

Cardiac Troponin Levels in Dogs with Dilate Cardiomyopathy

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Summary

This study was designed to determine plasma cTnI and cTnT concentrations in dogs with DCM and to investigate the value of these two parameters in this type of heart disease. For this purpose, 11 dogs with DCM diagnosed, via electrocardiographic and echocardiographic examinations nearby apparent clinical heart disease symptoms were studied. The correlation coefficients between serum CK, cTnI and cTnT values were determined insignificant and no important relationship was calculated between these values. High cTnI levels were determined for 10 of 11 (90.9%) dogs with DCM, while high cTnT levels were determined for 7 of 11 (63.6%) dogs with DCM. It is suggested that cardiac Troponins, especially cTnI is a strong candidate as a cardiac biomarker in animals.

Keywords: *Troponin, Creatinin Kinase, Cardiomyopathy, Dog*


Dilate Kardiyomiyopatili Köpeklerde Kardiyak Troponin Seviyeleri

Özet

Bu çalışmada, DCM'li köpeklerde plazma cTnI ve cTnT konsantrasyonlarının belirlenmesi ve bu tip kalp hastalıklarında bu iki parametrenin öneminin araştırılması amaçlanmıştır. Bu amaçla, belirgin klinik kalp hastalığı semptomlarının yanısıra, elektrokardiyografik ve ekokardiyografik muayenelerle DCM teşhisi konmuş farklı ırklardan 11 köpek kullanıldı. Serum CK, cTnI ve cTnT değerleri arasındaki korelasyon katsayıları önemsiz olarak hesaplandı ve bu değerler arasında önemli bir ilişki saptanmadı. DCM'li 11 köpeğin 10'unda (%90.9) yüksek cTnI seviyeleri gözlenirken, 7'sinde (%63.6) yüksek cTnT seviyeleri belirlenmiştir. Sonuç olarak, kardiyak Troponinlerin, özellikle cTnI'nın hayvanlarda önemli bir kardiyak belirleyici olmaya aday olduğu düşünülmektedir.

Anahtar sözcükler: *Troponin, Kreatinin Kinaz, Kardiyomiyopati, Köpek*

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INTRODUCTION

Dilated cardiomyopathy (DCM) is a term describing myocardial dysfunction characterised by reduced contractility, occurring with or without arrhythmias. DCM is the most frequent form of primary myocardial diseases and the third most common cause of heart failure ¹. A number of biochemical markers of cardiac injury have been investigated including aspartate dehydrogenase (AST), creatine kinase (CK), lactate dehydrogenase (LDH) and myoglobin (M). While being reasonably sensitive indicators of cardiac cell necrosis, these markers suffer from lack of specificity, since their circulating levels rise by hepatic disease, skeletal muscle injury or renal disease ^{2,3}. In human medicine, the cardiac biomarkers, cardiac troponin T (cTnT) and I (cTnI), and the cardiac isoenzyme of creatine kinase (CK-MB) are used extensively to diagnose and provide valuable prognostic information in patients with ischemic, traumatic, and septic myocardial injury and necrosis. The cardiac troponin (Tn) complex is comprised of three subunits (I, T, and C), regulating the excitation-contraction coupling of the cardiac sarcomeric proteins. TnI regulates the calcium dependent interaction between myosin and actin in the striated muscle. It prevents contraction in the absence of calcium and calcium binding TnC. cTnT binds the complex to tropomyosin ⁴. Acute and/or chronic cardiac injury induces release of these subunits into the circulation ⁵. In normal control patients, blood levels of cTnT or cTnI are very low or below the level of detectability of most assay systems in both humans ⁶, and animals ⁷. Elevated levels are detectable in blood within four hours, reach a peak within 12 to 24 hours, and then slowly decline over the next 5 to 20 days, depending on the degree of initial damage ⁸.

cTnI is 100% specific for the heart i.e., it is never expressed by skeletal muscle ⁹. The structure of cTnI is highly conserved across species, and assays for human cTnI have been validated in the dog ¹⁰. Besides, the homology between Tn's is about 95% among mammals, and therefore commercial diagnostic kits designed for use in humans also provide excellent results in other animals ³. It has proven to be a highly specific and sensitive marker for myocardial cellular damage and necrosis in many mammalian species ¹⁰,

demonstrating a higher specificity than CK-MB with better sensitivity and specificity than cTnT ¹¹. Also, cTnI has been validated as a biomarker for cardiotoxicity in numerous animal models including dogs ¹².

There are various studies reporting increased cTnT and cTnI concentrations in dogs with a variety of heart diseases including mitral valve regurgitation, trauma, gastric dilatation and volvulus, myocarditis, subaortic stenosis and DCM ¹³⁻¹⁷. Cardiac troponins are not uniformly elevated in dogs with heart disease ^{9,14,17}. As there is apparent myocardial injury in DCM, level correlated to the stage of disease, cardiac troponins may be a good prognostic reflector ^{1,17,18}. Therefore more studies should be performed to outline the exact levels of cardiac troponins in dogs. This study is designed to determine plasma cTnI and cTnT concentrations in dogs with DCM and to investigate the value of these two parameters in this type of heart disease.

MATERIAL and METHODS

Dogs admitted to the study were selected from patients referred to the clinics of Faculty of Veterinary Medicine of Istanbul University, with complaints including exercise intolerance, dry cough, oedema, upper respiratory system problems. They were subjected to electrocardiographic (PETAS) and echocardiographic (Schimadzu SDU-350A) examinations following physical examination. The study group consisted of 11 dogs with DCM were diagnosed on the basis of historical information and instrumental examinations. The subjects represented a wide range of breeds and generally older age (5-13) and were from both gender. From these animals, a 5 ml blood sample was obtained and collected into a plain tube. This blood was left to clot at room temperature for at least 30 minutes and then centrifuged at 3000 rpm for 10 minutes. Serum samples obtained from dogs diagnosed with DCM (n=11) were assayed for cTnI, cTnT and CK-MB concentrations by immunoassay system using Troponin T Cardiac Reader (Roche) and Troponin I enzyme Immune System (Roche) and spectrophotometric method (CK-MB Sprinayt). Values less than 0.05 (ug/L) were considered negative for cTnT ¹⁹ and values less than 2.00 (ng/ml) were considered negative for cTnI ¹⁰ according to the test procedure

(expressed in ng/ml).The correlation test was applied to determine the phenotypic correlation coefficients between CK, cTnI and cTnT 20.

RESULTS

Age, breed and sex distribution and serum CK-MB, cTnI and cTnT values of the dogs are presented in *Table 1*. Electrocardiographic examinations as well as the echocardiographic examinations of the dogs revealed apparent dilate cardiomyopathy. Echocardiography indicated poor systolic wall and septal motions, fractional shortening, increased systolic dimensions, decreased ejection fraction. Electrocardiographic findings were variable. Some QRS complexes suggested left ventricular dilation, some were widened with a sloppy R wave descent and ST-T coving and intraventricular conduction disturbances were also reported.

The correlation coefficients between serum CK, cTnI and cTnT values were determined insignificant and no important relationship was calculated between these values.

High cTnI levels were determined for 10 of 11 (90.9%) dogs with DCM, while high cTnT levels were determined for 7 of 11 (63.6%) dogs with DCM.

Table 1. Age, breed, sex distribution and serum CK-MB, cTnI and cTnT values of the dogs with DCM.

Table 1. DCM'li köpeklerin yaş, ırk, cinsiyet dağılımı ve serum CK-MB, cTnI ile cTnT değerleri

Age	Breed	Sex	CK-MB (U/L) (*)	cTnI (ng/ml) (**)	cTnT (ug/L) (***)
13	ACS [1]	Female	33	1.68	0.03
10	ACS [1]	Male	41	2.2	0.03
7	Pincher	Female	35	2.42	0.3
8	Boxer	Male	37	2.10	0.05
10	ACS [1]	Female	43	2.68	0.05
8	Boxer	Male	38	2.3	0.09
9	Terrier	Female	34	2.2	0.07
11	Kangal [2]	Female	191	2.1	0.05
5	ACS [1]	Male	44	2.1	0.03
12	Dalmatian	Male	33	2.80	0.01
9	Pointer	Male	199	2.08	0.07
X			66.18182	2.241818	0.070909

(*) Reference Value ⁽²¹⁾ 4.9-6.3U/l

(**) Reference Value ⁽¹⁰⁾ <2.00 ng/ml

(***) Reference Value ⁽¹²⁾ <0.05ug/L

[1] American Cocker Spaniel

[2] Anatolian Shepherd Dog

DISCUSSION

DCM is often referred to as being breed-specific for Boxers, Doberman Pinschers, English Cocker Spaniels and other breeds ¹⁸. Generally large breeds are known to be commonly affected with dilated cardiomyopathy ¹⁸, but in the present study 6 of 11 (54.5%) dogs were of small breeds. Nevertheless, most of the dogs were of breeds identified with DCM predisposition ¹⁸, with four American Cocker Spaniels and two Boxers. Average ages of the dogs were 9.27 and this was concordant with the tendency of DCM to old dogs.

Atrial fibrillation was the most common arrhythmia similar to the findings of Tidholm and Jonsson ¹⁷. For Boxers, correlations were found between serum cTnI concentration and number of VPCs/24 h and between concentration and the grade of arrhythmia ²². On the basis of this suggestion, in our study, arrhythmia was a common finding, and this fact may have contributed to the high cTnI values.

Schober et al.⁷ found elevated Tn concentrations in cats with cardiomyopathy and in dogs with dilated cardiomyopathy or suspected myocardial contusion. Several recent studies performed on animals reported a higher diagnostic performance of cTnT than CK in detecting damage to the heart muscle ²³. This is concordant with the present study, where only two of 7 dogs presenting high cTn values reflected high levels of CK activity. Besides, O'Brien et al.³ reported myofibrillar CK-MM and mitochondrial CK activities and Tn-T content were 25% lower in the dogs with IDCM than in controls, whereas cytosolic CK-MB activity was 50% lower. They reported that deficiency of CK-MB activity is a component of and may have a central role in the energy deficiency characteristic of failing myocardium in DCM of Doberman Pinschers and a side-result of their study was the observation that, in dogs CK-MB activity and Tn-T content of myocardium are sufficiently high to be candidate serum markers for active cardiac injury. Nevertheless, levels of MB-CK can be increased in patients with skeletal muscle injury or renal failure in the absence of myocardial injury; causing diagnostic confusion ², so more specific markers may be used for the diagnosis of cardiac disease. Also it should be considered that normal cTnT and

cTnI in the presence of increased CK-MB and total CK reflects non-cardiac injury, likely skeletal muscle injury²⁴.

Sato et al.²⁵, reported a relatively high percentage of patients to be positive for cTnT during the course of dilative cardiomyopathy. It has been reported that the return to normal levels of cTnT following therapy also has a beneficial impact on prognosis, with survival times that are significantly higher. Our results are not similar with those found for humans as high cTnT levels were determined for 7 of 11 (63.6%) dogs with DCM, but are similar to the results of Tarducci et al.¹⁹, whom reported that the positive test results observed in non-hypokinetic, myocardiopathic subjects implies that the measurement of cTnT is a diagnostic tool that is valuable for the identification of myocardial damage, even in animals that would not otherwise be suspected to have myocardial damage on the basis of clinical and instrumental examination. In addition, O'Brien et al.²⁶ tested and supported the hypotheses that serum cTnT was widely applicable in laboratory animals as a biomarker of cardiac injury arising from various causes; that it increased in proportion to severity of cardiac injury; and that it was more cardiospecific than CK or LD isozyme activities. In canine and rat models of myocardial infarction, cTnT concentration increased 1.000- to 10.000-fold and was highly correlated with infarct size within 3.h of injury. In our study, seven of eleven dogs (63.63%) with DCM presented high cTnT values. The normal cTnT values in four dogs with DCM may be attributed to the low concentrations of cTnT at the myocardial level, as reported by Tarducci et al.¹⁹ and O'Brien et al.²⁴ for Dobermans affected with idiopathic dilative cardiomyopathy.

Furthermore, De Francesco et al.²⁷ concluded that dogs with congestive heart failure and those with skeletal muscle trauma and dogs with neoplasia receiving high-dose doxorubicin chemotherapy may have increased serum cTnT concentration, which may be suggestive of myocardial damage.

Many authors suggested that measurement of blood cTnI levels may be a useful aid in the diagnosis of dogs with suspected heart disease and in indicating the severity of heart failure such as O'Brien et al.²⁴ whom reported that myocardial cTnI is 30% lower in dogs with heart failure because of idiopathic dilated cardiomyopathy

compared with those with nonfailing hearts. Also Spratt et al.²⁸, concluded that healthy dogs had very low or undetectable blood cTnI levels, as did dogs with congenital heart disease, however, cTnI levels were significantly elevated in dogs with acquired mitral valve disease, dilated cardiomyopathy and pericardial effusion and blood cTnI levels also varied with severity of heart failure. Furthermore, Pelander et al.²⁹ and Oyama et al.³⁰ found that some dogs with DCM, or mitral valve deficiency (MVD) and congestive heart failure (CHF), also had elevated cTnI levels, although a large number of symptomatic dogs had low blood Tn levels. Similarly in human medicine many authors like Missov et al.⁶, addressed the usefulness of cardiac Tn I as a sensitive and specific molecular marker of congestive heart failure in patients with severely reduced left ventricular performance. La Vecchia et al.¹¹, reported that cTnI represents a highly sensitive marker for myocardial cell death as cardiac cell death had been shown to occur in heart failure and has been implicated as one of the mechanisms responsible for progression of the disease. These are consistent with the present study's findings, where high cTnI levels were determined for 10 of 11 (90.9%) dogs with DCM.

Early myocardial disease can be difficult to diagnose even with the benefit of echocardiography. Early myocardial disease in dogs, especially DCM, also can be difficult to detect on physical examination (or with echocardiography). Measurement of blood cTnI levels are accepted a useful aid in the diagnosis of dogs with suspected heart disease-especially myocardial infarction-, and in indicating the severity of heart failure. Although myocardial infarction is believed to be a very rare presentation in veterinary medicine, since it is consistent with findings that myocardial hypertrophy associated with heart failure involves a complex process of myocyte apoptosis and remodeling³¹, we believe that determination of cTnI levels will bring great benefit in identifying dilatative or hypertrophic cardiomyopathy.

It is suggested that, in dogs with clinically apparent DCM, the cTnI concentration is correlated with survival¹⁴. Dogs with elevated cTnI were three times more likely to be euthanized or die versus dogs with lesser concentrations¹⁴. This suggestion fits to our study, in which high cTnI levels were determined for 10 of 11 (90.9%) dogs with DCM.

Monitoring plasma cTnI and to some extent cTnT concentrations could be sensitive, non-invasive, and cost-effective methods for evaluating therapy in patients with congestive heart failure, dilate cardiomyopathy or myocardial diseases that cause cardiomyocyte damage. Therefore cardiac Tn's, especially cTnI are a strong candidate as a cardiac biomarker in animals and further studies should be performed to obtain more specific reference values correlated with the stage of DCM in dogs, so that cardiac troponins may be a prognostic indicator and a guide towards optimal therapy in DCM.

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