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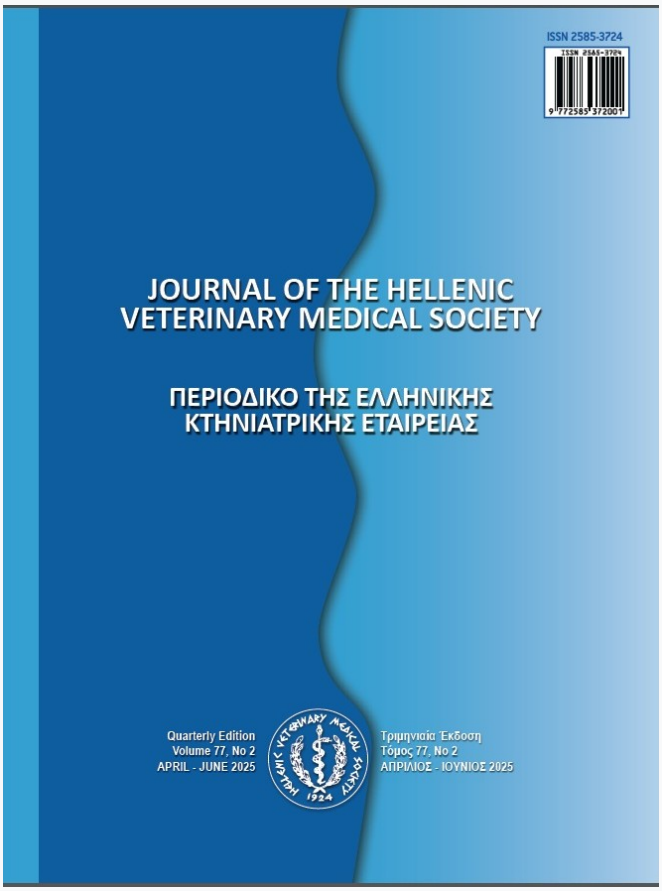

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Evaluation of MR-proADM and Copeptin Levels in Dogs with Heart Disease

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ABSTRACT: The measurement of copeptin and MR-proADM for the detection of cardiac dysfunction in humans has come to the fore in recent years. Although troponin and NT-proBNP have been extensively studied, copeptin and MR-proADM have not been studied in dogs. In this study, copeptin and MR-proADM levels were examined in dogs with heart disease. The study included groups B1 (n=15), B2 (n=13) and C (n=22), which were diagnosed with heart disease and classified according to ACVIM, and the control group (n=20), which was classified as healthy (group A). Blood count, biochemistry, electrolyte values, electrocardiographic and echocardiographic findings as well as serum levels of troponin I, troponin T, NT-proBNP, copeptin and MR-proADM were evaluated. In this study, copeptin levels were 71.12±30.06 ng/L in the control group, 85.88±26.25 ng/L in the B1 group, 113.35±36.07 ng/L in the B2 group and 344.63±453 ng/L in theC group. The MR-proADM values were 10.91±5.77 in the control group, 13.55±5.41 ng/L in B1, 14.16±3.94 ng/L in B2 and 54.72±88.89 ng/L in the C group. The increase in copeptin and troponin T in groups B2 and C and in MR-proADM, NT-proBNP and troponin I in group C proved to be statistically significant ($p < 0.05$). The values of troponin T and MR-proADM were moderately negatively correlated in group B2 (r=59.7%), while the values of troponin I and copeptin were moderately positively correlated in group C (r=44.6%). Furthermore, in group C, copeptin and MR-proADM values were more positively correlated than average (r=68.6%). Similar to human studies, copeptin and MR-proADM were significantly elevated in dogs with advanced heart disease. Copeptin and MR-proADM biomarkers can be used in conjunction with other cardiac biomarkers.

Keyword: Dog; copeptin; MR-proADM; heart disease.

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INTRODUCTION

Quality of life and longevity depend on early diagnosis of canine heart disease. Cardiac biomarkers are increasingly being used for the diagnosis of heart conditions, particularly for critically ill patients with respiratory problems (Langhorn and Wilesen, 2016).

Adrenomedullin (ADM) is expressed in kidney, lung, cerebrovascular, gastrointestinal and endocrine tissues (Peacock, 2014). ADM is also produced in the cardiovascular system (Nakamura et al., 1997). ADM has numerous biological effects such as maintaining cardiovascular homeostasis, regulating fluid-electrolyte balance, cardiovascular tissue development and preventing infections (Nicholls, 2004; Nakamura et al., 1997; Nishikimi et al., 1995). It reduces arterial pressure, increases heart rate and cardiac output, activates the sympathetic and renin-angiotensin systems and suppresses aldosterone production in heart attack and heart failure (Nicholls, 2004; Nicholls et al., 2003).In addition to its cardiac effects, ADM also has metabolic effects such as antioxidant, anti-apoptotic and inhibition of insulin secretion in beta cells (Betowski and Jamroz, 2004). However, reliable measurement of ADM is difficult due to the presence of a binding protein and its extreme physical properties. MR-pro-ADM, the more stable mid-regional fragment of the ADM precursor, directly reflects the amount of rapidly degraded active ADM peptide; in addition, MR-pro-ADM has a longer half-life and is more stable than ADM, so MR-pro-ADM is more reliable than ADM and is commonly used in clinical practice (Lorubbio et al., 2018).

Copeptin is the C-terminal part of the vasopressin precursor. It is released into the bloodstream by the pituitary(Szinnai et al., 2007). Physiological processes such as osmotic stimulation, heart failure, hypovolemia or stress stimulate the secretion of copeptin and vasopressin (Christ-Crain et al., 2022). One study reported that copeptin levels increased with increasing severity of disease in patients with hypervolemic volume status, heart failure and renal failure (Nigro et al., 2011). Elevated copeptin levels indicate adverse outcome and increased mortality in patients withheart failure (Gegenhuber et al., 2007; Stoiser et al., 2006; Zhong et al., 2017). A strategy that uses both troponin T and copeptin markers has been demonstrated to quickly and accurately exclude myocardial infarction (Tentzeris et al., 2011).

With the onset of heart failure, the levels of adrenomedullin, arginine vasopressin, brain natriuretic peptide and troponin increase (Nicholls et al., 2003). The measurement of mid-regional pro-adrenomedullin (MR-proADM) and copeptin levels has gained importance in the detection of cardiac dysfunction in human medicine in recent years (Kelly et al., 2008; Nigro et al., 2011; Pousset et al., 2000; Rademaker et al., 2003). Levels of B-type natriuretic peptide (pro-BNP), MR-proADMand copeptin were comparatively evaluated in patients with acute heart failure, and as a result of the study, they were all found to have similar prognostic value (Gegenhuber et al., 2007).

Although troponins and pro-BNP are frequently used as cardiac biomarkers for diagnostic purposes in veterinary cardiology, MR-proADM and copeptin have not yet found routine use in veterinary medicine and there are very few studies on this topic (Jougasaki et al., 2001, Riegger, 1988). The correlation of copeptin and MR-proADM levels with troponin and N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels in both healthy and diseased dogs was investigated for the first time in this study.

MATERIALS AND METHODS

The study was conducted in 20 healthy dogs (group A) and 50 dogs with heart disease. The dogs with heart disease were divided into groups B1 (n=15), B2 (n=13) and C (n=22) according to the standards of the American College of Veterinary Internal Medicine (ACVIM) (Keene et al., 2019). The ACVIM guidelines for heart disease state: stage B; heart disease is present but the patient has never developed clinical signs of congestive heart failure (CHF) (stage B is subdivided into stage B1; asymptomatic patients without radiologic or echocardiographic signs of cardiac remodeling with a vertebral heart score (VHS) < 10.5, Left atrial-to-aortic ratios (LA/Ao) <1.5 and normal left ventricular end-diastolic dimensions and stage B2;-asymptomatic patients that demonstrate radiographic and echocardiographic evidence of left-sided heart enlargement with VHS >10.5 and echocardiographic evidence of enlarged left ventricular end-diastolic dimensions or LA/Ao >1.5); stage C; patients with current or previous CHF with concomitant structural heart disease.

All studies were performed with owner consent and according to animal welfare guidelines approved by the ethics committee of our department (decision dated 09/06/2020, no. 19).

Patients with a history of lung, kidney or liver failure, sepsis, severe dermatological diseases, autoimmune diseases, advanced oral and gum diseases, diagnosed blood parasites and neoplastic diseases were excluded from the study. Also, in the patient group, animals that had previously taken vasodilator, anticoagulant, positive inotropic and antiarrhythmic drugs such as angiotensin-converting enzyme inhibitors due to heart disease were not included in the study.

A digital x-ray unit SMS-CM-N (EcoRay, Korea) was used for laterolateral chest radiographs. During the X-ray examination of the heart, the heart size was determined by calculating the vertebral heart score (VHS) of each patient was calculated as the sum of the long and short-axis distances drawn from the cranial border of the fourth thoracic vertebra according to the recommended technique developed by Buchanan and Bücheler (Buchanan and Bücheler, 1995). Radiographic signs of pulmonary edema (e.g. left atrial enlargement, pulmonary venous congestion, interstitial/alveolar pulmonary infiltrates) were evaluated.

Echocardiographic examinations were performed using the SIUI Apogee 3500V Ultrasound Imaging Device and the SI Multifrequency P3F14C Cardiac Phased Array Probe (1.7-4 MHz frequency) with optimal gain (0-100 dB), depth (1.6-30.8 cm) and pulse repetition frequency (0.25-25 KHz) (Shantou Institute of Ultrasonic Intstruments, China). The echocardiographic examinations of the dogs participating in the study were analyzed according to the recommendations of America Society of Echocardiography (Thomas et al., 1993). LA/Ao were derived from the right parasternal short axis two dimension (2D)view, using an inner-edge-to-inner-edge method obtained at the level of the aortic valve in early ventricular diastole (Hansson et al., 2002; Thomas et al., 1993). IVS (interventricular septal thickness), LVID (left ventricular inner dimension), LVPW (left ventricular posterior wall thickness), systolic and diastolic measurements on right parasternal short-axis M-mode images were determined (Boon, 2011; Thomas et al., 1993). FS% (fractional shortening) and EF% (ejection fraction) were automatically calculated using the Teicholz formula (Teichholz et al., 1976). Pulmonary artery Doppler measurements were performed on the right parasternal heart base image. Mitral inflow measurements were performed on the AP4C image (apical four chambers), Ao-Doppler measurements on the AP5C image (apical five chambers) and tricuspid inflow measurements on the AP4C image (Boon, 2011; Oyama et al., 2004; Thomas et al., 1993). Three consecutive measurements were averaged for each variable.

A 3 mL blood sample was collected from the cephalic vein. A complete blood count was performed using the Idexx ProCyte Dx Model blood scanner (IDEXX Laboratories, USA). The biochemical and electrolytic analyses were checked using the Idexx Catalyst One device (IDEXX Laboratories, USA). Serum biochemistry included glucose (GLU), creatinine (CREA), blood urea nitrogen (BUN), total protein (TP), albumin (ALB), globulin (GLOB), alanine aminotransferase (ALT) and alkaline phosphatase (ALKP). In the electrolyte measurement, the parameters chlorine (Cl), potassium (K) and sodium (Na) were measured.

Serum canine troponin I, troponin T, NT-proBNP, copeptin and MR-proADM were measured by ELISA using appropriate ELISA kits Bioassay Technology Laboratory, 2024). The absorbance measurement at 200-999 nm was performed with the EPOCH microplate spectrometer (BioTek Instruments, America).

IBM SPSS Statistics 22 software was used for statistical analysis of the study results. The normal distribution conformity of the parameters was evaluated using the Kolmogorov-Smirnov and Shapiro-Wilks tests. The Oneway-Anova test was used to compare quantitative data and assess normally distributed parameters between groups. The Tukey-HDS test was utilised to identify the quantitative data that differed between the groups. Meanwhile, the Kruskal-Wallis test was employed to compare parameters between groups that did not exhibit a normal distribution. The qualitative data was compared using the chi-square test. Relationships between parameters that did not conform to the normal distribution were examined using Spearman-Rho correlation analysis. Significance was assessed at the $p < 0.05$ level.

RESULTS

Group A comprised nine female and eleven male dogs. Group B1 had 7 female and 8 male dogs, Group B2 had 5 female and 8 male dogs, and Group C had 7 female and 15 male dogs. The average age of group A (4.55±2.46 years) was significantly lower than that of the other groups B1, B2 and C (7.6±2.5, 8.85±3 and 7.68±3.46 years respectively) (mean± SD) ($p<0.05$). Body weights in groups A, B1, B2 and C averaged 8, 9, 10.5 and 10.3 kg, respectively.

All patients in groups B1 and B2 had degenerative mitral valve disease. In group C, 10 patients had mitral valve disease and clinical signs of CHF. The others, in addition to CHF; 6 patients had degenerative mitral valve disease, 2 had hypertrophic cardiomyopathy, 1 had dilated cardiomyopathy, 1 had mitral valve dysplasia, 1 had tricuspid valve dysplasia and 1 had patent ductus arteriosus. In addition to the clinical signs of left-sided heart failure, we observed a history of tachypnoea, restlessness, dyspnoea or cough in group C.

The mean VHS in the control group (10.1±0.6) was statistically significantly lower (mean± SD) than in group B1 (11.06±0.34), group B2 (11.22±0.46) and group C (11.85±1.07) ($p < 0.05$). When evaluating the mean pulmonary edema between the groups, it was found that pulmonary edema increased with increasing degree of heart failure ($p<0.05$).

Echocardiographic examinations showed that the values of IVSs (interventricular septum thickness at end –systole) and AV (aortic valve) were significantly increased in groups B1, B2 and C, the values of IVSd (interventricular septum thickness at end –diastole), LVIDd (left ventricular internal dimension at end –diastole), LVPWd (left ventricular posterior wall thickness at end –diastole), LVIDs (left ventricular internal dimension at end –systole), Ao (aorta) root and LA (left atrium) were significantly increased in groups B2 and C and the values of EPSS (E-point-to-septal separation), La/Ao, MV E (mitral valve E-point) and MV A (mitral valve A-point) were significantly increased in group C compared to the other groups ($p < 0.05$). The LVPWs (left ventricular posterior wall thickness at end -systole) values in group B2 were statistically significantly higher than in the other groups ($p < 0.05$). In terms of EF, groups B1 and B2 were statistically significantly higher than the control group and group C ($p < 0.05$) (Table 1).

Although haematocrit and haemoglobin levels were within normal limits, they were statistically significantly lower in group C ($p<0.05$) (Table 2).

The values of troponin I, NT-proBNP and MR-proADMwere statistically significantly higher in group C ($p < 0.05$). Troponin T and copeptin levels were statistically significantly higher in groups B2 and C than in the control and B1 groups ($p < 0.05$) (Table 3).

There was a negative, moderate correlation between MR-proADMand troponin T levels in group B2 (r=-59.7%) ($p<0.05$).In group C, there was a pos-

Table 1. Evaluation of the groups in terms of echocardiographic parameters.

Parameter	Group A	Group B1	Group B2	Group C	
IVSd (cm)	0.62^{a}±0.15	0.79^{a}±0.31	0.82^{b}±0.24	0.79^{b}±0.22	0.041*
LVIDd(cm)	2.57^{a}±0.64	2.75^{a}±0.7	3.37^{b}±0.62	4.27^{b}±1.25	0.000*
LVPWd(cm)	0.66^{a}±0.17	0.81^{a}±0.27	0.88^{b}±0.22	0.83^{b}±0.22	0.022*
IVSs (cm)	0.88^{a}±0.18	1.19^{b}±0.33	1.26^{b}±0.29	1.29^{b}±0.28	0.000*
LVIDs(cm)	1.49^{a}±0.48	1.44^{a}±0.35	1.82^{b}±0.5	2.58^{b}±1.22	0.000*
LVPWs(cm)	0.85^{a}±0.21	1.09^{a}±0.36	1.28^{b}±0.22	1.06^{a}±0.34	0.001*
EF (%)	77.07^{a}±7.82	83.21^{b}±5.87	81.64^{b}±7.24	74.09^{a}±15.48	0.039*
FS (%)	42.87±6.	47.65±5.57	46.66±7.91	41.51±10.57	0.077
EPSS(cm)	0.28^{a}±0.09	0.34^{a}±0.13	0.33^{a}±0.16	0.53^{b}±0.47	0.040*
Ao root (cm)	1.28^{a}±0.31	1.4^{a}±0.5	1.61^{b}±0.47	1.54^{b}±0.38	0.044*
LA (cm)	1.59^{a}±0.38	1.69^{a}±0.47	2.24^{b}±0.64	3.49^{b}±1.23	0.000*
LA/Ao (cm)	1.24^{a}±0.13	1.27^{a}±0.18	1.41^{a}±0.19	2.31^{b}±0.7	0.000*
PV (cm/sn)	76.94±15.19	85.22±12.7	92.83±17.89	89.78±26	0.124
AV (cm/sn)	88.52^{a}±19.46	112.55^{b}±29.29	123.86^{b}±33.15	116.03^{b}±46.12	0.007*
MV E Vel (cm/sn)	63.56^{a}±14.37	67.09^{a}±19.18	74.03^{a}±16.42	119.35^{b}±49.47	0.000*
MV A Vel (cm/sn)	60.2^{a}±13.85	59.78^{a}±16	65.8^{a}±12.95	89.14^{b}±41	0.011*

Kruskal Wallis test *$p<0.05$, (mean ± SD), ^{a,b}: The difference between groups with different letters in the same row is statistically significant.

Table 2. Evaluation of the groups in terms of haemogram and biochemistry parameters.

Parameter	Group A	Group B1	Group B2	Group C	
RBC (M/µL)	6.79±0.73	6.95±0.96	6.62±0.89	6.3±0.75	[1]0.091
HCT (%)	43.42[a]±4.62	44.88[a]±7.11	42.61[a]±5.58	39.68[b]±4.99	[1]0.036*
HGB (g/dL)	16.14[a]±1.75	16.63[a]±2.52	15.61[a]±1.72	14.76[b]±1.85	[1]0.032*
MCV (fL)	64.16±2.2	64.44±3.2	64.49±3.36	63.03±2.43	[1]0.318
MCH (pg)	23.83±0.88	23.93±1.81	24.7±3.99	23.45±0.85	[2]0.244
MCHC (g/dL)	37.18±0.82	37.13±2.17	34.68±7.5	37.2±0.84	[2]0.486
WBC (K/µL)	10.28±2.77	12.43±6.39	23.36±43.6	11.49±4.26	[2]0.766
PLT (K/µL)	233.75±94.43	266±117.19	251.31±149.18	263.41±124.47	[1]0836
GLU (mg/dL)	106.65±8.69	103.07±15.58	109.08±14.64	109.95±18.64	[1]0.551
CREA (mg/dL)	1.02[a]±0.26	0.87[b]±0.35	0.75[b]±0.26	0.84[b]±0.26	[2]0.045*
BUN (g/dL)	15.48±5.08	16.07±3.99	14.46±6.83	19.73±9.56	[2]0.334
TP (g/dL)	6.96±0.67	7.14±0.67	6.88±0.47	6.65±0.71	[1]0.156
ALB (g/dL)	3.45±0.38	3.47±0.65	3.33±0.36	3.21±0.41	[1]0.267
GLOB (g/dL)	3.52±0.59	3.67±0.63	3.57±0.38	3.45±0.6	[1]0.705
ALT (U/L)	59.5±27.5	70.93±29.08	65.38±24.84	55.86±23.76	[2]0.290
ALKP (U/L)	57.45±53.37	56.13±50.59	86.77±84.76	83.18±103.36	[2]0.871
Na (mmol/L)	151.15±5.09	153.8±5.54	153.08±5.25	151.23±8.25	[2]0.518
K (mmol/L)	4.68±0.48	4.8±0.71	4.95±0.35	4.74±0.43	[2]0.389
Cl (mmol/L)	116.6±2.95	117.27±4.35	117.15±4.49	117.18±5.63	[2]0.998

[1]Oneway ANOVA test [2]Kruskal Wallis test, *p<0.05,(mean ± SD), [a,b]: The difference between groups with different letters in the same row is statistically significant.

Table 3. Evaluation of the groups in terms of cardiac panel parameters.

Parameter	Group A	Group B1	Group B2	Group C	
Troponin I (ng/ml)	2.41[a]±0.68	2.96[a]±0.98	2.49[a]±1.72	4.19[b]±3.16	0.006*
Troponin T (ng/ L)	21.97[a]±6.2	31.42[a]±10.35	40.09[b]±19.86	64.78[b]±49.29	0.000*
NT-proBNP (ng/ L)	231.38[a]±67.12	235.03[a]±74.36	200.46[a]±160.07	367.35[b]±288.6	0.049*
Copeptin (ng/ L)	71.12[a]±30.06	85.88[a]±26.25	113.35[b]±36.07	344.63[b]±453	0.000*
MR-pro-Adrenomedullin(ng/ L)	10.91[a]±5.77	13.55[a]±5.41	14.16[a]±3.94	54.72[b]±88.89	0.000*

Kruskal Wallis test *p<0.05, (mean ± SD), [a,b]: The difference between groups with different letters in the same row is statistically significant.

itive, moderate correlation between copeptin and troponin I (r=44.6%) and a positive, good correlation between MR-proADMandcopeptin levels (r=68.6%) (p<0.05).

DISCUSSION

In recent years, MR-proADM and copeptin levels have become increasingly important in cardiac diagnostics and pathophysiology in human medicine.

Many studies have found that adrenomedullin and copeptin levels increase in direct proportion to the severity of heart failure (Kelly et al., 2008; Nigro et al., 2011; Pousset et al., 2000; Rademaker et al., 2003).

Left atrial enlargement, exercise intolerance, pulmonary edema and coughing are common in dogs with progressive heart disease (Devi et al., 2009). While no clinical signs were observed in healthy animals in this study, which is consistent with other studies, we found that left atrial dilatation, cardiomegaly in group B2, additionallypulmonary edema in group C were observed.

Dilation of the left atrium and increase in LA/Ao ratio, which develop as a result of increased pulmonary capillary pressure and internal pressure in the left atrium in cardiac disease, have been reported in many studies (Nakamura et al., 2017). In another study, dogs with mitral valve disease were examined and it was reported that the LA/Ao ratio in stage C was above 1.7 (Borgarelli et al., 2015). In this study, we observed that left atrial dilatation developed in conjunction with the increase in atrial pressure as heart failure progressed. When Haggström et al. (1995) investigated the size of the LVIDd in mitral valve disease, they reported that left ventricular end-diastolic diameter increased according to the severity of heart failure. In another study, the increase in LVIDd size in heart failure was found to be useful for predicting mortality in dogs (Moonarmart et al., 2010). In our study, we observed that LVIDd increased with increasing severity of heart failure.

Studies have shown that increases in peak E-wave velocity and E/A ratio in dogs are associated with poor prognosis in heart disease (Borgarelli et al., 2012). In our study, there was an increase in E-wave peak velocity and E/A ratio in stage C dogs compared to healthy dogs. It is known that the EPPS value changes in diseases such as DCM in dogs (Holler and Wess, 2014). In our study, we found that EPSS was high in stage C patients, and we believe that this is due to the increase in pressure and size in all heart chambers as a result of heart failure.

Routinely applied estimates of ventricular systolic function, EF and FS, are not reliable assessments of systolic function in dogs with MMVD (Bonagura and Schober, 2009). Similarly our data showed that theventricular values (IVSd, LVID, LVPWd) were increased specially in groups B2 and C and EF values increased only in group B1 and B2 significantly.

Anemia is common and has been associated with the severity of heart failure in people with CHF (Horwich et al., 2002). Although they were within normal hematologic limits, we found that HCT and hemoglobin levels were significantly lower in group C than in the other groups. Therefore, we felt that this should be considered in advanced heart failure.

Prosek et al (2007) reported that the concentration of cardiac troponin I in plasma increases in dogs with heart disease, reflecting the severity of the disease. In our study, we observed an increase in troponin I concentration, and we found the highest values in group C, in which advanced heart failure was detected. However, the fact that we also found high plasma troponin I concentrations in healthy dogs is consistent with the opinion of Ruaux et al. (2015), who stated that troponin I concentrations are highly individualised and do not necessarily differ statistically significantly between healthy and diseased animals. We found that the troponin I concentration in group C was statistically significantly higher than in the other groups.

Oyama and Sisson (2004) reported that troponin I levels in dogs with mitral disease were moderately correlated with ventricular wall thickness whereas strongly correlated with left ventricular and atrial size. Our results were similar to those of the researchers, and we found that troponin I levels were higher in group C.

Previous studies have shown that cardiac troponin T is a marker for myocardial necrosis and can be used to predict hospitalisation and death from cardiac causes (Omland et al., 2009). Plasma troponin T levels of 0.02 ng/mL and above have been found in patients with heart failure in whom clinical symptoms are observed (Healey et al., 2003). In our study, we obtained similar data to the researchers and found that there was a significant increase in troponin T in groups B2 and C with increasing disease severity compared to the other groups.We also found that the biomarkers troponin I and troponin T increased simultaneously and showed a moderate positive correlation (r=59.6%).

Previous studies have found significantly increased levels of NT-proBNP and proANP in dogs with advanced heart failure (Häggströmet al., 2000; Oyama et al., 2008). The finding of high NT-proBNP levels in dogs with advanced heart failurein our study is consistent with previous studies. We believe that this increase is due to chronic damage to cardiac tissue, especially in dogs with CHF. In dogs,

NT-proBNP concentrations have been reported to be highly correlated with mortality rate, LA/Ao ratio, and LV systolic parameters (Noszczyk-Nowak, 2011). Takemura et al (2009) reported that plasma NT-proBNP concentration increased significantly with an increase in VHS and LA/Ao ratio. The study showed similar data to others, with group C having significantly higher left atrial size and LA/Ao ratio and significantly higher NT-proBNP levels.

Many human studies have reported that adrenomedullin and copeptin levels are elevated in patients with haematologic, biochemical or electrolyte imbalances such as anaemia, infectious diseases, renal failure, hemorrhagic and septic shock, liver cirrhosis, elevated portal pressure, hyponatremia and hypochloremia(Nigro et al., 2011; Sola et al. 2016). Dogs with various diseases were excluded from the study to avoid influencing the biomarker values analysed. Cardiac troponin I and NT-proBNP are commonly used to diagnose and assess the prognosis of heart disease. In the guidelines of the American College of Cardiology Foundation/American Heart Association, both natriuretic peptides and troponin tests are recommended for prognostic stratification (class I, level of evidence: A), while other biomarkers reflecting various pathophysiologic aspects of heart failure, including myocardial wall stress, hemodynamic abnormalities, inflammation, myocyte injury, neurohormonal upregulation and myocardial remodeling, and extracellular matrix turnover, have been assigned a level of evidence: IIb recommendation (Yancy et al., 2013).

Many studies have reported that copeptin is an informative biomarker for the prognosis of heart disease and its importance for risk stratification has been demonstrated (Gegenhuber et al., 2007; Neuhold et al., 2008; Tentzeris et al., 2011). Researchers have reported that vasopressin causes worsening of heart failure due to disproportionate water retention, hyponatremia and vasoconstrictive effects (Neuhold et al., 2008; Tentzeris et al., 2011). Copeptin levels measured in human patients with and without heart failure averaged 24.7 pmol/L and 12.8 pmol/L, respectively (Dieplinger et al., 2009). In a similar study, patients with acute heart failure had copeptin levels of 24.2 pmol/L in survivors and 55.2 pmol/L in non-survivors (Maisel et al., 2011). In a study of patients with heart failure, copeptin levels were monitored for one year; the results were 20.8 pmol/Lin survivors, 31.1 pmol/L in patients rehospitalised with a heart failure diagnosis, and 33.8 pmol/L in non-survivors (Stoiser et al., 2006). In our study, copeptin levels were 71.12 ng/L (0.159 pmol/L) in the healthy group, 85.88 ng/L (0.190 pmol/L) in the B1 group, 113.35 ng/L (0.253 pmol/L) in the B2 group and 344.63 ng/L (0.772 pmol/L) in the C group. There is no study investigating the level of copeptin in the serum of dogs. The study found that copeptin levels were lower in dogs than in humans, but similar to previous studies in humans, copeptin levels increased with disease severity.

Balling et al (2018) found that copeptin levels were higher in males, regardless of age. In our study, copeptin levels were higher in groups B2 and C, and group C was predominantly male.

Jeong et al. (2020) reported that the combination of troponin I and copeptin improves the diagnostic performance of acute myocardial infarction in patients. In our study, we found that the combination of troponin I and copeptin was positively correlated in group C, which represents the advanced stage of heart failure, which is consistent with previous studies.

Researchers have reported that MR-proADM has prognostic value in acute severe dyspnea and heart failure and predicts mortality risk (Lenka et al., 2018; Xue et al., 2013). In humans, the normal MR-proADM level was determined to be 0.33 nmol/L on average, while in acute myocardial infarction it was measured at 0.73 nmol/L (Morgenthaler et al., 2005). In another study, MR-proADM levels of 0.71 nmol/L were measured in survivors and 1.31 nmol/L in deceased patients with acute myocardial infarction (Khan et al., 2007). Xue et al. (2013) reported that MR-proADM levels above 0.72 nmol/L predicted mortality risk in heart failure. In our study, MR-proADM concentrations were 10.91±5.77 ng/L (0.037 nmol/L) in the healthy group, 13.55±5.41 ng/L (0.046 nmol/L) in the B1 group, 14.16±3.94 ng/L (0.049 nmol/L) in the B2 group and 54.72±88.89 ng/L (0.189 nmol/L) in the C group. Although tissue adrenomedullin levels have been studied in experimental canine heart disease, serum levels have not yet been examined. Although the levels determined in our study were very low compared to humans, a significant increase in MR-proADM levels was observed in patients who came to the clinic with severe symptoms such as dyspnea and cough, especially in stage C heart failure, similar to the results of human studies. We believe that this increase, similar to the results reported by the investigators, is mediated by

the vasodilatory and diuretic effects of adrenomedullin to regulate cardiovascular hemostasis and that MR-proADM levels increase in direct proportion to disease severity.

In humans, the combination of MR-proADM and copeptin is more effective than troponins and natriuretics in diagnosing acute heart failure and predicting mortality (Peacock et al., 2011). Similarly, our study showed that MR-proADM correlated with copeptin levels in patients with advanced heart failure (r=68.8%).

CONCLUSION

While the importance of copeptin and MR-proADM in human heart disease is well known, the clinical relevance of these biomarkers in canine heart disease has not yet been established. In this study, we have shown that copeptin and MR-proADM parameters can be used to identify patients with advanced heart failure in dogs. Furthermore, our results show that in dogs with advanced heart disease, copeptin and MR-proADM increase simultaneously with troponins, which are known to increase with cardiac damage. This is important for understanding the pathologic mechanism in heart failure. In addition, monitoring copeptin and MR-proADM levels can help improve diagnosis and treatment.

We believe that monitoring copeptin and MR-proADM levels in heart disease and other conditions will become increasingly important in the coming years.

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