Serum Cardiac Troponin I Concentrations in Cats with Lower Respiratory Diseases

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Abstract

Objectives: Determination of serum cardiac troponin I (cTnI) levels in cats with lower respiratory diseases (LRDs) and evaluation of clinical variables associated with increased serum cTnI concentrations in cats with LRDs.

Materials and Methods: 46 cats. Serum cTnI concentrations were measured in 23 cats with LRDs and compared with 23 healthy normal cats.

Results: Sixty-nine percent of cats (16 of 23) with LRDs had elevated cTnI levels (cTnI > 0.16 ng/mL). The mean and range of plasma cTnI in cats with LRDs were significantly ($P < 0.001$) greater than healthy control cats. There was no correlation between respiratory signs, type of LRDs, and radiographic patterns of LRDs with plasma cTnI concentrations in cats with LRDs.

Conclusion: Serum cardiac troponin I concentration was significantly increased in cats suffering from LRDs when compared to control group. Measurement of cTnI levels in cats could not be used as a stand-alone test to differentiate LRDs from cardiac disease in cats.

Keywords: Cardiac Diseases; Cardiac Biomarkers; Feline

Abbreviations

Tn: Troponin; cTn: Cardiac Troponin; cTnI: Cardiac Troponin I; cTnT: Cardiac Troponin T; LRDs: Lower Respiratory Diseases; D-CD: Cardiac Dyspnea; D-NCD: Non-Cardiac Dyspnea; Scr: Serum Creatinine

Introduction

Troponin (Tn) is an actin and myosin binding protein regulating striated muscle contraction. TnI, TnT, and TnC are three different forms of this protein. TnI and TnT are divided in two distinct tissue specific isoforms, including cardiac and skeletal muscle isoforms. Car

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diac Troponin I (cTnI) has not been identified outside myocardial tissue while small amounts of cardiac Troponin T (cTnT) can be found in skeletal muscle [1]. The very small quantity of cTnT in skeletal muscle does not cross over the assay [2]. Calcium binding between actin and myosin is controlled by the regulatory activity of cTnI and cTnT. Troponins are intracellular proteins and their presence in the blood stream is the hallmark of cellular injury and cell membrane rupture. Most of the troponin in the cell is structurally bounded (structural Troponin) and just a small amount of troponin is free cytosolic form. After cardiomyocyte injuries the cytosolic pool is released quickly followed by sustained and continuous release of structurally bounded troponin which may last for days to weeks. However, 2 to 3 hours following a cardiac insult a rise in serum troponin level can be seen which usually reached to peak concentration during 18 to 24 hours.

It is generally accepted that human assays for cardiac troponin can be used for most species [3]. There is 96% homology between feline and human troponin genes. Nevertheless, the reference range must be established for each assay individually. In recent years increasingly sensitive assays have been developed and the term "high sensitivity" has been used to call them [4]. The upper limit of cTnI levels in healthy cats, according to different studies, must not exceed 0.14 to 0.17 ng/ml in heparinized plasma. The cTnI range for normal cats is from 0.03 to 0.16 ng/mL, with a mean of 0.04 ng/mL and a median of 0.03 ng/mL (SD = 0.0387 ng/mL) [2].

In human medicine, the main reason for the measurement of cTnI is for the diagnosis of myocardial infarction and other ischemic heart diseases [5]. In cats, however, serum cTnI elevation is seen mostly in hypertrophic cardiomyopathy (HCM) [6-8], hyperthyroidism, and renal insufficiency [9]. In one cohort study in cats with hypertrophic cardiomyopathy, cardiac troponins measured at first admission, were significantly related to death. In this study a significant prognostic contribution was identified for cTnT however, there was no significant correlation between changes in cardiac troponin and myocardial thickness in a feline patient suffering from HCM [10].

Increase troponin in respiratory diseases has been postulated [5]. In human, increases in cardiac troponin occur in 10% to 50% of patients with pulmonary embolism (PE) [11]. In human, PE has been reported as the most common non-acute coronary syndrome cause of increased cTn [12]. Acute respiratory distress syndrome (ARDS) is also associated with increased cTn in human. In one study, 35% of ARDS patients had increased cardiac biomarkers [13]. Cardiac troponin is used commonly in human as a prognostic factor in respiratory disease. Elevations of cTnT in critically ill human patients admitted to ICU for acute respiratory disease are independently associated with in-hospital, short-term and long-term mortality [14]. Furthermore, elevated cTnI and cTnT levels in human with acute PE, are strong indicators of high risk of short-term death and adverse outcome events [15].

In veterinary medicine, however, investigations of the role of serum cardiac troponin are mainly limited to evaluating the sensitivity and specificity of cTnI in feline hypertrophic cardiomyopathy (HCM) [16] and to distinguish the congenital from acquired heart disease and to determine the severity of congestive heart failure (CHF) in dogs [2,6-8]. Three studies [17-19] in cats are now present in which the usefulness of cTnI to differentiate cardiac versus non-cardiac cause of dyspnea has been investigated. In cats with dyspnea related to CHF, the median plasma concentration of cTnI was significantly higher than those with non-cardiac dyspnea. This study [17] demonstrated that CHF as the cause of dyspnea in cats could be ruled in or ruled out with additional diagnostic testing, including echocardiography, in more than 50% of the study cats. According to their study, measurement of cTnI in cats with dyspnea may be useful as a diagnostic method to differentiate cardiac causes from non-cardiac causes of dyspnea [17]. In one study [20] in 48 dogs, circulating concentrations of atrial natriuretic peptide (NT-proANP), B-type natriuretic peptide (BNP), endothelin-1 (ET-1), and cardiac troponin-I (cTnI) have been evaluatedto distinguish cardiac and non-cardiac causes of dyspnea. Comparison of plasma concentrations (geometric mean, range) of cTnI did not reveal a significant difference between two groups (cardiac dyspnea and non-cardiac dyspnea group), whereas a similar comparisonof NT-proANP, BNP, and ET1 between two groups of dogs showed a significant difference between two groups. NT-proANP had the highestsensitivity (95.5%) and specificity (84.6%) for distinguishing between dogs with cardiac and non-cardiac causes of dyspnea [20].Nevertheless, there is no comprehensive study in veterinary medicine regarding the changes of cardiac troponin in cats with respiratorydisease and without any cardiac disease.

Aim of the Study

The aim of the current study is to evaluate the changes in plasma cTnI levels in cats with lower respiratory diseases and to find any possible correlations between cTnI level and various clinical and radiologic findings. To this end, plasma cTnI concentration was measured in cats with primary lower respiratory diseases (LRDs) and in normal healthy cats. We hypothesized that cTnI concentration should be higher in cats with LRDs in comparison with normal cats.

Materials and Methods

Animals

Lower respiratory disease group (LRD group)

In total 23 privately-owned cats with LRDs enrolled in the present study between Aprils to December 2018. Breeds consisted of 5 Persian cats; one Scottish fold; and 18 domestic shorthair cat (DSH). All the cats which were referred to 5 veterinary clinics in Tehran for respiratory problems were evaluated first and those with signs of LRDs including dyspnea, cough, tachypnea, orthopnea, respiratory effort and shallow breathing selected for further clinical investigations. Presence of crackle and or wheezes on auscultation were also indicators for possible lower respiratory pathology. Selected cats evaluated further by thoracic radiography and complete blood count to confirm LRDs. Serum creatinine (Scr) and blood urea nitrogen (BUN) were also measured to evaluate the presence of acute uremia. Exclusion criteria were a) presence of other organ diseases b) cats younger than 6 months of age and c) cats older than 10 years old.

Control group

Twenty-three healthy, privately-owned cats were included in the control group. Controls were selected based on history and clinical examination with similar age range as our target group. All controls were evaluated completely similar to target group to confirm their general health and been selected from those referred to clinics for other reasons than health problems (e.g. for grooming or vaccination).

Clinical evaluation

Signalment (breed, age, gender, neutered status, and body weight), history, physical examination findings, thoracic radiography, Scr, BUN and complete blood count were recorded. Lateral and dorsoventral thoracic radiographs were obtained to confirm and distinguish different pulmonary problems. Auscultation of lung and heart sounds were performed. Thorough evaluation of respiratory signs and type of dyspnea (inspiratory versus expiratory) was performed. Presence or absence of hypoxia and cyanotic mucus membrane were determined. To exclude other organ diseases a comprehensive history taking was obtained and a complete physical examination, including measuring core body temperature, evaluation of mucus membrane color and capillary refill time, evaluation of the strength and quality of femoral pulse, and careful palpation of abdominal organs was done. All clinical procedures were done by an experienced small animal internist and all the data were recorded for each case in distinct predesigned forms.

Blood sampling

Fasting blood samples for the measurement of cTnI, Scr, and BUN were collected from all cats and analyzed within 12 hours. Blood was centrifuged 30 minutes after being left to stand at room temperature in 1 ml serum gel tube to measure Scr and BUN. The serum was then chilled at 4°C for up to 12 hours before sending to a commercial laboratory. To analyze serum cTnI level 1 ml of blood was collected in a plain tube and centrifuged immediately. The serum was then chilled at -18°C and was transported in frozen cool packed ice to a comercial laboratory. The laboratory was blinded to patient history and the aim of the study.

Troponin assay

Plasma concentrations of cTnI were analyzed as previously described [2,3]. High-sensitivity assay was used to measure cTnI levels in blood samples. High-sensitivity assay detects free and complex serum troponin through two-site sandwich immunoassay method. According to previous studies [2] the cTnI range for normal cats is from < 0.03 to 0.16 ng/mL, with a mean of 0.04 ng/mL and a median of 0.03 ng/mL.

Statistical analysis

The Pearson and Spearman's ratio (a nonparametric correlation test) were applied to test a probable association between the Troponin levels and the other variables. The one-sample Kolmogorov-Smirnov test was implemented to evaluate the normality of the distribution of troponin concentrations. The Mann-Whitney test was applied to compare the median of Troponin concentrations between the normal and cats with lower respiratory disease. PASW statistics (SPSS Version 24, IBM Co; New York 10504-1722, United States) and MedCalc software (Version 18.2.1) were used to analyze the variables. Confidence level was considered 95%, and a P-value for statistical significance was considered less than 0.05.

Results

The one-sample Kolmogorov-Smirnov test showed that the normality of Troponin distribution was rejected in involved (D = 0.229, P = 0.0028) and normal (D = 0.3513, P < 0.0001) cases. The median of Troponin was estimated in normal (0.100 to 0.110 ng/ml, MED =0.1) and in cats with LRDs (0.1570 to 0.4955 ng/ml, MED = 0.24) with 95% confidence interval. The quartile of Troponin was 0.1 ng/ml in normal and 0.1350 ng/ml in cats with LRDs. Therefore, the Mann-Whitney U test was used to compare the median of Troponin concentrations between the normal cats and cats with LRDs (P = 0.00) (Figure 1). In addition, the Mann-Whitney U test was used to compare the range of Troponin concentrations between the normal (Range ± interquartile range = 0.20 ± 0.01 ng/ml) and cats with LRDs (Range ± interquartile range =1.64 ± 0.47 ng/ml) (P = 0.00) (Figure 2). The Pearson correlation test revealed that there was no correlation between troponin concentrations and age, weight, heart rate, respiratory rate, WBC, Neutrophils, Lymphocytes, Basophils, Eosinophils, and Monocytes. Since the upper tolerance limit at the 90th percentile as well with 90% confidence was considered as 0.16 ng/mL (based on Margaret., et al. 2001), there was no correlation between troponin ranges and mucous membrane color of the gum, sex, breed, castration, vaccination, place, polyuria/polydipsia, diarrhea, vomiting, capillary refill time, body condition score, anorexia, respiratory wheezing and Crackling Sound, ascites, dehydration, dyspnea, cough, respiratory efforts, Shallow breath, tachypnea, pulmonary patterns in graph, and chest disorder in graph levels. However, there was a well-defined correlation between LRDs and troponin levels in cats. Troponin level was markedly increased in cats with lower respiratory diseases. Sixteen cats with LRDs had troponin level greater than 0.16 ng/mL.
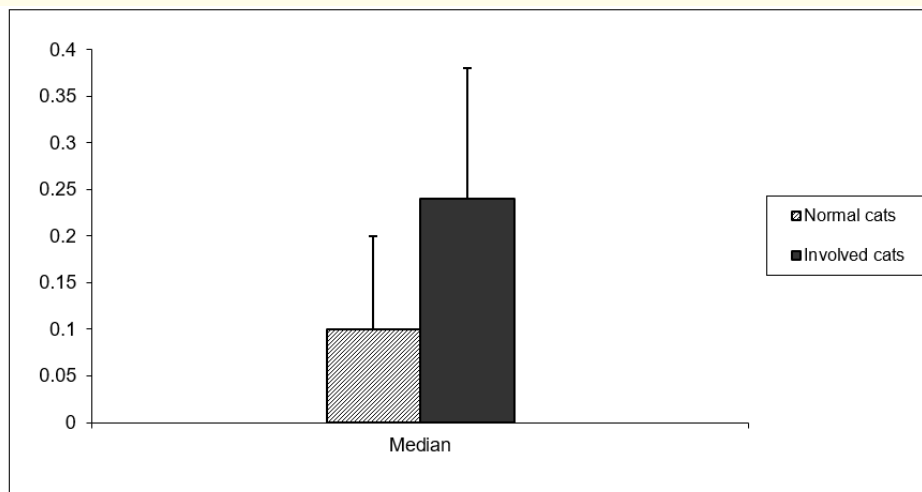

Figure 1: *Comparing the median of Troponin concentrations between the normal*
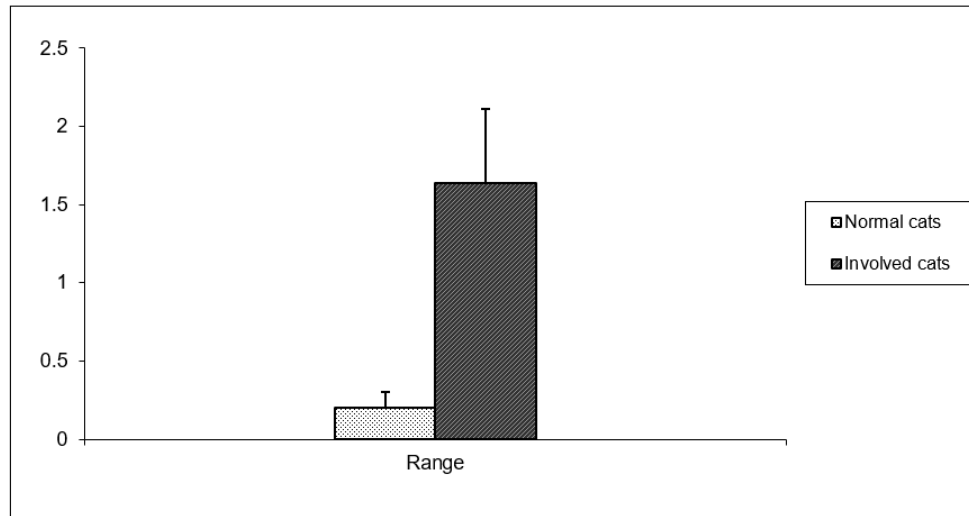*and cats with lower respiratory diseases (p = 0.00).*

Figure 2: Comparing the range of Troponin concentrations between the normal and cats with lower respiratory diseases (P = 0.00).

Discussion

The results of our study demonstrate that the mean and range of plasma cTnI in cats with LRDs is significantly (P < 0.001) greater than healthy cats. Furthermore, we demonstrated that 69.5% of cats (16 of 23) with LRDs have elevated cTnI levels (cTnI > 0.16 ng/mL). However, there was no correlation between respiratory signs, type of lower respiratory diseases, and radiographic patterns of lower respiratory diseases in cats with plasma cTnI concentrations.

Previous studies in cats have shown that cTnI concentration increases in myocardial disease and CHF [6,7]. Three more studies [17-19] have explored that cTnI concentration in cats could be used to distinguish cardiac from non-cardiac causes of dyspnea. These studies demonstrated that the cTnI concentration in dyspneic cats with cardiac disease (D-CD) was significantly higher than dyspneic cats with non-cardiac disease (D-NCD). These studies suggested that measurement of cTnI concentrations is a useful diagnostic test in cats evaluated for an unknown cause of dyspnea. In one of these studies [17] 64% of D-CD cats had cTnI concentrations greater than 66 ng/mL, whereas none of the D-NCD cats had cTnI concentrations above this level. In other study, all the D-CD cats had cTnI concentrations greater than 0.2 ng/mL whilst 50% of D-NCD cats had cTnI concentrations lower than 0.2 ng/mL, but 50% had values that exceeded the reference limit for healthy cats (> 0.17 ng/mL). Furthermore, in this study, none of the D-NCD cats had cTnI concentrations above 1.42 ng/mL hence based on their results cTnI could be a useful test to define and differentiate the etiologic origin of dyspnea in cats [17]. Investigator in another study [18] explored that cTnI concentration greater than 0.81 ng/mL could be used to discriminate D-CD cats with a sensitivity and specificity of 65.2% and 90.0%, respectively [18]. The different analyzers were used in each of these studies therefore, absolute values could not be established to differentiate cardiac from non-cardiac causes of dyspnea in cats.

Despite the fact that receiver operating characteristic (ROC) analyses of the 2 studies were similar and both studies suggest good specificity of the cTnI assay, the results from the other study [19] using the Immulite® cTnI assay revealed significant overlap in plasma cTnI concentrations between D-CD and D-NCD cats. Based on this study [19], measurement of cTnI concentrations should not be employed as a stand-alone test to differentiate cardiac from non-cardiac causes of dyspnea in cats and discriminatory tests such as echocardiography and radiography should additionally be used for this purpose.

Our results are similar to the latter study [19], in which three cats with lower respiratory diseases alone had cTnI concentrations greater than 1.42 ng/mL. Two of these cats had pneumonia and one had severe asthma. Echocardiography and thoracic radiography in these cats revealed no cardiac diseases. Two of these cats had severe dyspnea and tachypnea and one had severe cough. All the cats with cTnI levels greater than 1.42 ng/mL suffered from severe debilitating lower respiratory diseases. These findings showed that even cats with non-cardiac diseases could have cTnI concentrations greater than 1.42 ng/mL.

Seven cats with lower respiratory diseases had cTnI concentrations between 0.32 to 0.86 ng/mL. One of these cats had pulmonary edema, 4 cats had pneumonia, and 2 of them had bronchopneumonia. Cough, tachypnea and dyspnea was seen in 4, 2 and 2 of these cats respectively. All of these cats had moderate to severe lower respiratory diseases. However, there was no statistical correlation between the severity of lower respiratory diseases and cTnI concentrations in our study, but cats with higher levels of cTnI in their blood had more profound and devastating disease.

Five cats with lower respiratory diseases had cTnI levels between 0.17 to 0.32 ng/mL. Pneumonia and bronchopneumonia was seen in 3 and 2 of these cats respectively. Neither cough nor tachypnea was seen in these cats, however 4 out of 5 had mild to moderate dyspnea. Apparently, the severity of the LRDs in these cats was lower than cats with higher levels of cTnI concentrations (cTnI > 0.32 ng/mL). Furthermore, the duration of the disease plays an important role in the extent of cTnI elevation in blood. Cats with prolonged lower respiratory diseases are more prone to myocardial hypoxia and subsequent elevation in blood cTnI levels than those with a brief period of the disease. Increase in cTnI concentrations in cats with pneumonia and bronchopneumonia is due to decreased capacity of the lungs to exchange oxygen with blood and subsequent hypoxia.

The similarity between the current study with previous studies [17-19] in cats with dyspnea is that the median range of cTnI is significantly higher in cats with CHF than cats with respiratory disease alone. This is a valuable finding, however, its clinical applicability in distinguishing cardiac disease from respiratory disease in cats with dyspnea is very limited. Natriuretic peptides, NT-proANP and NT-proBNP, have been investigated to distinguish between cardiac from non-cardiac causes of respiratory distress in cats [21]. Based on a recent study [21], the diagnostic accuracy of NT-proBNP measurement was more than the measurement of cTnI in differentiating D-CD and D-NCD cats. Similar studies in dogs [20] revealed that Plasma NT-proANP, BNP, and endothelin-1, but not cTnI, appear useful for distinguishing between dogs with cardiac and non-cardiac causes of dyspnea, with plasma NT-proANP having the highest sensitivity (95.5%). We can speculate that the combination of cTnI and NT-proBNP assays could be used to distinguish cats with primary lower respiratory diseases from those with heart disease. Also, ancillary diagnostic tools like echocardiography and or radiography could be employed in adjunction to differentiate the primary respiratory disease from cardiac disease in dogs and cats.

Our results indicate that lower respiratory diseases in cats should be considered as one of the causative agents of increased cTnI levels, therefore increased cTnI levels in circulation is not always associated with cardiac disease in cats. This could be in some part as a result of close anatomical and functional relationship between the heart and lungs. Lower respiratory diseases could result in a hypoxic state in body with subsequent decrease in myocardial oxygen supply. Hypoxia, due to lower respiratory system dysfunction, could impose cardiac cells to a sub-lethal injury and could result in troponin release in the blood stream from damaged cardiac cells. However, troponin release in the blood due to respiratory disease is not as much as its release in CHF of primary cardiac disease [22].

Conclusion

In summary, although the median of plasma cTnI levels in cats with lower respiratory disease is higher than normal cats, this value is not as high as in cats with cardiac diseases. Differentiating cardiac from non-cardiac causes of cTnI elevations in cats necessitates the use of ancillary tests like echocardiography and radiography. Similarly, cTnI measurement could not be used as a stand-alone test to differentiate cardiac dyspnea from non-cardiac dyspnea in cats. Evaluating the role of other cardiac biomarkers in conjunction with cTnI levels in cats with lower respiratory disease as well as studying other forms of cardiac troponins particularly cTnT should be done in future studies.

Conflicts of Interest

We confirm that there are no conflicts of interest for current manuscript.

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