present study.

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