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Serum cardiac troponin I in canine syncope and seizures

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Abstract *Objective:* To determine if serum cardiac troponin I (cTnI) concentration distinguishes between cardiogenic syncope and collapsing dogs presenting with either generalized epileptic seizures (both with and without cardiac disease) or vasovagal syncope.
Animals: Seventy-nine prospectively recruited dogs, grouped according to aetiology of collapse: generalized epileptic seizures (group E), cardiogenic syncope (group C), dogs with both epileptic seizures and cardiac disease (group B), vasovagal syncope (group V) or unclassified (group U).
Methods: Most patients had ECG (n = 78), echocardiography (n = 78) and BP measurement (n = 74) performed. Dogs with a history of intoxications, trauma, evidence of metabolic disorders or renal insufficiency (based on serum creatinine concentrations >150 μmol/L and urine specific gravity <1.030) were excluded. Serum cTnI concentrations were measured and compared between groups using non-parametric statistical methods. Multivariable regression analysis investigated factors associated with cTnI. Receiver operator characteristic curve analysis examined whether cTnI could identify cardiogenic syncope.

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Results: Median cTnI concentrations were higher in group C than E (cTnI: 0.165 [0.02—27.41] vs. 0.03 [0.01—1.92] ng/mL; $p<0.05$). Regression analysis found that serum cTnI concentrations decreased with increasing time from collapse ($p=0.015$) and increased with increasing creatinine concentration ($p=0.028$). Serum cTnI diagnosed cardiogenic syncope with a sensitivity of 75% and specificity of 80%.
Conclusions: Serum cTnI concentrations were significantly different between groups C and E. However, due to the overlap in cTnI concentrations between groups cTnI, measurement in an individual is not optimally discriminatory to differentiate cardiogenic syncope from collapse with generalized epileptic seizures (both with and without cardiac disease) or vasovagal syncope.

Abbreviations

ANOVA	analysis of variance
BP	blood pressure
CSF	cerebrospinal fluid
cTnI	cardiac troponin I
GA	general anaesthesia
Group B	group with both cardiac disease and seizures
Group C	group with cardiogenic syncope
Group E	group with epilepsy
Group U	group in which no specific diagnosis was reached (unclassified)
Group V	group with vasovagal syncope
MRI	magnetic resonance imaging
ROC	receiver operator characteristic
SE	status epilepticus
SUDEP	sudden unexpected death in epilepsy
TLOC	transient loss of consciousness
USG	urine specific gravity

Introduction

The two major mechanisms of collapse with transient loss of consciousness (TLOC) are global cerebral hypoperfusion leading to syncope, and asynchronous discharge of cerebral neurons causing a seizure. Syncope and seizures can mimic one another in veterinary patients [1—3]. In human patients, this can also occur, with a consequent high rate of misdiagnosis, particularly as clinical examination on affected patients is often unremarkable at presentation [4—11]. Examples of disorders which may lead to seizure activity and can be confused with syncope include idiopathic epilepsy, intracranial disease, encephalopathies and metabolic disorders such as hypocalcaemia, hypoxia or hypoglycaemia. In human patients, studies suggest that one in four patients with 'epilepsy' may be misdiagnosed [4—8].

In veterinary medicine, published case reports illustrate the challenges of differentiating syncope from seizures [1,3]. Structural cardiac disease and arrhythmias (with or without underlying structural cardiac disease) can cause syncope [2,12]. Methods for differentiating benign causes of weakness and fainting from malignant cardiac arrhythmias, which may degenerate into ventricular fibrillation or cardiac arrest, are clinically important but currently relatively limited. Clinicians are often reliant on an incomplete medical history.

One cardiac biomarker, cardiac troponin I (cTnI), is useful for detecting cardiac myocyte damage [13] and is easy to sample. Increased circulating troponin concentrations have been associated with both cardiac [14,15] and non-cardiac conditions [16—22]. Studies in humans show that epileptic patients with generalized tonic—clonic seizures do not have raised circulating cTnI concentrations [23—26]. However, increased troponin concentrations may occur with more severe seizures, such as status epilepticus (SE), possibly due to seizure-associated catecholamine release causing tachyarrhythmias, coronary ischaemia and thereby myocardial injury [27]. There is little published information on the association of serum cTnI concentration with naturally occurring seizures in dogs, other than single case reports [3,28] and an oral presentation.[e] It is important to gain information regarding cTnI measurements following syncope or seizures to enable correct interpretation of data.

There are no data available regarding the clinical utility of serum cTnI for differentiating cardiac causes of syncope from seizures in dogs. Circulating

[e] Kim J, Song R, Lee D, Lee H, Park J & Park C. Changes of serum cardiac troponin I concentration in 13 dogs with intracranial disorders. J Vet Intern Med 2012; American College of Veterinary Internal Medicine Research abstract C-29.

cTnI concentrations are frequently increased in dogs with underlying heart disease and arrhythmias [14,15,29—32] and therefore would be expected to be higher in dogs with cardiogenic syncope. There may be a role for cTnI in the evaluation and diagnosis of dogs collapsing with TLOC.

The aim of the study was to examine the clinical utility of serum cTnI for differentiation of cardiogenic syncope from collapsing dogs with either epileptic seizures (with and without cardiac disease) or vasovagal syncope. It was hypothesized that dogs with cardiac syncope would have higher troponin concentrations than collapsing dogs with seizures or other non-cardiac causes.

Animals, materials and methods

The study protocol was approved by the University of Cambridge and University of Liverpool ethical review committees. Owners provided informed written consent before participation of their dogs in the study.

Dogs with episodes of collapse with TLOC were prospectively and consecutively recruited at three referral centres between February 2011 and May 2013. The major inclusion criterion was the history of a collapsing episode, involving TLOC, within seven days of presentation. Dogs were eligible for inclusion whether or not they had received prior medication. Full history was taken and clinical examination performed, in particular evaluating for the presence of a murmur or arrhythmia. Dogs undergoing investigations for suspected seizures had full neurological examination recorded. The following were also recorded: breed, age (years), gender (male/female), neutered status (yes/no), body weight (kilograms), time since collapse (the length of time, in hours, between the collapse and the time at which blood samples were taken on presentation), any medications being currently administered (yes/no). For patients with seizures, the following were also recorded: number of seizures during the seven days before presentation (low = 1—2 seizures; medium = 3—5 seizures; high = 6 or more seizures) and seizure length in minutes (if more than one seizure had occurred, then average seizure length was recorded).

Complete blood count, serum biochemistry and electrolytes were measured to screen for concurrent and causative disease in all the patients. The laboratories were blinded to patient history. Fasting blood samples for measurement of creatinine concentration (μmol/L) were collected and analysed within 24 h. Blood samples for the troponin assay were collected into 1-mL plain tubes from all dogs. Plain tube samples were separated by centrifugation immediately after clotting. The serum was stored for up to 12 h at −18 °C then transported on ice to a commercial laboratory (IDEXX Laboratories,[f] Wetherby) for analysis. Before analysis, the frozen serum was allowed to thaw slowly at room temperature. Concentrations of cTnI were measured using a two-site sandwich immunoassay[g] to detect free and complexed troponin. The use of this assay has been reported [33—35] and validated [36] previously for canine samples. The laboratory reference range is ≤ 0.07 ng/mL. The assay's lower limit of detection is 0.01 ng/mL. Dogs were assessed for cardiac disease on the basis of clinical examination, BP, electrocardiography (ECG) and echocardiography. For patients with seizures, cardiac investigations were always performed before general anaesthesia (GA), except when difficult to control patients arrived in SE. Status epilepticus patients had cardiac investigations and repeat thoracic auscultation delayed until 24 h following recovery from anaesthesia.

Dogs with suspected seizures underwent neuroimaging. Magnetic resonance imaging (MRI) scans[h] with gadolinium contrast were obtained in three planes of orientation (dorsal, sagittal and transverse) under GA. Pre-contrast and post-contrast T1-weighted and T2-weighted images were acquired. In some patients, additional sequences (pre-contrast and post-contrast FLAIR, gradient echo T2* and STIR) were carried out to better define the underlying brain pathology. In dogs without suspected raised intracranial pressure, cisternal cerebrospinal fluid (CSF) analyses were performed for diagnosis. CSF samples were chilled at 4 °C up to 12 h before transportation at ambient temperature to a commercial laboratory (Carmichael Torrence Diagnostic Services Ltd, W. Yorks.) for analysis. Additional tests, such as Toxoplasma and Neospora serology, were performed according to the judgement of the attending clinician.

If an arrhythmia was identified or suspected as a cause of collapse, patients underwent 24 h ambulatory ECG recording (Holter monitoring).[i] Holter diaries were kept by owners or veterinary staff

[f] Accredited for analytical testing BS ISO/IEC 17025:2005.

[g] Access Systems AccuTnI® Assay, Beckman Coulter Inc, Fullerton, CA. The AccuTnI® assay uses two mouse-derived monoclonal antibodies directed against the 24—40 and 41—49 amino acid sequences of cTnI.

[h] Vet-MR Grande; Esaote, Florence, Italy.

[i] The system used for the 24 h Holter recordings was the Lifecard CF digital Holter recorder (Spacelabs Healthcare, Issaquah, WA). The Holter analysis was performed using Spacelabs Pathfinder software.

detailing timings of activities such as exercise, sleep or collapse. Suspected (neurally mediated) vasovagal patients were definitively diagnosed by characteristic ECG changes obtained at the time of collapse, i.e. sinus tachycardia immediately followed by bradycardia and sinus pauses, consistent with the cardio-inhibitory form of neurocardiogenic syncope [12,37]. Vasovagal patients had no echocardiographic evidence of structural cardiac disease which could cause the collapse episodes. In patients for whom congestive heart failure or respiratory pathology was suspected, thoracic radiographs (lateral and dorsoventral views) were obtained. Abdominal ultrasound and blood tests to exclude infectious causes of myocarditis[j] were offered in dogs with ventricular tachycardia, raised serum troponin concentrations and no detectable structural cardiac disease. Systolic BP (mmHg) using a Doppler device[k] was measured from the metacarpal artery according to protocol [38].

Electrocardiography was performed by the use of a routine six-lead ECG machine.[l] The six limb leads were recorded simultaneously for a minimum of 20 consecutive RR intervals, at a paper speed of 50 mm/s, gain of 10 mm/mV. Patients underwent full standard echocardiographic examination without sedation, according to published recommendations [39] using various phased array probes (1.5—11 MHz), as appropriate for the patient, with harmonic imaging.[m] An ECG was recorded simultaneously and full M-mode, colour and spectral Doppler studies were recorded and analysed to reach an echocardiographic diagnosis in each case. For each variable, the mean of three measurements was calculated from consecutive cardiac cycles.

Definitive diagnosis of the cause of seizures was made by evaluation of all contributing evidence. Dogs were diagnosed with idiopathic epilepsy if they were younger than six years old at seizure onset, had recurrent seizures and were normal on interictal neurological and laboratory examination. They had to have no evidence of neurological disease, other than seizures, during their lives. This was further supported by normal results of CSF analyses and advanced imaging (MRI) with gadolinium contrast. Brain tumour was diagnosed with advanced imaging (MRI) and meningoencephalitis diagnosis based on MRI and CSF results. Cluster seizures were defined as two or more seizures occurring within a 24 h period in which the patient regained consciousness between seizures [40].

Dogs were grouped according to aetiology of collapse (Fig. 1): generalized epileptic seizures with no evidence of cardiac disease (group E), cardiogenic syncope with no evidence of neurological disease (group C), dogs with both generalized seizures and cardiac disease (group B), vasovagal syncope (group V) or unclassified (group U), for which the cause of collapse could not be reliably ascribed to either cardiac or neurological disease, or for which a diagnosis was not reached. Group E had to be free of cardiac disease based on examination, BP, ECG and echocardiography.

Dogs collapsing due to trauma or metabolic disorders were excluded. Patients with a history of intoxications or renal insufficiency were excluded (based on serum creatinine concentrations exceeding the upper limit of the laboratory reference range [>150 μmol/L] and USG measurement [<1.030]). A flow chart giving numbers of dogs undergoing specific investigations is shown (Fig. 2).

Statistical analysis

Results were analysed using commercially available software.[n] Data were assessed graphically for normality and by the Kolmogorov Smirnov test. Most data were not normally distributed, so descriptive data were given as median (range). Age distribution, body weights, concentrations of creatinine and cTnI of the groups were compared. For comparison between groups, the one-way ANOVA test was applied if data were normally distributed, with multiple pairwise testing by the Holm—Sidak test. For non-normally distributed data, the Kruskal—Wallis ANOVA on ranks was applied, with multiple pairwise testing by the Dunn's method. To compare categorical data, the chi-squared test was used to assess the difference of proportions of sex and neutered status between the various groups.

The cTnI data (n = 66 observations) were log transformed for the regression analysis to achieve a normal distribution. To determine which variables were significantly associated with the measured

[j] *Anaplasma phagocytophilum* antibody, *Bartonella henselae* antibody, *Borrelia burgdorferi* antibody, *Ehrlichia canis* antibody, *Toxoplasma gondii* IgM and IgG, *Neospora caninum* serology, Distemper IgG titre. Carmichael Torrence Diagnostic Services Ltd, W Yorks, UK.

[k] CAT Doppler BP Kit, Thames Medical, West Sussex, UK or Parks Model 811-B Doppler device, Aloha, Oregon.

[l] Esaote P80 Power, Esaote, Florence, Italy or Seca CT8000P ECG, Imotek International Ltd, Huntingdon, Cambridgeshire, UK.

[m] Esaote Piemedical MyLab 40 Vet, Esaote, Florence, Italy or Vivid S6 echocardiography machines GE Medical Systems, Wauwatosa, WI.

[n] SigmaPlot® 12 software (Systat Software Inc, London, UK), Minitab (version 16.2.3), Minitab Ltd., Coventry, UK and STATA 14 (StataCorp LP, College Station, Texas, USA).

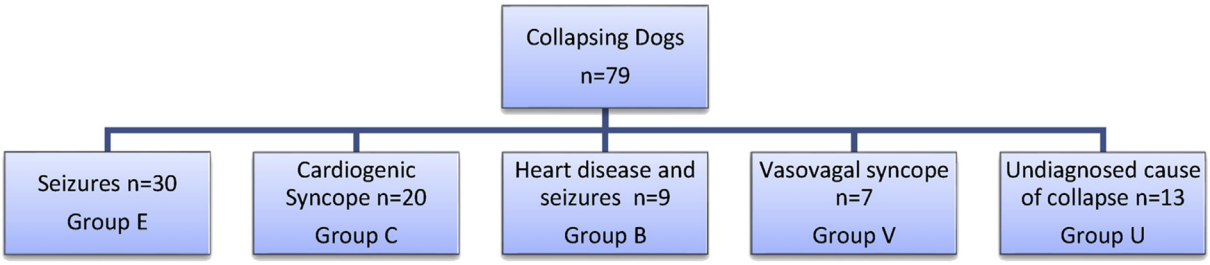

Figure 1 Flow chart depicting group categorization of dogs included in the study.

troponin concentrations, univariable regression was performed. A multivariable backwards stepwise linear regression analysis was then performed to further explain the variation in troponin values, with the model being initially populated with all available variables. The stepwise procedure was as follows: i) if removal of one or more variables was associated with a p value of >0.2, then the variable with the largest associated p value was discarded; ii) if inclusion of any of the discarded variables was

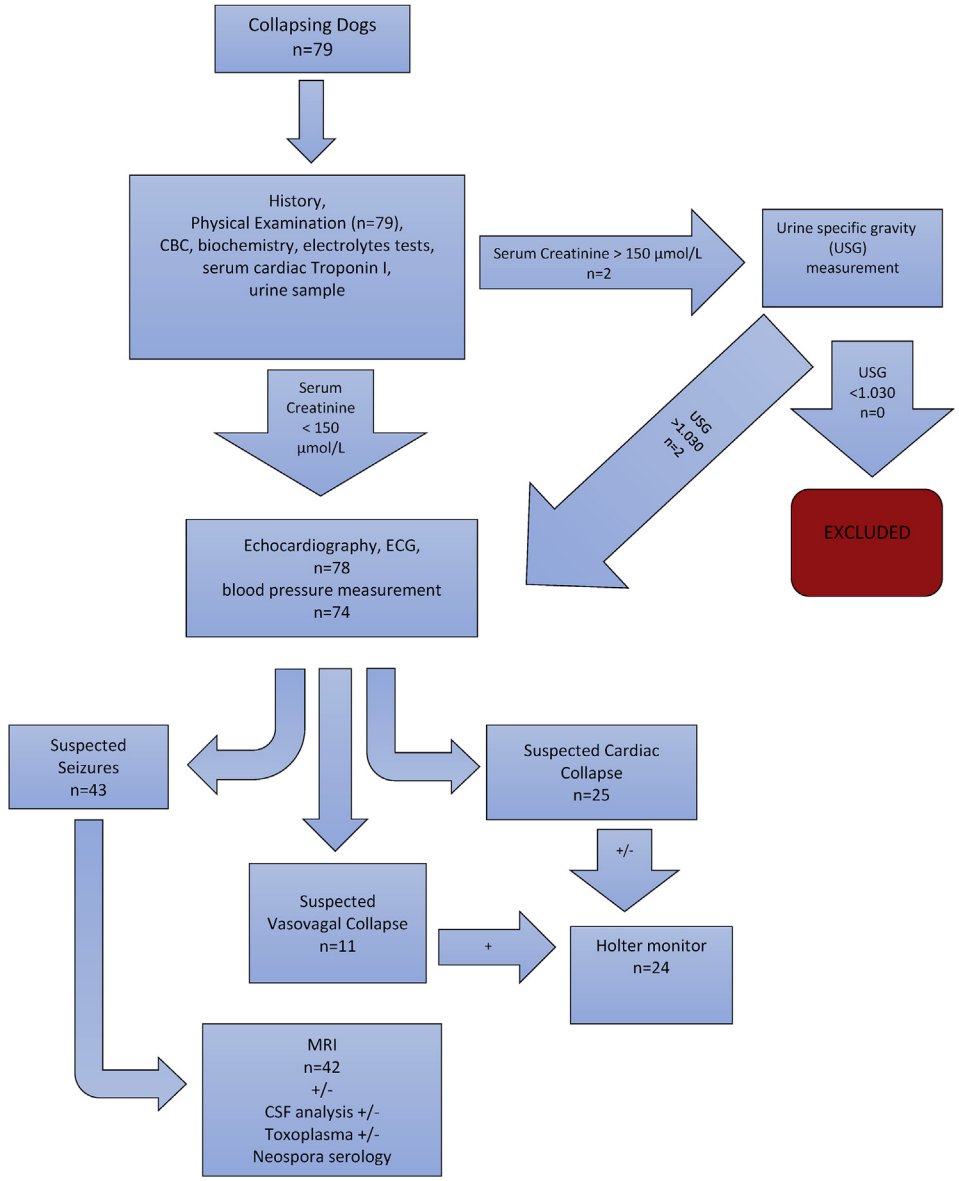

Figure 2 Flow chart depicting investigations performed according to suspected diagnosis.

associated with a p value of <0.05, then the variable with the smallest associated p value was added back into the model; iii) steps i) and ii) were repeated until no further variables could be added or discarded. Variables examined were serum creatinine concentration (μmol/L), disease group, age (years), gender (male or female), neutered status (entered as binary variables yes or no), body weight (kg), time since collapse to blood sampling (hours), medication (being administered at, or shortly before, presentation), systolic BP (mmHg). The chosen regression model was re-run after removing any variables with non-significant coefficients ($p>0.05$) as judged by their Wald statistics, and the effect of removing these variables was assessed. For the purpose of the analysis, samples in which cTnI concentrations were below limit of detection of the assay were ascribed a value of 0.01 ng/mL. For all statistical comparisons, a P value of <0.05 was accepted as statistically significant.

Receiver operator characteristic (ROC) curve analysis was used to determine the ability of cTnI concentration to discriminate between cardiac and non-cardiac causes of collapse. Area under curve and 95% confidence intervals of the ROC analysis for cTnI were calculated. Visual examination of the ROC curves was used to select cut-off values considered to provide an optimal combination of sensitivity and specificity. The positive and negative predictive values were calculated, assuming the prevalence of cardiogenic syncope was 30%, i.e. the same as we found in this study.

Results

Seventy-nine dogs were enrolled in the study and grouped according to aetiology of collapse with TLOC (Fig. 1). Breed and signalment are shown in Table 1. The boxer was the most represented of the pedigree breeds ($n = 10$). There were significant differences in age and time since collapse between the groups (Table 1).

The majority of dogs had ECG ($n = 78$), echocardiographic examination ($n = 78$) and BP ($n = 74$) performed. One patient (3.8 years of age) with generalized epileptic seizures (group E) had no BP, ECG or echocardiographic examination performed, but had no murmur or arrhythmia on cardiac auscultation. The troponin concentration for this patient was within the laboratory reference range. There were five BP readings not recorded. Group E comprised 27 dogs with idiopathic epilepsy, two with brain tumours (suspected meningioma and suspected glioma) and one with necrotizing meningoencephalitis. Five of the 30 group E dogs had cluster seizures. One dog in group E was suspected to have exercise-induced epilepsy. This case has been described previously [3]. Three epileptic patients had SE during the 24 h before presentation, only one requiring GA for seizure control. In group E, all but one dog were unremarkable on ECG and echocardiographic examination. The abnormality detected (mild left ventricular dilation) was attributed to bradycardia. Four of the five patients with cluster seizures were ranked as having a high number of seizures. All three SE patients were ranked as high.

Group C (cardiogenic syncope) comprised 14 dogs with arrhythmias (six without and eight with underlying structural heart disease detected on echocardiography), three dogs with neoplastic pericardial effusion and cardiac tamponade, one with cardiac neoplasia causing secondary right ventricular outflow tract obstruction, one with myxomatous degeneration of the mitral valve, left-sided congestive heart failure and systolic pulmonary hypertension and one dog with dilated cardiomyopathy. In group B (dogs with both epileptic seizures and cardiac disease), all nine were believed to have collapsed as a result of epilepsy and not cardiac disease. In group V (vasovagal syncope), only two of the seven dogs were definitively diagnosed based on history and Holter analysis. The remaining five had suspected vasovagal syncope (based on typical history, breed or Holter analysis). There were 13 dogs for whom no diagnosis was reached (group U).

The length of time between the collapse and blood sampling was recorded in each case apart from one group U dog (Table 1). Nine group C patients were receiving medication at, or shortly prior to, presentation (Table 1). These included furosemide, benazepril, pimobendan, lidocaine, sotalol and digoxin. In group E, 18 patients were taking anticonvulsants at presentation. These included phenobarbitone, levetiracetam, potassium bromide, gabapentin and diazepam. Other medications included oral antibiotics ($n = 1$) and injectable dexamethasone ($n = 1$). Medications administered to dogs in other groups included glucosamine, propentofylline, corticosteroids and non-steroidal anti-inflammatory drugs. There was no significant difference in BP between the five groups ($p=0.197$) (Table 1). Thirteen group U patients were not definitively diagnosed and therefore were excluded from further statistical analysis. There was no significant difference in serum creatinine concentration between the four groups E, C, B and V (Table 2). Two epileptic dogs had raised serum creatinine concentrations (>150 μmol/L); however, free catch urine samples obtained at blood sampling

Table 1 Signalment and collapse group assignment.

	Total n = 79	E (epilepsy) n = 30	C (cardiogenic syncope) n = 20	B (both heart disease and epilepsy) n = 9	U (unclassified) n = 13	V (vasovagal) n = 7	p-Value
Age (years)							
Median & range	5 0.3—15	3.9[a,b] 0.3—10.5	7.7[a] 0.8—15	4.5 0.8—12.3	8.8[b,c] 2—15	2[c] 0.7—9.5	p<0.001
Sex							
Male	n = 42	n = 16	n = 11	n = 4	n = 7	n = 4	p=0.986
Female	n = 37	n = 14	n = 9	n = 5	n = 6	n = 3	
Reproductive status							
Neutered	n = 53	n = 19	n = 15	n = 6	n = 11	n = 2	p=0.122
Entire	n = 26	n = 11	n = 5	n = 3	n = 2	n = 5	
Weight (kg)							
Median & range	16.4 0.7—78.5	15.1 5.5—49.2	25 8.2—78.5	12.1 6.3—40	15.7 3.2—36	24.7 0.7—31.8	p=0.258
Most commonly represented breeds							p=0.011
Boxer	n = 10	1	2	2	2	3	
Cavalier King Charles spaniel	n = 9	2	1	4	2	—	
Labrador retriever	n = 7	6	1	—	—	—	
West Highland white terrier	n = 5	—	1	2	2	—	
Border collie	n = 4	2	2	—	—	—	
Cross breed	n = 4	1	2	—	1	—	
Time since collapse (hours)							
Median & range	48 0—168 n = 78	42 0—168	26 2—168	48 8—144	72 4—168 n = 12	120 48—168	p=0.022
% Receiving medications at, or shortly before, presentation	47% 37/79 n = 79	60% 18/30	45% 9/20	56% 5/9	31% 4/13	14% 1/7	p=0.163
Systolic BP (mmHg)	140	144	135	136	153	133	p=0.197
Mean & range	80—180 n = 74	100—178 n = 29	80—179 n = 19	120—162 n = 9	125—180 n = 10	106—168 n = 7	

Time from collapse to presentation, medications prescribed and systolic blood pressure on presentation, according to group.
Pairs sharing the same superscript (age) are significantly different from each other.

Table 2 Serum cardiac troponin I (cTnI), and serum creatinine concentrations according to group, p value given.

	Total n = 66	E n = 30	C n = 20	B n = 9	V n = 7	p-Value	# Animals above rr
Creatinine (μmol/L)							
Mean & range	94 48–153	90 48–153	102 65–142	85 62–102	101 74–108	p=0.132	n = 2
cTnI (ng/mL)							
Median & range	0.05 0.01–27.41	0.03[a,b] 0.01–1.92	0.165[a,b] 0.02–27.41	0.04 0.02–0.21	0.05[b] 0.01–0.08	p=<0.001	n = 24

E, epilepsy group; C, cardiogenic syncope group; B, both cardiac disease and epilepsy; V, vasovagal syncope group; rr, laboratory reference range.
Pairs sharing the same superscript are significantly different from each other.

were concentrated (USG >1.030). As both patients had repeat blood tests at separate dates with creatinine concentrations <150 μmol/L, the changes were attributed to dehydration. Median cTnI values were significantly different between the four groups (Table 2). Overlap between groups was, however, present. The epileptic patient with the highest serum troponin concentration arrived in SE and required GA for seizure control.

Univariable and multivariable linear regression analysis were performed using the logarithmically transformed data for cTnI (n = 66). Univariable results showed a strong association of log cTnI concentration with disease group (p<0.001), age (p=0.001) and time from collapse (p=0.039). There was no significant association with body weight (p=0.058), creatinine concentration (p=0.065), blood pressure (p=0.19), neutered status (p=0.24), sex (p=0.69) or whether on medication (p=0.49). The backwards stepwise regression retained creatinine concentration (p=0.028), disease group (p<0.001), time from collapse (p=0.015) and medication (p=0.148; Table 3). The regression coefficients indicated that cTnI increased with increasing creatinine and with medication, whilst it decreased with increasing time from collapse (p=0.015). Removal of medication from the final model had little effect on the coefficients for the remaining two variables, but the significance for group remained at p<0.001, whereas that for creatinine changed to p=0.048. ROC analysis was used to estimate the optimum cut-off value of serum cTnI concentrations to discriminate between cardiac and non-cardiac causes of collapse with TLOC. This provided a test with a sensitivity of 75% and specificity of 80%; further characteristics are shown in Table 4.

Table 3 Results of the multivariable regression analysis.

Log cTnI	Coefficient	p Value	(95% Confidence interval)
Creatinine (μmol/L)	0.02	0.028	(0.002 to 0.034)
Group[a]		<0.001	
B	−1.46	0.007	(−2.51 to −0.41)
E	−1.97	<0.001	(−2.73 to −1.22)
V	−1.50	0.014	(−2.68 to −0.31)
On medication	0.48	0.148	(−0.18 to 1.13)
Time from collapse	−0.01	0.015	(−0.15 to −0.002)
Constant	−3.28	0.002	(−5.33 to −1.24)

[a] Baseline level is C; Group E, epilepsy group; B, both cardiac disease and epilepsy; V, vasovagal syncope group.

Table 4 Sensitivity and specificity of classification for the serum cTnI cut-off values, including receiver operator characteristic (ROC) curve area.

Groups	cTnI cut-off value	Correctly classified	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value (95% CI)	Negative predictive value (95% CI)	ROC Area (95% CI)
C vs. all other except U	>0.08 ng/mL	78.8%	75 (51–91)	80 (66–91)	63%[a] (41–82)	88%[a] (74–96)	87% (76–94)

C, cardiogenic syncope; U, unclassified; all other groups include cTnI, cardiac troponin I; CI, confidence interval.
[a] Prevalence = 30.3% (calculated from this study).

Discussion

This study confirms that, as hypothesized, dogs with cardiac syncope have higher cTnI concentrations than collapsing dogs with seizures and other non-cardiac causes of collapse with TLOC. To the authors' knowledge, this is the first time this has been shown in collapsing dogs. However, there was overlap between the cardiac syncope and seizure groups, so this biomarker is not discriminatory. The results demonstrate that serum cTnI concentrations are more markedly elevated in cardiogenic syncope compared with non-cardiac causes of collapse such as epilepsy. It would be expected that there is greater myocardial injury in dogs with cardiac disorders compared with dogs with seizures. The majority of cardiogenic syncope patients had arrhythmias, with a large proportion having underlying structural heart disease. Possible mechanisms for troponin elevation in these patients are numerous and include increased wall stress causing subendocardial injury, effects of neurohormones on myocytes, or tachyarrhythmias causing reduced coronary perfusion. It is notable that the two dogs with the highest troponin concentrations in group C had life-threatening ventricular tachycardia. Neither had clinically significant structural heart disease. Tests for infectious causes of myocarditis were negative. Unfortunately, both pets' owners declined abdominal ultrasound to help exclude systemic disease as a cause of ventricular tachycardia, although one patient was only 10 months old.

Of the group E dogs, those with the highest serum cTnI concentrations had cluster seizures. The patient with the highest serum troponin concentration arrived in SE, required GA for seizure control and showed reversible ECG and echocardiographic changes. Following treatment and, whilst receiving anti-convulsants, a sinus bradycardia (60 beats/min) was recorded on ECG. Echocardiography showed mild left ventricular dilation, trivial mitral and tricuspid regurgitation, but no structural valve abnormalities. The echocardiographic changes seen were attributed to the bradycardia. Repeat echocardiography performed three weeks later in normal sinus rhythm (120 beats/min) was unremarkable, indicating that the changes were reversible. It is unclear whether the changes detected were a result of anti-epileptic medications or a direct result of the cluster seizures.

The presence of myocardial injury with seizure activity could be expected given the apnoea, tachycardia, increased myocardial oxygen consumption and excess catecholamine release associated with seizures [41]. It has been hypothesized that seizures may initiate an autonomic storm which may have both parasympathetic and sympathetic effects [42]. This may subsequently trigger cardiac dysrhythmias and, in humans, sudden unexpected death in epilepsy (SUDEP) [43]. Recent ECG studies show that 20% of human patients experience abnormally slow heart rates during seizures, culminating in 16% with asystole [43]. This may be due to brainstem autonomic dysfunction of parasympathetic outflow leading to sinus and AV nodal block. Another hypothesis is that some epileptic patients have brain and cardiac ion channelopathies [43]. In human patients, bradyarrhythmias are mostly associated with temporal lobe seizures [44]. Cortical stimulation of the left insular cortex and amygdalae can also elicit bradycardias [45].

There are a few publications reporting increased circulating cTnI concentrations following GA in healthy dogs, with older dogs seemingly being more affected [46,47]. Overall, 14—55% of the dogs were reported as having increased cTnI concentrations post—GA, with variations in sampling ranging from 6 to 24 h after discontinuation of GA [46,47]. Some dogs were reported as having cTnI concentrations which decreased following GA [46,47], whereas one study reported no significant difference following GA [48]. In this study, only one SE patient required GA for seizure control in which cTnI sampling was delayed until 24 h following GA. This same patient had the highest serum cTnI concentration in group E. It is possible that the GA affected serum cTnI concentrations, resulting in elevated concentrations. However, two other group E patients with cluster seizures also had elevated cTnI concentrations (according to the laboratory reference range) despite not requiring GA for seizure control. Elevated cTnI concentrations may be due to cardiac necrosis lesions, caused by catecholamines released directly into the heart of the dogs with cluster seizures via neural connections. Catecholamines released directly into the myocardium are more toxic than those reaching the heart via bloodstream [42]. Further studies involving larger numbers of patients with cluster seizures, pre- and post—GA, would be required to further investigate this association with serum cTnI concentrations.

In group V, the five suspected vasovagal patients did not collapse wearing the Holter monitor but had a consistent history or were typical breeds (brachycephalic). This highlights the challenge faced obtaining a definitive diagnosis in patients

with intermittent collapse. Studies demonstrate the low diagnostic yield of Holter analysis; in one study only 24% of the patients collapsed during the Holter recording [12]. The recording was helpful in establishing a diagnosis 42% of the time [12]. The five patients with suspected vasovagal syncope were included in group V, despite not being definitively diagnosed as there was no clear evidence of any cardiac, metabolic or neurological disease which could have contributed to the collapse. They were mainly typical breeds (one boxer, one pug, one English bull terrier), had evidence on a Holter recording of a rapid transition from sinus rhythm to sinus bradycardia ($n = 1$) during a stumbling episode (but did not collapse), or had the typical history, i.e. brief collapse following intense excitement with rapid recovery ($n = 5$). These cases were not excluded from the analysis due to the strong suspicion by the attending clinician that they were indeed collapsing due to vasovagal syncope. In group B, all the dogs collapsed due to seizures and many had incidental mitral valve disease. Having an unclassified group (group U) was expected given the intermittent nature of collapse. It has been shown previously that definitively diagnosing collapse in dogs remains challenging. However, most dogs with collapse of an unknown cause carry a good prognosis, with the exception of boxers, possibly due to the presence of concurrent disease (e.g. arrhythmogenic right ventricular cardiomyopathy or neoplastic disease) [2,49,50].

Disease group and time from collapse to blood sampling were independently associated with cTnI concentrations. Time from collapse to blood sampling was negatively associated with cTnI concentration in all groups, reflecting the release kinetics and elimination of troponin. It illustrates the importance of inclusion criteria and highlights that the results of this study are only valid if clinicians collect cTnI serum within seven days following collapse. In this study, the time between the collapse episode and blood sampling varied. Considering the release kinetics of troponin, it is possible that peak circulating troponin concentrations were missed, particularly in patients presenting days after collapsing. Serial troponin measurements, commencing immediately after collapsing would have been preferable and might have shown improved diagnostic accuracy.

Serum creatinine was positively and independently associated with cTnI concentration. This was a particularly interesting finding as patients with renal insufficiency (creatinine concentrations >150 μmol/L and USG <1.030) were excluded. To the authors' knowledge, this relationship between cTnI and creatinine concentration has not been previously reported in patients without evidence of renal insufficiency. If further studies confirm this as a true association, its cause is unclear. It may be due to troponin possibly being eliminated by the renal system, or perhaps due to subclinical myocardial damage rather than altered excretion. In dogs with renal dysfunction, it has previously been shown that circulating cTnI concentrations are increased [17,18]. Subclinical myocardial damage may include direct myocardial injury caused by uremic toxins [51].

Inspection of the ROC curve indicates assay sensitivity and specificity at any given cut-off value and the ROC analysis for distinguishing between the cardiac and non-cardiac groups gave an area under curve of 0.87. This would suggest that the biomarker may help discriminate cardiogenic syncope from non-cardiac causes of collapse, such as epileptic seizures. This could be useful considering how the various clinical situations mimic one another. However, inspection of the cTnI values shows overlap between groups C and groups B, E and V, suggesting that the test should not be used alone. Of the 30 dogs with seizures, five have raised cTnI concentrations according to laboratory reference range and also using the optimal cut-off value of 0.08 ng/mL (Tables 2 and 4). Three of these patients should be easily identified as epileptic on history alone, due to the presence of cluster seizures. At the same cut-off (0.08 ng/mL), of the 20 group C patients, 15 are correctly classified (true positives), leaving five group C patients undetected, despite having cardiac causes of syncope (false negatives). This highlights a limitation of the test (i.e. limited sensitivity and specificity) and means that measurement of serum cTnI concentration should not be the sole test used for diagnosing dogs with cardiogenic syncope. However, it may assist practitioners, in conjunction with other cardiac investigations such as ECG, to help differentiate cardiogenic syncope from dogs with generalized seizures or vasovagal syncope. A low (<0.08 ng/mL) cTnI concentration may help exclude a cardiogenic cause of syncope.

There are numerous limitations with this study, intrinsic to clinical studies. Idiopathic epilepsy is a diagnosis of exclusion. Some dogs with seizures may have been incorrectly classified. Two epileptic dogs had MRI evidence of brain tumours; however, no post-mortem examinations were performed. All but one dog underwent a full cardiac evaluation which eliminated significant cardiac disease as a cause of troponin elevation in the non-cardiac groups. The group E patient which had

no cardiac investigations had no auscultatory abnormalities and troponin concentrations were within the laboratory reference range. As systemic disease of non-cardiac origin, especially inflammatory, neoplastic and renal disease also frequently leads to elevated cTnI concentrations, excluding patients with such diseases based on history, examination and blood tests was an important component of this study. No patients had myocardial biopsies or post-mortem examinations to confirm myocardial cellular damage. Most study cases had a history of collapse, yet the direct effect of blunt chest trauma on troponin release was not taken into account. The act of collapsing, particularly if during high intensity exercise, could conceivably result in thoracic trauma, and trauma can cause troponin release [52,53]. However, other than the collapse itself, no dog in this study had a history of trauma.

Some group sizes are small, affecting the power of the statistical analyses. It is possible that some patients were misclassified according to their group categorization, e.g. only two group V dogs were definitively diagnosed as having the cardioinhibitory form of vasovagal collapse. Small patient numbers in group C ($n = 20$) with a variety of underlying disease processes may reduce the overall significance of the results. Effect of neoplasia in group C was not examined, with four patients having neoplastic involvement. The series of patients presented here represent a referral population which may differ from the general practice canine population. Effect of breed was not assessed due to small numbers and diverse breeds.

The results of this study only apply to the troponin analyser used [34]. The influence of daily variation on biomarker concentration and effect of storage conditions on troponin stability were not assessed. However, all blood samples were stored and handled in a careful, easily reproducible, consistent manner to minimize analytical issues.

Conclusions

Patients collapsing with a cardiac cause have significantly increased troponin concentrations compared with epileptic or vasovagal patients; however, the overlap in troponin concentrations reduces the discriminatory power of the test in an individual dog. Therefore, troponin assays should not be used as stand-alone tests, but in combination with other diagnostic investigations, such as ECG and echocardiography. Future large prospective studies investigating the possible prognostic value of cTnI in syncopal dogs are warranted.

Conflicts of Interest

The authors do not have any conflicts of interest to disclose.

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