TITLE: Serum cardiac troponin I concentrations in dogs with generalised seizures

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Key:

[a] Troponin I Immulite® 1000 Siemens Medical Solutions.

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

[c] Access Systems AccuTnI® Assay, Beckman Coulter Inc, Fullerton, CA.

[d] Prism 7 for Windows version 7.02, Graphpad software inc.

[e] IBM SPSS Statistics Version 22.0.0.1

SUMMARY

Objectives: To determine if serum cardiac troponin I (cTnI) concentrations measured with both a first generation assay (FG-cTnI) and a high-sensitivity assay (hs-cTnI) were greater in dogs with generalized seizures than in controls and to identify any clinical variables associated with cTnI concentrations.

Methods: This prospective study investigated 30 dogs following generalized seizures and 30 healthy controls. Serum cTnI concentrations were measured using two commercially available assays and the correlation of clinical factors with concentrations was examined.

Results: Serum concentrations (median [range]) were higher in dogs after a seizure compared to controls when measured by both assays (FG-cTnI (0.07, [0.02-3.05] vs 0.05 [0.02-0.13] ng/mL; $p=0.014$) and hs-cTnI (0.03 [0.01-1.92] vs 0.02 [0.01-0.05] ng/mL; $p<0.001$)). The predictors most significantly influencing cTnI were an increasing number of seizures ($p<0.001$) and increasing age ($p<0.001$). Both predictors were positively associated with increasing concentrations of troponin I.

Clinical significance: Serum cTnI concentrations were significantly elevated in canine patients with generalized seizures when compared to controls and concentrations were higher in dogs that experienced more seizures. This association may indicate that generalized seizures are associated with damage to the myocardium.

Keywords: epilepsy, death, biomarker, myocardial damage

Introduction

Seizures have a dramatic effect on the autonomic nervous system and people with epilepsy have an increased risk of sudden unexpected death; termed "Sudden Unexpected Death in Epilepsy" or SUDEP (Tigaran *et al.* 2003, Jansen & Lagae 2010). Seizures can lead to short-term alteration of cardiac rhythm, intracardiac conduction, apnoea and cardiac ischaemia in people (Alehan *et al.* 2009, Velagapudi P *et al.* 2012). It has been suggested that an increased number of generalized seizures leads to an increased risk of SUDEP (Ryu *et al.* 2015, Scorza *et al.* 2016). In rats with experimentally induced seizures, plasma cardiac troponin I (cTnI) concentrations are elevated following seizures (Metcalf *et al.* 2009, Bealer *et al.* 2010). This was hypothesized to be a result of seizure-induced cardiac myofilament damage and the authors suggested routinely measuring cTnI concentrations following seizure activity (Metcalf *et al.* 2009). It has also been suggested that human epileptic patients at risk of SUDEP should have serum troponin concentrations measured following a seizure (Dupuis *et al.* 2012). Known SUDEP risk factors include an increased number and frequency of seizures, as well as an increased number of anti-epileptic medications (Lhatoo *et al.* 2015, Ryu *et al.* 2015).

Seizure activity may lead to increased sympathetic nervous discharge, secretion of adrenaline and noradrenaline, and reduced parasympathetic activity in human patients (Blumhardt *et al.* 1986, Tigaran *et al.* 2003, Mayer *et al.* 2004, Velagapudi *et al.* 2012). It has been suggested that the resultant increase in heart rate, blood pressure (BP) and myocardial contractility collectively increase myocardial oxygen demand (Alehan *et al.* 2009). Therefore, it is possible that prolonged seizure activity might impose a perfusion/demand mismatch of sufficient severity to produce sub-endocardial ischaemia (Tigaran *et al.* 2003). There is also evidence to suggest that direct neural connections to the heart can produce cardiac necrosis lesions in dogs with neurological disease as catecholamines reaching the heart directly via neural connections may be much more toxic than those reaching the heart via the bloodstream (Kolin & Norris 1984, Samuels 2007).

In dogs, measurement of cTnI concentrations can be used to detect myocardial cell injury caused by a variety of causes, including cardiac disease such as mitral valve disease, dilated cardiomyopathy, sub-aortic stenosis and pericardial disease (Oyama & Sisson 2004, Spratt *et al.* 2005), and noncardiac

disease such as pyometra (Hagman *et al* 2007), snake bites (Pelander *et al* 2010), renal failure (Sharkey *et al* 2009), pancreatitis (Serra *et al* 2010), canine babesiosis (Lobetti *et al.* 2002), ehrlichiosis (Diniz *et al.* 2008), immune-mediated haemolytic anaemia (Gow *et al.* 2011), parvoviral enteritis (Kocaturk *et al.* 2012), leishmaniasis (Silvestrini *et al.* 2012), dirofilariosis (Carretón *et al.* 2014) and gastric dilation-volvulus (Schober *et al* 2002). There is little published information on the association of serum cTnI concentration and naturally occurring seizures in dogs, other than isolated case reports (Kent *et al.* 2010, Snyder *et al.* 2010, Navarro-Cubas *et al.* 2011, Motta & Dutton 2013), an oral presentation describing a series of cases (Kim *et al.* 2012) and one study (Dutton *et al.* 2016). It has been shown that cTnI concentrations in patients with cardiogenic syncope are significantly increased as compared with epileptic or vasovagal patients, however overlap in levels between groups decreases the discriminatory power of the test for individual dogs (Dutton *et al.* 2016). No studies have evaluated cardiac troponin concentrations in a population of dogs with seizures that have no evidence of underlying cardiac or metabolic disease and compared them to a healthy control population of dogs. It is important to have information concerning the response of cTnI concentrations following a seizure, to enable correct interpretation of serum concentrations obtained from patients following a paroxysmal event (such as a seizure or syncopal episode).

The primary aim of the current study was to compare serum cardiac troponin I concentrations in dogs following generalized seizures to concentrations obtained from an age matched control population of healthy dogs. Secondary aims of the study included identifying whether clinical variables, such as age and seizure number, were associated with serum troponin concentrations and comparing troponin concentrations obtained using different cardiac troponin assays. We hypothesized that dogs with generalized seizures would have higher circulating serum cardiac troponin I concentrations, compared to control dogs.

Materials and Methods

The study protocol was approved by the University of Cambridge ethical review committee.

77 Dogs with generalized seizures and healthy controls were prospectively and consecutively recruited at
78 two referral centres between February 2011 and May 2013. The dogs with seizures included newly
79 diagnosed dogs and those already receiving treatment. To be included as a case a dog must have
80 experienced a generalized seizure within seven days of presentation and undergone full cardiac
81 investigations, including BP measurement, electrocardiography (ECG) and echocardiography without
82 a cardiac abnormality being discovered. Dogs had to be free from other disease likely to lead to altered
83 serum troponin concentration such as pancreatitis, immune-mediated haemolytic anaemia and renal
84 insufficiency, based on history, clinical examination, USG measurement, complete blood count, serum
85 biochemistry and electrolytes. Dogs were excluded if they showed evidence of renal insufficiency
86 (based on elevated serum creatinine concentrations (>150 μmol/L) and low urine specific gravity (USG)
87 measurement (<1.030)), metabolic disorders that could have caused the seizures (hypocalcaemia or
88 hypoglycaemia), or had a history of recent trauma or intoxication.

89 Healthy control animals consisted of staff pets undergoing blood sampling for screening before
90 vaccination, blood donation or elective surgery. They had to have no abnormalities detected on full
91 clinical, including neurological, examination. They had no history of seizures and were free of any
92 disease likely to lead to elevated serum cTnI concentrations. They also had to be free of cardiac disease
93 based on clinical examination, BP, ECG and echocardiography.

94 Signalment (breed, age, sex, neutered status, bodyweight), history (including time since seizure, in
95 hours, until blood sampling) and current medication (yes/no) were recorded. For the patients with
96 seizures, the number of seizures during the seven days prior to presentation and seizure length in
97 minutes (if more than one seizure had occurred, then the average seizure length) were noted. Full
98 neurological examination was performed.

99 Fasted blood samples for measurement of creatinine concentration (μmol/L) were collected from all
100 dogs and analysed within 24 hours. Blood was collected into 1 mL serum gel tubes and separated by
101 centrifugation 30 minutes after being left to stand at room temperature. Serum was then chilled at 4ºC
102 for up to 12 hours before transportation at ambient temperature to a commercial laboratory (Carmichael

Torrance Diagnostic Services (CTDS) Ltd, W Yorks) for analysis. Free catch urine samples were obtained the same day as blood sampling and USG measured. Laboratories were blinded to patient history.

Samples analysed using the first generation cardiac troponin I assay (FG-cTnI) were handled as described for serum creatinine and analysed using a previously described chemiluminescent immunoassay system[a] (O'Brien *et al.* 2006). The cTnI was detected using an enzyme-conjugated polyclonal anti-troponin I antibody following protein binding onto beads coated with murine anti-troponin I antibody, using Immulite® Analyser. Both antibodies recognise epitopes between amino acids 33 and 110 of cTnI. Sample handling for the high sensitivity cardiac Troponin I assay (hs-cTnI) involved collecting blood into 1 mL plain tubes. Plain tube samples were separated by centrifugation immediately after clotting. The serum was then stored for up to 12 hours at -18ºC before transportation in frozen cool packs to a commercial laboratory (IDEXX Laboratories[b], Wetherby). The high-sensitivity assay (AccuTnI® assay) is a two-site sandwich immunoassay[c] which detects free and complexed troponin. The assay uses two mouse-derived monoclonal antibodies directed against 24-40 and 41-49 amino acid sequences of cTnI. This assay has been reported (Adin *et al.* 2006, Hezzell *et al.* 2012) and validated (Oyama & Solter 2004) previously for canine samples. The laboratory reference range was cTnI < 0.15 ng/mL for the FG-cTnI assay and ≤ 0.07 ng/mL for the hs-cTnI assay. The assays' lower limits of detection (LOD) were 0.02 ng/mL (FG-cTnI) and 0.01 ng/mL (hs-cTnI). Samples for measuring serum cTnI concentrations by both methods were taken from patients at presentation. For patients with seizures, cardiac investigations occurred prior to general anaesthesia (GA), except any difficult to control status epilepticus (SE) patients, which had cardiac investigations delayed until 24 hours following GA. Systolic BP (mmHg) using a Doppler device was measured according to an established protocol (Brown *et al.* 2007). Five readings were taken and the mean calculated. Electrocardiography used a routine six-lead ECG machine (Esaote P80 Power or Seca CT8000P), with a period of acclimatisation beforehand. Six limb leads were recorded simultaneously for a minimum of 20 consecutive RR intervals, at a paper speed of 50 mm/second, gain of 10 mm/mV.

Patients underwent full 2D echocardiographic examination without sedation, according to published recommendations (Thomas *et al.* 1993) using phased array probes (1.5-11 MHz) with harmonic imaging (Esaote Piemedical MyLab 40 Vet or Vivid S6 echocardiography machines). An ECG was recorded simultaneously. Full M-mode, colour and spectral Doppler studies were recorded and analysed. All cardiac investigations were performed by the same resident in cardiology, working under the supervision of a Royal College of Veterinary Surgeons (RCVS) specialist in veterinary cardiology.

Dogs with suspected seizures underwent appropriate imaging. Magnetic resonance imaging (MRI) scans (0.25 T; Vet-MR Grande, Esaote) with gadolinium contrast were obtained in three planes of orientation (dorsal, sagittal and transverse) under GA. Pre- and post-contrast T1-weighted and T2-weighted images were acquired. In some cases, additional sequences (pre- and post-contrast FLAIR, gradient echo T2* and STIR) were carried out to better define the underlying brain pathology. Additional tests, such as Toxoplasma and Neospora serology, were performed by the attending clinician depending on the individual case. All neurological examinations were performed by European College of Veterinary Neurology board-certified neurologists or their residents. Definitive diagnosis of the cause of seizures was made by evaluation of all contributing evidence with idiopathic epilepsy being a diagnosis of exclusion (Berendt *et al.* 2015). Dogs with idiopathic epilepsy were younger than six years at seizure onset, had recurrent seizures and were normal on inter-ictal neurological and laboratory examination. They had no evidence of neurological disease, other than seizures during their lives up until presentation. Dogs were grouped into healthy controls (group C) or those with generalized seizures but no evidence of cardiac disease (group S).

Data were analysed using commercially available software[de]. Continuous variables were checked to see whether they came from a normal distribution using a Shapiro-Wilk test. In descriptive statistics continuous variables from a normal distribution are reported as mean (+/- SD) those not from a normal distribution as median (range). Cardiac troponin I concentrations below the LOD of the assays used were ascribed the value of the limit of detection; 0.01 ng/ml for the hs-cTnI assay, and 0.02 ng/ml for the FG-cTnI assay.

Simple linear regression was performed with cTnI concentration as the dependent variable. The following predictor variables were initially assessed individually for an association with the dependent variable; presence of seizures (y/n), seizure number (during seven days prior to presentation), seizure length (minutes), serum creatinine concentration (µmol/L), age (years), sex (male or female), neutered status (neutered/entire), bodyweight (KG), time since seizure (hours), whether anti-convulsant medication was being administered at presentation and systolic BP (mmHg). For dogs recorded as having > 10 seizures within seven days of presentation, the seizure number was arbitrarily allocated to 11. This allowed dogs with frequent seizures, but where owners were not certain of the exact number of seizures suffered, to be entered into the analysis. Those predictor variables demonstrating an association with the dependent variable with $P < 0.2$ in the univariable analysis were taken forward to the multivariable analysis. The multivariable linear regression analysis was performed with Log10 of cTnI concentrations obtained from all dogs (those with seizures and controls) as the dependent variable. Two sets of analyses were performed, one using concentrations obtained with the FG-cTnI assay and one using those from the hs-cTnI assay. The analysis was performed in a backward stepwise manner with the variable showing the highest p value excluded from the model at each step until all remaining variables had a p value less than 0.05. Final models were assessed for adequacy of fit using the adjusted R-square. The residuals of the final models were checked to confirm that they adequately met the model assumptions. Model assumptions were normality, linear relationship with an additive effect of the predictors, homoscedasticity and independence of the errors. The first assumption was checked using visual inspection of a histogram and normality tests of the residuals; to ensure the other assumptions were met the plots of the residuals were examined against fitted values and against their order in the dataset.

Concentrations of cTnI obtained using the two different assays were correlated using a Spearman's rank correlation and compared using Wilcoxon signed rank test. The latter analysis was performed using all values obtained from all dogs, including those with values ascribed at the limit of detection of the assay. The comparison was then repeated including only those animals where concentrations measured using both assays were above 0.02 ng/ml i.e. above the LOD of both assays. A Bland Altman plot of the

difference in concentration obtained between the two assays plotted against the average of the two assays was made.

For all analyses P values of < 0.05 were accepted as statistically significant.

Results

Sixty dogs were enrolled, consisting of 30 dogs with seizures (group S) and 30 control dogs (group C). One dog with seizures was excluded from all statistical analyses except the comparison of cTnI concentrations obtained using the different assays as it had no BP, ECG and echocardiographic examination and therefore did not fully meet the entry criteria. Signalment and other baseline characteristics are summarised in Table 1. The distribution of breeds was different between groups (Table 1). Group S comprised 26 dogs with primary (idiopathic) epilepsy, two with brain tumours (suspected meningioma and suspected glioma), and one with necrotising meningoencephalitis. Three dogs had SE during the 24 hours before presentation, one of which required GA for seizure control. The median number of seizures in group S was two (range 1 – 11). The median seizure length was three minutes (Table 1).

In group S, 17 dogs were taking anti-convulsants at presentation. Medications included phenobarbitone (Epiphen; Vetoquinol), levetiracetam (Keppra; UCB), potassium bromide (Epilease; Vet Plus Ltd), gabapentin (Neurontin; Pfizer) and diazepam (Diazepam Rectubes; Wockhardt UK). Other medications included oral antibiotics (n=1) and injectable dexamethasone (n=1) (Dexadreson; Intervet UK Ltd). No control dogs received medication. All but one of the dogs had normal ECGs and echocardiographic examinations. An echocardiographic abnormality was detected in one case in group S (mild left ventricular dilation) but this was attributed to the presence of sinus bradycardia (heart rate of 60 bpm). Repeat echocardiography performed three weeks later when the patient was in normal sinus rhythm (120 beats/minute) was unremarkable.

Serum concentrations of cTnI (median [range]) were higher in dogs after a seizure compared to controls when measured by both assays (FG-cTnI assay, dogs with seizures median 0.07 [0.02-3.05] vs control dogs median 0.05 [0.02-0.13]ng/ml; p=0.014 (Figure 1) and hs-cTnI assay, dogs with seizures median

0.03 [0.01-1.92] vs control dogs 0.02 [0.01-0.05]ng/ml; p<0.001. Seven dogs (1 with seizures and 6 control dogs) had troponin concentrations at or below the LOD of the FG assay. 17 dogs (3 with seizures and 14 control dogs) had troponin concentrations at or below the LOD of the hs assay. As the distribution of troponin concentrations was skewed, logarithmic transformation was required in order to create a variable suitable for inclusion as the dependent variable in the univariable and multivariable analyses. The results of the simple linear regression with a single predictor are reported (Table 2). The final models of the multivariable linear regression analyses indicated that an increasing number of seizures (p<0.001) and increasing age (p<0.001) were significantly associated with higher concentrations of cTnI measured using both the FG-cTnI and hs-cTnI assays and both predictors had positive regression coefficients (Table 3). The adjusted R-square value was greater for the model in which concentrations obtained with FG-cTnI assay were used as the dependent variable (adjusted R-square 0.48 with FG-cTnI assay and 0.44 with hs-cTnI assay). The residuals of the multiple regression analysis were normally distributed for the final model using FG-cTnI as the dependent variable but not for the final model using hs-cTnI. For these reasons the FG-cTnI model is the one for which the associations are reported.

The troponin concentrations measured using the FG-cTnI assay correlated well with those values of the hs-cTnI assay r = 0.82 (p<0.001). However, the values obtained with the FG-cTnI assay were significantly higher than those obtained using the hs-cTnI assay. This was the case when data from all 60 dogs was analysed and when only those 23 dogs for which values were greater than 0.02 ng/ml when measured by both assays were compared (P < 0.001 for both comparisons). The Bland Altman plot suggested that values obtained with the FG assay were consistently higher than those obtained with the hs assay (Figure 2).

Discussion

Our data show that dogs with generalized seizures have higher circulating serum cTnI concentrations compared with healthy control dogs. The results also indicate that the degree of troponin elevation is independently associated with number of seizures experienced and the patient's age. These results are similar to those from a study of human patients which demonstrated that cTnI concentration increased

with increased seizure number (Hajsadeghi *et al.* 2009). The presence of myocardial injury in the setting of seizure activity could be expected given the apnoea, tachycardia, increased myocardial oxygen consumption and excess catecholamine release associated with seizures which has been observed in experimental rat models (Metcalf *et al.* 2009). It is important to know that dogs which have recently experienced seizures may have elevated cTnI concentrations as seizures, which can be challenging to differentiate from syncope, are another cause of elevated cTnI in dogs without primary cardiac disease. A previous study suggested that serum cTnI is significantly higher in dogs following syncope compared with epilepsy in the absence of cardiac disease, although significant overlap compromised the diagnostic utility of cTnI for differentiation of these causes of episodic collapse (Dutton *et al.* 2016). The present study demonstrated similar findings in the dogs following seizures and, in addition that cTnI concentrations increase with increasing number of seizures and age. These findings suggest that a diagnosis of cardiac syncope should not be based on cTnI alone and that further research is warranted in finding diagnostic tests which could help differentiate cardiac syncope from epileptic seizures (Dutton *et al.* 2016).

Elevated cTnI concentrations may also be due to cardiac necrosis caused by catecholamines released directly into the myocardium via neural connections, as suggested by experimental models and studies on human patients with intracranial lesions (Burch *et al.* 1969, Kolin & Norris 1984, Shivalkar *et al.* 1993). It is also possible that myocardial fibrosis, such as that shown to occur in human SUDEP patients, may occur particularly following repetitive autonomic stimulation, i.e. with an increased number of seizures (Earnest *et al.* 1992, Natelson *et al.* 1998). In humans, an increased number of seizures has been shown to be a risk factor for SUDEP (Lhatoo *et al.* 2015). It is therefore possible that an increasing number of seizures might be causative for increased myocardial injury, although further studies are necessary to investigate this possibility. As well as relevance to SUDEP, this is an argument for improved seizure control.

In the population we describe, circulating cTnI concentrations increased with age consistent with previous canine studies (Oyama & Sisson 2004, Ljungvall *et al.* 2010, Hezzell *et al.* 2012). One possible cause is that in the aged heart, even in the absence of demonstrable cardiovascular disease, there is

gradual loss of cardiac myocytes. The presence of troponin in the circulation may therefore represent myocyte death, or turnover, which may be a normal consequence of ageing (Oyama & Sisson 2004). Another possibility is decreasing renal clearance of troponin with increasing age. However, in this study, patients with chronic kidney disease (creatinine concentrations >150 μmol/L and USG <1.030) were excluded and no association between cTnI concentrations and creatinine was observed. Further studies are required to investigate why troponin concentrations are positively associated with age in the absence of renal dysfunction. Multivariable analysis adjusts the analysis for the effect of age and therefore the effect of seizure number is independent of the effect of age.

We measured serum troponin I concentrations using two different assays generating similar results in the multivariable analyses. The FG-cTnI assay was able to detect cTnI in the serum of a greater proportion of the dogs with fewer results below the LOD of the assay, despite the assay having a higher limit of detection. The LOD reported for the FG-cTnI assay used in the current study was 0.02 ng/ml; this is lower than the LOD previously reported for this assay (Spratt *et al.* 2005). This was the LOD reported by the commercial laboratory that ran the analyses and chosen on the basis of good dilutional linearity of the assay down to concentration below 0.02 ng/ml demonstrated by the laboratory's own validation studies (personal communication).

The FG-cTnI assay demonstrated less clustering of values than those obtained with the hs-cTnI assay. Perhaps as a result of this the data obtained using the FG-cTnI assay resulted in a slightly higher R-squared value for the final model using this as the dependent variable. Values obtained with the FG-cTnI assay were significantly higher than the hs-cTnI values for the same patients. Explanations for higher cTnI values include different target amino acids for each analyser and differences in antibody specificity for free and complexed troponin (James *et al.* 2006). In human patients, various troponin assays have been compared and some were shown to be superior to others (James *et al.* 2006). Our study confirms that, despite close correlation, the two troponin assays cannot be used interchangeably. This has been shown in a previous veterinary study (Adin *et al.* 2006). The R-squared value indicates the proportion of the variance in the dependent variable that is predicted by the independent variables included in the model. In general, the higher the R-squared value, the better the model fits the data. The

289 data obtained with the FG-cTnI assay resulted in a slightly higher R-squared value for the final model.
290 It also better fulfilled the assumptions of the analysis with normally distributed residuals.
291 In this study, a control population was included and patient numbers were higher than in a previously
292 reported study on serum cTnI with seizures (Kim *et al.* 2012). A more homogenous population of dogs
293 was included here, the majority suffering from primary (idiopathic) epilepsy. The other dogs included
294 were also free of significant metabolic disease. The advantage of this being that the effect of variables
295 such as seizure number and length can be analysed without underlying disease processes confounding
296 the results. The disadvantage is that the effect of different types of underlying disease upon troponin
297 concentration cannot be compared. As troponin concentrations can change with noncardiac disease,
298 eliminating chronic kidney disease, immune-mediated haemolytic anaemia, sepsis, pyometra,
299 respiratory disease and pancreatitis on history, clinical examination and blood tests was an important
300 component of this study.

301 This study has a number of limitations. None of the patients had myocardial biopsies, coronary
302 angiography or post-mortem examinations to confirm myocardial cellular damage and rule out other
303 possible causes of cTnI release. The cases presented here represent a referral population, which may
304 differ from the population of dogs seen in general practice. The breed distribution of the control and
305 seizure groups were different and the effect of breed on cTnI concentrations was not examined. This is
306 unlikely to have had an effect on the overall findings but is a potential confounder. The results of the
307 study only apply to the two troponin analysers used and the results cannot be used interchangeably. The
308 effect of GA on troponin concentration was not assessed in this study, although only one patient required
309 GA for seizure control, therefore had cTnI sampling delayed until 24 hours following GA. This same
310 patient had the highest serum cTnI concentration in group S and had sinus bradycardia at 60 bpm. It is
311 unclear whether the changes detected were a result of anti-epileptic medications, GA or a direct result
312 of the high number of seizures. It is possible that the GA affected serum cTnI concentrations, resulting
313 in elevated levels. However, two other epileptic patients also had elevated cTnI concentrations
314 (according to the laboratory reference range) and yet did not require GA for seizure control.
315 Interestingly, both patients suffered a high (> 10) number of seizures prior to blood sampling. Excluding

316 the data of the one patient that underwent anaesthesia from the multivariable analysis did not
317 substantially alter the results obtained. Further studies involving larger numbers of patients with
318 seizures, pre- and post-GA, would be required to further investigate this association with serum cTnI
319 concentrations. Finally, it is possible that in three dogs (two with brain tumours and one with necrotising
320 meningoencephalitis) suffering seizures, that the underlying disease process may have had an effect on
321 the serum troponin concentrations, rather than the seizures themselves affecting the results. Larger
322 studies would be required to study the effects of individual disease processes on troponin concentrations
323 as, so far, only individual case reports are available (Snyder *et al* 2010, Navarro-Cubas *et al* 2011).
324 In conclusion, our results suggest that serum troponin concentrations are elevated in dogs with seizures
325 when compared to healthy controls. The elevation is independently associated with number of seizures,
326 when adjusted for the influence of age. The identification of elevated cTnI concentrations is important
327 as it suggests that myocardial injury might occur secondary to seizure activity, which could have clinical
328 implications for epileptic patients. In addition, the positive relationship between seizure number and
329 serum cTnI concentration is likely to further reduce the diagnostic utility of serum cTnI for
330 differentiation of syncope and seizure activity (Dutton *et al.* 2016).

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332 No conflicts of interest have been declared.

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Table 1. Baseline characteristics from both groups of dogs.		
	S (Seizure) n= 29	N (normal controls) n=30
Age (years).	4.0 (0.3-10.5)	4.4 (0.6-15.0)
Male	16 (55%)	12 (40%)
Neutered	18 (62%)	23 (77%)
Bodyweight (KG).	14.9 (5.5-49.2)	14.4 (3.2-40.8)
Serum Creatinine (umol/L).	82 (48-153)	90 (63-140)
Systolic BP (mmHg).	144 (100-178)	134 (100-180)
Breed Categories.		
Other pure breed	15 (52%)	5 (17%)
Cross breed	1 (3.4%)	8 (27%)
Labrador retriever	5 (17%)	4 (13%)
Jack Russell terrier	1 (3.4%)	7 (23%)
Staffordshire bull terrier	3 (10%)	1 (3.3%)
Cavalier King Charles spaniel	2 (6.9%)	1 (3.3%)
Border collie	2 (6.9%)	1 (3.3%)
Springer spaniel	0 (0%)	3 (10%)
Number of seizures	2 (1-11)	NA
Duration of seizures (minutes)	3 (0.5-30)	NA
Interval between last seizure and sample collection (hours)	36 (0-168)	NA
Receiving anticonvulsant medication	17 (59%)	0 (0%)

Table 2. Results of Simple Linear Regression Analysis for log(FG-cTnI) (n=59).				
Variable (unit of measurement or comparator category)	Coefficients B	P - value	95 % Confidence Interval for B	
			Lower	Upper
Seizure (no)	-0.299	**0.004**	-0.499	-0.099
Seizure number during 7 days prior to presentation	0.086	**< 0.001**	0.056	0.116
Seizure duration (minutes)	0.018	**0.006**	0.005	0.030
Creatinine concentration (µmol/L)	-0.002	0.449	-0.007	0.003
Age (years)	0.049	**0.003**	0.018	0.079
Sex (male)	-0.051	0.640	-0.266	0.165
Neuter (entire)	0.065	0.580	-0.168	0.298
Weight (kg)	0.000	0.950	-0.010	0.009
Time since last seizure (hours)	-0.002	0.293	-0.005	0.002
Medication (yes)	0.356	**0.002**	0.138	0.574
Blood pressure (mmHg)	0.005	**0.064**	0.000	0.010

Table 3. Results of Linear Regression Models for log(FG-cTnI) (n=59).					
			Confidence Intervals		
		Coefficient	Lower 95%	Upper 95%	P - value
log(FG-cTnI)	Number of Seizures	0.084	.057	.110	<0.001
Adjusted R-square = 0.48	Age (Years)	0.046	.022	.070	<0.001
	Constant	-1.507	-1.646	-1.367	<0.001

Figure Legend

Figure 1. Box and whiskers plot showing serum cardiac Troponin I concentrations measured using the first generation assay in the dogs that had experienced seizures (n=29) and control dogs (n=30). The whiskers indicate the range of values obtained, the box extends from the 25th to the 75th percentile, the horizontal bar in the box represents the median. Concentrations were significantly higher (Mann-Whitney; P = 0.014) in the dogs that had recently experienced seizures. Note the vertical axis is plotted on a logarithmic scale.

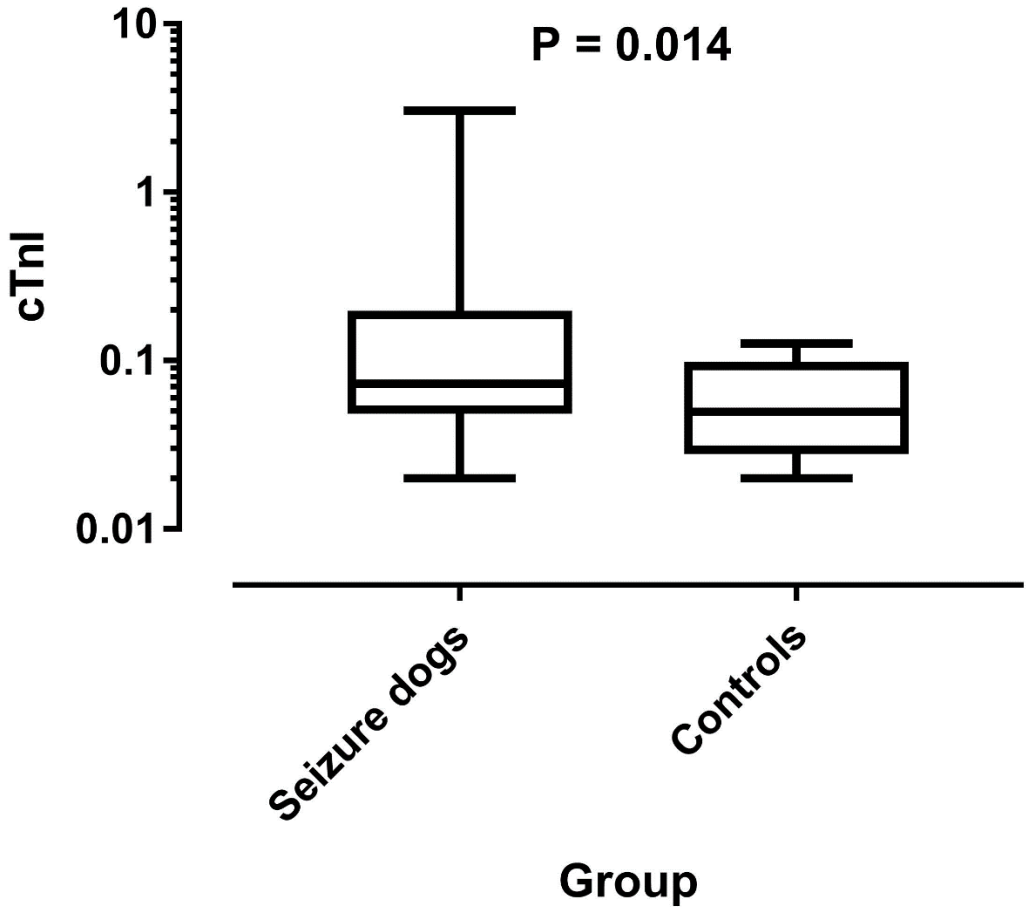

Figure 2. A Bland-Altman plot comparing concentrations of cardiac troponin I obtained by the two assays. The difference in concentration obtained by subtracting the concentration obtained using the high-sensitivity (hs) assay from the concentration obtained using the first generation (FG) assay is plotted (on the vertical axis) against the average of the two concentrations obtained (on the horizontal axis). The plot illustrates a consistently positive difference which appears to increase in a near linear fashion suggesting that the concentrations obtained by the FG assay are always higher and by a consistent factor. Note the horizontal axis is discontinuous in order to include one data point with high concentrations. Bias has been shown as a horizontal dashed line. The upper and lower 95% limits of agreement are shown as dotted lines.