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Cardiac Troponin-I Concentration in Dogs with Cardiac Disease

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Cardiac troponin-I (cTnI) is a highly sensitive and specific marker of myocardial injury and can be detected in plasma by immunoassay techniques. The purpose of this study was to establish a reference range for plasma cTnI in a population of healthy dogs using a human immunoassay system and to determine whether plasma cTnI concentrations were high in dogs with acquired or congenital heart disease, specifically cardiomyopathy (CM), degenerative mitral valve disease (MVD), and subvalvular aortic stenosis (SAS). In total, 269 dogs were examined by physical examination, electrocardiography, echocardiography, and plasma cTnI assay. In 176 healthy dogs, median cTnI was 0.03 ng/mL (upper 95th percentile = 0.11 ng/mL). Compared with the healthy population, median plasma cTnI was increased in dogs with CM (0.14 ng/mL; range, 0.03–1.88 ng/mL; $P < .001$; n = 26), in dogs with MVD (0.11 ng/mL; range, 0.01–9.53 ng/mL; $P < .001$; n = 37), and in dogs with SAS (0.08 ng/mL; range, 0.01–0.94 ng/mL; $P < .001$; n = 30). In dogs with CM and MVD, plasma cTnI was correlated with left ventricular and left atrial size. In dogs with SAS, cTnI demonstrated a modest correlation with ventricular wall thickness. In dogs with CM, the median survival time of those with cTnI >0.20 ng/mL was significantly shorter than median survival time of those with cTnI <0.20 ng/mL (112 days versus 357 days; $P = .006$). Plasma cTnI is high in dogs with cardiac disease, correlates with heart size and survival, and can be used as a blood-based biomarker of cardiac disease.

Key words: Cardiac biomarkers; Cardiomyopathy; Subaortic stenosis; Valvular disease.

Thoracic radiography, electrocardiography (ECG), and echocardiography are used routinely to diagnose and evaluate dogs with heart disease, but despite performance of these tests, uncertainties about severity of disease, response to treatment, and prognosis of individual patients often persist. Moreover, even with the use of cardiac diagnostics and medical therapy, the morbidity and mortality of dogs with heart disease remains high.[1–6] The development of a cardiac-specific blood-based biomarker may be of particular use in clinical canine medicine. Biomarkers, defined as ''a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic intervention,''[7] provide information regarding level of disease exposure, extent of injury, and prognosis.[8] The use of biomarkers for noncardiac organ systems is familiar and well-accepted—an example is the measurement of blood urea nitrogen concentration to assess renal function. In human cardiac medicine, biomarker substances such as cardiac troponin and atrial and B-type natriuretic peptides have found increased use as a result of studies indicating their diagnostic, monitoring, and prognostic utility.[9–12]

Previous studies in veterinary species have reported cardiac troponin concentrations in healthy dogs,[13] cats,[13] and horses,[14] and in animals with a variety of diseases including gastric dilatation and volvulus,[15] babesiosis,[16] blunt trauma,[17] infarction,[18] congestive heart failure,[19] and hypertrophic cardiomyopathy.[20,21] Collectively, these studies suggest that circulating cardiac troponin concentrations are high in patients with heart disease and have potential as a marker of heart injury.

The cardiac troponin complex is composed of 3 subunits (cTnI, cTnT, and cTnC) that help regulate excitation-contraction coupling of the sarcomeric proteins. cTnI is the inhibitory component and prevents interaction between actin and myosin until intracellular calcium is bound by cTnC. cTnI is normally bound to the actin filament via cTnT, but in response to sarcomeric injury, it detaches and is released into the cytosol and extracellular space.[22] The detection of high concentrations of circulating cTnI or cTnT is viewed as a specific indicator of myocardial injury and cellular necrosis.[23,24] This relationship is demonstrated in dogs with experimental coronary artery occlusion in which the concentration of circulating cTnI is proportional to the extent of cardiac injury.[18,a] Both cardiac and skeletal muscle utilize a troponin complex to mediate contraction, but the 2 isoforms, cardiac and skeletal, are antigenically distinct from each other.[25–27] The close homology of cardiac isoforms among mammalian species permits rapid and accurate measurement of canine cTnI concentrations using immunoassays developed for humans.[b] Thus, cTnI is an attractive potential biomarker because of its highly sensitive and specific indication of myocardial damage in both humans and animals.

Cardiac troponin assays are used routinely in human medicine, where they are regarded as a diagnostic standard of care in subjects suspected to have acute myocardial infarction.[28] Recently, there has been increasing interest in expanding the use of troponin assays to patients with chronic heart disease of primarily nonischemic origin (eg, dilated and hypertrophic cardiomyopathy, systemic hypertension). Previous studies indicate that many of these patients have mild increases of cardiac troponin consistent with chronic, low-level, and persistent myocardial damage.[29–38] Within this patient group, one-time or serial measurement of cardiac troponins can help quantify the degree of myocardial injury, monitor progression of disease, assess response to therapy, and offer prognosis.[36–42]

The purpose of this study was to evaluate plasma cTnI in canine patients with naturally occurring heart disease. In particular, we sought to establish a reference range for plas-

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ma cTnI in a population of healthy dogs, to determine cTnI concentrations in dogs with acquired heart disease or subaortic stenosis, to assess cTnI in relation to disease severity, and to evaluate the clinical potential of cTnI as a prognostic indicator in dogs with cardiomyopathy.

Materials and Methods

Dogs presented to the cardiology service of the Veterinary Teaching Hospital of the University of Illinois from August 2001 to October 2003 were eligible for entry into the study. The cardiology service also solicited healthy animals from Doberman Pinscher breeders, hospital employees, and students during this time period. Enrollment was based on owner consent and the availability of the dog for ECG and ultrasound examination. The study protocol was approved by the University of Illinois Institutional Animal Care and Use and Committee. Dogs were enrolled into 1 of 5 groups based on the following criteria:

(1) Healthy non-Doberman Pinscher dogs: dogs with normal cardiac auscultation, 10-lead ECG, 2-dimensional and color flow Doppler echocardiogram, and M-mode echocardiographic left ventricular and atrial measurements within a normal reference range[43];
(2) Healthy Doberman Pinschers: Doberman Pinscher dogs with normal cardiac auscultation and 10-lead ECG, absence of ventricular arrhythmias during a 5-minute lead II rhythm strip, normal 2-dimensional and color flow Doppler echocardiogram, M-mode echocardiographic left ventricular diastolic dimension (LVDd) ≤46 mm, and left ventricular systolic dimension (LVDs) ≤38 mm[c];
(3) Cardiomyopathy (CM): dogs referred to the cardiology service for evaluation of suspected CM, including those with ventricular arrhythmias or with clinical signs of dyspnea, tachypnea, ascites, syncope, lethargy, or exercise intolerance; echocardiographic evidence of dilated cardiomyopathy (Doberman Pinschers: LVDd >46 mm and LVDs >38 mm; other breeds: fractional shortening ≤23%); Boxer dogs with sustained or nonsustained ventricular tachycardia of right ventricular morphology;
(4) Degenerative mitral valve disease (MVD): dogs with a left apical systolic murmur; thickened, nodular, or prolapsing mitral valve leaflets on 2-dimensional echocardiogram; moderate to severe mitral valve regurgitation subjectively determined by color flow Doppler study (turbulent color flow jet occupying greater than one-third of the left atrial chamber);
(5) Congenital subaortic stenosis (SAS): dogs with a left or right basilar systolic murmur, echocardiographic visualization of a subaortic ring or ridge or Doppler left ventricular outflow tract velocity >2.1 m/s in the absence of clinically relevant concurrent valvular or shunting defects.

Dogs with either CM or MVD were further divided into subgroups based on the presence or absence of congestive heart failure (CHF). CHF (ie, pulmonary edema, pleural effusion, or ascites) was determined by evaluation of thoracic radiographs or ultrasonography. Dogs with CM also were divided into subgroups based on the presence or absence of arrhythmias on ECG (eg, atrial fibrillation, ventricular premature beats, ventricular tachycardia). Echocardiographic measurements of the left ventricle and atrium were indexed to the measured diameter of the aortic root.[44] Echocardiographic examinations were performed without sedation using a System V or VIVID7 echocardiographic machine.[d] ECGs were performed using a PageWriter XLi unit.[e]

Blood samples were collected in 8-mL blood tubes containing 143 U of sodium heparin.[f] Samples were centrifuged within 30 minutes after collection and the plasma was separated and stored at −70°C for batched cTnI analysis. cTnI plasma concentration was determined using the human Access AccuTnI assay.[g] The AccuTnI assay uses a chemiluminescent sandwich ELISA technique and previously has been shown to have great sensitivity and specificity for canine cTnI with a lower limit of detection of 0.02 ng/mL.[b]

Survival time of dogs in the CM group was calculated as the time from cTnI sampling to time of death, euthanasia, end of the study follow-up period (March 2004), or their last contact before being lost to follow-up. Dogs that were euthanized during this time period were treated as uncensored data, whereas dogs that were still alive at the end of the study or lost to follow-up were treated as right-censored observations.

Statistical Analysis

Statistical analysis was performed using personal computer–based statistical software.[h] Baseline characteristics between patient groups and the control population were compared using either analysis of variance and Bonferroni multiple comparison or a Kruskal-Wallis and Dunn posthoc test. Median cTnI concentrations among groups were compared using the Kruskal-Wallis test and Dunn posthoc test. The correlation between cTnI and echocardiographic parameters was evaluated using Spearman rank correlation. Survival times of dogs with CM with cTnI >0.20 ng/mL versus dogs with CM with cTnI <0.20 ng/mL were compared using Kaplan-Meier and log-rank analysis. Significance was defined as $P < .05$.

Results

Patient Population

The study population comprised 269 dogs. Of these, 176 dogs, including 81 Doberman Pinschers, were determined as healthy. Twenty-six dogs were diagnosed with CM, 37 dogs with MVD, and 30 dogs with congenital SAS. The signalment and echocardiographic data from each patient group are presented in Table 1. Compared with healthy subjects, dogs with CM and MVD had significantly increased LVDd and left atrial to aortic root (LA:Ao) dimensions. Dogs with CM had increased LVDs and decreased fractional shortening compared with healthy dogs, whereas dogs with MVD had increased fractional shortening compared with healthy dogs. The distribution of breeds in the CM and SAS populations reflected previous literature that indicated a high incidence of CM in Doberman Pinschers, Boxers, and giant breeds, and a high incidence of SAS in Golden Retrievers, Boxers, and Rottweilers.[45] Within the CM group, 7 dogs (27%) had CHF and 19 dogs (73%) had ventricular arrhythmias or atrial fibrillation. Within the MVD group, 10 dogs (27%) had CHF. In the CM group, 25 of 27 dogs (93%), and in the MVD group, 18 of 37 dogs (49%) were receiving medical therapy at time of cTnI sampling. Treatment included a wide variety and combination of drugs including furosemide, thiazide diuretics, angiotensin-converting enzyme inhibitors, digoxin, beta-blockers, spironolactone, amlodipine, and diltiazem.

Plasma cTnI Concentrations

Results of plasma cTnI assays are shown in Figure 1. The median cTnI of all healthy dogs was 0.03 ng/mL (range, 0.01–0.15 ng/mL). The median cTnI of healthy Doberman Pinschers was not significantly different from that of healthy dogs of other breeds ($P > .05$). The statistical description of plasma cTnI in the 5 patient groups is presented in Table 2. The upper 95th percentile for all 176 healthy dogs was 0.11 ng/mL. The median cTnI concentrations in dogs with CM, MVD, and SAS were significantly greater than the median cTnI in healthy dogs (Fig 1, Table 2). The median cTnI in dogs with CM was 0.14 ng/mL

Table 1. Patient signalment and echocardiographic data from 269 dogs.

	Healthy		Cardiac Disease		
	Non-Dobermans	Dobermans	CM[a]	MVD	SAS
Number	95	81	26	37	30
Sex					
Male	42	28	15	20	19
Female	53	53	11	17	11
Age (years)	4.5 (3.1)	5.8 (3.1)	8.1 (2.6)[cd]	11.2 (2.1)[c]	3.0 (2.8)
Echocardiogram					
LVDd:Ao	1.66 (1.43–1.78)	1.48 (1.37–1.65)	2.13 (1.85–2.54)[cd]	2.29 (1.96–2.69)[c]	1.62 (1.49–2.00)
LVSd:Ao	1.12 (0.98–1.16)	1.10 (1.01–1.25)	1.89 (1.57–2.30)[cd]	1.32 (1.15–1.63)[c]	1.21 (0.98–1.43)
%FS	30.0 (26.0–35.7)	25.2 (21.7–29.2)[c]	13.5 (7.1–18.3)[cd]	40.9 (33.7–47.1)[b]	31.5 (24.9–36.5)
LA:Ao	1.03 (0.90–1.16)	0.95 (0.82–1.1)	1.4 (1.08–1.62)[cd]	1.67 (1.25–2.02)[c]	1.19 (1.01–1.49)
IVSd:Ao	0.41 (0.37–0.45)	0.34 (0.32–0.37)[c]	0.36 (0.32–0.43)	0.43 (0.39–0.51)	0.49 (0.39–0.60)
LVPWd:Ao	0.41 (0.37–0.47)	0.35 (0.31–0.39)[c]	0.36 (0.30–0.49)	0.45 (0.37–0.53)	0.48 (0.39–0.56)
V_{LVOT}	N/A	N/A	N/A	N/A	3.39 (2.78–5.02)
CHF					
Yes/No	N/A	N/A	7/19	10/27	N/A
Arrhythmias					
Yes/No	N/A	N/A	19/7	N/A	N/A
Breeds	Great Danes = 12		Doberman = 15	Cocker spaniel = 5	Golden Retriever = 10
	Golden Retriever = 11		Great Dane = 4	Maltese = 4	Boxer = 9
	Boxer = 6		Boxer = 4	Shih Tzu = 3	Rottweiler = 5
	Labrador Retriever = 5		Rottweiler = 1	Dachshund = 3	Other purebred = 6
	Other purebred = 19		Labrador Retriever = 1	Other purebred = 15	
	Mixed breed = 42		Mixed breed = 1	Mixed breed = 7	

[a] CM patients were also compared with healthy Doberman Pinschers. Results listed as mean (SD) for age and median (25th–95th percentile values) for echocardiographic measurements. N/A = not applicable. LVDd, left ventricular diastolic dimension; Ao, aorta; LVSd, left ventricular systolic dimension; FS, fractional shortening; LA, left atrium; IVSd, interventricular end-diastolic thickness; LVPWd, left ventricular posterior wall end-diastolic thickness; V_{LVOT}, maximum velocity of blood flow in the left ventricular outflow tract.

[b] $P < .05$ versus healthy non-Doberman Pinschers.

[c] $P < .001$ versus healthy non-Doberman Pinschers.

[d] $P < .05$ versus healthy Doberman Pinschers.

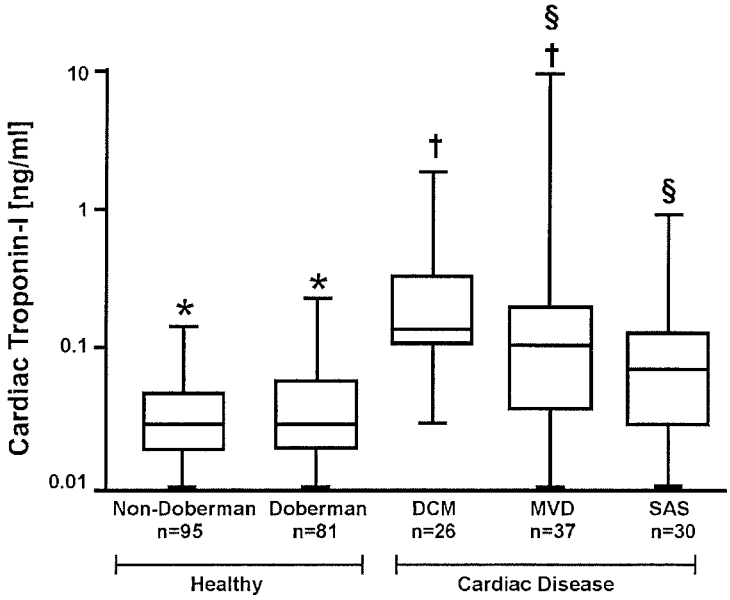

Fig 1. Plasma concentrations of cardiac troponin-I (cTnI) in healthy non-Doberman Pinschers, healthy Doberman Pinschers, and dogs with cardiomyopathy (CM), mitral valve disease (MVD), and congenital subaortic stenosis (SAS). The median value is shown as the horizontal line within boxes that bound the 25th and 75th percentiles. The whiskers represent the range of values obtained. Columns with different symbols have median values that are statistically different (CM versus SAS, $P < .05$; all others, $P < .001$).

Table 2. Statistical description of cTnI concentrations (ng/mL) in 269 dogs using the AccuTnI assay.

	Sample Size	Range	Percentiles					
			5th%	25th%	Median	75th%	90th%	95th%
Healthy								
Non-Doberman Pinschers	95	0.01–0.15	0.01	0.02	0.03	0.05	0.08	0.09
Doberman Pinschers	81	0.01–0.23	0.01	0.02	0.03	0.06	0.10	0.14
All healthy	176	0.01–0.23	0.01	0.02	0.03	0.05	0.08	0.11
Cardiac Disease								
CM	26	0.03–1.88	0.04	0.12	0.14	0.34	1.08	1.57
MVD	37	0.01–9.53	0.02	0.04	0.11	0.19	0.95	7.44
SAS	30	0.01–0.94	0.01	0.03	0.08	0.12	0.23	0.41
All cardiac disease	93	0.01–9.53	0.02	0.04	0.11	0.21	0.84	1.57

CM, dilated cardiomyopathy; MVD, mitral valve disease; SAS, subaortic stenosis.

(range, 0.03–1.88 ng/mL; $P < .001$ versus both healthy Doberman Pinschers and healthy non-Doberman Pinschers). The median cTnI in dogs with MVD was 0.11 ng/mL (range, 0.01–9.53 ng/mL; $P < .001$ versus healthy non-Doberman Pinschers). The median cTnI in dogs with SAS was 0.08 ng/mL (range, 0.01–0.94 ng/mL; $P < .001$ versus healthy non-Doberman Pinschers). Among dogs with heart disease, those with CM had significantly higher median cTnI concentrations versus those with SAS ($P < .05$). Of the 93 dogs with heart disease, 44 (47%) had cTnI concentrations above the 95th percentile reference value of healthy dogs. In dogs with CM and MVD, there was no difference in median cTnI in dogs with and without CHF (CM with CHF, 0.32 ng/mL versus CM without CHF, 0.14 ng/mL; $P = .258$; MVD with CHF = 0.14 ng/mL versus MVD without CHF = 0.11 ng/mL; $P = .230$). Dogs with CM exhibited no difference in the median cTnI in those with and without arrhythmias (with, 0.14 ng/mL versus without, 0.14 ng/mL; $P = .642$).

Within the group of 176 healthy dogs, a modest but significant positive correlation was detected between cTnI and age ($\rho = 0.488$, $P < .0001$; Fig 2). There was no correlation between cTnI and any of the echocardiographic measurements in the healthy population ($P > .05$). In dogs with CM and MVD, cTnI had a modest but significant correlation with LA:Ao, LVDd, and LVDs (Table 3). In dogs with SAS, cTnI had a modest but significant correlation with the LA:Ao and the diastolic thickness of the left ventricular wall and interventricular septum (Table 3).

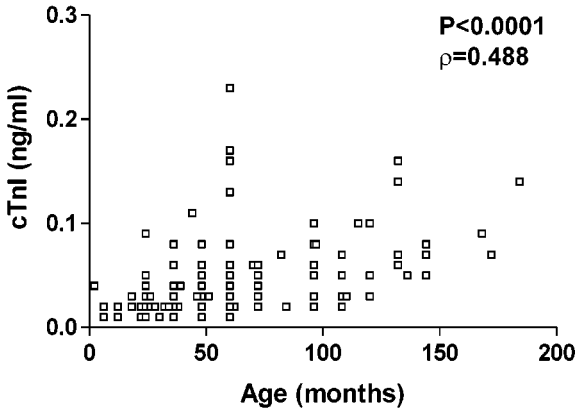

Fig 2. Correlation between plasma concentration of cardiac troponin-I (cTnI) and age in 176 healthy dogs (non-Doberman Pinscher and Doberman Pinscher groups). There was a significant positive correlation between patient age and cTnI concentration.

Survival of Dogs with CM

Within the population of 26 dogs with CM, 11 had cTnI >0.20 ng/mL and 15 had cTnI <0.20 ng/mL. Within the group with higher cTnI, 6 dogs died, 3 were euthanized, and 2 were lost to follow-up. Within the group with lower cTnI, 3 died, 4 were euthanized, 4 were lost to follow-up, and 4 were still alive at the end of the study period. Of the 7 euthanasias performed, 5 were due to worsening heart disease (eg, intractable heart failure, severe activity intol-

Table 3. Correlation between cardiac troponin-I concentrations, patient age, and echocardiographic data.

	ρ	*P*
CM		
Age	0.129	0.527
LVDd/Ao	0.695	0.0001
LVDs/Ao	0.664	0.0003
LA/Ao	0.634	0.0007
MVD		
Age	0.045	0.791
LVDd/Ao	0.569	0.0007
LVDs/Ao	0.436	0.012
LA/Ao	0.385	0.030
SAS		
Age	0.139	0.482
LVDd/Ao	0.363	0.068
LVDs/Ao	0.328	0.101
LA/Ao	0.588	0.006
IVSd/Ao	0.400	0.042
LVPWd/Ao	0.547	0.004
V_{LVOT}	0.165	0.412

LVDd/Ao = left ventricular end-diastolic to aortic root diameter ratio, LVDs = left ventricular end-systolic to aortic root diameter ratio, LA/Ao = left atrial to aortic root diameter ratio, IVSd/Ao = interventricular septum end-diastolic thickness to aortic root diameter ratio, LVPWd/Ao = left ventricular posterior wall end-diastolic thickness to aortic root diameter ratio, V_{LVOT} = maximum velocity of blood flow in the left ventricular outflow tract.

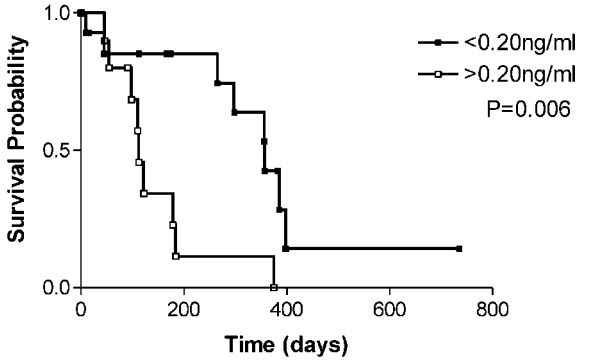

Fig 3. Kaplan-Meier survival curves in dogs with cardiomyopathy. Dogs with cardiac troponin-I levels >0.20 ng/mL (n = 15) had longer survival times than dogs with cardiac troponin-I levels <0.20 ng/mL (n = 11; $P = .006$; log-rank test).

erance, owner-perceived loss of quality of life) and 2 were due to gastric dilatation and volvulus (1 in each group). Log-rank analysis identified a significant difference in survival time between dogs with cTnI >0.20 ng/mL and those with cTnI <0.20 ng/mL (hazard ratio, 3.27; 95% CI, 1.59–16.2; $P = .006$) (Fig 3). The median survival time of dogs with cTnI >0.20 ng/mL was 112 days versus 357 days in dogs with cTnI <0.20 ng/mL. The difference in survival time between the 2 groups remained significant regardless of the censoring status of the euthanized animals (uncensored versus right-censored).

Discussion

Damage and disintegration of the cardiac sarcomere causes release of cTnI into the cytosol and escape into the interstitial space.[26] Cardiac lymphatics act to clear cTnI, but when overwhelmed by the amount of tissue damage, seepage into the general circulation occurs. In humans, cardiac troponin is used to diagnose and evaluate myocardial injury secondary to acute infarction and is an established biomarker of cardiac necrosis.[28] Cardiac necrosis is a prominent feature of both acute and chronic heart disease[46–49], and cardiac troponin can be high in patients with chronic disease of a primarily nonischemic origin (eg, idiopathic dilated cardiomyopathy).[38] In these patients, increases in cTnI typically are below the threshold for diagnosis of an acute ischemic event and are most consistent with the presence of a limited amount of cardiac injury and necrosis.[29,30,36,40–42,50,51] Because dogs with heart disease possess many of the same cellular abnormalities found in humans (including myocyte lysis, atrophy, vacuolization, and necrosis)[52–56], we hypothesized that the concentration of circulating cTnI in healthy dogs would be low, but that cardiac disease would elicit a release of excessive quantities of cTnI into the circulation, thus serving as a detectable biomarker of cardiac injury.

Cardiac troponin is tightly bound to the sarcomeric actin filament, and without cardiac damage, circulating cTnI concentrations in healthy subjects are low.[25,26] Our finding that healthy dogs have cTnI near or below the lower detection limit of the assay agrees with previous reports in human[57,58] and veterinary medicine.[13,14,19,20] Specifically, our median cTnI of 0.03 ng/mL in 176 healthy dogs is comparable to that of a previous study that reported a median cTnI of 0.02 ng/mL in 41 clinically healthy dogs.[13] Our results expand on this previous work by involving a larger number of dogs, confirming normal cardiac function by ECG and echocardiographic examination, and establishing a breed-specific range in healthy Doberman Pinschers. Together, these studies demonstrate agreement of results performed using 2 different cTnI immunoassay systems.[i]

An unexpected finding in our healthy population involved the relationship between age and plasma cTnI. The aged heart, even in the absence of demonstrable cardiovascular disease, loses up to 35% of its total myocyte number.[59] The reason for this loss is unknown but may be related to degenerative changes in the coronary vasculature, decrease in capillary density, and subsequent ischemic injury.[60,61] The presence of cTnI in the circulation therefore may represent myocyte death or turnover as a normal consequence of aging. Alternatively, increased cTnI may herald developing myocardial disease. This latter hypothesis may be particularly applicable within the population of ostensibly healthy Doberman Pinschers. Four dogs within this group possessed cTnI concentrations between 0.16 and 0.23 ng/mL, values that were well above the upper 95th percentile of our study (0.11 ng/mL). To further explore the possibility of undetected myocyte damage and the development of overt cardiomyopathy, serial cardiac examinations and cTnI measurements are being performed in these dogs.

Noncardiac parameters also could affect cTnI in clinically healthy subjects. The concentration of circulating cTnI is a balance between its release from the myocardium; leakage into the general circulation; degradation by serum proteases; and clearance by the kidney, liver, and reticuloendothelial system. In humans with chronic renal failure (serum creatinine >8.0 mg/dL), troponin concentrations can be high due to decreased renal clearance,[57] but this effect apparently is more significant for cTnT measurement than for cTnI.[62] We did not specifically perform renal function tests in our study population, but given the high prevalence of chronic renal disease in older dogs, it is possible that impaired clearance of cTnI influenced our findings.

Our study expands the findings of previous studies performed by DeFrancesco et al,[19] which revealed increased cTnT in 3 dogs with naturally occurring heart failure. Our results indicate that cTnI is increased in dogs with CM, MVD, and SAS, and represents the first report of cTnI in a large population of dogs with primary cardiac disease. Forty-four of 93 dogs (47%) with heart disease had increases above the 95% upper limit of the healthy controls. This finding compares favorably with reports by LaVecchia et al[42] and Perna et al[40,41] that estimate between 15 and 55% of human patients with chronic heart disease have mild increases of cardiac troponin. The median cTnI in our affected population was significantly higher than that in healthy dogs but still 5 times lower than the diagnostic cutoff for acute infarction in humans (0.5 ng/mL),[j] and 5 to 2,000 times lower than the concentrations found in dogs with experimentally induced acute myocardial infarction.[a] The cTnI concentrations found in our study are similar to those found in human subjects with chronic myocardial disease. Missov et al[30] reported a mean cTnI of 0.072 ng/mL

in patients with chronic heart failure versus 0.025 ng/mL in controls. Similarly, Horwich et al[38] reported a mean cTnI of 0.29 ng/mL in 50 patients with CM versus ≤0.04 ng/mL in controls. We performed cTnI tests using a highly sensitive second-generation immunoassay. The range of cTnI values in our study population suggests that earlier veterinary studies involving dogs with gastric dilatation and volvulus,[15] babesiosis,[16] and cardiac contusions[17] may have been hindered by the use of immunoassay systems with relatively low sensitivity. These studies employed immunoassays with lower limits ranging from 0.3 to 0.5 ng/mL. In our study, the majority of dogs with heart disease had concentrations <0.30 ng/mL. This finding suggests that use of cTnI assays with high sensitivity is needed to reduce the potential for false negative results.

Insofar as cTnI is an established marker for myocardial cell death, and lacking any extracardiac event capable of causing its release into the circulation,[25] the most likely explanation for our findings is the presence of a limited amount of cellular damage and cardiac myocyte necrosis. Myocyte death, whether through necrosis, apoptosis, or autophagy is a feature of heart failure in both humans and animals, and is believed to play a key role in the development and progression of cardiac dysfunction.[63–66] Previous studies in humans with chronic heart disease[30,36–38,42,51] indicate that mild increases of cardiac troponin likely indicate ''ongoing subclinical myocyte degeneration associated with deterioration of clinical status.''[36] Although we did not obtain histological confirmation in our population, the causal relationship between canine myocyte injury and high cardiac troponin is supported by previous work involving both experimentally induced and naturally occurring disease. Ricchiuti et al[18] and Remppis et al[67] reported that increases in circulating cTnI and cTnT correlated with the extent of myocardial infarction and necrosis in dogs undergoing surgical occlusion of the coronary circulation. Similarly, Lieb et al[a] reported high cTnI in a population of 7 dogs with experimental infarction. In that study, cTnI reflected severity of disease insofar as dogs with lesser degrees of infarction and necrosis demonstrated only mild increases in circulating troponin. Myocyte necrosis associated with high cTnI also has been reported in dogs with naturally occurring disease. Lobetti et al[16] examined 3 dogs with high cTnI that died from babesiosis infection and found evidence of coronary emboli in 1 dog and both coronary emboli and myocardial necrosis in the remaining 2. Schober et al[15] reported variable degrees of myocardial fibrosis, necrosis, hemorrhage, and edema in 4 dogs with high cardiac troponin that died as a result of gastric dilatation and volvulus. Collectively, these data strongly support the assertion that cardiac troponin is a marker of myocardial cellular injury in dogs with heart disease. Although the inciting causes of cellular damage and cTnI release have not been elucidated, putative mechanisms may involve oxidative stress, neurohormonal factors, calcium-handling abnormalities, inflammatory cytokines, and mechanical stress.[68–70] A limitation of our study is the absence of histological examination and confirmation of cellular damage. Further study involving postmortem histology or antemortem myocardial perfusion and viability techniques should be considered.

In dogs with congenital SAS and high cTnI, we speculate that cellular damage due to microvascular disease and subsequent ischemia plays a prominent role in the pathophysiology of cardiac injury. Dogs with severe disease suffer from intimal hyperplasia, smooth muscle hypertrophy, and arteriosclerosis of the coronary vasculature, the end result being diminished tissue perfusion, regional ischemia, and myocyte necrosis.[71–73] In the clinical setting, 2 specific findings (ECG ST segment change and ultrasonic hyperechogenicity of the myocardium) often are interpreted as indicators of ischemic damage.[74–76] It is interesting that in our study, 8 of 30 dogs with SAS (27%) had high cTnI but only 1 of these dogs demonstrated an ST segment change ≥0.2 mV in amplitude. If ischemia-induced cell necrosis was truly present in these 8 dogs, our results indicate that detection of the underlying pathology was better accomplished by cTnI than by ECG.

The ideal cardiac biomarker would be detected in proportion to the severity of underlying disease.[7] Traditionally, the severity of CM and MVD is assessed by a combination of radiographic, electrocardiographic, and echocardiographic techniques. In particular, the extent of left ventricular hypertrophy on echocardiography is commonly used as a surrogate measure of disease severity. In the current study, cTnI in dogs with CM and MVD demonstrated a significant correlation with both left atrial and left ventricular dimensions as measured by echocardiography. Furthermore, in both groups of dogs, cTnI correlated with a measure of cardiac systolic function, the left ventricular end-systolic dimension (LVDs). Our results are compatible with those of Perna et al[40] who reported increased diastolic and systolic left ventricular dimensions in human patients with mildly increased cTnT, those of Horwich et al[38] who reported lower cardiac index and ventricular ejection fraction in patients with high concentrations of cTnI, and those of La Vecchia et al[42] who observed a trend between cTnI and left ventricular ejection fraction in 10 human patients with chronic heart disease ($r = -.530$; $P = .11$). Our findings suggest that progressive hypertrophy and loss of contractile function are accompanied by increasing amounts of cellular damage and cTnI release.

In dogs with SAS, cTnI concentration had a modest positive correlation with the thickness of both the interventricular septum and left ventricular posterior wall but not to the maximum instantaneous pressure gradient across the obstructive lesion. A previous study showed the maximum pressure gradient to be a useful but imperfect predictor of disease outcome.[77] The predictive value of other echocardiographic parameters, such as wall thickness or outflow tract area ratios in dogs, has yet to be determined.[75,78] In both humans and animals with pressure overload, mortality is likely associated with the degree of left ventricular concentric hypertrophy, development of microvascular disease, and resultant ischemia.[71,74] If so, cTnI assay may have prognostic value and help direct potential medical or interventional therapy in individual dogs. Our results indicate that further study of the relationship among cTnI concentration, survival, histological changes, and other echocardiographic indices in dogs with SAS may be useful.

In dogs with CM, MVD, and SAS, the correlation between cardiac hypertrophy and cTnI was significant but moderate, and not all dogs with evidence of clinically rel-

evant heart enlargement had high cTnI. The lack of strong correlation between these 2 parameters is not completely unexpected given the differences in the pathophysiological events they represent. Ventricular hypertrophy primarily is a result of increased wall stress, slippage of myocardial fibers, and thickening or elongation of myocardiocytes,[79] whereas cardiac troponin release indicates degradation of sarcomeric proteins, loss of membrane integrity, and death of specific myocardial cells. Obviously, the 2 processes are interrelated such that increases in myocardial wall stress can induce myocyte necrosis, and loss of viable cells places further stress on the remaining tissue. Nonetheless, if cTnI assay has the capacity to accurately reflect the end consequence (ie, cell death) of the underlying disease process, then it provides information that is fundamentally different than the evaluation of global heart size by conventional radiographic and echocardiographic examination. The use of cTnI testing to quantify the extent of cellular damage, coupled with the traditional evaluation of heart size, arrhythmia, and global cardiac function may offer clinicians a more comprehensive method for evaluating cardiac disease. Our results suggest that cTnI can be used as a marker of cardiac disease in dogs, but the full clinical utility of cTnI requires additional study. Particularly important is the relationship of plasma cTnI to gross and histological necropsy findings, other circulating biomarkers (eg, atrial and B-type natriuretic peptides), response to medical therapy, and prognosis.

Worsening cardiac function is typified by increasing degrees of salt and water retention.[46] Although our results demonstrated a correlation between heart size and cTnI concentration, median cTnI in dogs with CM and MVD with overt CHF was not significantly different from results in dogs without CHF. Two previous veterinary studies indicated that patients with CHF have higher cTnI concentrations than those without CHF. DeFrancesco et al[19] reported mild increases of cTnT in 3 of 10 dogs with CHF versus 5 dogs with asymptomatic disease. In cats with HCM, those with CHF ($n = 6$) had higher cTnI concentrations than those without CHF ($n = 9$).[20] These studies involved only small numbers of patients, and further investigation is warranted. In human medicine, the relationship between troponin concentration and CHF is not clear. Sato et al[36] studied patients with CM and reported that increased cardiac troponin was independent of filling pressures, whereas Horwich et al[38] found a significant correlation between the two. It is interesting that Goto et al[37] found that medical treatment and successful clinical resolution of CHF did not necessarily result in a lowering of cTnI. This finding was particularly intriguing. It suggested that cTnI is reflective of an underlying and persistent pathologic process rather than a manifestation of the contemporary state of a patient's hemodynamics. If so, cTnI should be a useful prognostic indicator in patients with chronic heart disease independent of their clinical response to CHF treatment. This hypothesis is supported by the work of La Vecchia et al,[42] who found that cTnI was a significant independent predictor of 3-month mortality in 34 human patients with chronic heart disease. Moreover, cTnI was superior to echocardiographic heart size, cardiac rhythm, serum sodium concentration, and clinical response to medical treatment in predicting outcome. La Vecchia et al[42] found that patients who presented with cTnI >0.3 ng/mL had nearly a 7-fold greater risk for death than those with lower concentrations (hazard ratio, 6.86; 95% CI, 1.32–35.4; $P = .013$). Sato et al,[36] Setsuta et al,[39] and Perna et al[40,41] reported a similar predictive value of cTnT in patients with chronic heart disease.

In our population of 26 dogs with CM, the prognostic value of cTnI concentration was demonstrated. Dogs with cTnI >0.20 ng/mL had a 3-fold greater risk for death or euthanasia than dogs with lower concentrations (Fig 3). Our results extend those of Schober et al,[15] who found that cTnT and cTnI predicted survival in dogs with cardiac arrhythmia due to gastric dilatation and volvulus. To our knowledge, ours is the first report indicating that cTnI concentrations have a prognostic value in dogs with CM, but several cautionary statements are pertinent. Our study was not prospectively designed to evaluate survival, and the number of animals that actually died (as opposed to being euthanized) was relatively small in each group. Euthanasia can produce unique problems when attempting to analyze survival in veterinary studies and no corrective method is entirely satisfactory.[80] Ultimately, we chose to use a combined endpoint of either death or euthanasia when analyzing the survival curves. We note that the results of the survival analysis did not change significantly when we right-censored euthanized dogs and reanalyzed the curves. Also, our survival analysis did not attempt to account for the fact that dogs were receiving a wide variety of medical therapy at time of cTnI sampling. Despite these obstacles, the prognostic value of cTnI in our study population compares favorably with the aforementioned studies in humans, and the prognostic value of one-time or serial troponin assessment warrants continued investigation.

Footnotes

[a] Lieb C, Fernandes B, Fedewa M, et al. Correlation of canine troponin I levels with size of myocardial infarct (Abstract). Vet Pathol 2002; 39:614

[b] Oyama MA, Solter PF. Validation of a commercially available human immunoassay (AccuTnI, Beckman Coulter, Inc.) for the measurement of canine cardiac troponin I (Abstract). J Vet Intern Med 2003;17: 437

[c] O'Grady MR, Horne R. Outcome of 103 asymptomatic Doberman pinschers: incidence of dilated cardiomyopathy in a longitudinal study (Abstract). J Vet Intern Med 1995;9:199

[d] GE Medical Systems, Waukesha, WI

[e] Hewlett Packard, Andover, MA

[f] Monoject #8881–320751, Sherwood Medical, St. Louis, MO

[g] Beckman Coulter, Inc., Fullerton, CA

[h] Prism 3.0 for Windows, GraphPad Software, San Diego CA

[i] The report by Sleeper et al used the Stratus CS fluorometric analyzer (Dade Behring, Newark, NJ) with a lower limit of detection of human cTnI of 0.03 ng/mL

[j] Access Immunoassay System Assay Manual 1.1, 2003

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