Serum Cardiac Troponin I in Canine Syncope and Seizure

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I would like to dedicate this dissertation to

my husband and sons for their love

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Abbreviations

AMI	Acute myocardial infarction
ANOVA	Analysis of variance
AUC	Area under curve
BP	Blood pressure
CI	Confidence intervals
CSF	Cerebrospinal fluid
cTnC	Cardiac troponin C
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
DCM	Dilated cardiomyopathy
Group B	Group with both cardiac disease and seizures
Group C	Group with cardiogenic syncope
Group CS	Group with cluster seizures
Group E	Group with epilepsy
Group N	Group of normal control dogs
Group S	Group without cluster seizures
Group U	Group whereby no diagnosis was reached (unclassified)
Group V	Group with vasovagal syncope
hscTnI	High-sensitivity cardiac troponin I
LOD	Limit of detection
MRI	Magnetic resonance imaging
PH	Pulmonary hypertension
ROC	Receiver operator curve analysis
SE	Status epilepticus
SUDEP	Sudden unexpected death in epilepsy
SVT	Supraventricular tachycardia
TLOC	Transient loss of consciousness

Abstract

Objective: The purpose of this prospective study was to determine if serum cardiac troponin I (cTnI) and high-sensitivity cardiac troponin I (hscTnI) concentrations were elevated in dogs collapsing with generalised epileptic seizures compared to a control population. In addition, to determine whether serum cTnI and hscTnI concentrations could distinguish cardiogenic syncope from generalised epileptic seizures in collapsing dogs.

Animals, materials and methods: The study prospectively recruited 109 dogs comprising a control population and 79 dogs collapsing with either generalised epileptic seizures or syncope. Serum cTnI and hscTnI were measured using commercially available assays and concentrations in the normal and collapsing groups of dogs compared using non-parametric statistical tests.

Results: Dogs with naturally occurring epileptic seizures compared with control dogs had significantly higher median concentrations of cTnI (0.073 [range 0.02-3.05] vs 0.05 [range 0.02-0.126] ng/ml; $p=0.024$) and hscTnI (0.03 [range 0.01-1.92] vs 0.02 [range 0.01-0.05] ng/ml; $p<0.001$). Furthermore, median cTnI and hscTnI concentrations were significantly higher in dogs with cardiogenic syncope than those with epileptic seizures (cTnI: 0.505 [range 0.105-77.3] vs 0.073 [0.02-3.05] ng/ml] $p<0.05$; hscTnI: 0.165 [0.02-27.41] vs 0.03 [0.01-1.92] ng/ml; $p<0.05$).

Conclusions: Serum cTnI and hscTnI concentrations were significantly elevated in canine epileptic patients with generalised seizures compared to the control population. Serum cTnI and hscTnI concentrations were significantly different in the cardiogenic syncope group compared to the group with epileptic seizures. However, the overlap in troponin concentrations between the two groups reduces the clinical efficacy of the assay for differentiating cardiogenic syncope from generalised epileptic seizures in dogs.

1. 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 seizure. Syncope and seizure can mimic one another with a consequent high rate of misdiagnosis, particularly as affected patients are often normal at the time of presentation (Grubb *et al* 1991, Linzer *et al* 1994, Scheepers *et al* 1998, Smith *et al* 1999, Zaidi *et al* 2000, Chadwick & Smith 2002, Sheldon *et al* 2002, Werz 2005, Penning *et al* 2009, Barnett *et al* 2011, Motta & Dutton 2013). Examples of disorders that 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 association with insulinoma, sepsis, paediatric patients, toy breed dogs or hepatic disease). In human patients, studies suggest that one in four patients with 'epilepsy' may be misdiagnosed (Grubb *et al* 1991, Linzer *et al* 1994, Scheepers *et al* 1998, Smith *et al* 1999, Zaidi *et al* 2000).

In veterinary medicine, published case reports illustrate the challenges of differentiating syncope from seizures (Penning *et al* 2009, Motta & Dutton 2013). It is important to correctly distinguish causes of weakness and fainting from malignant cardiac arrhythmias which can degenerate into ventricular fibrillation or cardiac arrest. Clinicians are often reliant on incomplete owner history. One cardiac biomarker, cardiac troponin I (cTnI), is useful for detecting cardiac myocyte damage (Wess *et al* 2010) and is easy to sample. It is important to have information concerning the response of cTnI following syncope or seizure, to enable correct interpretation of data.

Studies in humans have shown that epileptic patients with generalised tonic-clonic seizures do not have raised circulating cTnI levels (Woodruff *et al* 2003, Alehan *et al* 2009, Hajsadeghi *et al* 2009, Eskandarian *et al* 2011). There is little published information on the association of serum cTnI concentration with naturally occurring seizures in dogs, other than single case reports (Kent *et al* 2010, Motta & Dutton 2013) and an oral presentation (Kim *et al* 2012). There are no data available regarding the clinical utility of serum cTnI for differentiating cardiac causes of syncope from seizures in dogs. Both cardiac syncope (Stafford Johnson *et al* 2004, Borgarelli *et al* 2008) and increased cTnI in dogs with underlying heart disease are associated with increased risk of death (Wells & Sleeper 2008, Fonfara *et al* 2010a, Hezzell *et al* 2012). It is therefore possible that cardiac syncope may be

associated with increased cTnI. There may be a role for cTnI and hscTnI in the evaluation and diagnosis of dogs collapsing with TLOC. These biomarkers may help to distinguish dogs collapsing with epileptic seizures from those with cardiogenic syncope.

1.1 Definitions of Syncope, Collapse, Epilepsy and Seizures

Syncope, collapse, epilepsy and seizures have specific definitions. Collapse may be defined as a state of unintentional prostration arrived at due to pathological mechanisms (Wray 2005). Syncope is a sudden, transient loss of consciousness due to global cerebral hypoperfusion associated with loss of postural tone (collapse) followed by spontaneous recovery (Kittleson 1998, Rush 1999, Kapoor 2000, Moya *et al* 2009). Syncope can easily be mistaken for seizures.

Seizures are a physical manifestation of a paroxysmal transient event caused by asynchronous discharge of cerebral neurons and typically have motor activity (Thomas 2010). Seizures have a pro-dromal phase and a post-ictal phase, however these signs are not always present (Quesnel 2005). With generalised seizures, the first clinical signs indicate initial involvement of both cerebral hemispheres (Thomas 2010). Consciousness may be impaired and motor manifestations are bilateral. The animal typically loses consciousness and falls to its side in opisthotonus with extended limbs (Thomas 2010). Collapse is therefore sometimes confusingly used interchangeably to describe syncope and seizure activity.

Epilepsy is a group of heterogeneous conditions that share a common feature: chronic, recurring seizures (Thomas 2010). Not all seizures are associated with epilepsy. For example, a seizure can be the reaction of a normal brain to a transient insult, such as intoxication or metabolic disorder. Symptomatic epilepsy occurs when the seizures are caused by an identifiable structural lesion of the brain, such as a tumour (Thomas 2010). Idiopathic epilepsy occurs when there are chronic recurring seizures with no underlying structural brain lesion or other neurological signs (Thomas 2010).

1.2 Prevalence of Syncope and Epilepsy

A survey of a veterinary medical database revealed a syncopal prevalence of 0.15% (Ware 2002). In contrast, a study conducted at Liverpool University showed that 6.3% of all cases

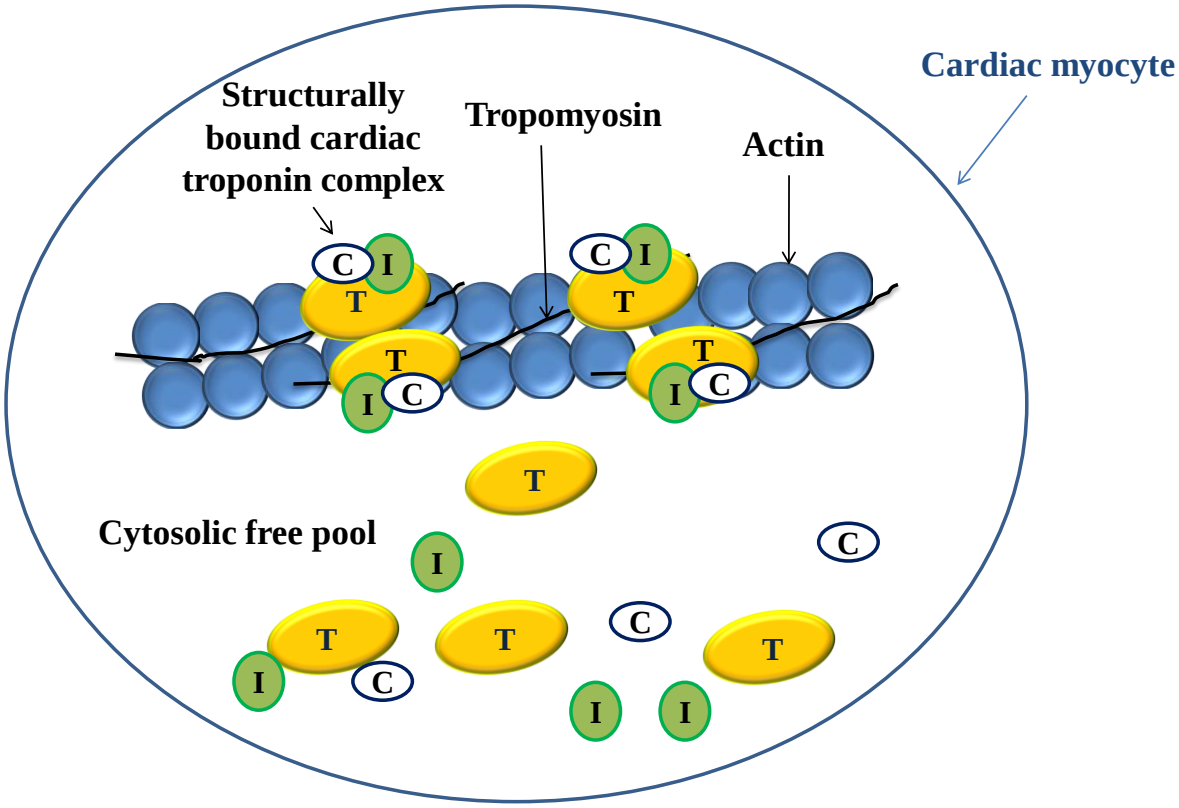
admitted to the hospital were due to collapse and 1.8% of cases had syncope (James *et al* 2008). Another study reports that 20.6% of dogs with myxomatous mitral valve disease have a history of syncope, the prevalence increasing with disease progression (Borgarelli *et al* 2008). The prevalence of epilepsy in the general dog population is estimated to be 1-2% (Saito *et al* 2001, Gulløv *et al* 2011).

Over two years, (September 2009 until September 2011), the prevalence of canine collapse in 2,854 referral cases presenting to ChesterGates Referral Hospital was 5.6%. Of all cases admitted to the hospital, 1.4% had seizures and 0.5% were syncopal.

1.3 Cardiac Troponin I Structure and Function

Cardiac troponin I is a subunit of the troponin protein complex which plays a role in myocardial contraction. The troponin complex, an intracellular cardiac myofibrillar protein, was first described in 1946 (Bailey). The troponin complex consists of three integrated proteins: cardiac troponin C (cTnC), cardiac troponin T (cTnT) and cTnI. Cardiac troponin T anchors the complex to the tropomyosin strand of the thin actin filament (Figure 1). Cardiac troponin C binds calcium ions released from the sarcoplasmic reticulum, and cTnI inhibits the enzymatic hydrolysis of adenosine triphosphate that powers cardiac myocyte contraction (Shave *et al* 2010).

Approximately 3-4% of cTnI exists freely in the cardiac myocyte cytoplasm as a soluble cytosolic pool (Adams *et al* 1994, Bleier *et al* 1998). The remainder is bound via tropomyosin to actin within the sarcomere filament and is released as a result of proteolytic degradation (De Gennaro *et al* 2008, Giannoni *et al* 2009) (Figure 1). This cellular distribution determines release kinetics, with free cytosolic proteins being released earlier. The slower release of structurally bound troponin follows, which results in a sustained elevation in the circulation (Wells & Sleeper 2008). These contractile proteins are released from the myocardium in proportion to the degree of tissue injury and disruption of myocyte membranes (Metzger & Westfall 2004).



Abbreviations; C: Cardiac troponin C, T: Cardiac troponin T, I: Cardiac troponin I.

Figure 1: Diagrammatic representation of cardiac troponins existing in structurally bound forms and in a free cytosolic pool within a cardiac myocyte.

Permission received from the authors (Korff *et al* 2006, Agewall *et al* 2011), BMJ and Oxford Journals.

Cardiac troponin I was found to be a highly sensitive and specific marker of cardiac myocyte injury in the mid-1990s and is now part of the gold standard for diagnosing myocardial infarction in humans (Thygesen *et al* 2012). The recent development of hscTnI assays permits detection of very low circulating levels and has resulted in improved early diagnosis of acute myocardial infarction (AMI) and risk stratification in people (Jaffe 2011). Few studies have assessed hscTnI in veterinary medicine (Ljungvall *et al* 2010, Ljungvall *et al* 2011, Hezzell *et al* 2012, Falk *et al* 2013). Immunoassays for human cTnI are effective in a wide range of animal species because the structure and function of troponins are highly

conserved across species (O'Brien *et al* 1997, Rishniw *et al* 2004). Human assays have been used and validated in dogs (Sleeper *et al* 2001, Oyama & Solter 2004, Adin *et al* 2005). Canine studies have shown that plasma cTnI concentrations vary between different analysers (Adin *et al* 2006). Each analyser uses different target amino acids and has varying degrees of affinity for either free or complexed cTnI. Results between analysers are therefore not considered interchangeable (Adin *et al* 2006).

The half-life of troponin and its complex in the canine circulation is approximately two hours (Katus *et al* 1989, Jaffe *et al* 1996, Dunn *et al* 2011). In humans with AMI, this protein is detectable in the blood 3-12 hours after cardiac injury, peaks at 1-2 days, and dissipates after 5-10 days (Eisenman 2006). Experimental canine models with AMI showed release times similar to those observed in clinical human patients although the peak was attained earlier (range 10–16 hours) (Cummins & Cummins 1987). One explanation for this was a more rapid development of necrosis in the experimental situation as compared to human patients with AMI (Wells & Sleeper 2008). The sustained elevation of cTnI for several days after AMI is likely due to ongoing release from damaged myocytes rather than impaired elimination (Wells & Sleeper 2008). Elimination of troponins is thought to involve clearance by the reticuloendothelial system (Wells & Sleeper 2008, Mohammed & Januzzi 2010). There is also some evidence that troponins may be broken down into small fragments then renally excreted (Freda *et al* 2002, Wells & Sleeper 2008).

In dogs, ischaemic heart disease is considerably less common than in humans (Boswood 2009). However, cardiac troponins can be used to detect myocardial cell injury caused by a variety of other aetiologies such as nonischaemic cardiac disease (Oyama & Sisson 2004, Spratt *et al* 2005) and noncardiac disease for example pyometra (Hagman *et al* 2007), snake bites (Pelander *et al* 2010), renal failure (Sharkey *et al* 2009), pancreatitis (Serra *et al* 2010) and gastric dilation-volvulus (Schober *et al* 2002). With reference to cardiac collapse in dogs, elevated cTnI concentrations have been reported in dogs with arrhythmogenic right ventricular cardiomyopathy and dilated cardiomyopathy (Oyama & Sisson 2004, Spratt *et al* 2005, Baumwart *et al* 2007, Wess *et al* 2010), symptomatic bradyarrhythmias and myocarditis (Church *et al* 2007, Trafny *et al* 2010, Fonfara *et al* 2010b), aortic stenosis (Oyama & Sisson 2004), pulmonary hypertension (Guglielmini *et al* 2010) and pericardial effusion (Shaw *et al* 2004, Linde *et al* 2006, Chun *et al* 2010).

1.3.1 Cardiac Troponin I Concentrations associated with Seizures

The hypothesis that patients with generalised tonic-clonic seizures do not have raised circulating cTnI levels, has been tested in human patients with naturally occurring seizures (Woodruff *et al* 2003, Alehan *et al* 2009, Hajsadeghi *et al* 2009, Eskandarian *et al* 2011) and experimental rat models (Metcalf *et al* 2009, Bealer *et al* 2010). The studies have produced conflicting results. Metcalf *et al* (2009) showed elevation of cTnI in rats with seizure activity of more than 30 minutes duration. They investigated the effects of prolonged seizure activity on cardiac function and susceptibility to arrhythmias. The results of this study showed that intense seizure activity increased blood pressure (BP), heart rate and d*P*/d*t* consistent with the activation of the sympathetic nervous system. Ten to 12 days following the experimentally induced seizure activity, QT-intervals were significantly increased therefore increasing susceptibility to sudden death. Their data suggested that seizure activity produces tachycardic ischaemia following activation of the sympathetic nervous system, resulting in cardiac myofilament damage, arrhythmogenic alterations in cardiac electrical activity, and increased susceptibility to ventricular arrhythmias. Additionally, that cTnI should be routinely measured and the electrical activity of the heart evaluated following prolonged seizure activity.

Bealer *et al* (2010) studied the cardiac effects of prolonged seizure activity on rat models. In this study, seizures were associated with increased QT-interval, increased QT dispersion, elevated cTnI concentrations and increased susceptibility to experimentally induced arrhythmias. Their data suggest that ion channel remodelling may contribute to cardiac mortality in the period following intense seizure activity.

In contrast, studies in humans suggest that epileptic patients with generalised tonic-clonic seizures do not have raised circulating cTnI levels (Woodruff *et al* 2003, Alehan *et al* 2009, Hajsadeghi *et al* 2009, Eskandarian *et al* 2011). Seizure length was recorded in only one study (Woodruff *et al* 2003), with the mean duration being 105 seconds which is shorter than the prolonged experimentally induced seizures (Metcalf *et al* 2009, Bealer *et al* 2010).

It is well known that people with epilepsy have an increased risk for sudden unexpected death (SUDEP) (Tigaran *et al* 2003, Jansen & Lagae 2010). Seizure related tachyarrhythmias, bradyarrhythmias and ictal asystole are thought to play an important role in the pathophysiology of SUDEP (Nei *et al* 2000, Jansen & Lagae 2010). The seizure related

cardiac arrhythmias are facilitated by the increase in sympathetic nervous discharge, increased secretion of adrenaline and noradrenaline, and reduced parasympathetic activity (Velagapudi *et al* 2012).

Brobbey & Ravakhah (2004) reported the first case of elevated cTnI in a human patient with no evidence of myocardial infarction after a 20 minute generalised seizure. Concomitant increases in heart rate, BP, myocardial contractility, along with tonic muscle contraction due to seizure activity may collectively increase myocardial oxygen demand (Alehan *et al* 2009). Therefore, a prolonged and isolated seizure can impose a perfusion/demand mismatch that may be severe enough to produce sub-endocardial ischaemia in the absence of coronary flow impairment (Tigaran *et al* 2003, Alehan *et al* 2009). Hajsadeghi *et al* (2009) showed no elevations of post-ictal cTnI levels in epileptic patients with healthy cardiovascular systems. However they did find a significant correlation between serum level of cTnI and number of epileptic seizures.

To date, there have been no studies performed in seizuring dogs, without evidence of underlying cardiac or metabolic disease, testing the hypothesis that epileptic seizures are associated with elevated cTnI concentrations. Some data have, however, been reported in abstract form (Kim *et al* 2012). This population of dogs included patients with head trauma and inflammatory disease, such as meningoencephalitis. Inflammatory disease has been shown to raise serum cTnI levels in two separate case reports of dogs with meningitis-arteritis (Snyder *et al* 2010, Navarro-Cubas *et al* 2011). In addition, head trauma in dogs has been associated with myocardial necrosis (King *et al* 1982) and cardiac arrhythmia (Haley *et al* 2010).

In conclusion, there appear to be contradicting reports on whether the effects of seizures raise cTnI levels in rat models, human and canine patients. There is perhaps an association with seizure length or frequency and cTnI concentration. In line with the majority of human studies, it is possible that canine cTnI concentrations are not raised after short uncomplicated seizures which can be mistaken for cardiac syncope.

1.3.2 Cardiac Troponin I Concentrations with Cardiogenic Syncope

To date, the diagnostic utility of cTnI in collapsing dogs has not been investigated. Reed *et al* (2010) tested the hypothesis that there are increased concentrations of cTnI in syncopal human patients presenting to the Emergency Department (ED). Reed *et al* reported that a normal troponin concentration would assist in the identification of patients that could be safely discharged early after admission. A more sensitive cTnI assay was used in a later study (Reed *et al* 2012) whereby the majority of patients admitted from the ED with syncope had detectable plasma troponin concentrations. It was suggested that cTnI may play a future role in the risk stratification of patients with syncope.

1.3.2.1 Arrhythmias and Cardiac Troponin I Concentration

It has been suggested that arrhythmias are the most common cause of syncope in dogs (Kittleson 1998, Rasmussen 2011). There are several human (Agewall *et al* 2011) and canine studies (Church *et* al 2007, Fonfara *et al* 2010b) reporting raised cTnI associated with various arrhythmias which may lead to collapse. The underlying cause of the arrhythmia, such as cardiac fibrosis or inflammation may be one possible mechanism for troponin elevation. The arrhythmia itself may also decrease coronary artery perfusion, cause hypoxic myocardial damage, resulting in cTnI release (Trafny *et al* 2010). In human patients, elevated troponin concentrations are sometimes observed after episodes of supraventricular tachycardia (SVT), even in healthy individuals (Redfearn *et al* 2005, Patanè *et al* 2009). The most likely mechanism for troponin elevation following tachycardia is shortening of diastole with subsequent subendocardial ischaemia (Agewall *et al* 2011). Other possibilities include either increased myocardial oxygen demand or coronary artery vasospasm. In animal studies, myocardial stretch is believed to represent another possible mechanism for tachycardia-mediated troponin elevation as there is a direct association between a parallel rise in natriuretic peptide and troponin concentrations in patients with various tachycardias (Qi *et al* 2000, Agewall *et al* 2011). Hessel *et al* (2008) showed, in an experimental study, that cTnI was released from viable cardiomyocytes by a stretch related mechanism mediated by integrins, mechanotransducer molecules that link the extracellular matrix to the intracellular cytoskeleton.

1.3.2.2 Pulmonary Hypertension and Cardiac Troponin I Concentration

Pulmonary hypertension (PH) is a common cause of syncope in dogs (Ware 2002). Precapillary PH is defined as PH which results from abnormalities on the arterial side of the pulmonary vascular system and postcapillary PH is typically associated with left-sided heart disease. A study by Guglielmini *et al* (2010) showed serum cTnI to be high in dogs with either precapillary or postcapillary PH.

1.3.2.3 Pericardial Disease and Cardiac Troponin I Concentration

Pericardial disease can cause significant elevations in troponin levels in both human (Brandt *et al* 2001) and canine patients (Shaw *et al* 2004, Spratt *et al* 2005, Linde *et al* 2006). Proposed mechanisms of troponin release include epicardial inflammation, decreased coronary perfusion during cardiac tamponade and possible myocardial necrosis associated with neoplastic pericardial effusions (Agewall *et al* 2011).

1.3.2.4 Obstruction to Flow and Cardiac Troponin I Concentration

Oyama & Sisson (2004) found that median plasma cTnI was significantly greater in dogs with sub-aortic stenosis and that cTnI demonstrated a modest correlation with ventricular wall thickness. Myocardial hypertrophy and remodelling causing reduced capillary density in the subendocardium with regional ischaemia is a possible mechanism for cTnI release in dogs with sub-aortic or pulmonic stenosis (Saunders *et al* 2009).

1.3.2.5 Myocardial Failure and Cardiac Troponin I Concentration

Myocardial failure as a result of dilated cardiomyopathy (DCM) is a possible cause of syncope in dogs (Ware 2002, O'Grady & O'Sullivan 2004, Wray 2005). Martin *et al* (2010) found the presence of collapse in dogs with DCM had a significant negative association with survival. Oyama & Sisson (2004) found that median plasma cTnI was increased in dogs with cardiomyopathy and that 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.

To summarise, elevated serum cTnI concentrations have been reported in association with a variety of canine cardiac disorders which may result in syncope.

1.4 Aims and Objectives

The main aim of this study was to establish whether there was evidence of raised hscTnI and cTnI concentrations in a population of dogs collapsing with naturally occurring epileptic seizures compared to a control population. Another aim of the study was to examine the clinical utility of serum cTnI and hscTnI for differentiating cardiogenic syncope from epileptic seizures.

2. MATERIALS AND METHODS

2.1 Patient Population

Dogs were prospectively and consecutively recruited. They presented with either episodes of collapse with TLOC (n=79) or were healthy control dogs (n=30). The cases were not age, bodyweight or breed matched. They presented at one of two private practices, ChesterGates Referral Hospital, Cheshire (n=68) or Northwest Surgeons, Cheshire (n=39) in the period February 2011 – May 2013. Two dogs were seen at The University of Liverpool Small Animal Teaching Hospital and investigated by a European College of Veterinary Internal Medicine Companion Animal board-certified cardiologist. All neurological examinations were performed by European College of Veterinary Neurology board-certified neurologists or their residents. The study protocol was approved by the University of Cambridge and University of Liverpool ethical review committees. Owners provided informed written consent prior to participation of their dogs in the study.

2.1.1 Controls

Healthy control animals consisted of 30 dogs all examined and investigated by the author. Most dogs were staff animals and were undergoing blood sampling for other purposes such as screening as part of an annual health check before vaccination, blood transfusion or elective surgery such as neutering. They were free from cardiac disease or any other disease likely to lead to alteration in serum troponin concentration on the basis of a history, physical examination, complete blood count, serum biochemistry and electrolytes result (see Appendix 1), BP measurement, electrocardiographic (ECG) and echocardiographic examination. Dogs were excluded if there was a history of collapse or seizures.

2.1.2 Collapsing Groups

Seventy-nine dogs presenting to either the neurology or cardiology departments for collapse with TLOC were prospectively and consecutively recruited in the period February 2011 – May 2013. They comprised dogs receiving treatment as well as dogs at the first presentation. Inclusion criterion was the history of a collapsing episode involving TLOC within seven days of presentation. Dogs were excluded if their cause of collapse was trauma or metabolic

disorders. Cases with evidence of renal insufficiency were excluded based on serum creatinine concentrations that exceeded the upper limit of the laboratory reference range (>150 umol/L) and urine specific gravity measurement (<1.030). Cases with a history of intoxications were excluded.

Dogs were then grouped according to aetiology of collapse: generalised epileptic seizures with no evidence of cardiac disease, cardiogenic syncope with no history of epilepsy, dogs with both generalised epileptic seizures and cardiac disease, vasovagal syncope or unclassified (where the cause of collapse could not be reliably ascribed to either cardiac or neurological disease, or for which a diagnosis was not reached).

The group with epileptic seizures had to be free of cardiac disease based on physical examination, BP measurement, ECG and echocardiographic examination. All cardiac cases were examined and had BP measurement, ECG, echocardiographic examination and, where appropriate, Holter examination to aid diagnosis.

2.2 Background Characteristics and Clinical Signs

Full history was taken and clinical examination performed. In the case of the epileptic dogs, full neurological examination was performed and recorded (see Appendix 2). In addition, the following were recorded: breed, age (years), gender (male/female), reproductive status (neutered: yes; entire: no), bodyweight (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) were recorded. In the case of the epileptic patients, the following were also recorded: number of seizures during the seven days prior to 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 the average seizure length was recorded).

2.3 Investigations

The following measurements were recorded; serum creatinine, cTnI and hscTnI concentrations, systolic BP measurement, ECG and echocardiography results.

2.3.1 Biochemistry and Biomarkers

Complete blood count, serum biochemistry and electrolytes were carried out to screen for concurrent and causative disease in all cases (see Appendix 1). Blood samples were taken by jugular venipuncture. The laboratories were blinded to the patient history.

2.3.1.1 Serum Creatinine Concentration

Starved blood samples for measurement of creatinine concentration (umol/L) were collected from all 109 cases enrolled onto the study and analysed within 24 hours. The samples were handled as described below for serum cTnI.

2.3.1.2 Serum Cardiac Troponin I and High-sensitivity Cardiac Troponin I

Blood samples for both troponins were collected from all 109 dogs, at least once. Of the 79 dogs with collapse, 15 were sampled twice, and four on three occasions. The length of time (hours) between the collapse and time at which the blood sample taken recorded in each case.

Sample handling (cTnI & hscTnI)

Blood was collected into 1ml serum gel tubes and 1ml plain tubes.

Sample handling: serum cTnI

The serum gel tube samples were separated by centrifugation 30 minutes after being left to stand at room temperature. They were centrifuged at 12,000 g for 150 seconds. The samples were then chilled at 4ºC for up to 12 hours before transportation at ambient temperature to a commercial laboratory (Carmichael Torrence Diagnostic Services Ltd, W Yorks) for analysis using a previously described chemiluminescent immunoassay system [a] (see Appendix 3) (O'Brien *et al* 2006). The cTnI was detected by using enzyme conjugated polyclonal anti-troponin I antibody following protein binding onto beads coated with murine anti-troponin I antibody, using Immulite® Analyser. Both antibodies recognize epitopes between amino acids 33 and 110 of cTnI. The lower limit of detection (LOD) of the assay was 0.02 ng/ml.

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

Sample handling: serum hscTnI

The plain tube samples were separated by centrifugation at 12,000 g for 150 seconds immediately after clotting. The serum was stored up to 12 hours at -18ºC before being transported in frozen cool packs to a commercial laboratory (IDEXX Laboratories [b], Wetherby) for analysis. Prior to analysis, the frozen serum was allowed to thaw slowly at room temperature. Concentrations of hscTnI were measured by a two-site sandwich immunoassay [c] to detect free and complexed troponin (see Appendix 3). The AccuTnI® assay uses two mouse-derived monoclonal antibodies directed against the 24-40 and 41-49 amino acid sequences of cTnI. The lower LOD of the assay was 0.01 ng/ml. The use of this assay has been reported (Adin *et al* 2006, Ljungvall *et al* 2010, Hezzell *et al* 2012) and validated (Oyama & Solter 2004) previously for canine samples.

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

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

2.3.2 Cardiac Investigations

All dogs received a complete physical examination on arrival by the author, in particular evaluating for the presence of a murmur or arrhythmia. The dogs also had cardiac investigations which included systolic BP measurement, electrocardiography and echocardiography. Cardiac investigations were always performed prior to general anaesthesia, the exception being those patients arriving in status epilepticus (SE). SE patients had cardiac investigations and repeat thoracic auscultation delayed until 24 hours following recovery from anaesthesia. The patients underwent full standard 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 during echocardiography. Full M-mode, colour and spectral Doppler studies were performed in order to reach an echocardiographic diagnosis in each case. For each variable, the mean of three measurements was calculated from consecutive cardiac cycles.

Twenty-four hour Holter recordings were offered to all cases. The system used was the Lifecard CF digital Holter recorder (Spacelabs Healthcare). It was attached to each dog using three electrodes (two on the left chest wall, one on the right) following skin preparation

(shaving a vertical strip of fur on either side of the precordium then wiping with alcohol). Holter diaries were kept by either owners or veterinary staff detailing timings of activities such as exercise, sleep, syncopal or seizure episodes. The Holter analysis was performed using Spacelabs Pathfinder software by a cardiologist holding a Royal College of Veterinary Surgeons Diploma in Veterinary Cardiology. Thoracic radiographs (lateral and dorsoventral views) were obtained in those cases where the presence of congestive heart failure or respiratory pathology was suspected.

2.3.3 Systolic Blood Pressure Measurement

Systolic BP (mmHg) using a Doppler device (CAT Doppler BP Kit, Thames Medical or Parks Model 811-B Doppler device) was measured. Five BP readings were taken from the metacarpal artery using the protocol recommended in the American College of Veterinary Internal Medicine consensus statement (Brown *et al* 2007) and the mean calculated.

2.3.4 QT-interval Corrected for Heart Rate (QTc)

Electrocardiography was performed by use of a routine six-lead ECG machine (Esaote P80 Power or Seca CT8000P ECG). Patients were allowed a short period of acclimatisation before a paper-trace ECG recording was taken. The six limb leads were recorded simultaneously for a minimum of 20 consecutive RR intervals, at a paper speed of 50 mm/second and gain of 10 mm/mV.

For control dogs and patients with generalised epileptic seizures (with no cardiac disease), the QT-interval was measured from lead II, and recorded in each case as the mean value from 10 consecutive complexes. The QT-intervals were corrected for heart rate by using Fridericia's cube root formula ($QTc=QT/\sqrt[3]{RR\ interval}$) (Fridericia 1920). The RR interval used in this calculation was taken as the mean RR interval across the 10 preceding complexes. QT-interval was measured from the onset of the Q wave to the intersection between the maximal downslope of the terminal portion of the T wave to the isoelectric baseline. All measurements were taken to the nearest 0.25 mm of ECG paper, corresponding to a resolution of 5 ms duration.

2.3.5 Neurological Investigations

Magnetic resonance imaging (MRI) scans (Vet-MR Grande; Esaote) with gadolinium contrast were obtained in three planes of orientation (dorsal, sagittal and transverse) under general anaesthetic. 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. In those cases without suspected raised intracranial pressure, cisternal cerebrospinal fluid (CSF) analyses were performed to obtain a diagnosis. The CSF samples were chilled at 4ºC for up to 12 hours before transportation at ambient temperature to a commercial laboratory (Carmichael Torrence Diagnostic Services Ltd, W Yorks) for analysis (see Appendix 1). Additional blood tests such as Toxoplasma and Neospora serology were performed by the attending clinician depending on the individual case. Electroencephalography was also performed in one SE case under anaesthesia to assess for seizure activity.

2.4 Categorisation of Patients

2.4.1 Categorisation Part 1: Serum Cardiac Troponin I & High-sensitivity Cardiac Troponin I Concentrations in Epileptic Patients and Controls

Definitive diagnosis of the cause of the generalised epileptic seizures in each case was made by evaluation of all contributing evidence. Dogs with idiopathic epilepsy were all younger than six years old at seizure onset and had recurrent seizures, were normal on interictal neurological and laboratory examination. They did not have any evidence of neurological disease other than seizures at any time 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 necrotising meningoencephalitis diagnosis based on MRI and CSF results. The 30 epileptic patients were then further divided into dogs with and without cluster seizures (Figure 2). Cluster seizures were defined as two or more seizures occurring within a 24 hour period in which the patient regained consciousness between the seizures (Thomas 2010).

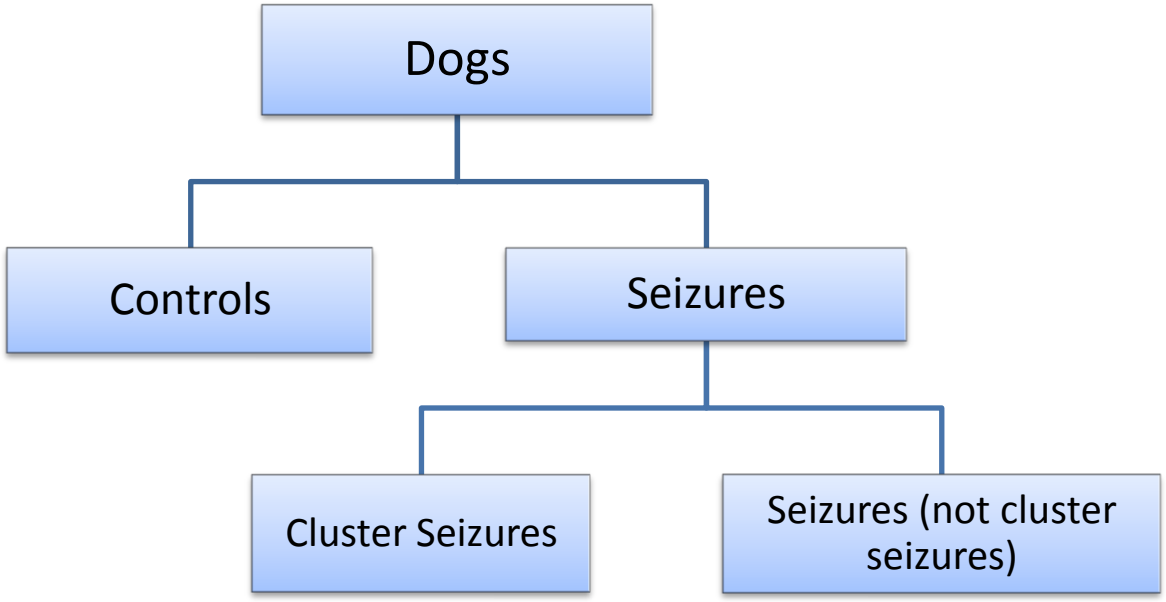


Figure 2. Hierarchy Charts Depicting Group Categorisation for Part 1.

2.4.2 Categorisation Part 2: Serum Cardiac Troponin I & High-sensitivity Cardiac Troponin I Concentrations in Patients with Syncope or Seizure

All cases presenting for collapse with TLOC were categorised according to the underlying cause following extensive investigations detailed in sections 2.3.1 – 2.3.5 (Figure 3).

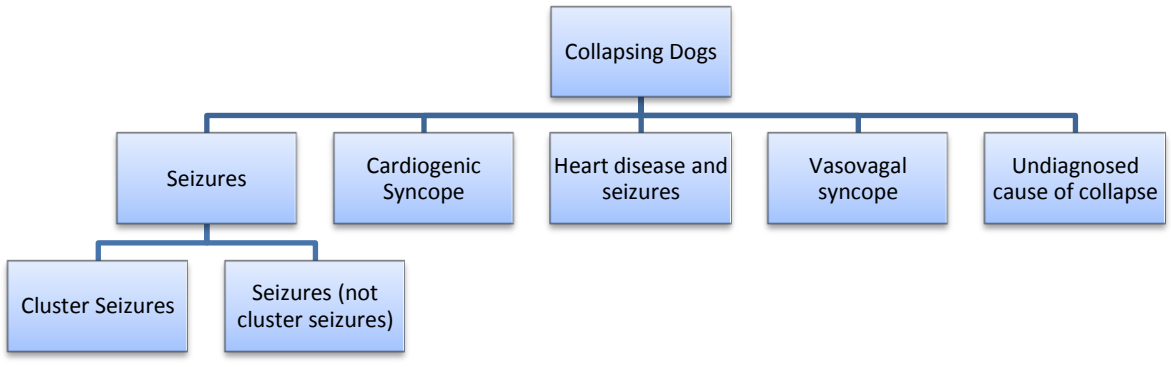


Figure 3. Hierarchy Charts Depicting Group Categorisation for Part 2.

2.5 Statistical Analysis

Results were analysed using SigmaPlot® 12 software (Systat Software inc) and Minitab (Version 16.2.3). The age distribution, bodyweights, concentrations of creatinine, cTnI and hscTnI of the groups were compared by the Mann-Whitney U tests (equality-of-populations rank test). Data were assessed graphically for normality. In most cases, data were non-parametric, in which case, analysis of variance (ANOVA) on Ranks was performed (Kruskal-Wallis) to identify significant difference among the groups ($p<0.05$). A chi-squared test was used to assess the difference of proportions of sex and also reproductive status between the various groups. The QTc-intervals were compared between the epilepsy and control groups using *t*-tests. BP measurements were compared between groups using *t*-tests or ANOVA. Results are tabulated and presented as box and whisker plots or bar charts as appropriate with the use of Weibull scale where necessary to improve visual interpretation. The cTnI and hscTnI data were log transformed for the regression analysis to achieve a normal distribution.

Part 1 cTnI & hscTnI concentrations with seizures

A multivariable stepwise linear regression analysis was performed to determine which of the following variables were significantly associated with each of the two troponin concentrations measured in the epileptic group. Variables examined were number of seizures (during the seven days prior to presentation), seizure length (minutes), serum creatinine concentration (umol/L), group, age (years), gender (male or female), reproductive status (entered as binary variables yes [neutered] or no [entire]), bodyweight (kg), time since seizure (hours), medication being administered at presentation (yes or no), systolic BP (mmHg), QTc (ms). Stepwise criteria were probability less than 0.05 for inclusion and greater than 0.2 for exclusion. A p value of <0.05 was considered significant.

Paired *t*-tests were used to assess the significance of changes in serum cTnI concentration after treatment of seizures in individual dogs. If the data were non-parametric, Wilcoxon Signed Rank test was performed.

Part 2 cTnI & hscTnI concentrations with collapse and TLOC

In all dogs, a multivariable stepwise linear regression analysis was performed to determine which variables were significantly associated with each of the two troponin concentrations measured. Variables examined were serum creatinine concentration (umol/L), disease group, age (years), gender (male or female), reproductive status (entered as binary variables yes [neutered] or no [entire]), bodyweight (kg), time since collapse (hours), medication (being administered at presentation), systolic BP (mmHg). Stepwise criteria were probability less than 0.05 for inclusion and greater than 0.2 for exclusion. A p value of <0.05 was considered significant. For the purpose of the analysis, samples in which the cTnI and hscTnI concentrations were below the LOD of the assays were ascribed a value of 0.02 ng/ml and 0.01ng/ml respectively.

The strength of the relationship between cTnI and hscTnI was assessed by Pearson's correlation coefficient. Receiver operator curve (ROC) analysis was used to determine the ability of cTnI and hscTnI concentration to discriminate between cardiac and noncardiac causes of collapse. The noncardiac causes were separated further into those with presence of heart disease and those without. Area under curve (AUC), standard error and 95% confidence intervals (CI) of the ROC analysis for cTnI and hscTnI were calculated. Sensitivity and specificity of classification for the hscTnI and cTnI cut-off values, with the highest percentage of correct classification were estimated for pairwise group comparisons of dogs belonging to the cardiac group and the noncardiac group. The positive and negative predictive values were calculated.

3. RESULTS

3.1 Patient Population

One hundred and nine dogs were enrolled onto the study. Age ranged from 0.3 to 15 years. Patients comprised 15 female, 40 female neutered, 18 male and 36 male neutered dogs. The majority were cross bred. The Labrador retriever was the most represented of the pedigree breeds (n=10); see Appendix 4, Table 1.

3.2 Disease Categorisation

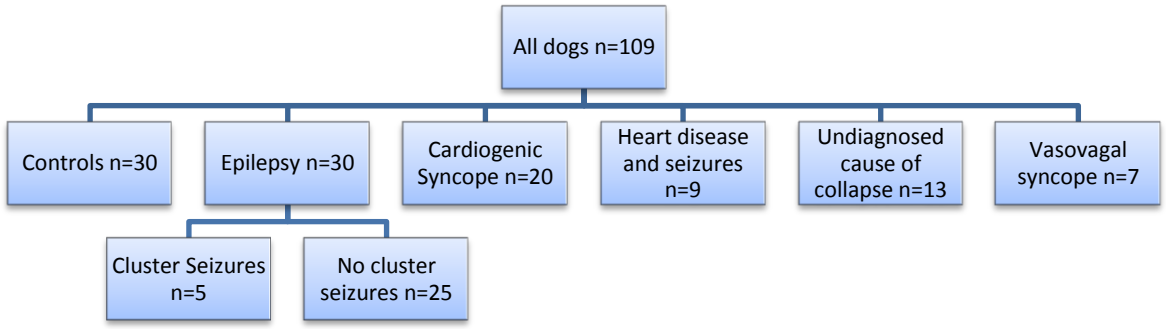


Figure 4. Dogs categorised by disease group.

Thirty control dogs, group N, were considered normal based on history, physical examination, results of cardiac investigations and laboratory tests. Seventy-nine cases were grouped according to aetiology of collapse with TLOC (see Figure 4).

The epilepsy group, E, comprised 27 dogs with idiopathic epilepsy, two dogs with brain tumours (suspected meningioma and suspected glioma) and one dog with necrotising meningoencephalitis. The 30 seizuring dogs were then divided into five with cluster seizures, group CS, and 25 without, group S. All five CS dogs had idiopathic epilepsy. In group S, one dog had a suspected diagnosis of idiopathic epilepsy which has been published as a case report (Motta & Dutton 2013). Three epileptic cases had SE during the 24 hours prior to presentation.

The cardiogenic syncope group, C, 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 dog with cardiac neoplasia causing secondary right ventricular outflow tract obstruction, one dog with myxomatous degeneration of the mitral valve, left-sided congestive heart failure and systolic pulmonary hypertension and one dog with myocardial failure (DCM).

Of the nine dogs with both cardiac disease and seizures, group B, all nine were collapsing as a result of epilepsy and not cardiac disease. Only two dogs in the group of seven with vasovagal syncope, group V, were definitively diagnosed based on history and Holter analysis (see Appendix 5). The remaining five patients had suspected vasovagal syncope. There were 13 dogs whereby no diagnosis was reached (unclassified group, U).

3.3 Background Characteristics and Clinical Signs Part 1a

3.3.1 Breed Specification

A total of 37 breeds were included (Appendix 4, Tables 1 and 2), the majority being cross bred. The most common epileptic breed was the Labrador retriever. In the control group, the Jack Russell terrier was most commonly represented. No statistical comparison of breeds between groups was undertaken as numbers were small and the breeds diverse.

3.3.2 Age, Gender, Reproductive Status and Bodyweight

Age range for group N was from 0.6 to 15 years and 0.3 to 10.5 years for group E. There was no significant difference between the ages ($p=0.487$) or bodyweights ($p=0.579$) of these two groups (see Table 1). The proportions of males ($p=0.411$) and neutered animals ($p=0.399$) were not significantly different between groups E and N.

Table 1. Background characteristics according to group. MW Mann Whitney Rank Sum, p value given.				
	Total n=60	N (normal controls) n=30	E (epilepsy) n=30	Test p value
Age (years). Median & range.	4 0.3-15	4.4 0.6-15	3.9 0.3-10.5	MW p=0.487
Percentage male. n=number.	47% n=28	40% n=12	53% n=16	Chi-square p=0.411
Percentage neutered.	70% n=42	77% n=23	63% n=19	Fisher's Exact test p=0.399
Bodyweight (kg). Median & range.	14.7 3.2-49.2	14.4 3.2-40.8	15.1 5.5-49.2	MW p=0.579

3.3.3 Time Since Collapse and Medications

The number of hours between the seizure and time at which blood samples were taken was recorded in each case (see Tables 2 and 3). One dog arrived in SE therefore 0 hours was recorded.

Table 2. Clinical findings according to group.			
	Total	N (normal controls)	E (epilepsy)
Time since collapse (hours). Median & range.	42 0-168 n=30	N/A	42 0-168 n=30
% receiving medications at first presentation.	30% n=60	0% n=30	60% n=30

In the epileptic group, 18 cases were taking anti-epileptic medications at presentation. These included phenobarbitone, levetiracetam, potassium bromide, gabapentin and diazepam. Other medications included oral antibiotics (n=1) and injectable dexamethasone (n=1). None of the control dogs were receiving medication at initial presentation (see Table 2).

3.3.4 Number of Seizures

For the purpose of statistical analysis, the number of seizures suffered by the patient during the seven days prior to presentation was ranked as either low (1–2 seizures), medium (3–5

seizures) or high (6 or more seizures). The breakdown and classification for each individual case is shown (Table 3).

Table 3. Descriptive table for number and length of seizures, and time since seizure in the group of 30 epileptic dogs.

CASE NUMBER	NUMBER OF SEIZURES (during 7 days prior to presentation)	GROUP (S or CS)	RANK (low, medium or high)	SEIZURE LENGTH (minutes)	TIME SINCE SEIZURE (hours)
1	6	S	high	0.5	1
2	3	S	med	2.0	144
3	1	S	low	1.0	23
4	2	S	low	5.0	96
5	2	S	low	1.0	12
6	4	S	med	4.0	96
7	1	S	low	5.0	72
8	1	S	low	5.0	48
9	4	S	med	0.5	72
10	1	S	low	10.0	96
11	2	S	low	3.0	24
12	1	S	low	7.0	30
13	1	S	low	20.0	6
14	3	S	med	2.0	120
15	1	S	low	3.0	36
16	2	S	low	5.0	24
17	1	S	low	5.0	168
18	2	S	low	1.0	72
19	1	S	low	30.0	96
20	1	S	low	3.0	168
21	1	S	low	30.0	120
22	2	S	low	2.0	48
23	1	S	low	20.0	120
24	7	S	high	2.0	2
25	6	S	high	0.5	3
26 (CS Case 1)	3	CS	med	1.0	14
27 (CS Case 2)	>10	CS	high	0.5	16
28 (CS Case 3)	9	CS	high	30.0	0
29 (CS Case 4)	12	CS	high	3.0	9
30 (CS Case 5)	>10	CS	high	30.0	24

S, epileptic patients with no cluster seizures; CS, epileptic patients with cluster seizures.

Eighteen patients suffered a low number of seizures, five patients were ranked in the medium category and seven in the high category. Four of the five epileptic patients with cluster seizures were ranked as high. All three SE patients were ranked as high. The median number

of seizures suffered by the epileptic patients was two, with numbers ranging between one and twelve. There was no history of seizures in the control group.

3.3.5 Seizure Length

The seizure length for all 30 epileptic patients ranged between half a minute and 30 minutes, with the median length being three minutes. For the purpose of statistical analysis, those seizures lasting seconds were categorised as half a minute (see Table 3).

3.4 Investigations

3.4.1 Cardiac Investigations

No significant ECG or echocardiographic abnormalities were detected in either of the two groups, other than one epileptic patient (group CS) that had arrived in SE. Following treatment and, whilst receiving anti-epileptic medications, a sinus bradycardia (60 beats/minute) was recorded on ECG examination (see Appendix 6). Echocardiographic examination showed mild left ventricular dilation (see Appendix 6) and trivial mitral and tricuspid regurgitation, with no detectable structural valve abnormalities. The echocardiographic changes seen were attributed to the bradycardia. Repeat echocardiography performed three weeks later in normal sinus rhythm (120 beats/minute) was unremarkable, indicating that the changes detected were reversible.

Twenty-four hour Holter recordings were performed in only 35 dogs as the majority of owners declined the test. Three dogs from group E suffered a seizure during the Holter recordings and sinus tachycardia was the predominant rhythm during two of the three episodes. No life-threatening cardiac arrhythmias were detected although one patient had occasional ventricular premature complexes (see Appendix 5).

3.4.2 Systolic Blood Pressure Measurement

One epileptic patient had cardiac examination only, with no cardiac investigations performed due to owner time constraints. As a result, one BP measurement was missing from the group E. This patient had full neurological examination and investigations performed. Thoracic auscultation was unremarkable with no murmur or arrhythmia detected.

Blood pressure values were not significantly different between groups E and N, p=0.442 (Table 4).

Table 4. BP measurement and QT-interval corrected for heart rate (QTc-interval).

	Total	N (normal controls)	E (epilepsy)	Test p value
Systolic BP (mmHg). Mean & range.	139 100-180 n=59	138 100-180 n=30	142 100-178 n=29	t-test p=0.442
QTc-interval (ms). Mean & range.	230.6 194-260 n=54	229.4 194-260 n=27	231.8 203-252 n=27	t-test p=0.526

3.4.3 QTc-interval

A total of fifty-four ECGs from the two groups were collected. Five ECGs were excluded from the study owing to an excessive baseline artefact that prevented accurate measurements. One epileptic patient had no ECG examination. There was no significant difference in QTc-interval between groups E and N (p=0.526) (Table 4).

3.4.4 Serum Creatinine Concentration

There was no significant difference in serum creatinine concentrations between groups E and N, p=0.636 (see Table 5). Two epileptic dogs had raised serum creatinine concentrations (>150 umol/L), however free catch urine samples obtained at the time of blood sampling were concentrated (urine specific gravity >1.030). As both cases had repeat blood tests at separate dates with creatinine concentrations <150 umol/L, the changes were attributed to pre-renal azotaemia, possibly a result of dehydration.

Table 5. Serum creatinine concentration according to group. MW Mann Whitney Rank Sum, p value given.

	Total n=60	N (normal controls) n=30	E (epilepsy) n=30	Test p value
Serum creatinine concentration (umol/L). Median & range.	85.5 48-153	90 63-140	82.5 48-153	MW p=0.636

3.4.5 Serum Cardiac Troponin I Concentration

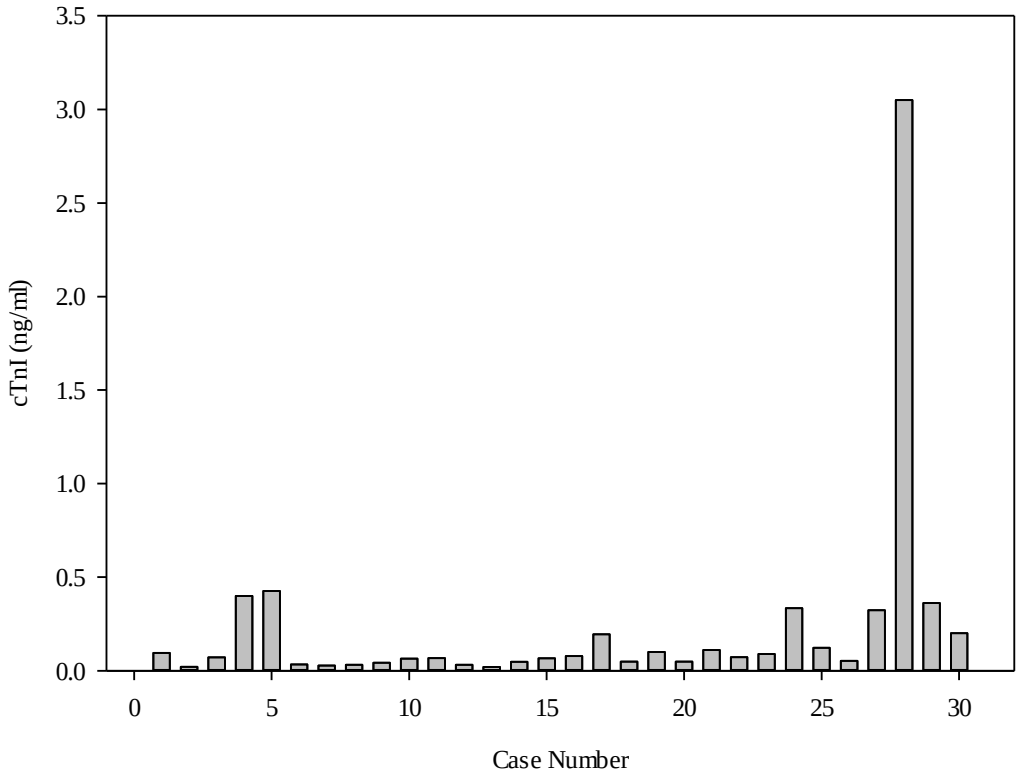


Figure 5. Vertical bar chart showing individual serum cTnI concentrations within the epilepsy group.

The serum cTnI concentrations ranged from 0.02 to 0.126 ng/ml in the control population and from 0.02 to 3.05 ng/ml in the epilepsy group (see Figure 5, Tables 6 and 7).

Table 6. Serum cardiac troponin I (cTnI) concentrations of the control and epileptic dogs.

Case Number	Controls (group N) n=30 Serum cTnI concentration (ng/ml)	Epileptics without cluster seizures (group S) n=25 Serum cTnI concentration (ng/ml)	Epileptics with cluster seizures (group CS) n=5 Serum cTnI concentration (ng/ml)
1	0.097	0.095	0.053
2	0.103	0.021	0.324
3	0.028	0.072	3.050
4	0.120	0.400	0.362
5	0.020	0.426	0.201
6	0.020	0.034	
7	0.110	0.028	
8	0.062	0.032	
9	0.020	0.043	
10	0.028	0.065	
11	0.108	0.068	
12	0.050	0.032	
13	0.037	0.020	
14	0.116	0.048	
15	0.041	0.067	
16	0.126	0.079	
17	0.058	0.195	
18	0.020	0.049	
19	0.048	0.100	
20	0.086	0.049	
21	0.044	0.111	
22	0.020	0.073	
23	0.120	0.089	
24	0.029	0.334	
25	0.020	0.123	
26	0.049		
27	0.058		
28	0.059		
29	0.039		
30	0.052		

When comparing serum cTnI concentrations between groups E and N, there was a statistically significant difference between them, p=0.024 (Table 7 and Figure 6).

Table 7. Serum cardiac troponin I (cTnI) concentrations according to group. MW Mann Whitney Rank Sum, p value given.				
	Total n=60	N (normal controls) n=30	E (epilepsy) n=30	Test p value
Serum cTnI concentration (ng/ml). Median & range.	0.059 0.02-3.05	0.05 0.02-0.126	0.073 0.02-3.05	MW p=0.024

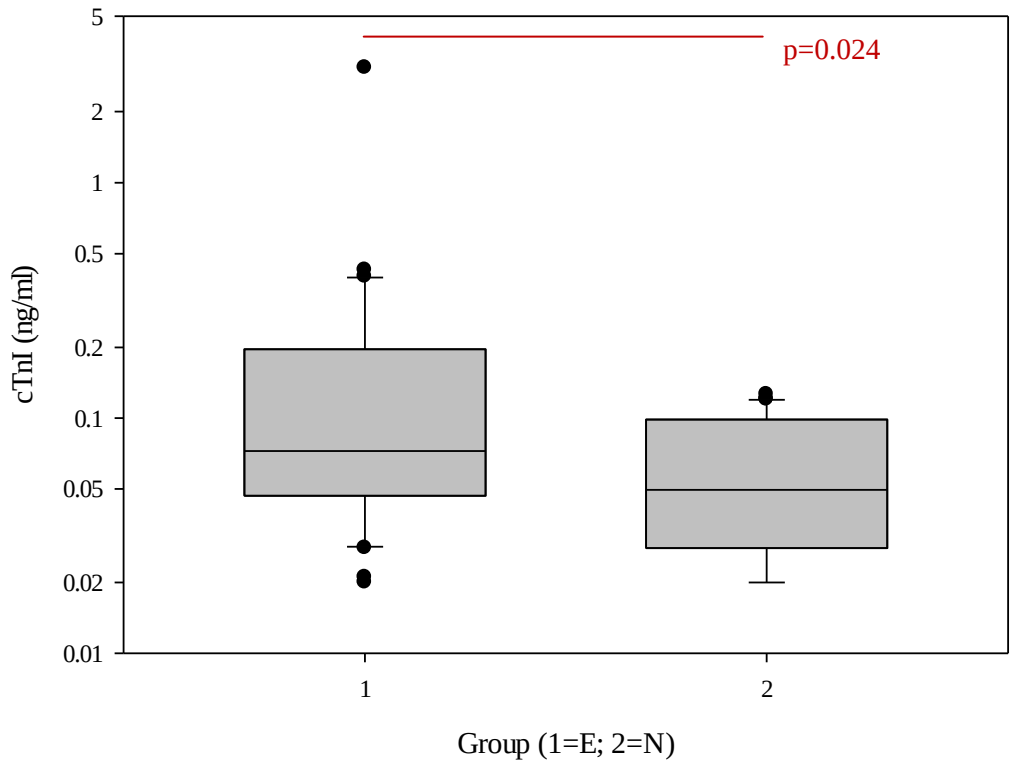


Figure 6. Weibull box plots to show comparison of serum cTnI concentrations between group E (n=30) and group N (n=30).

E, epilepsy group; N, control group.

The horizontal line in each box is the median value. The boxes are the 25th-75th percentiles, whiskers are 10th and 90th. The circles are outliers. Line joins pair with significant difference, p value given.

3.4.6 Serum High-sensitivity Cardiac Troponin I Concentration

The serum hscTnI concentrations ranged from 0.01 to 0.05 ng/ml in the control population and from 0.01 to 1.92 ng/ml in the epilepsy group (see Tables 8 and 9).

Table 8. Serum high sensitivity cardiac troponin I (hscTnI) concentrations of the control and epileptic dogs.

Case Number	Controls (group N) n=30 Serum hscTnI concentration (ng/ml)	Epileptics without cluster seizures (group S) n=25 Serum hscTnI concentration (ng/ml)	Epileptics with cluster seizures (group CS) n=5 Serum hscTnI concentration (ng/ml)
1	0.02	0.02	0.02
2	0.04	0.02	0.08
3	0.01	0.04	1.92
4	0.02	0.09	0.10
5	0.01	0.11	0.05
6	0.01	0.02	
7	0.04	0.02	
8	0.02	0.02	
9	0.01	0.01	
10	0.01	0.03	
11	0.04	0.03	
12	0.01	0.02	
13	0.01	0.01	
14	0.02	0.03	
15	0.01	0.02	
16	0.05	0.04	
17	0.01	0.07	
18	0.02	0.02	
19	0.02	0.03	
20	0.02	0.01	
21	0.01	0.03	
22	0.01	0.02	
23	0.04	0.04	
24	0.01	0.06	
25	0.01	0.06	
26	0.02		
27	0.02		
28	0.01		
29	0.02		
30	0.03		

When comparing serum hscTnI concentrations between groups E and N, there was a statistically significant difference between them, p=<0.001 (Table 9 and Figure 7).

Table 9. Serum high sensitivity cardiac troponin I (hscTnI) concentrations according to group.
MW Mann Whitney Rank Sum, p value given.

	Total n=60	N (normal controls) n=30	E (epilepsy) n=30	Test p value
Serum hscTnI concentration (ng/ml). Median & range.	0.02 0.01-1.92	0.02 0.01-0.05	0.03 0.01-1.92	MW p=<0.001

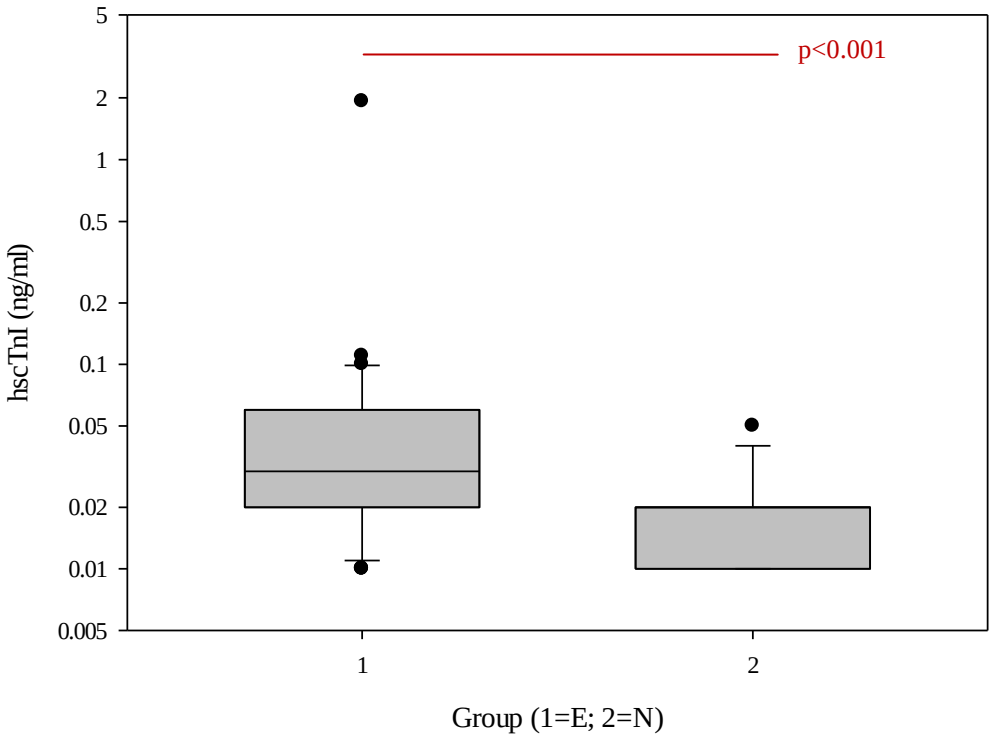


Figure 7. Weibull box plots to show comparison of serum hscTnI concentration between group E (n=30) and group N (n=30).

E, epilepsy group; N, control group.

The horizontal line in each box is the median value. The boxes are the 25th-75th percentiles, whiskers are 10th and 90th. One box is missing a whisker as the lower quartile is equal to the minimum value. The circles are outliers. Line joins pair with significant difference, p value given.

3.5 Background Characteristics and Clinical Signs Part 1b

In Part 1b, the epileptic patients were further divided into those with cluster seizures (group CS) and those without cluster seizures (group S).

Table 10. Background characteristics according to group, p value given.

	Total n=60	Group N n=30	Group S n=25	Group CS n=5	Test p value
Age (years). Median & range.	4 0.3-15	4.4 0.6-15	3.8 0.3-10.5	5.5 2.6-8.7	Kruskal Wallis rank test p=0.233
Percentage male. n=number.	47% n=28	40% n=12	52% n=13	60% n=3	Chi-squared test p=0.555
Percentage neutered.	70% n=42	77% n=23	64% n=16	60% n=3	Chi-squared test p=0.522
Bodyweight (kg). Median & range.	14.7 3.2-49.2	14.4 3.2-40.8	15.3 5.5-49.2	13.1 6.1-27.1	Kruskal Wallis rank test p=0.754

Group N, controls; Group S, epileptic patients with no cluster seizures; Group CS, epileptic patients with cluster seizures

3.5.1 Age, Gender, Reproductive Status and Bodyweight

There was no significant difference in age (p=0.233) or bodyweight (p=0.754) between groups CS, N and S. The proportions of males (p=0.555) and neutered animals (p=0.522) were not significantly different between groups CS, N and S (see Table 10).

3.5.2 Time Since Collapse and Medications

The number of hours between the seizure and time at which blood samples were taken, ranged from 0 to 24 hours in group CS, and from 1 to 168 hours in group S (see Table 11).

Table 11. Clinical findings according to group.

	Total	Group N (normal controls)	Group S (epileptic patients with no cluster seizures)	Group CS (epileptic patients with cluster seizures)
Time since collapse (hours). Median & range.	42 0-168 n=30	N/A	72 1-168 n=25	14 0-24 n=5
% receiving medications at first presentation.	30% n=60	0% n=30	56% n=25	80% n=5

In group CS, four patients were receiving medication at presentation which included phenobarbitone (n=4), levetiracetam (n=2), potassium bromide (n=2), diazepam (n=2) or gabapentin (n=1). Fourteen patients in group S were taking medication at initial presentation (see Table 11).

3.6 Investigations

3.6.1 Systolic Blood Pressure Measurement

There was no significant difference in BP measurement between groups N, S and CS (see Table 12).

Table 12. Systolic BP measurements according to group.
ANOVA, analysis of variance.

	Total	Group N (normal controls)	Group S (epileptic patients with no cluster seizures)	Group CS (epileptic patients with cluster seizures)	Test p value
Systolic BP (mmHg). Mean &range.	139 100-180 n=59	138 100-180 n=30	139 100-170 n=24	153 140-178 n=5	One Way ANOVA p=0.254

3.6.2 Serum Creatinine Concentration

There was no significant difference in serum creatinine concentration between groups CS, N and S, p=0.372 (see Table 13).

Table 13. Serum creatinine concentration according to group, p value given.

	Total n=60	Group N n=30	Group S n=25	Group CS n=5	Test p value
Serum creatinine concentration (umol/L). Median & range.	85.5 48-153	90 63-140	85 48-153	78 73-88	Kruskal Wallis rank test p=0.372

Group N, controls; Group S, epileptic patients with no cluster seizures; Group CS, epileptic patients with cluster seizures

3.6.3 Serum Cardiac Troponin I Concentration

There was a significant difference in serum cTnI concentration between groups CS, N and S, p=0.008 (see Table 14 and Figure 8).

Table 14. Serum cardiac troponin I (cTnI) concentrations according to group, p value given.

	Total n=60	Group N n=30	Group S n=25	Group CS n=5	Test p value
Serum cTnI concentration (ng/ml). Median & range.	0.059 0.02-3.05	0.05 0.02-0.126	0.068 0.02-0.426	0.324 0.053-3.05	Kruskal Wallis rank test p=0.008

Group N, controls; Group S, epileptic patients with no cluster seizures; Group CS, epileptic patients with cluster seizures

A pairwise multiple comparison procedure was performed (Dunn's Method) which showed that there were significant differences in serum cTnI concentration between groups CS and N ($p<0.05$). There was no significant difference in cTnI concentration between groups S and N ($p>0.05$) or between groups S and CS ($p>0.05$).

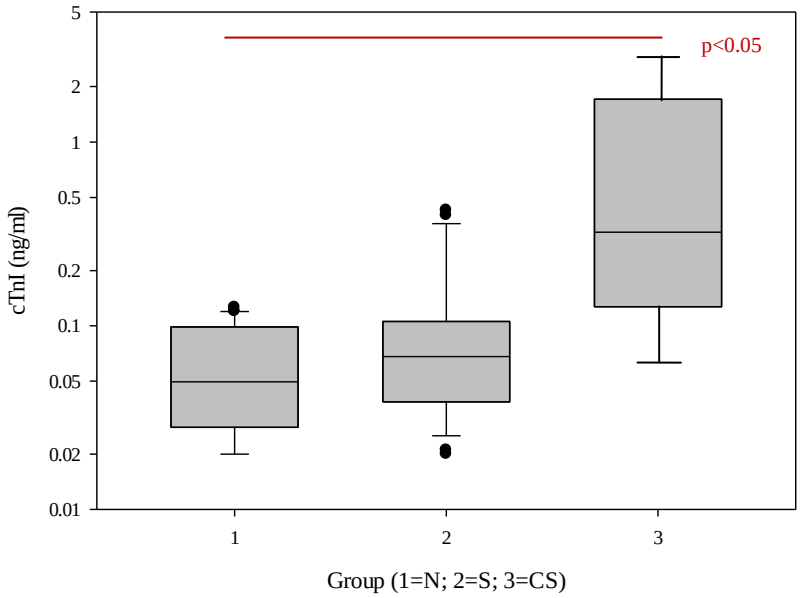


Figure 8. Weibull box plots to show comparison of serum cTnI concentrations between groups.

N, controls; S, epileptic patients with no cluster seizures; CS, epileptic patients with cluster seizures.

The horizontal line in each box is the median value. The boxes are the 25th-75th percentiles, whiskers are 10th and 90th. The circles are outliers. Line joins pair with significant difference, p value given.

3.6.4 Serum Cardiac Troponin I Concentration with Treatment of Seizures

Of the 15 dogs that were sampled more than once, five were epileptic with cluster seizures. All five dogs responded to anti-epileptic medications in the hospital and the seizures resolved. Four of the five dogs showed a reduction in serum cTnI concentration and one remained similar (this dog did not have raised cTnI levels according to the laboratory reference interval). The differences, however, did not reach significance, p=0.125 (Figure 9).

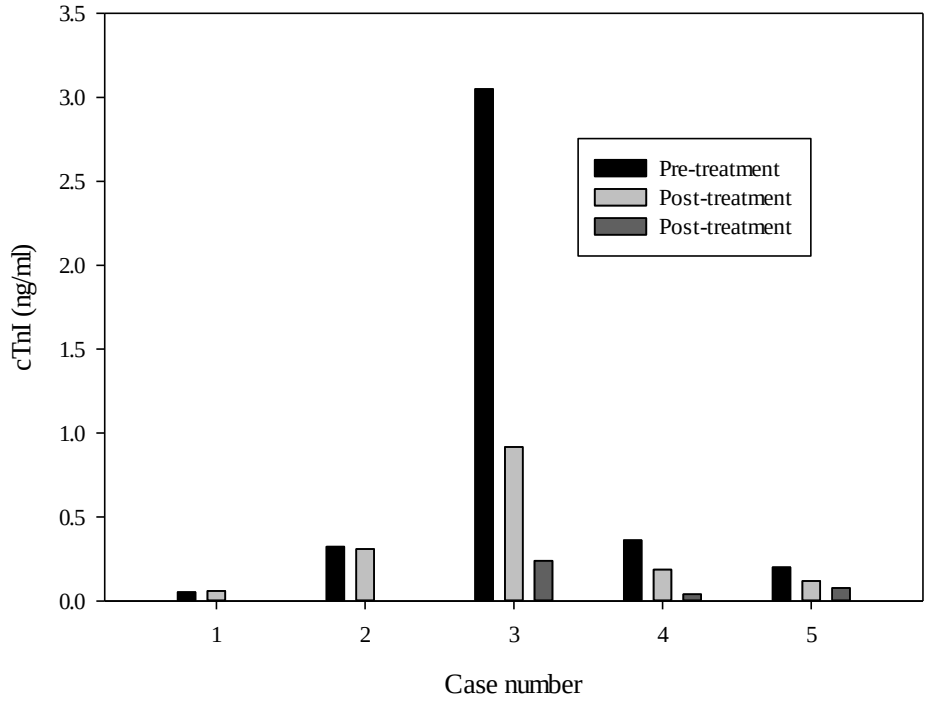


Figure 9. Serum cTnI concentrations before and after treatment of seizures for dogs with cluster seizures.

The length of time between presentation and repeat blood samples varied between patients (see Table 15).

Table 15. Length of time (days) between presentation (day 0) and repeat blood samples.

	First blood sample (day 0)	Second blood sample	Third blood sample
Case 1	0	1	N/A
Case 2	0	2	N/A
Case 3	0	4	26
Case 4	0	1	29
Case5	0	4	5

3.6.5 Serum High-sensitivity Cardiac Troponin I Concentration

The epileptic group was further divided into those with and without cluster seizures (see Table 16 and Figure 10).

Table 16. Serum high sensitivity cardiac troponin I concentrations (hscTnI) according to group, p value given.

	Total n=60	Group N n=30	Group S n=25	Group CS n=5	Test p value
Serum hscTnI concentration (ng/ml). Median & range.	0.02 0.01-1.92	0.02 0.01-0.05	0.03 0.01-0.11	0.08 0.02-1.92	Kruskal Wallis rank test p<0.001

Group N, controls; Group S, epileptic patients with no cluster seizures; Group CS, epileptic patients with cluster seizures

There was a significant difference in serum hscTnI concentration between the groups, N, S and CS, p=<0.001. A pairwise multiple comparison procedure was performed (Dunn's Method) which showed significant differences in hscTnI concentration between groups N and S, and between groups N and CS (p<0.05). There was no significant difference between groups CS and S (p>0.05).

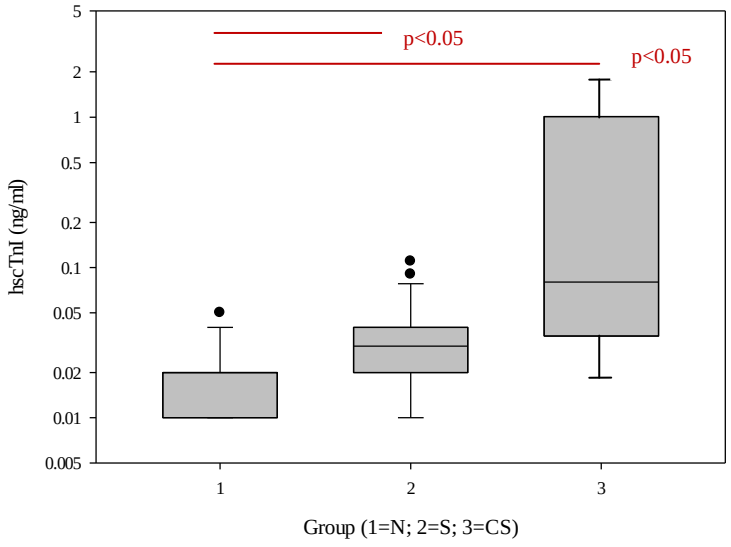


Figure 10. Weibull box plots to show comparison of serum hscTnI concentration according to group.

N, controls; S, epileptic patients with no cluster seizures; CS, epileptic patients with cluster seizures. The horizontal line in each box is the median value. The boxes are the 25th-75th percentiles, whiskers are 10th and 90th. One box is missing a whisker as the lower quartile is equal to the minimum value. The circles are outliers. Lines join pairs with significant difference, p values given.

3.7 Regression Analysis

As the serum cTnI concentrations were significantly different between groups CS and N ($p<0.05$) (see section 3.6.3), regression analysis was performed to examine which variables affected cTnI concentrations in the epileptic group.

3.7.1 Predictor Variables for log cTnI in the Epileptic Group

The cTnI concentrations were log transformed for the multivariable linear regression analysis to bring the data more in line with a normal distribution. Identification of those factors which most influenced log cTnI showed that the three variables seizure number, age and receiving medications significantly affected log cTnI concentration, $p<0.05$ (see Table 17). Seizure length, group, creatinine concentration, QTc-interval, time since seizure, bodyweight, reproductive status, gender and systolic BP did not significantly affect cTnI concentration ($p>0.05$).

Table 17. Analysis of variance for log cTnI, using Adjusted Sum of Squares (Adj SS) for tests.
DF (degrees of freedom), Seq SS (sequential sums of squares), Adj MS (adjusted mean of squares), F (f-statistic).

Source	DF	Seq SS	Adj SS	Adj MS	F	p value
Age	1	1.50622	0.97171	0.97171	14.2	**0.000**
Time from seizure	1	0.11004	0.18472	0.18472	2.7	0.107
Group (N, S, CS)	2	2.50166	0.19557	0.09778	1.43	0.250
Number of seizures	2	1.20642	1.21960	0.60980	8.91	**0.001**
Receiving Medications	1	0.29618	0.36443	0.36443	5.33	**0.026**
Reproductive status	1	0.16103	0.11674	0.11674	1.71	0.198
QTc-interval	1	0.12945	0.12945	0.12945	1.89	0.176
Error	44	3.01039	3.01039	0.06842		
Total	53	8.92139				

Group N, controls; Group S, epileptic patients with no cluster seizures; Group CS, epileptic patients with cluster seizures; Number of seizures ranked high, low, medium.

Standard deviation (S) = 0.272875 Correlation squared (R-Sq) = 61.92%

R-Sq (adj) = 55.94%

Table 18. Regression coefficient values.				
Term	Regression Coefficient	Standard Error Coefficient	T	p value
Constant	-0.4057	0.6569	-0.62	0.540
Age	0.04936	0.01310	3.77	**0.000**
Time from seizure	0.002153	0.001310	1.64	0.107
QTc-interval	-0.003891	0.002829	-1.38	0.176

The regression coefficient for age indicated that as age increased, cTnI concentration also increased, p=0.000 (see Table 18).

3.7.2 Predictor Variables for log hscTnI in the Epileptic Group

The hscTnI data were also log transformed for the multivariable linear regression analysis. The two variables, seizure number and age, significantly affected log hscTnI concentration, $p<0.05$ (see Table 19). Neutered status, seizure length, group, creatinine concentration, QTc-interval, time since seizure, bodyweight, receiving medications, gender and systolic BP did not significantly affect log hscTnI concentration ($p>0.05$).

Table 19. Analysis of variance for log hscTnI, using Adjusted Sum of Squares (Adj SS) for tests.
DF (degrees of freedom), Seq SS (sequential sums of squares), Adj MS (adjusted mean of squares), F (f-statistic).

Source	DF	Seq SS	Adj SS	Adj MS	F	p value
Group (N, S, CS)	2	2.96763	0.38939	0.19470	2.66	0.081
Age	1	1.10044	0.73326	0.73326	10.01	**0.003**
Weight	1	0.07582	0.12124	0.12124	1.65	0.205
Number of seizures	2	0.53111	0.62263	0.31131	4.25	**0.021**
Time from seizure	1	0.02996	0.09696	0.09696	1.32	0.256
Receiving Medications	1	0.22419	0.23372	0.23372	3.19	0.081
QTc-interval	1	0.09670	0.09670	0.09670	1.32	0.257
Error	44	3.22421	3.22421	0.07328		
Total	53	8.25006				

Group N, controls; Group S, epileptic patients with no cluster seizures; Group CS, epileptic patients with cluster seizures; Number of seizures ranked high, low, medium.

Standard deviation (S) = 0.270698 Correlation squared (R-Sq) = 60.92%

R-Sq (adj) = 52.93%

Table 20. Regression coefficient values.				
Term	Regression Coefficient	Standard Error Coefficient	T	p value
Constant	-0.8871	0.7858	-1.13	0.265
Age	0.04007	0.01267	3.16	**0.003**
Weight	0.005597	0.004351	1.29	0.205
Time from seizure	0.001512	0.001315	1.15	0.256
QTc-interval	-0.004039	0.003516	-1.15	0.257

The regression coefficient for age indicated that as age increased, hscTnI concentration also increased, p=0.003 (see Table 20).

3.8 Background Characteristics and Clinical Signs Part 2

3.8.1 Breed Specification

The collapse with TLOC breeds are outlined in Table 3, Appendix 4. The cavalier King Charles spaniel was the most common breed in group B. The boxer was most commonly represented in group V.

Table 21. Background characteristics according to collapsing group, p value given.							
	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	Test & p value
Age (years). Median & range.	5 0.3-15	3.9 0.3-10.5	7.7 0.8-15	4.5 0.8–12.3	8.8 2-15	2 0.7-9.5	One Way ANOVA (followed by Holm Sidak test) p<0.001
Percentage male. n=number.	53% n=42	53% n=16	55% n=11	44% n=4	54% n=7	57% n=4	Chi-squared test p=0.986
Percentage neutered.	67% n=53	63% n=19	75% n=15	67% n=6	85% n=11	29% n=2	Chi-squared test p=0.122
Bodyweight (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	Kruskal Wallis rank test p=0.258

3.8.2 Age, Gender, Reproductive Status and Bodyweight

Age ranged from 0.8 to 15 years for dogs in group C, 0.8 to 12.3 years in group B, 0.7 to 9.5 years in group V and from 2 to 15 years in group U (Table 21). When the ages of all the collapsing dogs were compared (Figure 11), the dogs in group C were significantly older than those in group E, p=0.015. There were significant differences in age between groups U and E, (p=0.003) and between groups U and V (p=0.023). The Pairwise Holm-Sidak test was used.

The proportions of males (p=0.986) and neutered animals (p=0.122) were not significantly different between the collapsing groups C, E, B, U and V (Table 21). There was no significant difference in bodyweight between the collapsing groups, p=0.258 (see Table 21).

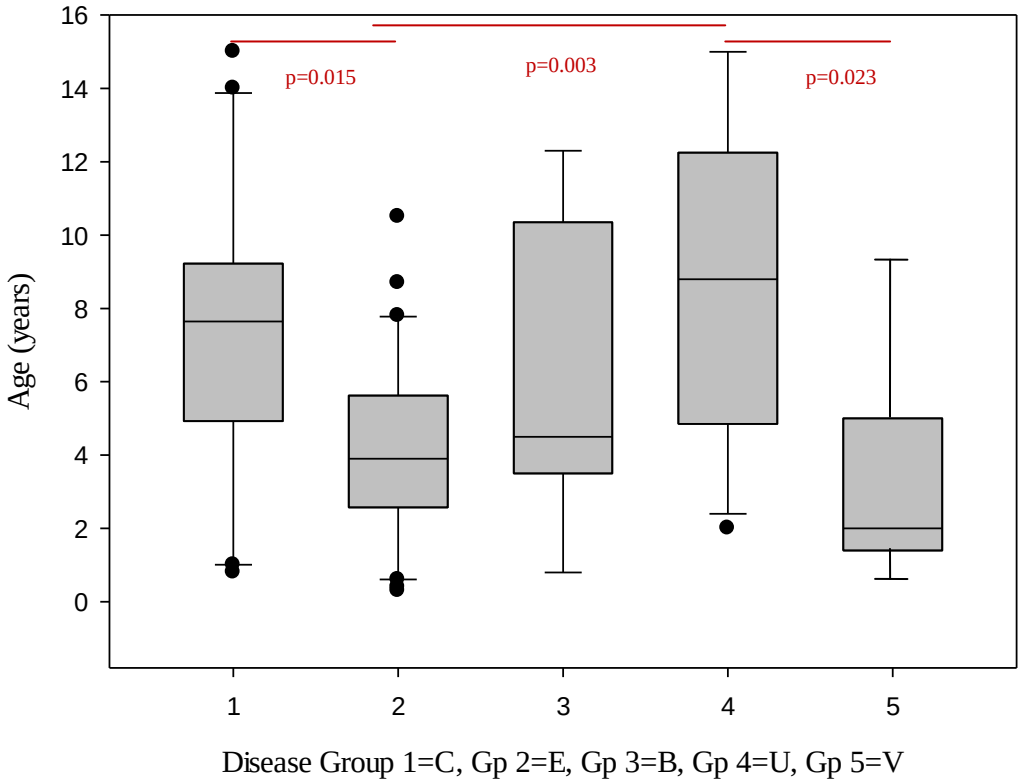


Figure 11. Box plots to show comparison of ages in different collapse with TLOC groups C (n=20), E (n=30), B (n=9), U (n=13) and V (n=7).

Group C, cardiogenic syncope; Group E, epilepsy; Group B, both cardiac disease and epilepsy; Group U, unclassified; Group V, vasovagal.

The horizontal line in each box is the median value. The boxes are the 25th-75th percentiles, whiskers are 10th and 90th. The circles are outliers. Lines join pairs with significant difference, p values given.

3.8.3 Time Since Collapse and Medications

The number of hours between the collapse and the time at which blood samples were taken was recorded in each case apart from one dog in the unclassified group (Table 22 and Appendix 7).

Table 22. Clinical findings according to group.

	Total	E (epilepsy)	C (cardiogenic syncope)	B (both heart disease and epilepsy)	U (unclassified)	V (vasovagal)
Time since collapse (hours). Median & range.	48 0-168 n=78	42 0-168 n=30	26 2-168 n=20	48 8-144 n=9	72 4-168 n=12	120 48-168 n=7
% receiving medications at first presentation.	47% n=79 37/79	60% n=30 18/30	45% n=20 9/20	56% n=9 5/9	31% n=13 4/13	14% n=7 1/7

Nine group C patients were receiving medication at first presentation (see Table 22). These included furosemide, benazepril, pimobendan, lidocaine, sotalol and digoxin. See section 3.3.3 for details regarding anti-epileptic medications administered to patients in group E. Medications administered to dogs in other groups included glucosamine, propentofylline, corticosteroids and non-steroidal anti-inflammatory drugs.

3.9 Investigations

3.9.1 Systolic Blood Pressure Measurement

One BP measurement was missing from group C and three from group U. There was no significant difference in BP between the five groups (p=0.197) (see Table 23).

Table 23. Systolic BP measurements according to group.
ANOVA, analysis of variance.

	Total	E	C	B	U	V	Test p value
Systolic BP (mmHg). Mean &range.	140 80-180 n=74	144 100-178 n=29	135 80-179 n=19	136 120-162 n=9	153 125-180 n=10	133 106-168 n=7	One way ANOVA p=0.197

E, epilepsy group; C, cardiogenic syncope group; B, both cardiac disease and epilepsy; V, vasovagal syncope group.

3.9.2 Serum Creatinine Concentration

There was no significant difference in serum creatinine concentration between the four groups E, C, B and V, p=0.132 (see Table 24). Thirteen group U patients were not definitively diagnosed therefore were excluded from further statistical analysis.

Table 24. Serum creatinine concentration according to group, p value given. ANOVA, analysis of variance.

	Total n=66	E n=30	C n=20	B n=9	V n=7	Test p value
Serum creatinine concentration (umol/L). Mean & range.	94 48-153	90 48-153	102 65-142	85 62-102	101 74-108	One Way ANOVA p=0.132

E, epilepsy group; C, cardiogenic syncope group; B, both cardiac disease and epilepsy; V, vasovagal syncope group.

3.9.3 Serum Cardiac Troponin I Concentration

The median cTnI values were significantly different between the four collapse with TLOC groups, p=<0.001 (see Tables 25 and 26).

Table 25. Serum cardiac troponin I concentrations (cTnI) according to group, p value given.

	Total n=66	E n=30	C n=20	B n=9	V n=7	Test p value
Serum cTnI (ng/ml) concentration. Median & range.	0.139 0.02-77.3	0.073 0.02-3.05	0.505 0.105-77.3	0.14 0.036-0.402	0.122 0.034-0.244	Kruskal Wallis rank test p=<0.001

E, epilepsy group; C, cardiogenic syncope group; B, both cardiac disease and epilepsy; V, vasovagal syncope group.

All pairwise multiple comparison procedures (Dunn's Method) isolated the groups that differed from each other. Comparing the cTnI concentrations in collapsing groups E, C, B and V, the concentrations in group C were significantly greater than those in groups E and V, p<0.05. The cTnI concentrations in animals in group C were not significantly different to those in group B, p>0.05.

There was no significant difference in serum cTnI concentrations between dogs in groups E and B, p>0.05 (Figure 12).

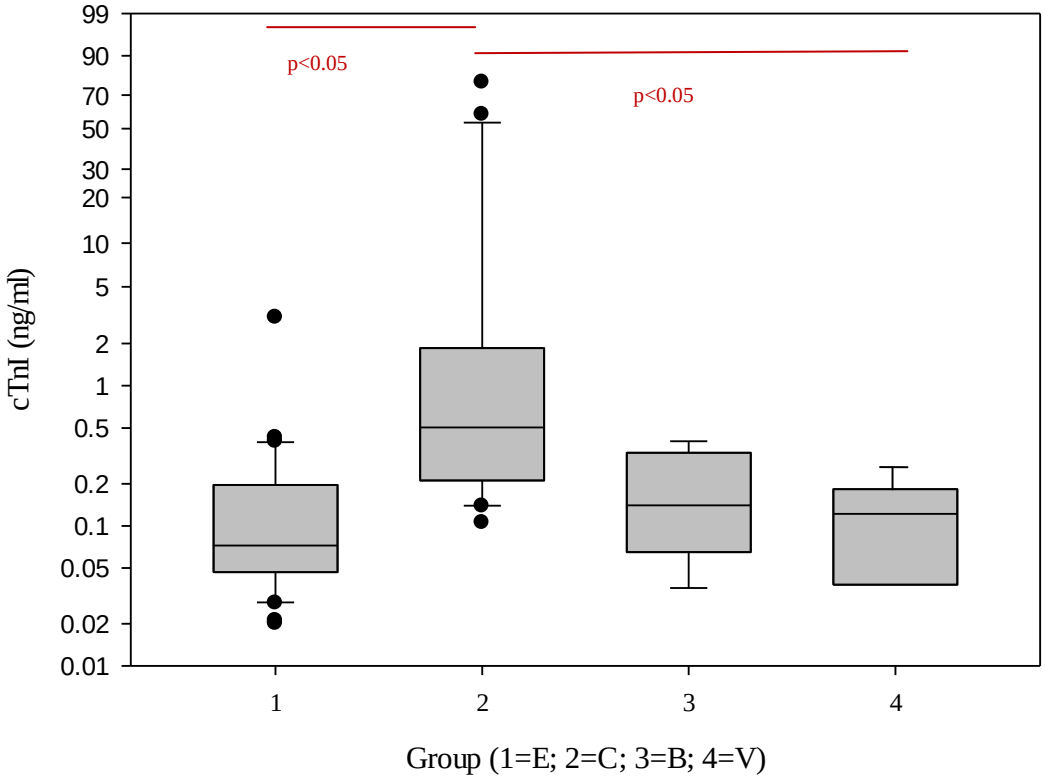


Figure 12. Weibull box plots comparing serum cTnI concentrations in collapsing groups.

E, epilepsy group; C, cardiogenic syncope group; B, both cardiac disease and epilepsy; V, vasovagal syncope group.

The horizontal line in each box is the median value. The boxes are the 25th-75th percentiles, whiskers are 10th and 90th. The circles are outliers. One box is missing a whisker as the lower quartile is equal to the minimum value. Lines join pairs with significant difference, p values given.

Table 26. Serum cardiac troponin I (cTnI) concentrations of the affected dogs according to aetiology of collapse with TLOC.

Case Number	Epileptics (group E) n=30 Serum cTnI concentration (ng/ml)	Cardiogenic syncope (group C) n=20 Serum cTnI concentration (ng/ml)	Both heart disease + epilepsy (group B) n=9 Serum cTnI concentration (ng/ml)	Vasovagal (group V) n=7 Serum cTnI concentration (ng/ml)
1	0.095	0.153	0.081	0.038
2	0.021	0.252	0.140	0.123
3	0.072	77.30	0.036	0.095
4	0.400	10.60	0.398	0.122
5	0.426	1.250	0.268	0.244
6	0.034	2.060	0.402	0.034
7	0.028	0.794	0.093	0.183
8	0.032	0.194	0.049	
9	0.043	0.253	0.164	
10	0.065	0.105		
11	0.068	0.502		
12	0.032	58.30		
13	0.020	0.508		
14	0.048	0.910		
15	0.067	4.290		
16	0.079	0.138		
17	0.195	0.248		
18	0.049	0.444		
19	0.100	0.199		
20	0.049	0.773		
21	0.111			
22	0.073			
23	0.089			
24	0.334			
25	0.123			
26	0.053			
27	0.324			
28	3.050			
29	0.362			
30	0.201			

3.9.4 Serum High-sensitivity Cardiac Troponin I Concentration

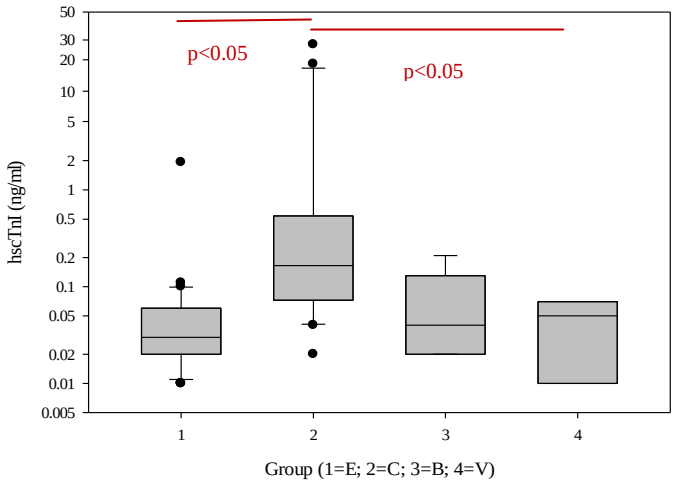
There was a significant difference in serum hscTnI concentration between groups E, C, B and V (Tables 27 and 28), p=<0.001.

Table 27. Serum high sensitivity cardiac troponin I concentration (hscTnI) according to group, p value given.

	Total n=66	E n=30	C n=20	B n=9	V n=7	Test p value
Serum hscTnI concentration (ng/ml). Median & range.	0.05 0.01-27.41	0.03 0.01-1.92	0.165 0.02-27.41	0.04 0.02-0.21	0.05 0.01-0.08	Kruskal Wallis rank test p=<0.001

E, epilepsy group; C, cardiogenic syncope group; B, both cardiac disease and epilepsy; V, vasovagal syncope group.

An all pairwise multiple comparison procedure (Dunn's Method) was performed and isolated the groups that differed from each other. The serum hscTnI concentrations in group C were significantly greater than those in groups E and V, p<0.05. The hscTnI concentrations in group C were not significantly different from those in group B, p>0.05. There was no significant difference in serum hscTnI concentration between dogs in groups E and B, p>0.05 (see Figure 13).



Lines join pairs with significant difference, p values given.

Figure 13. Weibull box plots comparing serum hscTnI concentrations in collapsing groups.
E, epilepsy group; C, cardiogenic syncope group; B, both cardiac disease and epilepsy; V, vasovagal syncope group.
The horizontal line in each box is the median value. The boxes are the 25th-75th percentiles, whiskers are 10th and 90th. The circles are outliers. Some boxes are missing whiskers as the upper quartile is equal to the maximum value or the lower quartile is equal to the minimum value.

Table 28. Serum high sensitivity cardiac troponin I (hscTnI) concentrations of the affected dogs according to aetiology of collapse with TLOC.

Case Number	Epileptics (group E) n=30 Serum hscTnI concentration (ng/ml)	Cardiogenic syncope (group C) n=20 Serum hscTnI concentration (ng/ml)	Both heart disease + epilepsy (group B) n=9 Serum hscTnI concentration (ng/ml)	Vasovagal (group V) n=7 Serum hscTnI concentration (ng/ml)
1	0.02	0.08	0.04	0.01
2	0.02	0.12	0.04	0.05
3	0.04	27.41	0.02	0.05
4	0.09	2.25	0.12	0.05
5	0.11	0.48	0.14	0.08
6	0.02	0.56	0.21	0.01
7	0.02	0.23	0.02	0.07
8	0.02	0.06	0.02	
9	0.01	0.04	0.05	
10	0.03	0.05		
11	0.03	0.14		
12	0.02	18.28		
13	0.01	0.15		
14	0.03	0.30		
15	0.02	1.32		
16	0.04	0.02		
17	0.07	0.07		
18	0.02	0.18		
19	0.03	0.12		
20	0.01	0.34		
21	0.03			
22	0.02			
23	0.04			
24	0.06			
25	0.06			
26	0.02			
27	0.08			
28	1.92			
29	0.10			
30	0.05			

3.9.5 Strength of the Relationship between cTnI and hscTnI

The serum cTnI and hscTnI concentrations of the thirty epileptic cases were compared and found to have good correlation using Pearson's correlation (correlation coefficient=0.989), $p<0.05$.

3.9.6 Regression Analysis

3.9.6.1 Predictor Variables for log cTnI in the Collapsing Groups

For consistency, the multivariable linear regression analysis was performed using the logarithmic values. The variables found to be significantly associated with log cTnI concentration were group (p=0.000), serum creatinine concentration (p=0.043) and time from collapse (p=0.005) (see Table 29). Age, receiving medications, bodyweight, reproductive status, gender and BP did not significantly affect cTnI concentration.

Table 29. Analysis of Variance for log cTnI, using Adjusted Sum of Squares (Adj SS) for Tests

DF (degrees of freedom), Seq SS (sequential sums of squares), Adj MS (adjusted means of squares), F (f-statistic)

Source	DF	Seq SS	Adj SS	Adj MS	F	p value
Time from collapse	1	2.7156	2.6183	2.6183	8.57	**0.005**
Serum creatinine concentration	1	4.2443	1.3031	1.3031	4.27	**0.043**
Group (E, C, B, V)	3	8.2050	8.2050	2.7350	8.95	**0.000**
Error	60	18.3314	18.3314	0.3055		
Total	65	33.4963				

Group E, epilepsy group; C, cardiogenic syncope group; B, both cardiac disease and epilepsy; V, vasovagal syncope group.

Table 30. Regression coefficient values.

Term	Regression Coefficient	Standard Error Coefficient	T	p value
Constant	-1.1215	0.3184	-3.52	**0.001**
Time from collapse	-0.004315	0.001474	-2.93	**0.005**
Serum creatinine concentration	0.007272	0.003521	2.07	**0.043**

The regression coefficient indicated that as serum creatinine concentration increased, then cTnI concentration also increased, p=0.043. As time from collapse increased, cTnI concentration decreased, p=0.005 (see Table 30).

Table 31. Least Squares Means for log cTnI		
Group	Mean	SE Mean
B	-0.810	0.1868
E	-1.022	0.1018
C	-0.168	0.1288
V	-0.846	0.2196
Group B, group with both cardiac disease and epilepsy; Group E, epilepsy group; Group C, cardiogenic syncope group; Group V, vasovagal syncope group.		

Table 32. Grouping Information Using Tukey Method and 95.0% Confidence			
Group	Number	Mean	Grouping
C	20	-0.168	A
B	9	-0.810	B
V	7	-0.846	AB
E	30	-1.022	B
Group C, cardiogenic syncope group; Group B, group with both cardiac disease and epilepsy; Group V, vasovagal syncope group; Group E, epilepsy group.			

Means that do not share a letter are significantly different (Table 32). Therefore group C were significantly different from groups E and B (see Tables 31 and Grouping column Table 32).

3.9.6.2 Predictor Variables for log hscTnI in the Collapsing Groups

The variables found to be significantly associated with log hscTnI concentration were disease group (p=0.000), serum creatinine concentration (p=0.050) and time from collapse (p=0.016) (see Table 33). Age, receiving medications, bodyweight, reproductive status, gender and systolic BP were not significantly associated with log hscTnI concentration.

Table 33. Analysis of Variance for log hscTnI, using Adjusted Sum of Squares (Adj SS) for Tests DF (degrees of freedom), Seq SS (sequential sums of squares), Adj MS (adjusted means of squares), F (f-statistic)						
Source	DF	Seq SS	Adj SS	Adj MS	F	p value
Time from collapse	1	1.9195	1.8642	1.8642	6.14	**0.016**
Serum creatinine concentration	1	3.8062	1.2184	1.2184	4.01	**0.050**
Group (E, C, B, V)	3	7.0626	7.0626	2.3542	7.75	**0.000**
Error	60	18.2172	18.2172	0.3036		
Total	65	31.0054				
Group E, epilepsy group; C, cardiogenic syncope group; B, both cardiac disease and epilepsy; V, vasovagal syncope group.						

Table 34. Coefficient values				
Term	Coefficient	Standard Error Coefficient	T	p value
Constant	-1.5895	0.3174	-5.01	**0.000**
Time from collapse	-0.003641	0.001469	-2.48	**0.016**
Serum creatinine concentration	0.007032	0.003510	2.00	**0.050**

As serum creatinine concentration increased, then cTnI concentration also increased, p=0.050 (see Table 34). As time from collapse increased, cTnI concentration decreased, p=0.016.

Table 35. Least Squares Means for log hscTnI		
Group	Mean	SE Mean
B	-1.230	0.1863
E	-1.439	0.1015
C	-0.650	0.1284
V	-1.317	0.2189
Group B, group with both cardiac disease and epilepsy; Group E, epilepsy group; Group C, cardiogenic syncope group; Group V, vasovagal syncope group.		

Table 36. Grouping Information Using Tukey Method and 95.0% Confidence			
Group	N	Mean	Grouping
C	20	-0.650	A
B	9	-1.230	AB
V	7	-1.317	AB
E	30	-1.439	B
Group C, cardiogenic syncope group; Group B, group with both cardiac disease and epilepsy; Group V, vasovagal syncope group; Group E, epilepsy group.			

Group C was significantly different from group E as no letter was shared (see Table 35 and Grouping column Table 36).

3.9.7 Receiver Operating Curve Analysis

Table 37. Receiver operating characteristic (ROC) Curve Area Report for serum cTnI and hscTnI concentrations.

Groups	Biomarker	AUC (area under curve)	Standard Error	95% Confidence Interval	p value
E vs. C	cTnI	0.89	0.05	0.80 to 0.98	**<0.0001**
E vs. C	hs cTnI	0.89	0.05	0.79 to 0.98	**<0.0001**
S vs. C	cTnI	0.94	0.03	0.88 to 1.0	**< 0.0001**
S vs. C	hs cTnI	0.93	0.04	0.84 to 1.0	**< 0.0001**
E vs. B	cTnI	0.64	0.10	0.44 to 0.84	0.2053
E vs. B	hs cTnI	0.64	0.11	0.42 to 0.85	0.2237
C vs. B	cTnI	0.84	0.08	0.69 to 0.99	**0.004033**
C vs. B	hs cTnI	0.81	0.08	0.65 to 0.98	**0.007735**
E vs. V	cTnI	N/A	N/A	N/A	N/A
E vs. V	hs cTnI	N/A	N/A	N/A	N/A
C vs. V	cTnI	0.93	0.05	0.83 to 1.03	**0.0009011**
C vs. V	hs cTnI	0.88	0.07	0.75 to 1.01	**0.003676**
B vs. V	cTnI	0.6	0.15	0.30 to 0.90	0.4914
B vs. V	hs cTnI	0.55	0.16	0.23 to 0.87	0.7508

E, epileptics; C, cardiogenic syncope; B; both heart disease and epilepsy; S, epileptics with no cluster seizures; V, vasovagal; cTnI, cardiac troponin I; hscTnI, high-sensitivity cardiac troponin I.

Serum cTnI and hscTnI concentrations are significantly able to discriminate between groups C and E, and between groups C and V (Table 37). However, the biomarkers cannot discriminate between groups B and E, or groups B and V. The ROC analysis was repeated for the cardiac group and the epileptic group but those dogs with cluster seizures had their values

omitted (group C versus group S). The AUC for cTnI is the same or higher than the AUC for hscTnI for all group comparisons (Table 37). See Figures 14, 15, 16 and 17 for ROC curves.

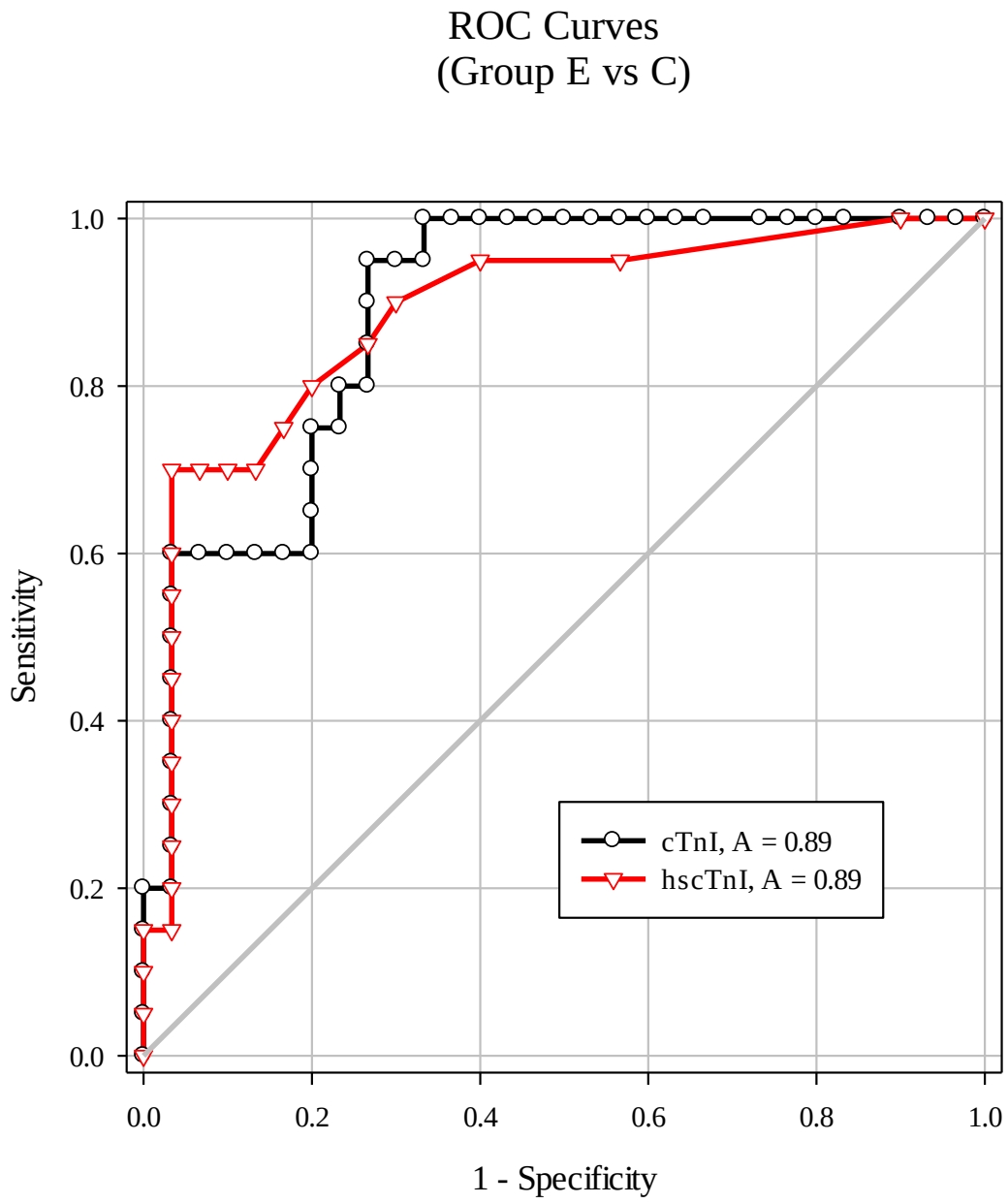


Figure 14. Receiver operating characteristic (ROC) curves for serum cardiac troponin I (cTnI) and high-sensitivity troponin (hscTnI) to distinguish dogs with epilepsy (n=30) E from those with cardiogenic syncope (n=20) C.

A=Area under curve

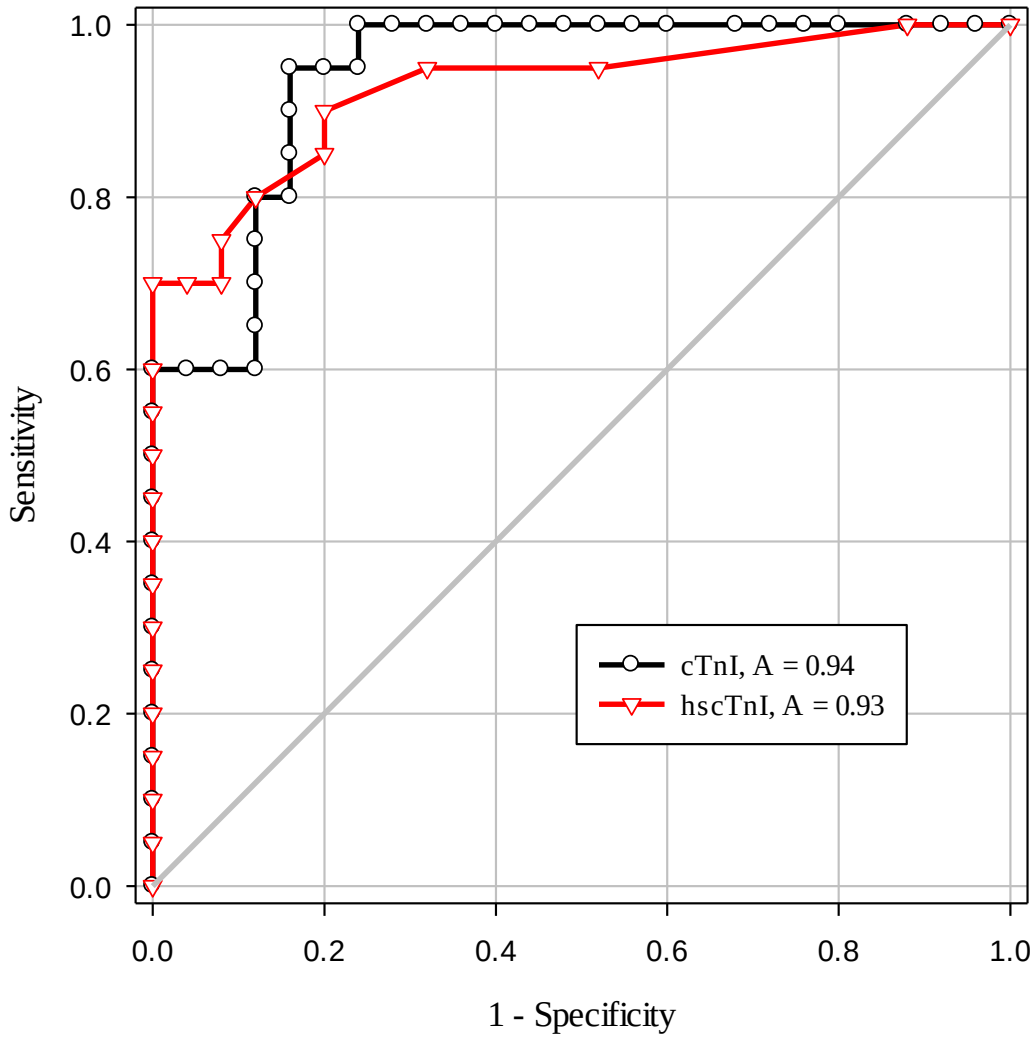


Figure 15. Receiver operating characteristic (ROC) curves for serum cardiac troponin I (cTnI) and high-sensitivity troponin (hscTnI) to distinguish dogs with epileptic seizures (no cluster seizures), S (n=25), from dogs with cardiogenic syncope, C (n=20).

A=Area under curve

.

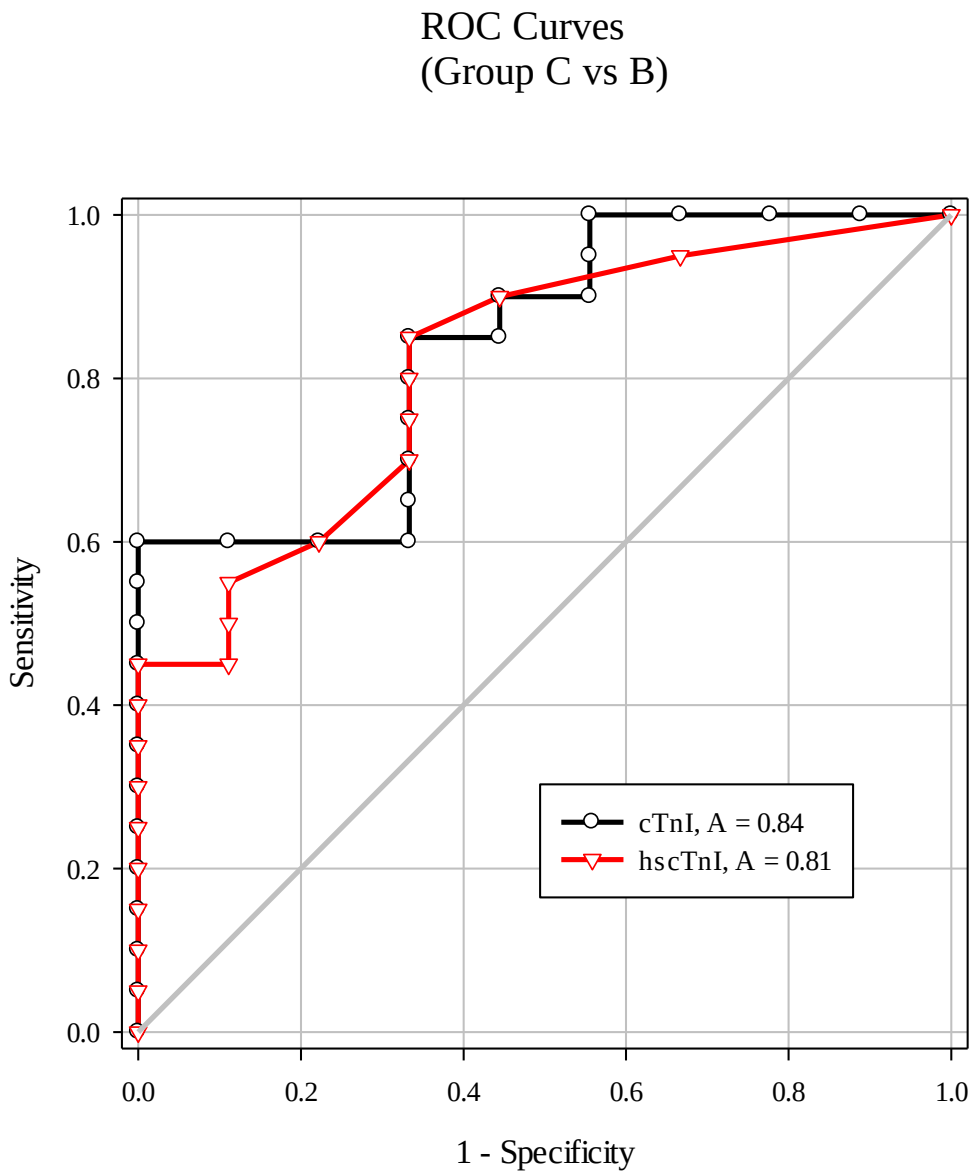


Figure 16. Receiver operating characteristic (ROC) curves for serum cardiac troponin I (cTnI) and high-sensitivity troponin (hscTnI) to distinguish cardiogenic syncope dogs, C (n=20) from dogs with both heart disease and epilepsy, B (n=9).

A=Area under curve

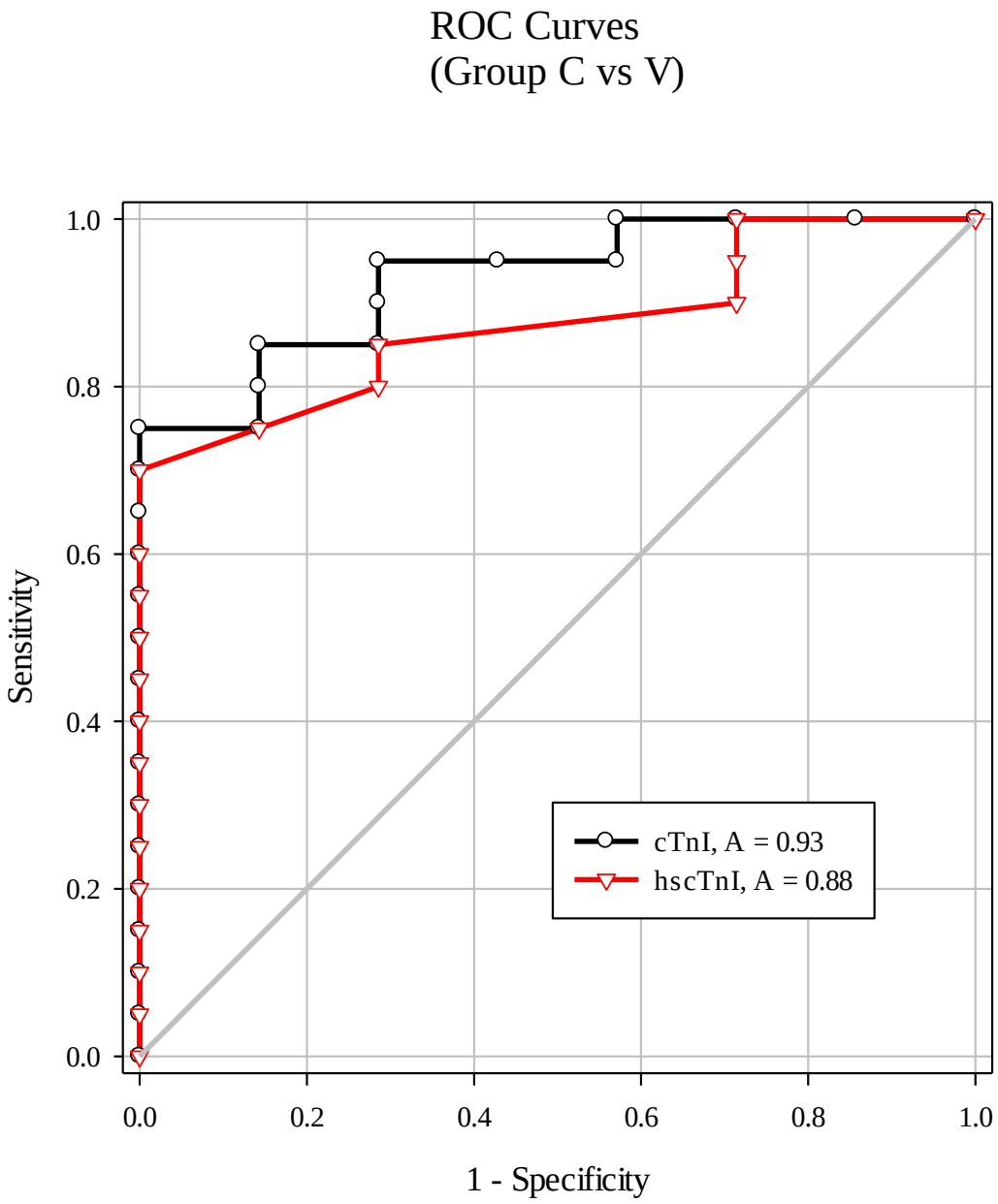


Figure 17. Receiver operating characteristic (ROC) curves for serum cardiac troponin I (cTnI) and high-sensitivity troponin (hscTnI) to distinguish the cardiogenic syncope dogs, C (n=20) from the vasovagal dogs, V (n=7) dogs.

A=Area under curve

3.9.8 Cut-Off Values

Table 38 shows pairwise group comparisons of dogs belonging to the cardiac group and dogs belonging to the noncardiac groups, with cut-off values that discriminate between cardiac and noncardiac causes of collapse with TLOC. The percentage correctly classified as cardiac, along with the sensitivity and specificity for the hscTnI and cTnI cut-off values, are shown.

Table 38. Sensitivity and specificity of classification for the serum cTnI and hscTnI cut-off values.

Groups	Biomarker	Cut-off value	Correctly Classified	Sensitivity (%)	Specificity (%)
E vs. C	cTnI	>0.4350 ng/ml	70%	60	97
E vs. C	hs cTnI	>0.1150 ng/ml	72%	70	97
S vs. C	cTnI	>0.4350 ng/ml	99%	60	100
S vs. C	hs cTnI	>0.115 ng/ml	99%	70	100
C vs. B	cTnI	>0.423 ng/ml	99%	60	100
C vs. B	hs cTnI	>0.22 ng/ml	99%	45	100
C vs. V	cTnI	>0.246 ng/ml	100%	75	100
C vs. V	hs cTnI	>0.10 ng/ml	100%	70	100
E, epileptics; C, cardiogenic syncope; B; both heart disease and epilepsy; S, epileptics with no cluster seizures; V, vasovagal; cTnI, cardiac troponin I; hscTnI, high-sensitivity cardiac troponin I					

Pre-test probability=0.02.

3.9.9 Positive and Negative Predictive Values

Assuming the prevalence of cardiogenic syncope is 2% (James *et al* 2008), the positive and negative predictive values have been calculated (see Table 39).

Table 39. Positive and negative predictive values.

Groups	Biomarker	Cut-off value	Positive Predictive Value	Negative Predictive Value
E vs. C	cTnI	>0.4350 ng/ml	27%	99.2%
E vs. C	hs cTnI	>0.1150 ng/ml	30%	99.4%
S vs. C	cTnI	>0.4350 ng/ml	100%	99.2%
S vs. C	hs cTnI	>0.115 ng/ml	100%	99.4%
C vs. B	cTnI	>0.423 ng/ml	100%	99.2%
C vs. B	hs cTnI	>0.22 ng/ml	100%	98.9%
C vs. V	cTnI	>0.246 ng/ml	100%	99.5%
C vs. V	hs cTnI	>0.10 ng/ml	100%	99.4%
E, epileptics; C, cardiogenic syncope; B; both heart disease and epilepsy; S, epileptics with no cluster seizures; V, vasovagal; cTnI, cardiac troponin I; hscTnI, high-sensitivity cardiac troponin I				

Table 40. Table showing overlap in serum troponin concentrations (cTnI) when comparing group E to group C.

Serum cTnI Range (ng/ml)	Number of dogs		cTnI cut-off	Sensitivity	Specificity	Correctly classified
	Epilepsy (Group E)	Cardiogenic Syncope (Group C)				
<0.091	18	0				
0.092-0.1454	4	2	≥ 0.092	100%	60%	62%
0.1455-0.199	1	3	≥ 0.1455	90%	73.3%	62%
0.200-0.504	6	5	≥ 0.200	75%	76.7%	62%
0.505-67.7	1	9	≥ 0.505	50%	96.7%	69%
67.8-99	0	1	≥ 67.8	5%	100%	99%

Table 40 shows the troponin concentrations in dogs with cardiogenic syncope and epilepsy. Test performance for identifying dogs with collapse of cardiac origin from dogs with collapse of noncardiac origin are reported for different troponin cut-off values. Sensitivity, specificity and percent correctly classified are reported.

4. DISCUSSION

The purpose of this study was to determine if serum cTnI and hscTnI concentrations were elevated in dogs collapsing with generalised epileptic seizures compared to a control population. This study provides evidence for epileptic patients having significantly raised serum cTnI and hscTnI concentrations when compared to a control population of healthy dogs. The results also demonstrate that serum cTnI and hscTnI concentrations show a more marked elevation in cardiac causes of collapse with TLOC compared to patients collapsing with epileptic seizures.

4.1 Part 1: Serum Cardiac Troponin I & High-sensitivity Cardiac Troponin I Concentrations in Epileptic Patients and Controls

The most important result from the study is identifying a noncardiac cause of troponin elevation, whereby concentrations in epileptic dogs are significantly raised when compared to a control population. When the epileptic patients are further divided into those patients with and without cluster seizures, only those with cluster seizures have significantly elevated serum cTnI concentrations compared to the control population. In contrast, serum hscTnI concentrations are significantly elevated in both groups of epileptic patients when compared to the control population. This difference may be due to the hscTnI assay being more sensitive and able to detect minor myocardial damage earlier than the less sensitive cTnI assay. The results also indicate that the degree of troponin elevation is independently associated with seizure number and also age. These results are similar to a human study which demonstrates that cTnI concentration increases with increased number of seizures (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 (Metcalf *et al* 2009).

Circulating hscTnI and cTnI concentrations are positively associated with age in this study which is in agreement with previous canine studies (Oyama & Sisson 2004, Ljungvall *et al* 2010, Hezzell *et al* 2012). An association was shown between serum cTnI concentration with patients receiving anti-epileptic medication on arrival. This may be a direct effect of medication on troponin concentration or perhaps a reflection of the severity of seizures in

patients taking multiple anti-epileptic medications, i.e. those with chronic refractory epilepsy. The only anti-epileptic medication reported to have cardiotoxic effects is gabapentin which may be pro-arrhythmic (Finsterer & Stöllberger 2011). Further studies are warranted to investigate whether anti-epileptic medications cause troponin release. This association between medication and serum troponin concentration was not shown with hscTnI, suggesting that the association with serum cTnI concentration may not be as significant as initially thought.

The epileptic patients in which serial samples were taken that showed improvement in clinical signs, all showed a reduction in serum cTnI concentration or similar concentration on second sampling. There were small patient numbers (n=5) and a significant conclusion could not be reached. However, although this trend toward lower serum cTnI concentrations following reduction in number of seizures did not reach significance, it seems to indicate that myocardial damage as a result of seizures is transient which reflects the findings in one study of human patients with severe refractory status epilepticus (Hocker *et al* 2013).

The epileptic patient with the highest serum troponin concentration showed evidence of reversible ECG and echocardiographic abnormalities. This case arrived in SE and required general anaesthesia for seizure control. It is unclear whether the cardiac changes detected (bradycardia and left ventricular chamber dilation) were a result of the anti-epileptic medications or a direct result of the cluster seizures. Bradyarrhythmias have been reported in human epileptic patients although are much less frequent than tachyarrhythmias (Velagapudi *et al* 2012). Repolarisation abnormalities were not detected in this study. The QTc-intervals in the epilepsy and control groups were not significantly different. This may be due to low case numbers. Further studies including a larger population of dogs with prolonged seizure activity are warranted to determine the arrhythmogenic effects of seizures in dogs.

In this study, a control population is included and the number of patients recruited is higher than in a previously reported study on serum cTnI with naturally occurring seizures (Kim *et al* 2012). A more homogenous population of dogs is included here, with the majority suffering from idiopathic epilepsy. This has the advantage that the effect of variables such as seizure number and length can be analysed without the underlying disease process tending to affect overall significance of the results. The disadvantage is that the effect of different types of underlying disease upon troponin concentration cannot be compared.

4.2 Part 2: Serum Cardiac Troponin I & High-sensitivity Cardiac Troponin I Concentrations in Patients with Syncope and Seizure

The results demonstrate that a more marked troponin elevation occurs in dogs with cardiac causes of syncope compared to collapse with epilepsy. It would be expected that there is greater myocardial injury in dogs with cardiac disorders compared to epileptic patients. The majority of cardiogenic syncope patients had arrhythmias, with a large proportion having echocardiographic evidence of underlying structural heart disease. Possible mechanisms for troponin elevation in these cases 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 the cardiac group had life-threatening ventricular tachycardia. Neither had clinically significant structural cardiac disease detected during echocardiography.

Disease group, serum creatinine concentration and time from collapse were all independently associated with cTnI concentration and this was also shown to be true for hscTnI concentration, demonstrating consistent results. Time from collapse was negatively associated with cTnI and hscTnI concentration reflecting the release kinetics and elimination of troponin. It also illustrates the importance of inclusion criteria, i.e. obtaining blood samples within seven days of a collapse episode. Serum creatinine concentration was positively and independently associated with serum cTnI and hscTnI concentration. The cause for this association is unclear, however, it may be due to troponin being eliminated by the renal system, or possibly due to subclinical myocardial damage rather than altered excretion. In dogs, it has been shown that circulating cTnI concentrations are increased in dogs with renal dysfunction (Porciello *et al* 2008, Sharkey *et al* 2009). The association between creatinine and troponin concentration was independent of medications, such as furosemide, and disease group. The latter point is notable as the presence of cardiac disease itself can influence renal function by decreasing renal perfusion. Further studies would be required to investigate this association.

Inspection of the ROC curve indicates assay sensitivity and specificity at any given cut-off value. ROC analysis to distinguish between cardiogenic syncope and epileptic groups gave an AUC of 0.89. Ideally, the AUC of 1.0 is a perfectly discriminatory test and the plot would rise from 0, go straight up to 1.0 then follow horizontally along the sensitivity line (Drobatz

2009). None of the ROC curves assume the perfect shape. Taking into account only those epileptic patients without cluster seizures, the AUC increases to 0.94 (cTnI) and 0.93 (hscTnI) and the shape of the curve improves. This would suggest that the biomarkers may help discriminate cardiogenic syncope from short epileptic seizures. This could be useful considering how the two clinical situations mimic one another, especially as cluster seizures should be easily identified on history alone. However, seven out of 30 epilepsy dogs (23%) and 15 out of 20 cardiac dogs (75%) have serum cTnI concentrations between 0.2 and 78 ng/ml. The higher sensitivity assay shows three out of 30 epilepsy dogs (10%) and 14 of 20 cardiac dogs (70%) have serum hscTnI concentrations between 0.1 and 28 ng/ml. This significant overlap between the two groups reduces the clinical utility of the assays. The overlap is still present after excluding those with cluster seizures.

The study shows that although serum troponin concentrations are different between the cardiac and epileptic groups, the overlap is too great for the tests to be used alone. Elevation of serum cTnI or hscTnI is not a sensitive indicator of cardiogenic syncope because a proportion of the epileptic dogs have raised troponin concentrations. This lack of sensitivity and specificity means that measurement of serum cTnI or hscTnI concentration is unlikely to be of value in the diagnosis of cardiogenic syncope. It is, however, important for clinicians to have data concerning the response of troponin in various clinical situations, such as following syncopal or seizure episodes.

4.3 Limitations

Idiopathic epilepsy is a diagnosis of exclusion and it is possible that some seizuring dogs were incorrectly classified. Two epileptic dogs had MRI evidence of brain tumours, however no post-mortem examinations were performed. All epileptic patients as well as control dogs had full cardiac investigations with the exception of one case. This eliminated significant cardiac disease as a possible cause of troponin elevation in all but one dog. This patient had no auscultatory abnormalities and troponin concentrations were within the laboratory reference intervals. Preclinical cardiac disease could not be excluded albeit unlikely in view of the dog's age (3.8 years). As troponin concentrations can fluctuate with other disease states, eliminating other diseases such as renal failure and pancreatitis on the basis of history, clinical examination and blood tests was an important component of this study. None of the patients had myocardial biopsies, coronary angiography 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. It is known that trauma can cause troponin release (Burgener *et al* 2006, De Gennaro *et al* 2008). A potential limitation of the study is the variation in time between blood samples being taken and the seizure occurring. This variation in time was a factor which could not be controlled. However, statistical analysis in Part 1 showed no significant association between time since seizure and serum troponin concentrations.

Some of the group sizes are small affecting the power of the statistical analyses. It is possible that some patients were misclassified according to their group categorisation, for example only two cases were definitively diagnosed in the vasovagal group. Small patient numbers in the cardiac group (n=20) with a large variety of underlying disease processes may reduce the overall significance of the results. The effect of neoplasia in the cardiac group was not examined, with four cases having neoplastic involvement. The series of cases presented here represent a referral population which may differ from the population of dogs seen in general practice. Although the majority of cardiac investigations were performed by the author, there were a small number of cases investigated by other cardiologists. The effect of breed was not assessed due to small numbers and diverse breeds. The group of three SE patients was small, therefore no statistical comparisons were made. However, all three patients were ranked as having a high number of seizures. Seizure number was considered in the statistical analysis.

It was beyond the scope of the study to assess the intra- and inter-assay coefficients, or to combine other biomarkers such as N-terminal pro B-type natriuretic peptide. However, the two different troponin assays were used with similar results which, in most cases, increased the significance of the results. The results of this study only apply to the two troponin analysers used (Adin *et al* 2006). The influence of daily variation on the biomarker concentration and effect of storage conditions on troponin stability were not assessed. However, all blood samples were stored and handled in a careful, consistent manner which is easily reproducible and minimises analytical issues such as fibrin interference. Previous studies have shown that cTnI is stable in serum samples for up to 48 hours (Wu *et al* 2009).

4.4 Conclusions

The results suggest that serum troponin concentrations are elevated in dogs with naturally occurring seizures compared to a healthy control population. The elevation that occurs is independently associated with number of seizures, age of the dog and, in the case of cTnI only, administration of anti-epileptic medication. The data available from dogs where sequential samples were taken suggest that, in an individual patient, a reduction in serum cTnI may occur with a reduction in the number of seizures and following anti-epileptic treatment. When comparing serum troponin concentrations in epileptic patients to those with a cardiac cause of syncope, a significant difference was found, with the cardiac group having higher concentrations. The overlap in troponin concentrations between the epileptic and cardiogenic syncope group reduces the clinical efficacy of the assay which therefore should not be used as a stand-alone test but in combination with other diagnostic investigations such as ECG and echocardiography. The results suggest that serum troponin concentrations are significantly elevated in syncopal dogs with life-threatening cardiac disease and arrhythmias compared to those cases with more benign causes of syncope. Although troponin as a prognostic indicator was not directly examined, further large-scale prospective studies, with troponin playing a role in the risk stratification of patients with syncope, are warranted.

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APPENDIX 1 – Laboratory tests

Biochemistry, Complete Blood Count and Electrolytes Tests (including reference intervals)

Total Protein	54-77 g/L
Albumin	25-40 g/L
Globulin	20-47 g/L
Albumin Globulin ratio	0.6-1.5
Sodium	142-157 mmol/L
Potassium	3.6-6.6 mmol/L
Na:K ratio	25-35
Total Calcium	2-3 mmol/L
Urea	3-9 mmol/L
Creatinine	40-150 umol/L
Alk Phos	0.1-150 U/L
ALT	5-66 U/L
AST	0-49 U/L
Total Bilirubin	0.1-9umol/L
Glucose	3.5-6.5 mmol/L
CK	0-190 U/L
Amylase	0-1800U/L

RBC	5-8.5 x 10^12/L
Hb	12-18 g/dl
HCT	37-55 %
MCV	60-80 fl
MCH	19-26 pg
MCHC	30.5-37 g/dl
Platelets	160-500 x 10^9/L
WBC	6-15 x 10^9/L
Neutrophils	3-11.5 x 10^9/L
Lymphocytes	1-4.8 x 10^9/L
Monocytes	0-1.3 x 10^9/L
Eosinophils	0-1.25 x 10^9/L
Basophils	0-0.2 x 10^9/L
Nucleated RBCs	0-0 10^9/L
Blood film examination	

Cerebrospinal Fluid (CSF) Analysis

CSF total protein	5-27 mg/dl
Fluid CK	0-40 IU/L
CSF RBCs	
CSF Nucleated Cells	
CSF cytology	

Infectious Agents

A. Phagocytophilum antibody, Bartonella henselae antibody, B. Burgdorferi antibody, Ehrilchica canis antibody, Toxo IgM, Toxo IgG, Neospora serology, Distemper IgG titre.

APPENDIX 2 – Neurological examination record sheet

Signalment

Owner.....Animal.....

Breed.....Age.....

Sex.....Weight……..

Medication (type, dose, last given)

……..……
…….....
.....

Diet (brand, dry-wet, allergies)

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…….....

Findings of Physical examination

Heart rate…….Body temperature…….....

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……..……...…

Neurologic Examination

Head

Seizures/Collapses (Y/N, place, exercise related or not)

……
…….....

Head pressing (Y/N)…….

Head tremors (Y/N)……

Mentation (describe)……

Head turn ((Y/N, direction)……

Head tilt (Y/N, direction)…….

Cranial nerves

	Left	Right		Left	Right
Olfaction(Y/N)			Menace(Y/N)		
Vision(Y/N)			Midrange pupil size(Y/N)		
Mydriasis(Y/N)			Miosis(Y/N)		
Direct pupillary light reflex(Y/N)			Consensual papillary light reflex(Y/N)		
Strabismus((Y/N,direction)			Enopthalmos(Y/N)		
Ptosis(Y/N)			Intranasal sensation(Y/N)		
Temporal/masseter atrophy			Jaw tone, range of motion		
Physiological nystagmus(Y/N)			Palpebral reflexes(Y/N)		
Positional nystagmus(Y/N,direction)			Aural reflexes(Y/N)		
Spontaneous nystagmus(Y/N, direction)			Drooping lip(Y/N)		
Swallow(Y/N)			Facial sensation		
Voice change(Y/N)			Hearing		
Regurgitation(Y/N)			Stridor(Y/N)		
Trapezius atrophy(Y/N)			Tongue atrophy		

Gait

Pacing(Y/N)…….....
Circling(Y/N, direction)……
Lame (leg, grade)……
Ataxic(generalised-forelimbs-hindlimbs)…….....
Paretic(mild-moderate-severe, mono-para)……
…….....
Paralysed (no voluntary movement)…….....
Ambulatory(Y/N)…….....
Nonambulatory(Y/N)…….....
Leaning/drifting to one side…….....

Postural reactions

Hopping (reduced, normal, side)
…….....
Conscious proprioception (leg)
…….....
Tactile placing
(table)…….....

Spinal reflexes

……
Muscle atrophy(Y/N,location)…….....…….
Voluntary urination(Y/N)…….....…….
Bladder tone…….....…….
Neck pain(Y/N)…….....……
Superficial sensation/cut(Y/N,location)…….....…….
Deep pain sensation(Y/N)…….....……
Cutaneous trunci(Y/N, location)…….....……..

Lesion location

……

Differential diagnosis

Degenerative (aquired)			
Anomalous (congenital or familial)			
Metabolic			
Nutritional			
Neoplastic			
Inflammatory or infectious			
Idiopathic			
Toxic			
Traumatic			
Vascular			

Further diagnostic tests

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Medication given (type, dose, date, time, adjustments during hospitalisation)

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APPENDIX 3 – Troponin assays

Troponin I Immulite® 1000 Siemens Medical Solutions Assay Information (cTnI)

IMMULITE®/IMMULITE® 1000 Troponin I

English

Intended Use: For *in vitro* diagnostic use with the IMMULITE and IMMULITE 1000 Analyzers — for the quantitative measurement of troponin I in serum, heparinized or EDTA plasma, as an aid in the diagnosis of acute myocardial infarction (AMI).

Catalog Number: **LKTI1** (100 tests), **LKTI5** (500 tests)

Test Code: **TPI** Color: **Light Gray**

CDC Test System Identifier Code: 10159
CLIA Complexity Category: Moderate

Summary and Explanation

Acute myocardial infarction (AMI) is usually diagnosed on the basis of chest pain, electrocardiographic changes, and elevations of markers of myocardial injury. The MB isoenzyme of creatine kinase (CK-MB) has been the preferred marker for two decades.[2] A study by Wu, et al. found excellent clinical sensitivity of the CK-MB assay between 6 and 24 hours following onset of AMI, with decreased sensitivity beginning in the 24 to 48-hour interval.[13] However, CK-MB levels can also increase in patients with acute or chronic muscle disease who lack apparent cardiac injury. In the same study, myoglobin, a muscle protein considered to be an early marker of AMI, became elevated within 6 hours of onset, achieved peak clinical sensitivity in the interval 6 to 12 hours following onset, and no longer offered diagnostic value by hour 24.[13] Myoglobin, although valuable for the early information it provides, also lacks specificity for cardiac injury. A marker specific for myocardial injury is therefore highly desirable.

Cummins, et al.[6,7] reported the release of cardiac troponin I (cTnI) in AMI. Many studies have focused on cTnI as a candidate marker with acceptable sensitivity and specificity for AMI and other cardiac diseases.

Troponin, a molecule that binds to the thin filament (actin) of striated muscle fibers, acts with intracellular calcium to control the interaction of the thin filament with the thick filament (myosin), thus regulating muscle contraction. Troponin consists of three subunits: T, which connects the troponin complex and tropomyosin (another cardiac muscle regulatory protein); I, which prevents muscle contraction in the absence of calcium; and C, which binds calcium.[8] Cardiac troponin I (MW 22.5 kDa) and the two skeletal muscle isoforms of troponin I have considerable amino acid sequence homology, but cTnI contains an additional N-terminal sequence[11] and is highly specific for myocardium.[1]

Clinical studies report several desirable features of cTnI as a marker of myocardial injury. cTnI rises early in AMI patients and attains levels that are clearly separated from baseline values, so that by 7 hours following onset, the cTnI test detects 95 percent of patients in whom AMI will be confirmed.[9] Plasma values of cTnI remain elevated for several days, providing a long window for detection of cardiac injury.[3,13] cTnI has also demonstrated value for predicting mortality risk in unstable angina and in non-Q wave myocardial infarction.[5]

cTnI has demonstrated equivalent diagnostic accuracy for AMI when compared with lactate dehydrogenase type 1 and CK-MB,[3,10] and may clarify diagnosis in contexts where elevated CK-MB cannot be attributed with certainty to cardiac injury alone.[3] These include surgery,[4] traumatic injury, renal failure, seizures, and skeletal muscle myopathies.[1]

In addition, a study on patients undergoing coronary artery bypass grafting (CABG) showed cTnI to be a sensitive marker for perioperative myocardial infarction (PMI); the peak concentration and time of peak both served as diagnostic criteria.[12]

Principle of the Procedure

IMMULITE/IMMULITE 1000 Troponin I is a solid-phase, enzyme-labeled chemiluminescent immunometric assay. The solid phase (bead) is coated with monoclonal murine anti-troponin I antibody. The liquid phase consists of alkaline phosphatase (bovine calf

Access Systems AccuTnI® Assay Information (hscTnI)

Access®
Immunoassay Systems

AccuTnI®

REF A78803

BECKMAN COULTER®

Intended Use

The Access AccuTnI assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of cardiac troponin I (cTnI) levels in human serum and plasma using the Access Immunoassay Systems to aid in the diagnosis and treatment of myocardial infarction and cardiac muscle damage.

Cardiac troponin I determination aids in the risk stratification of patients with unstable angina or non-ST segment elevation acute coronary syndromes with respect to relative risk of mortality, myocardial infarction, or increased probability of ischemic events requiring urgent revascularization procedures.

Summary and Explanation

Coronary artery disease is not only a leading cause of death in men and women in the US, but is also associated with other life-threatening complications.[1,2,3] The development of symptoms of coronary artery disease, which at times is unexpected and sudden, is associated with increased risk for adverse cardiac events such as death, myocardial infarction (MI), or hospitalization requiring urgent revascularization. Therefore, patients presenting with ischemic syndromes require prompt management. Specific and sensitive cardiac markers (troponin I and T) have been used in conjunction with other clinical findings and patient history information to better identify subjects with MI.[1,2,4,5,6] These specific cardiac markers have also been used to identify those patients at higher risk for short- and long-term adverse cardiac events (endpoints/outcomes).[1,7,8,9,10,11]

Cardiac troponin I is a contractile protein exclusively present in the cardiac muscle.[12,13] It is one of three subunits of the troponin complex (I, T, C), which with tropomyosin are bound to actin in the thin filament of the myofibril. cTnI is found as free troponin I (free TnI) and complexed with troponin C (binary IC), with troponin T (binary IT) or with both troponin C and troponin T (ternary ITC). Its physiological role is to inhibit the ATPase activity of the actin-myosin complex in the absence of calcium, and therefore, to prevent muscular contraction.[14] Three tissue isoforms have been identified:

- Fast troponin I and slow troponin I with molecular weights of 19,800 Da each, expressed in fast twitch and slow twitch skeletal muscle fibers, respectively.
- cTnI with a molecular weight of 24,000 Da contains an additional 31 amino acid residues in the N-terminal.

Sequencing of cTnI from mammals has shown important differences between the cardiac [15] and skeletal [16] forms. All three troponin I isoforms are encoded by different genes. The human cTnI exhibits only 52% and 54% amino acid sequence homology with the human fast and slow skeletal troponin I, respectively. The Access AccuTnI monoclonal antibody pair is selected to be cTnI specific. In addition, it has been well documented that skeletal muscle does not express cTnI, either during development or in response to stimuli.[17] Therefore, the absolute cardiospecificity of cTnI allows distinction between cardiac and skeletal injuries, and allows diagnosis of myocardial infarction distinct from muscle lesions (rhabdomyolysis, polytraumatism) and non-cardiac surgery.[17,18,19,20] Elevated troponin I levels have also been documented in cases of unstable angina (UA) [21] and congestive heart failure (CHF).[22]

cTnI levels in acute myocardial infarction (AMI) exhibit similar rise and fall patterns to those found in CK-MB. The collection of at least three blood samples during the early triage period has been recommended.[23] cTnI is 13 times more abundant in the myocardium than CK-MB and

APPENDIX 4 – Breed tables

Table 1. Distribution of breeds (all groups): 37 breeds total		
Affenpinscher 1	Cross breed 12	Pug 2
Airedale terrier 1	Doberman 1	Rhodesian ridgeback 1
Alaskan malamute 1	English bulldog 1	Rottweiler 2
Basset hound 2	English bull terrier 1	Saint Bernard 1
Bernese mountain dog 1	German spitz 1	Shetland sheepdog 1
Border collie 5	Golden retriever 2	Springer spaniels 4
Border terrier 3	Hungarian vizsla 1	Standard schnauzer 1
Boston terrier 1	Irish setter 3	Staffordshire bull terrier 4
Boxer 10	Jack Russell terrier 8	Tibetan terrier 1
Cairn terrier 1	Labrador retriever 11	West Highland white terrier 6
Chihuahua 1	Lhasa apso 1	Yorkshire terrier 2
CKCS 10	Miniature schnauzer 2	
Cocker spaniel 2	Poodle 1	

Table 2. Distribution of breeds amongst the epilepsy dogs (E) and control dogs (N)		
Breed	E	N
Labrador retriever	6	4
Staffordshire bull terrier	3	1
Springer spaniels	0	3
CKCS	2	1
Border collie	2	1
Border terrier	2	0
Irish setter	2	0
Pug	1	0
Jack Russell terrier	1	7
Cocker spaniel	1	1
Basset hound	1	0
Boxer	1	0
Cross breed	1	8
Hungarian vizsla	1	0
Yorkshire terrier	1	1
Poodle	1	0
Rottweiler	1	1
German spitz	1	0
Boston terrier	1	0
Affenpinscher	1	0
West Highland white terrier	0	1
Alaskan malamute	0	1

Table 3. Distribution of breeds amongst the collapse with TLOC cases.
E, epilepsy, C, cardiogenic syncope, B, both heart disease and epilepsy, U, unclassified, V, vasovagal

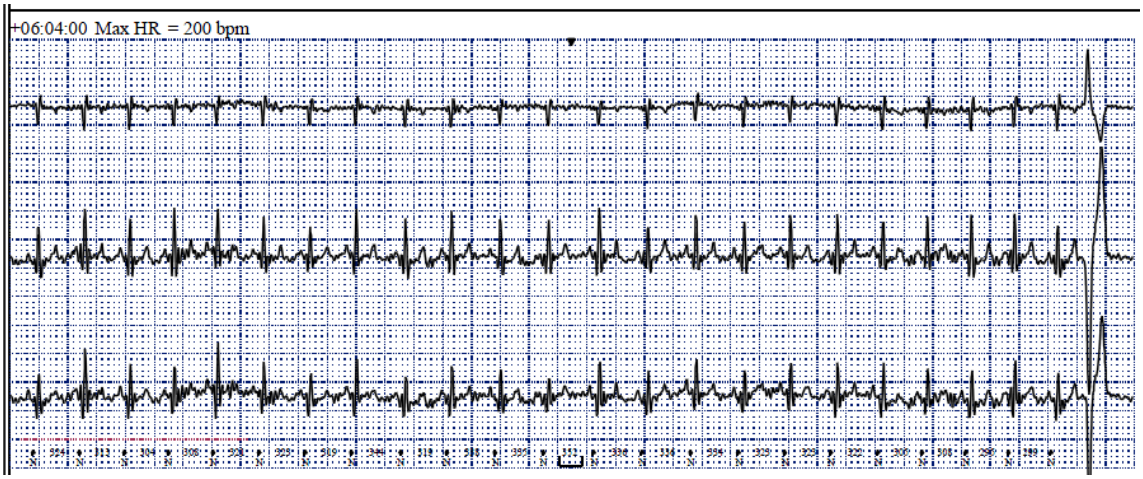
Breed	E	C	B	U	V
Labrador retriever	6	1			
Boxer	1	2	2	2	3
Border collie	2	2			
Golden retriever		1		1	
Doberman		1			
Bernese mountain dog		1			
Miniature schnauzer		1			1
Standard schnauzer		1			
West Highland white terrier		1	2	2	
Cross breed	1	2		1	
Irish setter	2	1			
Cavalier King Charles spaniel	2	1	4	2	
Saint Bernard		1			
Lhasa apso		1			
Rhodesian ridgeback		1			
Border terrier	2	1			
Shetland sheepdog		1			
Affenpinscher	1				
Boston terrier	1				
Staffordshire bull terrier	3				
German spitz	1				
Rottweiler	1				
Cocker spaniel	1				
Poodle	1				
Hungarian vizsla	1				
Yorkshire terrier	1				
Pug	1				1
Jack Russell terrier	1				
Basset hound	1		1		
English bull terrier					1
Cairn terrier					1
Tibetan terrier				1	
English bulldog				1	
English springer spaniel				1	
Chihuahua				1	
Airedale terrier				1	

APPENDIX 5 – Holter recordings

Seizuring patient (Group CS case number 1): The ECG traces below are from a 24 hour Holter recorded from a seizuring patient (CS case 1).

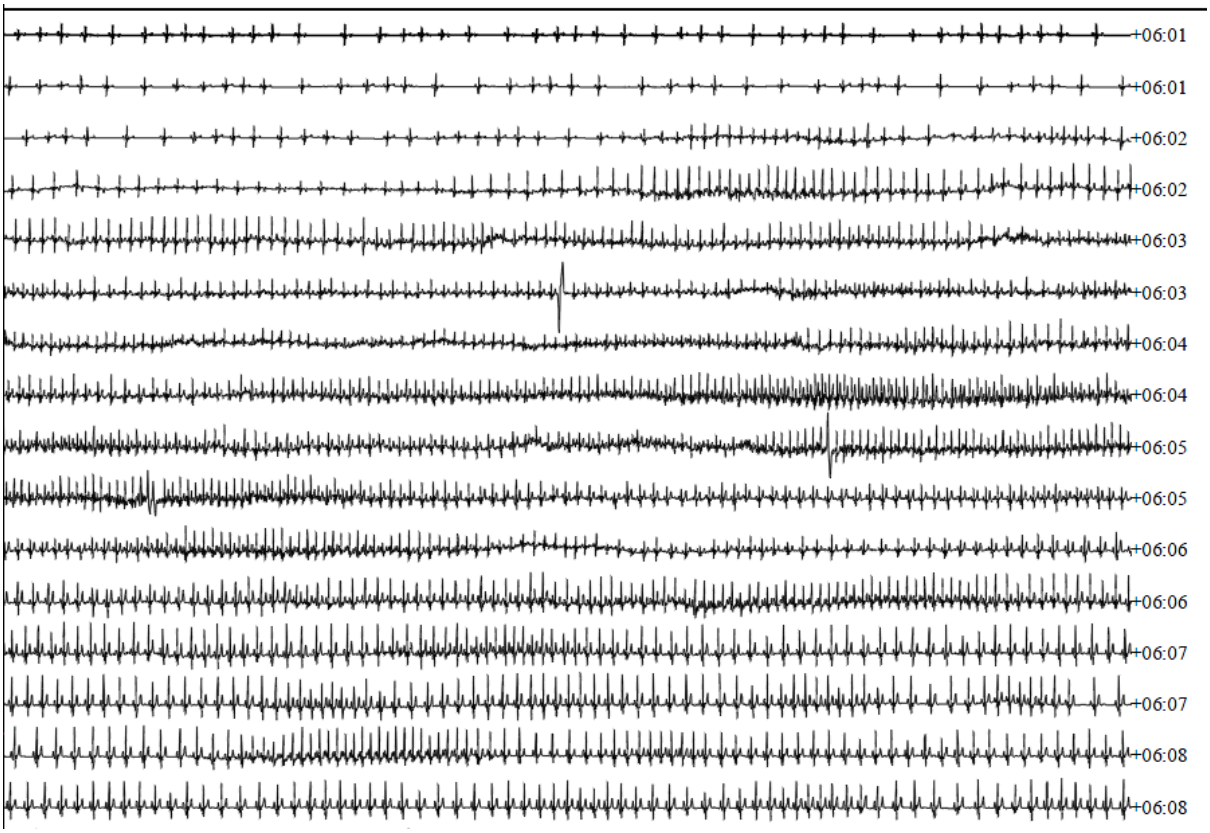
A. The ECG trace during the seizure episode which, according to the diary sheet commenced at 06.04, is shown below. The seizure duration was approximately 35 seconds and no anti-epileptic medications were administered. The maximum heart rate during the 24 hour ECG recording occurred during the seizure (200 beats/minute) at 06.04 and, despite the baseline artefact, the trace appeared to show sinus tachycardia.

A.



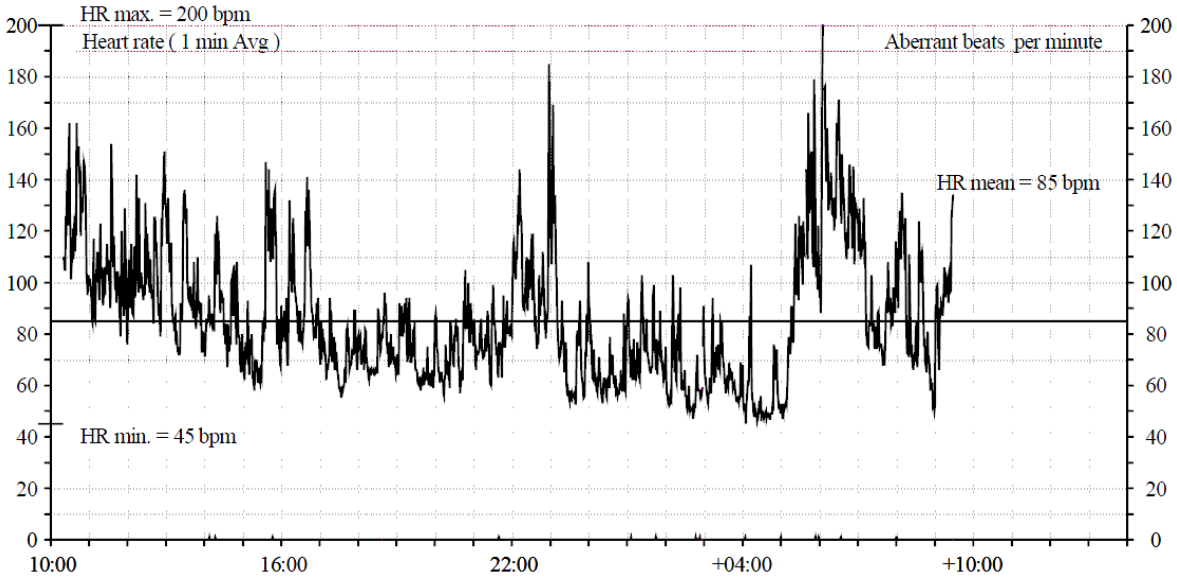
B. Occasional ventricular premature complexes can be seen throughout the ECG trace below. These occur singly and there is one couplet at 06.05 which, according the diary sheet, was during the post-ictal phase.

B.

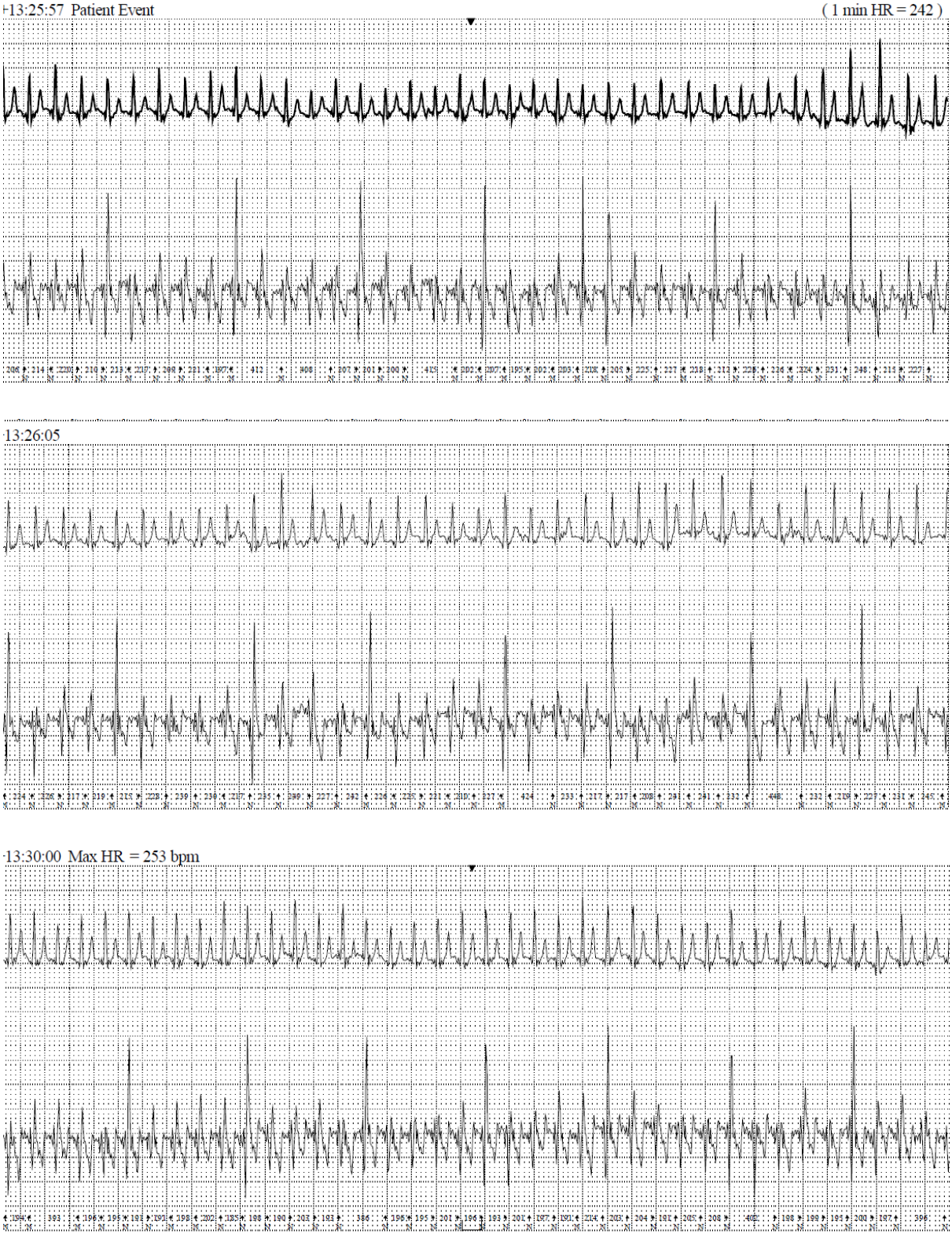


C. Tachogram from seizuring patient, Group CS case number 1, which shows the maximum heart rate occurred at 06.04 during the seizure episode.

C.

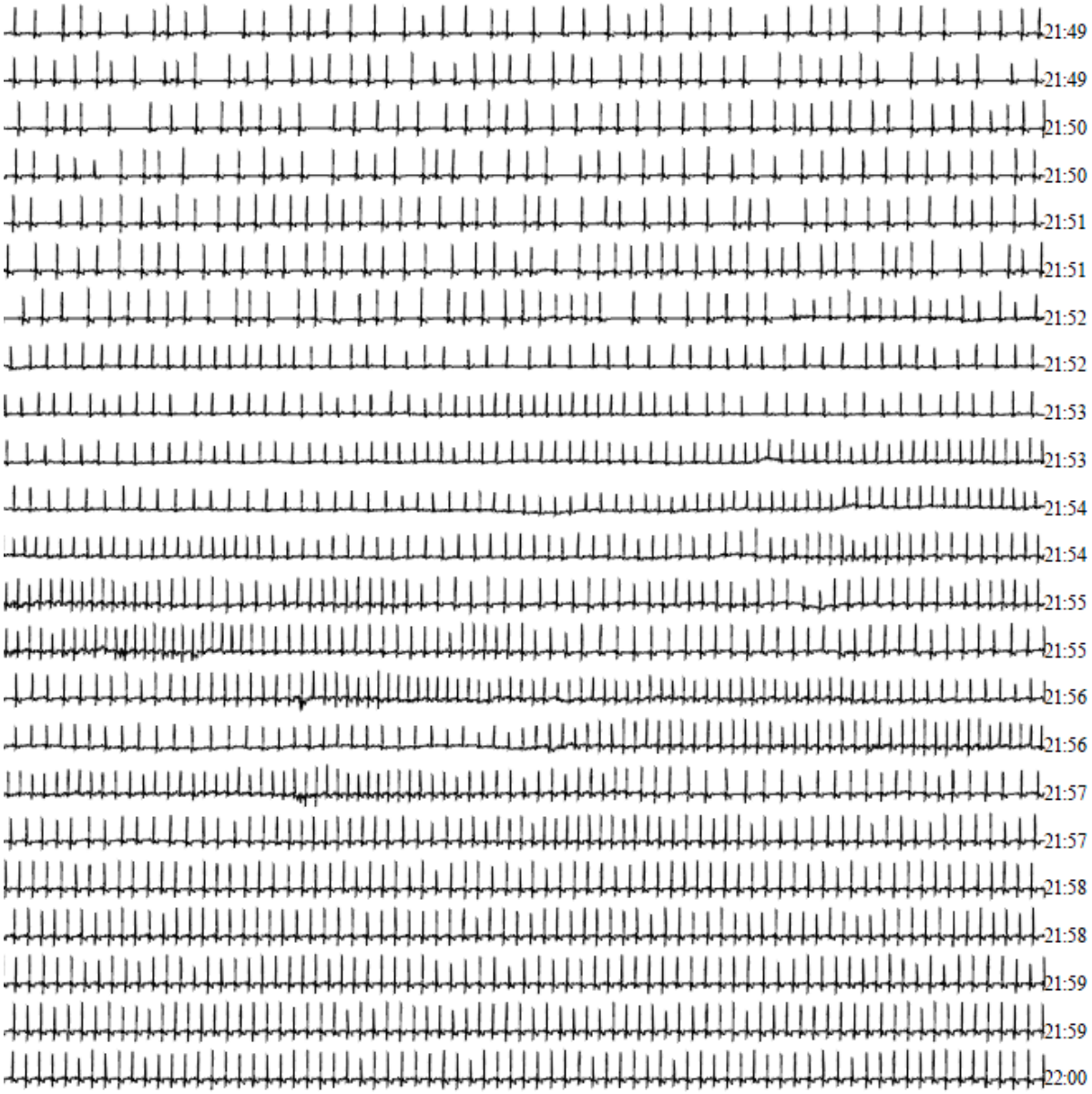


Seizuring patient (case number 20): The ECG trace below is from a Holter recorded from patient 20 with suspected exercise-induced seizures. The owner recorded that the seizure commenced at 13·26 and finished at 13·30 on the diary sheet. Sinus tachycardia at a rate reaching approximately 300 bpm is evident during the suspected exercise-induced seizure.



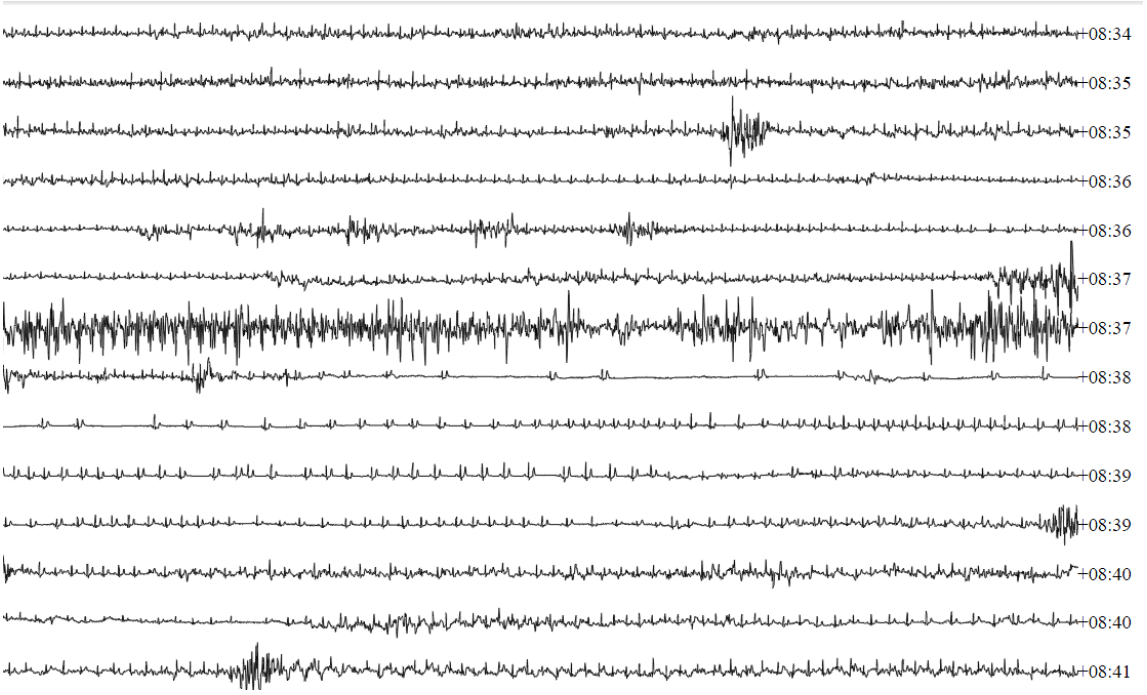
Seizuring patient (Group CS case number 2): The ECG trace below is from a 24 hour Holter recorded from a seizuring patient (CS case number 2).

According to the diary sheet, the seizure commenced at 21.54 and finished at 21.56. Sinus rhythm was recorded at 21.54 (see ECG trace below) and the maximum 1 minute mean heart rate during the episode was 155bpm.



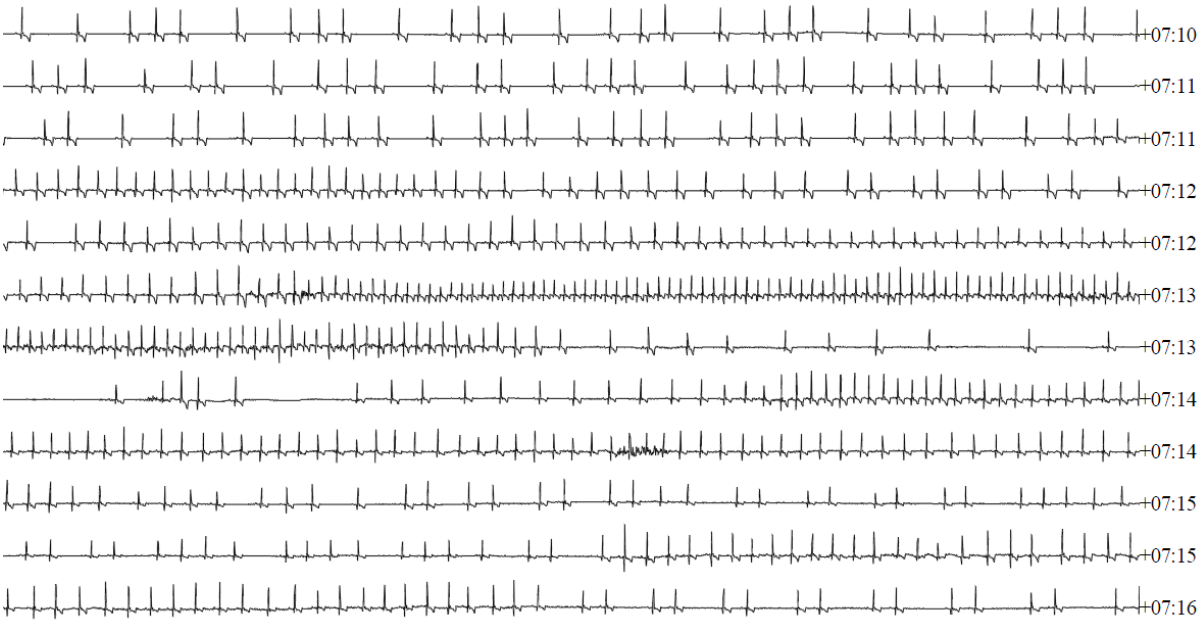
Vasovagal syncope patient (Group V, case number 5)

The ECG trace below is from a Holter recording during a collapse episode from vasovagal case number 5. Prior to the patient event (08.38) the trace shows sinus tachycardia with baseline artefact followed by a sudden change in heart rate and rhythm to sinus bradycardia with sinus pauses (and ventricular asystole) of up to 4.3 seconds before gradual resumption of sinus rhythm. These findings are suggestive of vasovagal syncope.



Vasovagal syncope patient (Group V, case number 2)

The ECG trace below is from a Holter recording from dog 2 during a collapse episode. Prior to the syncopal episode (07:13), the trace showed sinus tachycardia followed by a rapid transition to sinus bradycardia with a gradual return to sinus rhythm. This pattern is suggestive of a vasovagal episode.



APPENDIX 6 – ECG and echocardiographic exam results SE patient

Electrocardiography Report for the Status Epilepticus Patient (CS case number 3)

Species / Breed: Staffordshire bull terrier	Body Position: Right lateral
Age: 8 y 8 mth Sex: Mn	Date: 11th July 2011

Normal values

		DOG	CAT
Heart Rate	**60 bpm**	70 - 160 bpm (adults) Toy breeds <180 bpm Puppies < 220 bpm	160 - 240 bpm
Rhythm	**Sinus**	Sinus rhythm/arrhythmia	Sinus rhythm
P wave	**0.2 mV X 0.03 s**	< 0.4 mV x 0.04 s	0.2 mV x 0.04 s
P-R interval	**0.1 s**	0.06 - 0.13 s	0.05 s - 0.09 s
Rwave height	**1.6 mV**	2.5 mV (small breeds) 3.0 mV (large breeds)	0.9 mV (1.0 mV sternal)
QRS duration	**0.04 s**	0.05 s (small breeds) 0.06 s (large breeds)	0.04 s
Q-T interval	**0.24 s**	0.15 - 0.25 s	0.12 - 0.18 s
ST segment	**-**	Not elevated/depressed	Not elevated/depressed
T wave	**+ve, < 25%**	<25% height R wave	<0.3 mV
MEA	**+ 90 º**	$+40^{o}$ - $+100^{o}$	0^{o} - $+160^{o}$

NOTE: Amplitude measurements are based on lead II (unfiltered).

ECG diagnosis: Sinus bradycardia.

Echocardiography Results for the Status Epilepticus Patient

(CS case number 3)

Table 4. M-mode and Doppler echocardiographic (echo) measurements from status epilepticus Staffordshire bull terrier categorised as having cluster seizures (group CS).

	Initial echo results 24 hours following treatment of cluster seizures	Echo results 3 weeks after initial echo
LVIDd	49.4 mm	44.9 mm
PWd	9.9 mm	8.8 mm
IVSd	11.2 mm	11.0 mm
LVIDs	36.6 mm	32.3 mm
PWs	11.3 mm	11.5 mm
IVSs	13.5 mm	13.1 mm
EPSS	5.7 mm	5.3 mm
EF	51 %	54 %
FS	26%	28 %
MR velocity	5.49 m/s	N/A
MR max PG	120.8 mmHg	N/A
TR velocity	1 m/s	N/A
TR max PG	4 mmHg	N/A

LVID, left ventricular internal dimension; d, diastole; s, systole; PW, left ventricular free wall; IVS, interventricular septum; EPSS, E-point to septal separation; EF, ejection fraction; FS, fractional shortening; MR, mitral regurgitation; TR, tricuspid regurgitation; PG, pressure gradient;

Table 5. Two dimensional and indexed echocardiographic measurements from Staffordshire bull terrier categorised as having cluster seizures (group CS).

	Initial echo results 24 hours following treatment of cluster seizures	Echo results 3 weeks after initial echo
LA	24.9 mm	26.9 mm
Ao	22.3 mm	20.4 mm
LA:Ao ratio	1.1	1.3
ESVI	40.3 ml/m²	44.1 ml/m²
LVIDdALLO	1.9 (1.27 – 1.85)	1.73 (1.27 – 1.85)
LVIDsALLO	1.32 (0.71 – 1.26)	1.16 (0.71 – 1.26)

LA, left atrial diameter right parasternal short-axis view; Ao, aortic root diameter right parasternal short-axis view; LVID, left ventricular internal dimension; d, diastole; s, systole; ESVI, Simpson's-derived end-systolic volume index; ALLO, dimension after allometric scaling; (reference intervals for bodyweight in brackets) (Cornell *et al* 2004).

APPENDIX 7 –Time since collapse Part 2

Table showing the number of hours between the collapse episode and time at which blood samples were taken, according to group and case number.					
CASE NUMBER	Epileptics (group E) n=30	Cardiogenic syncope (group C) n=20	Both heart disease + epilepsy (group B) n=9	Unclassified (group U) n=12	Vasovagal (group V) n=7
1	1	168	8	168	120
2	144	168	24	24	168
3	23	2	120	72	72
4	96	12	48	24	144
5	12	24	43	168	96
6	96	12	144	168	48
7	72	72	96	72	144
8	48	24	24	36	
9	72	120	72	120	
10	96	72		4	
11	24	62		144	
12	30	4		72	
13	6	6			
14	120	18			
15	36	48			
16	24	26			
17	168	12			
18	72	26			
19	96	48			
20	168	144			
21	120				
22	48				
23	120				
24	2				
25	3				
26	14				
27	16				
28	0				
29	9				
30	24				