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Author(s)	Surachetpong, Sirilak Disatian; Teewasutrakul, Patarakrit; Rungsipipat, Anudep
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FULL PAPER

Serial measurements of cardiac troponin I (cTnI) in dogs treated with doxorubicin

Sirilak Disatian Surachetpong[1,*], Patarakrit Teewasutrakul[2] and Anudep Rungsipipat[3]

[1] Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand 10330

[2] Chulalongkorn Veterinary Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand 10330

[3] Companion Animal Cancer Research Unit, Department of Veterinary Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand 10330

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Abstract

The study aimed to evaluate whether cardiac troponin I (cTnI) and pulsed-wave tissue Doppler imaging (TDI) measurements can detect cardiac changes during doxorubicin therapy in dogs with various types of cancers compared to conventional echocardiography. Serial measurements of cTnI and conventional and pulsed-wave TDI echocardiography were performed in 12 dogs diagnosed with various types of cancers at day 0, weeks 3, 6, 9, and 12 prior to each doxorubicin injection. After treatment with doxorubicin, dogs had significantly increased cTnI levels at week 9 ($p = 0.027$) and 12 ($p = 0.027$) compared to normal untreated dogs. Dogs had increased cTnI levels during doxorubicin therapy ($p = 0.004$). Percent left ventricular ejection fraction (LVEF) and fractional shortening (FS) assessed by 2-dimensional and M-mode echocardiography significantly decreased at weeks 9 and 12. Pulsed-wave TDI derived myocardial performance index (MPI) increased significantly at weeks 9 and 12 compared to day 0 ($p = 0.028$ and 0.040, respectively). In conclusion, dogs treated with doxorubicin had increased cTnI levels. An increase in cTnI levels was detected before echocardiographic value changes. Serum cTnI can be a sensitive marker for detection of cardiotoxicity in dogs treated with doxorubicin.

Key Words: cancer, canine, cardiac marker, doxorubicin, tissue Doppler imaging

Introduction

Doxorubicin is an anthracycline drug. Anthracyclines and related compounds are effective in the treatment of several types of tumors found in dogs and cats.[22,25,38] However,

*Corresponding author: Dr. Sirilak Disatian Surachetpong, Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand 10330
Phone: +66-2-218-9412. E-mail: sirilakd27@gmail.com
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their use is limited secondary to irreversible cumulative dose-dependent cardiotoxic effects.[24] Doxorubicin can cause myocardial injury by the production of superoxides or by the activation of the anthracyclines to a free radical.[4] Dogs with a myocardial injury may finally develop irreversible myocardial dysfunction and heart failure.[11]

In humans, the endomyocardial biopsy is the goal standard method for monitoring the cardiotoxicity of doxorubicin[15]. However, the cardiac biopsy has not been routinely performed for cardiac monitoring in veterinary medicine. When administering doxorubicin, the conventional (two- dimensional and M-mode) echocardiographic monitoring of the cardiac function is recommended to be performed in humans[28] and dogs.[31,33] However, the standardized prescreening or ongoing monitoring echocardiographic guidelines have not been developed in veterinary medicine.

The left ventricular ejection fraction (LVEF) is a recommended method used to evaluate the systolic function in humans treated with anthracycline drugs.[28] The left ventricular ejection fraction mostly decreases secondary to systolic dysfunction induced by doxorubicin.[28] However, LVEF may not be sensitive enough to detect the early cardiac injury. A reduction of LVEF may be observed after the occurrence of an irreversible myocardial damage during a doxorubicin treatment.

In veterinary medicine, fractional shortening (FS) is a recommended method for evaluating dogs receiving doxorubicin.[16] Fractional shortening is rapid and easy to perform, but the interpretation of FS offers low specificity due to its strong dependence on preload and afterload.[20] The usefulness of FS in monitoring dogs treated with doxorubicin is uncertain. A previous study showed a decreased FS in doxorubicin-treated dogs[2], but another study failed to demonstrate the significant change of FS in dogs treated with a cyclic chemotherapy combination protocol including doxorubicin.[40] Because of the limitations of conventional echocardiography, a more sensitive and specific method to monitor and predict cardiac injury secondary to doxorubicin therapy is required.

In humans, cardiac troponin I (cTnI) is a cardiac biomarker widely used for evaluating myocardial cell damage that is increased in cases of cardiac ischemia, congestive heart failure, and myocarditis.[48] In veterinary medicine, cTnI is increased in cases of cardiac abnormalities and specific cardiac diseases such as dilated cardiomyopathy, right arrhythmogenic cardiomyopathy, and bradyarrhythmias, as well as in some extracardiac abnormalities such as gastric dilatation volvulus and snake bites.[26,44,49] Cardiac troponin I is a sensitive marker for detecting very early cardiac injury before cardiac dysfunction can be revealed by echocardiography.[13] When compared to other cardiac markers, such as atrial and B-type natriuretic peptides, cardiac troponin I seems to be a more suitable marker to monitor dogs treated with doxorubicin because myocardial cell death is likely to be the major pathology of doxorubicin-induced cardiotoxicity[36]. However, a few clinical studies have provided controversial results on changes in cTnI levels in dogs receiving doxorubicin. One prospective study has failed to detect changes of cTnI[41], whereas the other retrospective study showed a significant increase in cTnI levels in dogs receiving doxorubicin.[33] Thus, a study clarifying the advantage of cTnI as a monitoring marker in dogs treated with doxorubicin needs to be performed.

Tissue Doppler imaging (TDI) is an echocardiographic method for evaluating systolic and diastolic myocardial functions assessed by tissue velocities. It is a more sensitive method, which detects changes in cardiac function earlier compared to M-mode and two- dimensional echocardiography.[3,23] The TDI-derived myocardial performance index (MPI) or Tei is used to assess the global cardiac function.[30] MPI is calculated by using time intervals of TDI measurements. Unlike LVEF and FS, MPI is less influenced by preload and afterload.[30] Therefore, it may be a

useful method for detecting cardiac dysfunction in dogs treated with doxorubicin.

The hypothesis of this study was that the measurement of cTnI levels and TDI can be used as monitoring tools for cancer dogs treated with doxorubicin. The aim of this study was to evaluate whether cTnI and TDI measurements can detect cardiac changes during doxorubicin therapy in dogs with various types of cancers compared to conventional echocardiography.

Materials and Methods

This study was a prospective study. All dogs used in the study were patients of Chulalongkorn Small Animal Veterinary Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University, Thailand. The study was approved by the Faculty of Veterinary Science, Chulalongkorn University Ethics Committee (Animal Use Protocol Number 1431061). The owners had signed a written consent form before their dogs were included in the study.

Animals: Dogs included in the study were the patients at the Oncology Clinic, Chulalongkorn Small Animal Veterinary Teaching Hospital from January 2014 to May 2015. Dogs were diagnosed with any types of cancers and treated with doxorubicin. Types of cancers were confirmed by cytological and histopathological examinations and reported by veterinary pathologists from the Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Thailand. Before starting treatment, a full cardiologic examination including history taking, physical examination, radiography, electrocardiography, and echocardiography was performed. All findings from examinations were collected as baseline data. Signalment of each dog (age, gender, breed, and weight) was recorded. Inclusion criteria included 1) diagnosis of cancers via cytology and histopathology; 2) normal cardiac structure assessed by using two- dimensional echocardiography and normal systolic function assessed by following protocol: LVEF of over 40%, percent fractional shortening (%FS) over 25%, left ventricular dimension during systole within normal limit, and no regional wall motion abnormality function[27]; and 3) normal hematology and biochemistry profile values.[47] The exclusion criteria were as follows: 1) renal azotemia[29,34] 2) the administration of other anthracycline drugs; and 3) concurrent cardiovascular or other systemic diseases. All dogs treated with doxorubicin at a dosage of 30 mg/m^2 body surface area (BSA), 10 min IV bolus, every 3 weeks for a total of 5 treatments (at day 1 and weeks 3, 6, 9, and 12). A final accumulative dose of doxorubicin was 120 mg/m^2 BSA. Serial serum sample collections and echocardiography were performed on day 0, and every prior doxorubicin treatment at weeks 3, 6, 9, and 12.

Twenty healthy dogs of various breeds were used as controls. All of these dogs had normal physical examination findings and blood profile values. Dogs with abnormal electrocardiographic, radiographic, and echocardiographic findings were not included.

Echocardiography: Echocardiography was performed by an investigator (SS). Dogs were in right and left lateral recumbent position without sedation during the echocardiographic examination. M-mode and 2-dimensional echocardiography were done using an ultrasound machine (Samsung Madison, Eko7, Seoul, South Korea) with 2–4 and 4–12 MHz phased array transducers following the recommendation of the Echocardiography Committee of the Specialty of Cardiology of the American College of Veterinary Internal Medicine.[42] The two-dimensional echocardiography was performed on right parasternal long and short axis views to evaluate the cardiac structure abnormality. The M-mode echocardiography was done on right parasternal long axis left ventricular outflow view to measure cardiac chamber size and wall thickness: left ventricular end diastolic dimension (LVDd),

left ventricular end systolic dimension (LVDs), wall thickness of left ventricular free wall during diastole (LVWd) and systole (LVWs), interventricular septal thickness during diastole (VSd) and systole (VSs), and left atrium to aorta dimension ratio (LA/Ao ratio). Average values were calculated from three measurements of each parameter. M-mode echocardiographic values were indexed with Cornell's allometric normalized values (LVDd/body weight$^{0.294}$ and LVDs/body weight$^{0.315}$).[12] Percent FS was calculated by [LVDd-LVDs)/LVDdx100]. LVEF was measured using the modified Simpson's method on the left apical four-chamber view.[6] The end systolic and diastolic volumes (ESV and EDV) were indexed by body surface area.[14]

Tissue Doppler imaging (TDI) measurements: Pulsed-wave TDI was performed on the left apical four-chamber view. Real-time pulsed-wave TDI was superimposed on the gray scale using a narrow sector with high frame rates. The cursor was placed parallel to the longitudinal motion of the myocardium with the gate on the mitral annulus at the left ventricular free wall. The peak systolic (S'), early diastolic (E'), and late diastolic (A') tissue Doppler velocities were measured in three consecutive cycles, and the mean value was calculated. TDI-derived MPI was calculated by the following formula: [isovolumic contraction time (ICT') +isovolumic relaxation time (IVT')]/systolic wave duration.[18] To determine the between-day variability of the TDI measurements, the TDI data were obtained by the same echocardiographer (SS) on 3 different days from 5 dogs. The inter-examination variability was evaluated by repeated echocardiographic examinations of 5 dogs at 3 different time points on the same day by the same examiner (SS).

Radiography: Thoracic radiography from right lateral and ventrodorsal views was taken from each dog at week 0 and week 12 with a radiographic machine (Brivo DR-F, GE Healthcare, UK). The cardiac size was evaluated by measuring vertebral heart scale (VHS).[7] Pulmonary edema, pleural effusion, pulmonary vessel congestion, and caudal vena cava enlargement were evaluated.

Electrocardiography: Five-minute electrocardiography with a 12-channel ECG machine (CardiMax FX 7102, Fukuda Denshi, USA) was performed in the right lateral recumbent position at day 0 and weeks 3, 6, 9, and 12 to evaluate cardiac arrhythmias and ECG changes. ST-segment depression was defined by the depression of the ST-segment level >0.2 mV in lead II.[43] Duration and amplitude of P wave, QRS complex and T wave as well as PR and QT intervals were measured randomly and averaged from 5 consecutive waves. QT and corrected QT (QTc) intervals, were measured and calculated, using Van de Water's equation [$QTc = QT - 0.087 \times (RR - 1000)$].[45]

cTnI measurements: Serum was obtained from 2 ml venous blood samples by centrifuging at 1000 g for 15 minutes. cTnI levels were measured within 15 minutes after blood collection using an automated radioimmunoassay machine (TOSOH AIA 360, TOSOH Bioscience, South San Francisco, CA, USA) with a sandwich ELISA method. The detection range of the test is 0.01–50 ng/ml. Three different concentrations of cTnI control samples (0.1, 1.0, and 10 ng/ml) were prepared by spiking serum from that of a healthy dog. To evaluate the intra-assay precision, the samples were run 3 times in the same batch. To evaluate the inter-assay precision, the samples were run each day for 3 days. The linearity was evaluated by spiking pooled canine serum with cardiac troponin standards. The concentration of cTnI was ranged from 0.015 to 10 ng/ml. The unspiked serum was prepared from the same pooled canine serum.

Statistical analysis: Statistical analyses were performed using the computer-based statistical software (SPSS statistic 17.0, IBM, Chicago, IL, USA). Descriptive analysis was applied for

signalment information, including age, breed, and gender. Quantitative data were tested for normality by using the Shapiro-Wilk test. Data were presented as the mean ± standard deviation (SD) for normally distributed data and the median (25^{th}–75^{th} interquartile range) for non-parametric data. Changes of echocardiographic values and cTnI levels between day 0 and weeks 3, 6, 9, and 12 were compared using repeated ANOVA and Friedman's test. The multiple comparisons were performed by the Bonferroni method. The difference of echocardiographic values and cTnI levels between normal dogs and dogs treated with doxorubicin was analyzed by Mann-Whitney U test. The correlation between cTnI levels and accumulative doses of doxorubicin was performed using Spearman partial's rank correlation. A *p*-value less than 0.05 was considered statistically significant.

To analyze the between-day variability of TDI parameters, the SD within subjects (SD_{within}) and within-subjects CV were calculated. The SD_{within} was estimated by two-way ANOVA. The CV was calculated as the ratio of the SD_{within} to overall mean of measurements, expressed as a percentage. The inter-examination coefficient of variation (CV) of each TDI parameter was calculated from the mean and SD values from the 3 examinations of each dog. An overall CV for each TDI parameter was analyzed. A CV $<$15% was considered acceptable repeatability.[10] The intra- and inter-precision of cTnI assay were calculated as the CV. The linearity was determined via least-squares regression analysis. If the correlation coefficient was $>$0.95, the linearity of the assay was assumed.[27]

Results

Fifteen dogs were recruited into the study. Three dogs were excluded from the study due to the owner's decision to stop treatment. Data of 12 dogs are summarized in Table 1. Median (25^{th}–75^{th} interquartile range) body weight was 7.20 Kg (4.25–33.00 Kg). Median age was 10.5 years (10.0–12.0 years). Eight were female, and 4 were male. Dogs were diagnosed with mammary gland adenocarcinoma (n = 7), lymphoma (n = 2), leiomyosarcoma (n = 1), perianal adenocarcinoma (n = 1), and mesenchymal cell tumor (n = 1).

Normal dogs were 16 female and 4 male. Breeds included Shih Tzu (n = 5), Pomeranian (n = 4), Poodle (n = 3), French Bulldog (n = 2), Chihuahua (n = 1), West Highland White Terrier (n = 1), Beagle (n = 1), Miniature Pinscher (n = 1), and mixed breed (n = 2). The median body weight was 6.35 Kg (3.68–9.36 Kg), and the median age was 8.9 years (6.0–12.0 years). There was no statistical difference between age and weight of normal dogs and dogs treated with doxorubicin.

The linearity of cTnI assay used in this study was 0.976. The mean intra-assay and inter-assay precision were 4.2 ± 1.5% and 4.5 ± 1.7%. The median cTnI level of 20 normal dogs was 0.020 ng/ml (0.015–0.043 ng/ml). No significant difference of cTnI levels between normal dogs and dogs treated with doxorubicin at baseline or day 0 (p = 0.613). The median cTnI levels of dogs treated with doxorubicin at weeks 9 and 12 were significantly higher than that of normal dogs (p = 0.027 and 0.027, respectively) (Table 1). The cTnI levels were statistically different between time points (p = 0.004). With pairwise comparison, significant differences were found between day 0 and weeks 3, 6, 9, and 12 (p = 0.045, 0.045, 0.036, and 0.027, respectively) (Table 2). A weak correlation was found between cTnI levels and cumulative doses of doxorubicin (r = 0.271, p = 0.045).

With the Friedman test, the means of LVEF and FS of the entire dog population were statistically different between time points (Table 2). The p-value of post hoc analyses comparing the means of LVEF between day 0 and week 3, 6, 9, and 12 were 3.92, 2.30, 0.048, and 0.048, respectively. The p-value of post hoc analyses comparing the means of FS between day 0 and week 3, 6, 9, and 12 were 0.528, 2.696,

Table 1. Information of 12 dogs included in the study

Dog	Breed	Age (year)	Gender	Weight (Kg)	ECG	Cancer types	Other drugs
1	Shih Tzu	12	FS	4.4	Normal	Leiomyosarcoma, Uterine tumor	–
2	Miniature pinscher	12	FS	5.6	Normal	Adenocarcinoma, Mammary tumor	–
3	Shih Tzu	10	FI	4.2	Normal	Adenocarcinoma, Mammary tumor	–
4	Mixed	12	FS	9.5	Normal	Adenocarcinoma, Mammary tumor	–
5	Mixed	6	FS	6.4	ST segment depression at week 9	Multicentric Lymphoma	Prednisolone
6	Labrador retriever	11	MC	34	Normal	Perianal adenocarcinoma	–
7	German shepherd	10	FS	38	Normal	Adenocarcinoma, Mammary tumor	–
8	Golden retriever	10	MC	30	Normal	Adenocarcinoma, Mammary tumor	–
9	White highland white terrier	14	MI	8	VPC at week 9	Multicentric Lymphoma	Vincristine, cyclophosphamide
10	Poodle	14	FI	4.2	Normal	Malignant Mesenchymal tumor	Piroxicam
11	Golden retriever	8	MI	37.4	Normal	Adenocarcinoma, Mammary tumor	–
12	Pomeranian	10	FI	1.7	Normal	Adenocarcinoma, Mammary tumor	–

VPC = ventricular premature complex; FI = female intact; FS = female spray; MI = male intact; MC = male castrate

0.048, and 0.044, respectively.

Tissue Doppler systolic velocity (S'), early and late diastolic velocities (E' and A'), and the ratio of tissue Doppler early and late diastolic velocities ratio (E'/A') were not different between time points (Table 2). The E'/A' ratio of less than 1 was primarily found at week 3 in 2 dogs and week 6 in 1 dog. These dogs had E'/A' ratios that remained reduced by less than 1 during the rest of the treatment. The TDI-derived MPI decreased in week 9 and 12 compared to day 0 ($p = 0.028$ and 0.040, respectively). The inter-examination and between-day CV of each TDI parameters are presented in Table 3.

Left ventricular dimensions during diastole (LVDd) and systole (LVDs), left atrium to aorta ratio (LA/Ao), end diastolic and systolic volume indexes (EDVI and ESVI) were not different between time points (Table 2). No significant cardiac structural change was noticed on two-dimensional echocardiographic examination.

There was no significant change of mean VHS at day 0 (10.5 ± 0.2) and week 12 (10.8 ± 0.3) ($p = 0.205$). There was no sign of pulmonary edema, i.e. left-sided congestive heart failure or vascular enlargement, detected on radiographs at week 12 after doxorubicin therapy. None of the dogs had lung metastasis or intrathoracic lymph node enlargement before and after 12 weeks of doxorubicin therapy.

Duration and amplitude of P wave, QRS complex, and T wave as well as PR and QT intervals, were not different between time points (Table 2). Two dogs had ECG abnormalities

Table 2. Cardiac troponin I levels, electrocardiographic values and echocardiographic values at different time points

	Day0	Week3	Week6	Week9	Week12	*p*-value[#]
cTnI (ng/ml)	0.021 (0.017, 0.054)	0.048* (0.026, 0.079)	0.046* (0.034, 0.075)	0.053*, ± (0.038, 0.072)	0.053*, ± (0.029, 0.084)	0.004
LVEF (%)	62.21 ± 4.63	65.79 ± 5.33	66.48 ± 4.21	54.84 ± 5.26*	54.74 ± 5.47*	0.044
FS (%)	36.06 ± 4.30	37.19 ± 3.87	36.06 ± 2.95	28.07 ± 3.42*	25.27 ± 4.07*	0.045
S' (cm/s)	10.0 ± 1.3	12.6 ± 2.4	11.8 ± 1.4	12.2 ± 1.3	11.8 ± 1.2	0.825
E' (cm/s)	9.2 ± 2.0	12.0 ± 3.8	11.0 ± 4.0	12.8 ± 3.8	12.0 ± 3.8	0.289
A' (cm/s)	9.5 ± 2.3	12.0 ± 3.29	10.2 ± 2.7	11.7 ± 3.1	11.5 ± 2.8	0.345
E'/A'	1.00 ± 0.3	0.99 ± 0.3	1.06 ± 0.2	1.12 ± 0.3	1.04 ± 0.3	0.774
MPI	0.77 ± 0.14	0.70 ± 0.14	0.84 ± 0.12	0.90 ± 0.15*	0.91 ± 0.15*	0.019
ESVI (ml/m^2)	22.59 ± 3.75	26.89 ± 7.28	21.01 ± 4.54	23.39 ± 3.97	20.17 ± 2.50	0.681
EDVI (ml/m^2)	55.15 ± 5.42	56.97 ± 2.24	50.25 ± 5.97	50.07 ± 3.83	54.04 ± 5.09	0.785
LVDs (cm/kg)	0.87 ± 0.15	0.77 ± 0.27	0.78 ± 0.24	0.83 ± 0.24	0.91 ± 0.13	0.795
LVDd (cm/kg)	1.25 ± 0.29	1.17 ± 0.55	1.29 ± 0.26	1.27 ± 0.26	1.37 ± 0.23	0.576
LA/Ao	1.22 ± 0.06	1.28 ± 0.06	1.22 ± 0.04	1.29 ± 0.06	1.29 ± 0.09	0.787
P wave duration (sec)	0.04 ± 0	0.04 ± 0	0.04 ± 0	0.04 ± 0	0.04 ± 0	–
P wave amplitude (mV)	0.22 ± 0.04	0.19 ± 0.07	0.16 ± 0.08	0.19 ± 0.07	0.2 ± 0.07	0.350
PR interval (sec)	0.11 ± 0.02	0.11 ± 0.03	0.11 ± 0.02	0.10 ± 0.02	0.10 ± 0.02	0.410
QRS duration (sec)	0.04 ± 0	0.04 ± 0	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.429
QRS amplitude (mV)	1.08 ± 0.34	1.27 ± 0.39	1.42 ± 0.42	1.39 ± 0.43	1.38 ± 0.43	0.982
QTc duration (sec)	0.20 ± 0.04	0.19 ± 0.02	0.20 ± 0.03	0.22 ± 0.02	0.22 ± 0.03	0.762

cTnI values presented as median (25^{th}–75^{th} interquartile)
Other data presented as mean ± SD (standard deviation)
[#]comparison using repeated ANOVA and Friedman's test
[±]indicate significant difference at $p < 0.05$ compared to the normal group by the Mann-Whitney U test
*indicate significant difference at $p < 0.05$ compared to day 0 by the Wilcoxon signed rank test
cTnI = cardiac troponin I; LVEF = left ventricular ejection fraction; FS = fractional shortening; S' = systolic tissue Doppler velocity; E' = early diastolic tissue Doppler velocity; A' = late diastolic tissue Doppler velocity; E'/A' = the ratio of early to late diastolic tissue Doppler velocity; MPI = myocardial performance index (ICT'+IRT')/systolic wave duration; ESVI = end systolic volume index; EDVI = end diastolic volume index; LVDs = left ventricular dimension during systole; LVDd = left ventricular dimension during diastole; LA/Ao = the ratio of left atrium to aorta

Table 3. Repeatability of tissue Doppler imaging parameters

Parameters	Between-day variability (%)	Interexamination variability (%)
S'	8.4	9.6
E'	8.5	9.7
A'	8.5	7.6
E'/A'	7.5	8.8
MPI	6.5	7.7

S' = systolic tissue Doppler velocity; E' = early diastolic tissue Doppler velocity; A' = late diastolic tissue Doppler velocity; ESVI = end systolic volume index; E'/A' = the ratio of early to late diastolic tissue Doppler velocity; MPI = myocardial performance index (ICT'+IRT')/systolic wave duration

including ST-segment depression (>0.2 mV below the baseline on lead II) and occasional ventricular premature complex (VPC) (1–2 VPCs every 5 seconds). All dogs had hematology and biochemistry blood profile values within normal limit during the study period.

Discussion

The main finding of this study was an increase of cTnI levels in dogs treated with doxorubicin with functional but not cardiac structural changes assessed by conventional (two- dimensional and M-mode) and TDI echocardiography. In addition, an increase in cTnI levels was detected before the change of echocardiographic values suggesting that cTnI may be a more sensitive indicator to detect cardiotoxicity secondary to doxorubicin therapy.

Cardiac troponin I is a cardiac biomarker widely used to detect cardiac injury in human patients.[35] It is increasingly used in veterinary medicine. To evaluate whether the cTnI can be used as a monitoring tool in dogs treated with doxorubicin, the cTnI levels was measured serially during the treatment of doxorubicin. The present study showed that dogs had increased cTnI levels after receiving doxorubicin when compared to the median levels of normal dogs and baseline values at day 0.

An increase of cTnI levels in this study suggested an on-going effect of doxorubicin to the heart up to three weeks after administration. This result is in agreement with another study in humans that showed the persistent elevation of cTnI a month after completion of chemotherapy.[8] One dog in the present study had a cTnI level of 0.257 ng/ml at week 9 and 0.508 ng/ml at week 12. During the study period, this dog had echocardiographic values within normal limits[6] and no cardiac structural change. However, the dog developed dilated cardiomyopathy and congestive heart failure six months later, after stopping treatment. These findings suggest that doxorubicin may cause damage to the heart; however, the function of the heart may reserve, and the alterations in structure and function may not be detected until the cardiac injury is severe enough. Cardiac damage may become apparent months or years after receiving doxorubicin.

A weak correlation between cTnI levels and cumulative doses of doxorubicin was found in the present study. Previous reports in dogs indicated that a cumulative dose of doxorubicin ranging from 122 to 265 mg/m^2 BSA could induce cardiomyopathy and congestive heart failure in doxorubicin-treated dogs.[21,39] The median cumulative dose of doxorubicin was 150 mg/m^2 BSA before systolic failure was detected in dogs.[24] Some dogs could develop cardiac abnormalities at a cumulative dose of less than 90 mg/m^2 BSA.[24] However, none of the dogs in this study developed cardiomyopathy or congestive heart failure during the study period.

The method of doxorubicin administration is also associated with the severity of cardiotoxicity.[24] A ten-minute injection, a protocol of doxorubicin administration used in the present study, has been suggested to have a higher toxic effect than the slow rate constant infusion protocol.[24] Maybe that is the reason changes in echocardiography and cTnI levels were detected in this study but not in another study, which used the slow rate constant infusion protocol for doxorubicin injection.[41]

In this report, %FS decreased in dogs treated with doxorubicin. Percent FS is load-dependent. Therefore, it should not be used as a stand-alone method to determine systolic function. The Simpson method for evaluating LVEF and the left ventricular end systolic volume index (EVSI) is a better way to determine the systolic function of the left ventricle.[49] The LVEF declination was also found in dogs treated with doxorubicin in the present study. However, the EVSI was not changed during the study period.

Because of its high sensitivity in detecting systolic dysfunction, peak systolic tissue Doppler velocities (S') of the mitral annulus by pulsed-wave TDI has been suggested to be an adjunct method for monitoring doxorubicin-induced cardiomyopathy in humans.[28] Several human studies demonstrated that patients treated with anthracyclines had a decreased peak S' velocity assessed by TDI prior to or concomitant with the reduction in LVEF.[17] Although the decline of

LVEF was detected in the population of dogs in the present study, the change of peak S' velocities assessed by TDI was not demonstrated. The non-significant change of peak S' velocities during doxorubicin treatment in the present study are probably due to the measurements of peak S' velocities being performed in only one region that is the mitral annulus at the left ventricular free wall. Doxorubicin may cause a regional myocardial dysfunction but not in the whole heart.[1] Therefore, other advanced echocardiographic techniques, such as tissue speckle tracking, which can detect the myocardium function in several segments simultaneously, may be more sensitive and more suitable to detect the effects of doxorubicin on myocardial systolic function.[1]

Because the changes in tissue Doppler velocities including peak S', E', and A' velocities could not be detected in the present study, the technical limitations of TDI including angle dependence, passive motion, and reproducibility should be considered. In order to measure tissue Doppler velocities accurately the cursor was aligned parallel through the annulus to avoid underestimation of the Doppler signal in all dogs in this study. Also, the between-day and inter-examination CV of all TDI parameters were $<$15%, which was considered acceptable.[10]

The E'/A' ratio was less than 1 in 3 dogs during treatment suggesting that dogs treated with doxorubicin may develop left ventricular diastolic dysfunction. These findings were similar to those of a previous report.[41] The necropsy report on dogs with doxorubicin cardiotoxicity showed vacuole myocardial degeneration, myocytolysis, and fibrosis in the heart.[24] These abnormalities might cause diastolic dysfunction, same as systolic dysfunction. However, the effect of doxorubicin on diastolic function has not been elucidated yet, and it needs further investigation.

Myocardial performance index (MPI) or Tei index is another method that can be used to evaluate the regional myocardial function. The present study demonstrated the significant increase of TDI-derived MPI at week 9 and 12, suggesting a more sensitive property of this parameter than peak S', E', and A' velocities. Similarly, a previous study in humans showed that MPI index based on TDI worsened earlier than peak S' and E' velocities.[5]

Earlier studies reported a marked decrease in QRS amplitude in dogs treated with doxorubicin.[2,24,46] In contrast, the present study showed a non-significant increase in QRS amplitude. Prolonged QT intervals have been observed in humans treated with anthracycline drugs.[32] An increase by approximately 10 percent of QT intervals was presented in this study, but this increase was not statistically significant. Two dogs in this study had ECG abnormalities, including ST-segment depression and ventricular premature complex (VPC). The dog with ST-segment depression had an increase of cTnI levels (0.105 ng/ml at week 9 and 0.115 ng/ml at week 12), with a decrease of %FS and LVEF. However, the other dog with VPC had cTnI levels that did not differ from the baseline level (0.019 ng/ml at week 9 and 0.020 ng/ml at week 12). Previous studies suggested that these ECG changes may be transient or hemodynamically insignificant and do not correlate with the development of cardiomyopathy.[19]

Cardiac arrhythmias were reported in 18.9% (31/175) of dogs treated with doxorubicin in one study.[24] Types of ECG abnormalities including supraventricular and ventricular arrhythmias, conduction disturbances, and ST-segment slurring were reported. The most common abnormality was unifocal VPC.[24] ECG abnormalities could occur after dogs received a low doxorubicin dosage (90 mg/m^2 BSA)[24], which was the same as for dogs in the present study. One study reported that VPC in dogs treated with doxorubicin can progress to more severe arrhythmias, such as ventricular tachycardia or ventricular fibrillation that lead to asystole or sudden death.[32] Thus, ECG should be monitored in all dogs treated with doxorubicin.

One limitation of this study was the lack of a necropsy study that might have shown changes related to cTnI levels. Also, spectral Doppler

echocardiography was not performed in this study. Data from spectral Doppler echocardiography, particularly diastolic parameters, might provide more information on the diastolic function of these dogs. The major limitations of this study were the lack of data from the population of dogs with cancer not receiving doxorubicin and the low numbers of study dogs. The levels of other cardiac biomarkers, such as atrial natriuretic peptides (ANP) and B-type natriuretic peptides (BNP), were not measured in the present study. Thus, the usefulness of these markers and cTnI on monitoring dogs treated with doxorubicin could not be compared. In humans, the peak level of cTnI in circulation is detected within 72 hours of chemotherapy.[9] However, in this study, the peak cTnI levels could not be evaluated because the blood was not collected within 72 hours after the doxorubicin administration. Because none of the dogs in the present study developed heart failure secondary to doxorubicin administration during the study period, a cut-off level of cTnI for detecting doxorubicin- induced cardiomyopathy could not be determined. Also, the cardiac biopsy was not performed. Thus, the relationship between cTnI levels and the degree of cardiac tissue damage could not be evaluated. Further studies with a longer period of therapy, a higher accumulative dose of doxorubicin, and histopathological examinations of the myocardial tissue should be performed to determine the benefit of cTnI on a detection of doxorubicin cardiotoxicity.

In conclusions, dogs treated with doxorubicin may have the cardiac injury that can be assessed by an increase in cTnI levels. Also, cTnI levels increase before echocardiographic changes can be detected. Therefore, cTnI may be a potential candidate biomarker for monitoring cardiotoxic effects of doxorubicin in dogs. Further studies with a longer period of study and a larger number of dogs should be performed.

Conflict of interest

The authors declare no conflict of interest.

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