Cardiac Biomarkers, Acute Phase Proteins and Survival in Small Animals.

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II. ABSTRACT:

Blood-based biomarkers are commonly used in veterinary patients; however, cardiac blood testing is a relatively new concept in Small Animal Medicine and the correct use of these circulating substances remains uncertain. This Masters project includes two retrospective studies investigating the association between cardiac troponin I (cTnI) and survival in dogs and cats with cardiac disease and one prospective study exploring the relationship between cTnI, B-type prohormone natriuretic peptide (NT-proBNP) and acute phase proteins (APP) in cats with cardiac and non-cardiac diseases.

The first retrospective study included 94 dogs with cardiac diseases. Higher cTnI concentrations were associated with shorter median survival times. Dogs with cardiac disease and cTnI concentrations above the reference range (>0.16ng/ml) had a median survival time of 29 months whereas dogs with cTnI concentrations within the reference range (<0.16ng/ml) had a median survival time of 44 months. However, there was a significant overlap of cTnI concentrations between survivors and non-survivors. The contribution of arrhythmias to cTnI release remains uncertain as some dogs with severe arrhythmias and high cTnI values survived long periods.

The second retrospective study included 51 cats with cardiac disease, 9 with hyperthyroidism and 8 with systemic diseases. Increased cTnI concentrations were associated with shorter median survival time in cats with cardiac disease. Cats with cTnI concentrations above 5ng/ml survived a median of 1 month whereas cats with cTnI concentrations between 0.5-5ng/ml survived a median of 15 months.

Finally, in a prospective study, two cardiac biomarkers (cTnI and NTproBNP) and four APP (Serum Amyloid A, SAA; Haptoglobin, Hp; C-reactive protein, CRP; α_1-acid glycoprotein, AGP) were measured in 99 cats with cardiac and non-cardiac diseases. Signficantly higher concentrations of hscTnI, NT-proBNP and AGP were found in cats in congestive heart failure (CHF). Concentrations of cardiac biomarkers were commonly increased in cats with non-cardiac diseases. Some of these animals were non-anaemic, non-azotaemic and had normal echocardiographic examinations. No associations were found between cardiac and inflammatory markers.

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VI. LIST OF ABBREVIATIONS:

AGP	α_1-acid glycoprotein
AICM	Anthracycline induced cardiomyopathy
AID	Anaemia secondary to inflammatory disease
APP	Acute phase proteins
APR	Acute phase response
AMI	Acute myocardial infarction
ANP	Atrial natriuretic peptide
AST	Aspartate amino-transferase
ARVC	Arrhythmogenic right ventricular cardiomyopathy
ATE	Arterial thromboembolism
AUC	Area under the curve
BCT	Blunt chest trauma
BNP	Brain natriuretic peptide
CHF	Congestive heart failure
CK	Creatine Kinase
CM	Cardiomyopathy
CMhm	Candidatus Mycoplasma haemominutum
cMRI	Cardiac magnetic resonance
cTnI	Cardiac Troponin I/ Cardiac isoform of Troponin I
cTnT	Cardiac Troponin T
CRF	Corticotropin releasing factor
CRP	C-reactive protein
DCM	Dilated Cardiomyopathy
DIC	Disseminated intravascular coagulation
ECG	Electrocardiogram
Echo	Echocardiography
ECM	Endomyocarditis
ES-HCM	End-stage hypertrophic cardiomyopathy
FCoV	Feline Coronavirus
FECV	Feline enteric coronavirus
FIP	Feline infectious peritonitis

FIPV	Feline infectious peritonitis virus
GDV	Gastric dilatation-volvulus
Hb	Haemoglobin
Hp	Haptoglobin
HCM	Hypertrophic cardiomyopathy
HOCM	Hypertrophic obstructive cardiomyopathy
HSA	Haemangiosarcoma
HscTnI	High sensitivity cardiac troponin I
ICU	Intensive care unit
IMHA	Immune-mediated haemolytic anaemia
IPE	Idiopathic pericardial effusion
JDCM	Juvenile dilated cardiomyopathy
LD	Lactate dehydrogenase
LGE	Late gadolinium enhancement
LVEDDN	Left ventricular end-diastolic diameter
LVPWd	Left ventricular posterior wall in diastole
Mhf	Mycoplasma haemofelis
MVD	Mitral valve disease
NT-proBNP	B-type prohormone natriuretic peptide
PE	Pericardial effusion
PSVT	Paroxysmal supraventricular tachycardia
PWD	Portuguese water dog
RCM	Restrictive cardiomyopathy
RIA	Radioimmunoassay
ROC	Receiver operator characteristic curve
SAA	Serum Amyloid A
SAS	Subaortic stenosis
SGOT	Serum glutamic oxaloacetic transaminase
SIRS	Systemic inflammatory response syndrome
SPVC	Supraventricular premature complex
TnI	Troponin I
TnT	Troponin T
TnC	Troponin C

UCM	Unclassified cardiomyopathy
VHS	Vertebral heart score
VPC	Ventricular premature complex
IVSd	Interventricular septum in diastole
WBC	White blood cell count
WHO	World Health Organization

CONFLICT OF INTEREST DECLARATION

This study was partially funded by IDEXX Laboratories and ReactivLab Ltd. These sponsors participated in the analysis of the data but did not take part in the study design, sample collection, interpretation or statistical analysis.

ORIGINALITY DECLARATION

The work in this thesis was performed solely by the author except where the assistance of others has been acknowledged.

CHAPTER 1:

Introduction to Cardiac and Inflammation markers

1.INTRODUCTION

1.1 CARDIAC BIOMARKERS

Time to treatment plays a critical role in human patients with ischaemic coronary heart disease. Morbidity and mortality rates decrease significantly in those patients undergoing early intervention **[Moscucci and Eagle, 2006]**. In consequence, scientists have been looking for a reliable and sensitive blood marker that could give an early indicator of patients suffering from acute myocardial infarction (AMI).

The National Cancer Institute defines a "biomarker" as a biological molecule found in blood, body fluids or tissues that is a sign of a normal or abnormal process, or of a condition or disease. The Biomarkers Definitions Working Group stated in 2001 that a biological marker is a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention" **[Atkinson et al, 2001].**

1.1.1 HISTORY

When cardiomyocytes die the proteins inside the cells are released. Because the major function of the heart is pumping, the proteins involved in contraction and producing the energy to support it have been traditionally tested as candidates for cardiac biomarkers **[Ladenson, 2007]**.

The first cardiac biomarker used was serum glutamic oxaloacetic transaminase (SGOT), now called aspartate amino-transferase (AST). Increased concentrations of SGOT after AMI were reported in 1954 for the first time **[Karmen et al. 1955]**. It was the starting point for assaying substances discharged from necrotized myocardium into blood.

As SGOT became more widely used, increased concentrations in patients with liver damage, and thus the lack of specificity, were appreciated. Today, it is known that AST is not cardiac specific as it is found in liver, skeletal muscle, red blood cells and many other tissues **[Dolci and Panteghini, 2006]**.

In the 1960s, creatine kinase (CK) and lactate dehydrogenase (LD) and its isoenzymes were demonstrated to be possible markers of cardiac damage **[Wróblewski et al. 1956; Wróblewski et al. 1960]**. LD was found in nearly all human tissues but CK gave the cardiologist the opportunity to make a diagnosis of AMI within 24 hours of

admission. Rapid appearance, marked increase in the serum concentration after AMI and higher specificity when compared with AST and LD made CK the "first cardiac biomarker". However, CK was not fully specific and it was proven that CK was present in greater concentrations in skeletal muscle than in myocardium in the human body [Jaffe et al.1984, Brancaccio et al.2007].

Electrophoretic methods for separation of CK and LD isoenzymes were first described in 1972 [Roe et al. 1972]. The clinical value of CK isoenzymes stems from the fact that skeletal and myocardial muscle have different isoenzyme distribution. CK-MM is present in both but only trace levels of CK-MB are present in the skeletal muscle and a higher percentage in the myocardium [Wu, 2002]. CK-MB isoenzyme showed the highest diagnostic accuracy for AMI. The discovery of the relatively higher concentration of CK-MB in the myocardium (22% in myocardium, 1-3% in the skeletal muscle) made it the most important biomarker of cardiac injury in the 1980s [Dolci and Panteghini, 2006].

New immunoinhibition assays for CK-MB were soon developed. The availability of a rapid and automated assay for CK-MB determination made this test popular and widely adopted for several years. In 1979, the World Health Organization (WHO) included cardiac biomarkers in the triad of diagnostic tools for AMI [World Health Organization, 1979]. However, the results were far from satisfactory. The major problem was the high rate of false positive results. CK-MB was increased in 20% of patients following general surgery interventions in the absence of myocardial damage [Adams et al. 1993]. In the 1980s scientists started to measure the protein concentration instead of catalytic activity using antibody-assisted techniques. Radioimmunoassays (RIA) and sandwich assays were developed, but even if measured immunochemically as protein, CK-MB was not totally cardiac specific and did not differentiate the origin of the damage [Chan et al. 1985; Wu et al. 1985]. This prompted investigators to turn their attention to other cardiac proteins and the troponin era began.

In 1987, a RIA for detecting the subunit I of the troponin complex (TnI) was developed [Cummins et al. 1987] and, two years later, an enzyme-linked immunoassay for the T subunit (TnT) was designed [Katus et al. 1989]. However, these first-generation assays for the cardiac isoform of TnT (cTnT) showed significant cross reactivity with skeletal isoforms. Moreover, it was proven that cTnT

was re-expressed during diseases that involve skeletal muscle regeneration (muscular dystrophy, polymyositis or uremic myopathy) [Bodor et al. 1997; Muller-Bardoff et al. 1997]. In consequence, cTnI came into the limelight.

Clinical studies with isoform I (cTnI) led to a few surprises. While CK-MB returned to the baseline after a few days, cTnI was still detectable 7-10 days after the cardiac insult. This turned out to be an advantage to detect cardiac damage in patients seen late after their MI [Jaffe et al. 1996]. Secondly, troponins were shown to be elevated in about one third of patients in whom AMI was ruled out by WHO criteria. These patients were believed to have sustained "minor myocardial injury", later described as "unstable coronary disease" [Galvani et al. 1997]. The prognosis for these patients was definitively worse compared with those with troponin levels within the normal limits. The only indication of myocardial injury in these patients was the increase in cTnI concentration. Early therapy in patients with unstable coronary disease reduced the risk of major cardiac events, making early detection and risk stratification critical .Laboratory information classified as falsely positive only ten years before now had therapeutic implications.

Finally, the criteria for diagnosis of myocardial infarction changed in 2000, now defined as detection of a rise and/or fall of cardiac biomarker values (preferably cardiac troponin) with at least one of the following: symptoms of ischaemia, changes in the electrocardiogram (ECG), imaging evidence of new loss of viable myocardium/wall motion abnormality and identification of an intracoronary thrombus by angiography or autopsy [Alpert et al. 2000].

The discovery of Troponin as a biomarker changed the foundations of human cardiology. A simple blood test measuring a cardiac protein became the cornerstone for myocardial infarction diagnosis.

1.1.2 BIOCHEMICAL BACKGROUND

Troponins are muscle proteins that are only present in striated muscle [Wells et al. 2008]; they are restricted to the skeletal muscles and the heart. Troponins are not present in the smooth muscle [Collinson et al. 2001; Wells et al. 2008].

The striated pattern in skeletal and cardiac muscle results from the arrangement of the filaments within the myofibrils. Two types of these protein structures are identified, "thick" and "thin" filaments. The thick filaments are composed almost

entirely of the contracting protein “myosin”. The thin filaments contain the contractile protein “actin” as well as two other regulatory proteins: “tropomyosin” and “troponin” [Fox, 2006].

Troponins are a hetero-trimeric complex, which lies at regular intervals along the thin filament [Solaro et al. 2008]. This is shown in Figure 1.1. The complex consists of 3 interacting and functionally distinct proteins: troponin C, troponin T and troponin I. The structure resembles a tadpole, with the head comprising a dumb-bell-shaped troponin C, a globular troponin I and the C-terminal region of troponin T; the tail is made up of the N-terminal region of troponin T [Collinson et al. 2001].

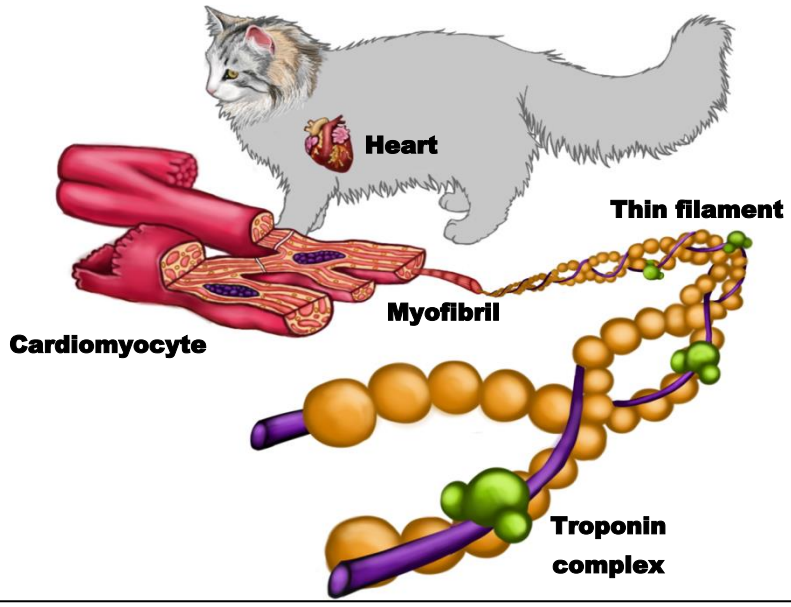

FIGURE 1.1 Structure of the muscle. The troponin complex (green),tropomyosin (purple) and actin (yellow) constitute the thin filaments. Thin and thick filaments (myosin) form cylindrical bundles known as myofibrils. The cytoplasm of cardiomyocytes is filled with myofibrils and they are responsible for contraction in the cardiac muscle.

The subunits or proteins that form the troponin complex have different function, structure and molecular weight. Troponin C (“C” for calcium, TnC) is the calcium-binding subunit of the troponin complex. Structural studies of TnC revealed a dumbbell-shaped molecule with two globular N and C-terminal domains connected by a central helical linker. Its molecular weight is 18 kDa. Troponin T (“T” for tropomyosin, TnT) binds the troponin complex to the tropomyosin and two regions are recognized, N-terminal region which attaches the troponin complex to

tropomyosin and C-terminal region which interacts with TnI and TnC in a calcium-dependent manner. This protein is heavy and is 37 kDa. Troponin I ("I" for inhibition, TnI) is the inhibitory subunit of the troponin complex and it is a rod-like flexible protein. Its molecular weight is 22 kDa **[Collinson et al.2001]**.

Many authors described troponin I as the most sensitive of the three subunits. Commonly, cTnI levels increase with less severe disease than cTnT, suggesting that cTnI is more sensitive to myocardial injury **[Langhorn et al. 2013]**. Several veterinary studies have measured both troponin T and troponin I in parallel in the same population of animals **[Burgener et al. 2006, Carretón et al. 2011, Langhorn et al. 2013 and Langhorn et al. 2014 (b)]**. In all of them cTnI was detectable and above the reference range in a higher proportion of the animals included in comparison with troponin T results. These authors hypothesized this could be due to a difference in protein size and molecular weight or because cTnT may have a structurally closer binding to the tropomyosin chain than cTnI.

1.1.2.1 Isoforms and specificity

Troponin C (TnC) is present in 2 isoforms. There are 2 known TnC genes, one encoding for the fast skeletal muscle isoform (fTnC) and the other expressing both cardiac (cTnC) and slow skeletal (sTnC) isoforms. The amino sequences of human cTnC, sTnC and fTnC are 66% identical. This homology between cardiac and skeletal isoforms reduces the cardiac specificity of TnC **[Gomes et al. 2002]**.

Troponin T (TnT) has multiple isoforms. Several are present in skeletal muscle and 4 isoforms exist in cardiac tissue, although only one is characteristic of the adult heart. The other 3 are expressed in fetal tissue and may be re-expressed during heart failure or in damaged skeletal muscle **[Wells et al. 2008]**. Studies have documented the lack of specificity of the capture antibody in the first-generation TnT assay. This assay detected both fetal cardiac isoforms and some skeletal muscle cTnT isoforms re-expressed in skeletal muscle in response to injury. These fetal isoforms were then characterized and a new antibody was developed. Experiments conducted by different groups concluded that cTnT had unique cardiac specificity that was equal to that of cTnI **[Thygesen et al.2010]**. However, it has been recently demonstrated that TnT expressed in diseased skeletal muscle is detected by the

antibodies used in both the fourth-generation and high-sensitivity assays **[Jaffe et al. 2011]**

Three isoforms exist for TnI. There are three known TnI genes, which encode for cardiac, fast and slow skeletal troponin isoforms (cTnI, fTnI and sTnI, respectively) **[Parmacek and Solaro, 2004]** . The amino acid sequences of these three isoforms are highly homologous with the exception of a unique 32-residue N-terminal extension that is only present in the cardiac one. This unique extension is present in all cTnI isoforms across species and is highly conserved among them. Both canine and feline troponin genes have been cloned and sequenced **[Rishniw et al. 2004]**. Compared with the human gene, canine cTnI had one additional amino acid, which is also detected in rodents (mouse and rat). This "extra" amino acid was also present in feline cTnI but cats lack of a single amino acid - Glutamate/ Glycine (position 4). Therefore there is a high degree of homology in the coding region among dogs, cats, humans, mice and rats and commercial human cTnI analyzers can be used to measure serum cTnI in dogs and cats. Unlike TnT, cTnI is not expressed after damage and regeneration in adult skeletal muscle. Therefore, cTnI is the most cardio-specific protein of the troponin complex and the main target of the analytical assays **[O´Brien et al. 1997].**

1.1.2.2. Function

Troponins are essential regulatory proteins of the muscle contractile apparatus. Striated muscle contraction is regulated by intracellular calcium concentration via the thin filament regulatory proteins: troponin and tropomyosin **[Solaro et al.2007]**. This system prevents interaction of actin and myosin in the absence of calcium. At rest, the concentration of calcium in the cytoplasm is very low due to calcium pumps in the sarcoplasmic reticulum. With low levels of calcium, troponin and tropomyosin are located on the outer domain of the actin, covering the myosin-binding sites. This is known as the "blocked state" **[Gomes et al. 2002].**

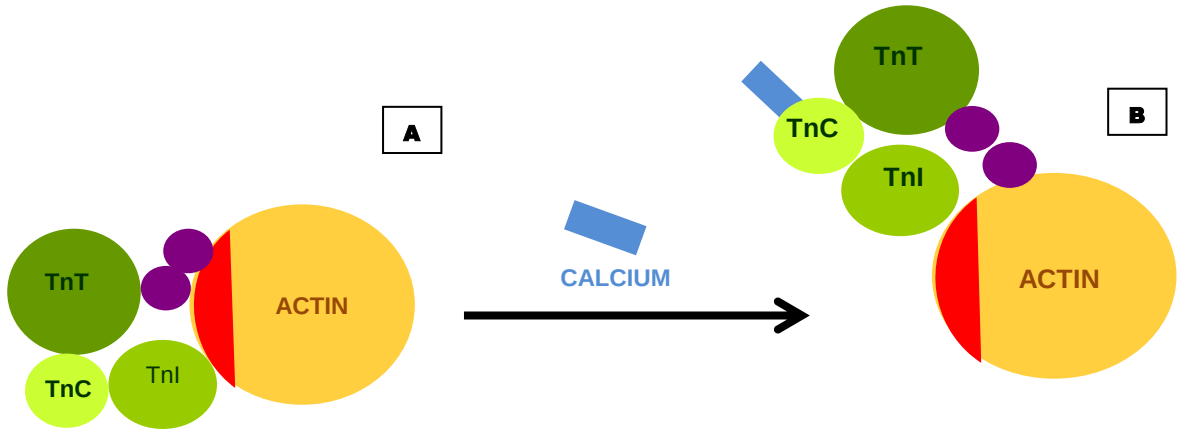

FIGURE 1.2. Calcium regulation of muscle contraction. Calcium induces a conformational change on Tn, exposing the myosin binding region of the actin (colored in red) which allows the interaction between the filaments. A, blocked state; B, open state.

On depolarization of the sarcolemma, calcium is released from the sarcoplasmic reticulum and binds to TnC. Because TnC does not interact directly with actin or tropomyosin, the signal must be transmitted via the other two troponin subunits (TnT and TnI). TnI and TnC bind with higher affinity in the presence of calcium, which triggers a conformational change and the tropomyosin is moved away from its blocking position on actin, changing to the "open state" **[Craig and Lehman, 2001; Geeves and Lehrer, 2002].** Figure 1.2 illustrates the structural changes in the Tn complex and thin filament following calcium activation.

1.1.2.3. Biomarker release

There are 2 populations of cTn within the cell. The majority of cTn is found as structural proteins in the contractile apparatus, bound within the thin myofilaments. However, a small percentage (2-4%) remains free in the cytosol **[Hickman et al. 2010, Lippi et al. 2011].** It is believed that the cytosolic pool is released first resulting in an early rise in blood and a rapid washout. The half-life of TnI in blood is 2 hours. A rapid rise and fall within 24 hours may be consistent with the release of this pool and "reversible damage". In contrast, if Tn levels remain elevated for 4-10 days, this might be due to continued breakdown of contractile proteins **[White, 2011]**. Figure 1.3 shows the mechanisms of troponin release in transient and permanent ischaemia.

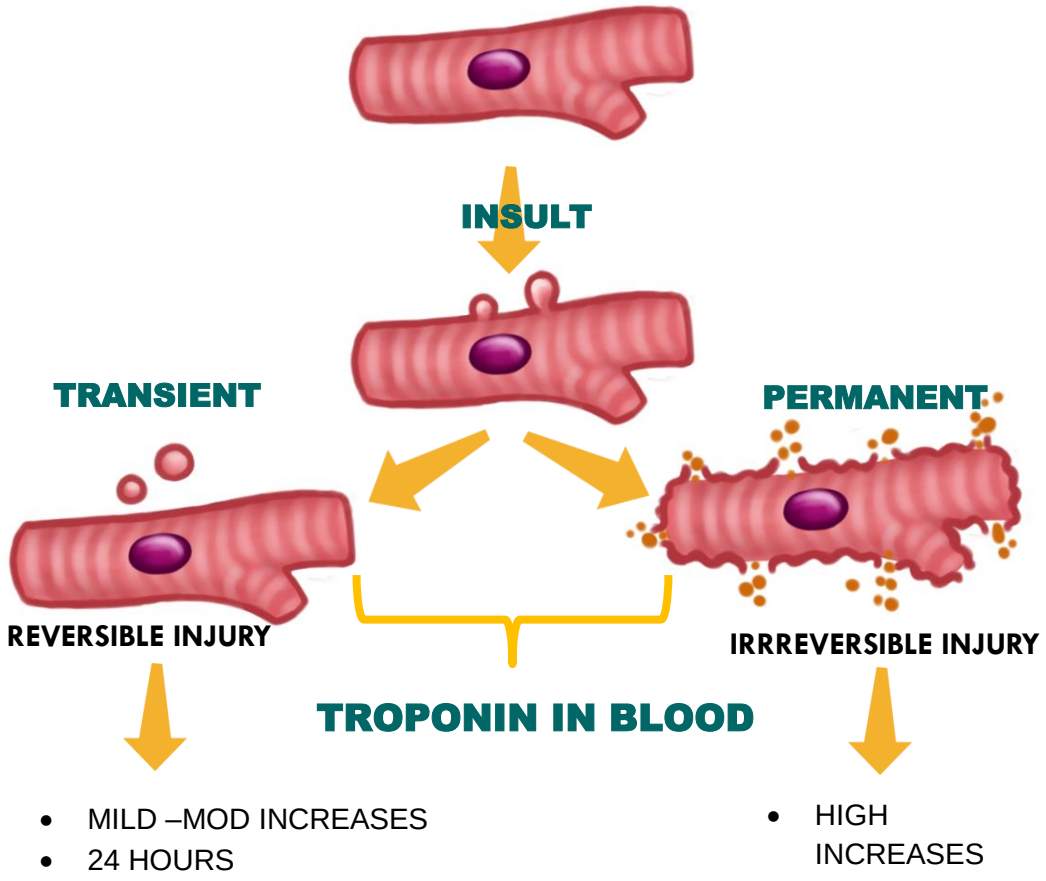

FIGURE 1.3. **Mechanisms of release of troponin in transient or permanent ischaemia.**

Proteolytic degradation is mandatory for the structural troponin to be released, whether the cytosolic pool could be released in the presence of ischaemia rather than necrosis alone is a matter of debate. Troponin has been proven to be elevated in several non-cardiac conditions. For example, 94% of athletes undergoing endurance sports had measurable high sensitivity troponin levels - this is thought to mirror increased membrane permeability rather than a clinically threatening myocardial injury [Lippi et al. 2011].

There are 6 potential major pathobiological mechanisms for troponin release:

1. **MYOCYTE NECROSIS:** ischaemia, inflammation, infiltrative disease, trauma and toxic causes (including sepsis) [French and White, 2004].
2. **APOPTOSIS:** programmed cell death [Narula et al.1996] .

3. **NORMAL MYOCYTE TURNOVER:** cardiac myocytes regenerate although they do it slowly (1% annual turnover at the age of 25). Whether such low-grade turnover results in release of troponin is unknown **[Bergmann et al. 2009]**.

4. **PROTEOLYTIC DEGRADATION:** proteolysis to create small fragments could allow these to pass through an intact membrane **[Gao et al.1997 and Feng et al.2001]**

5. **INCREASED WALL PERMEABILITY:** simulation of stretch-responsive integrins has been shown to result in release of troponin from intact cultured cardiomyocytes. Rat models have demonstrated that increased pre-load is associated with troponin release, independent of ischaemia **[Hessel et al.2008]**

6. **BLEBS:** cardiac myocytes develop blebs (active secretion vesicles) during anoxia, with release of cytoplasmic enzymes without cell necrosis **[Schwartz et al.1984].**

1.2 TROPONINS IN SMALL ANIMALS

1.2.1 TROPONINS IN CANINE MEDICINE

The literature reviewing the usefulness of troponins in canine patients in both cardiac and non-cardiac conditions is extensive, detailed and confusing. The lack of standardization of the cTnI assays employed in scientific research has resulted in a wide variety of proposed reference ranges, significant differences in detection limits of the assays and it is not uncommon to find controversial conclusions.

1.2.1.1 CARDIAC DISEASE

A. Pericardial disease

The most common cause of clinically significant pericardial effusions (PE) in dogs is neoplasia **[French, 2010]**, most often haemangiosarcoma. Due to the diagnostic challenge of finding small masses by echocardiography and the poor prognosis for haemangiosarcoma, three studies have explored the value of troponin in the diagnosis of cardiac neoplasia.

In one study, samples from 37 dogs with PE and 5 controls were collected prospectively. Eighteen dogs with HSA, 6 with idiopathic pericardial effusion (IPE), 1 heart base tumour and 1 mesothelioma were finally included; in 11 cases a definitive clinical or histopathological diagnosis was not made **[Shaw et al. 2004]**.

Dogs with PE had significantly higher concentrations of cTnI than did the controls. Dogs with HSA had significantly higher cTnI concentrations than did dogs with IPE. There was no difference in cTnT concentrations between the groups. Thus cTnI might be a useful marker by discriminating between idiopathic pericardial effusions and those caused by HSA.

In contrast, a second study concluded that cTnI could not act as a discriminator in dogs with PE. Twenty-five dogs with PE were included (neoplastic PE= 21; non-neoplastic PE= 4) and cTnI was measured in both pericardial effusion and peripheral blood. Dogs with HSA (n=14), heart base tumours (n=2), carcinomas (n=2), lymphoma (n=1) and mesothelioma (n=1) were included. The four dogs with non-neoplastic PE included fungal and bacterial pericarditis and lymphocytic-plasmacytic pericardial inflammation (possible foreign body).

No significant difference was identified between dogs with right atrial tumours (n=14) and other types of neoplasia (n=7). The median cTnI level in PE from dogs with neoplastic and non-neoplastic disease was not significantly different **[Linde et al. 2006]**.

A third study prospectively recruited 57 dogs, of which 18 had cardiac HSA (5 confirmed, 13 suspected), 14 had non-cardiac HSA, 10 had PE not caused by HSA and 15 had non-cardiac, non-HSA neoplasms. The plasma cTnI concentration was higher in dogs with cardiac HSA compared with dogs with HSA at other sites, dogs with other neoplasms and dogs with PE not caused by HSA. cTnI concentrations were therefore considered useful in identifying cardiac HSA in dogs with PE. Plasma concentrations above 0.25ng/ml indicated that HSA was likely in dogs with PE (sensitivity 81%, specificity 100%) and levels above 2.45ng/ml indicated cardiac involvement in dogs with HSA at other sites **[Chun et al. 2010].**

Finally, in a retrospective survival study of 120 dogs, most dogs with IPE had cTnI between 0.151 and 1.0 ng/ml whereas dogs with PE associated with neoplasia had cTnI levels above 1.01 ng/ml, suggesting that cTnI might be useful to distinguish the aetiology **[Fonfara et al. 2010].**

B. Cardiomyopathies

Dilated cardiomyopathy (DCM) is the most common type of cardiomyopathy in dogs. It is a disease of cardiac muscle of unknown origin, often familial, characterized by slowly progressive ventricular dilation and loss of cardiac contractibility. Approximately 25% of Irish Wolfhounds, 50% of male Doberman Pinschers and 33% of female Doberman Pinschers develop DCM. Sudden cardiac death caused by ventricular tachycardia-fibrillation occurs in at least 25-30% of affected Dobermans **[Oyama, 2008]**.

B.1) DOBERMAN PINSCHERS

Serum cTnI concentrations in Dobermans in various stages of cardiomyopathy (CM) have been evaluated. Dobermans (n=336) without evidence of systemic disease were recruited for this prospective study. Dogs were classified in 4 different groups: a "control group" (no clinical signs, no arrhythmia, no morphological changes), an "incipient group" (initially with no signs, no arrhythmia and normal echo at the time of examination, but abnormal at the next examination within 1.5 years), a "VPC

(ventricular premature complex) group" (no clinical signs, normal echo but >100 VPCs/24h on Holter), an "ECHO group" (no signs, <50 VPCs/24h but abnormal M mode measurements), a "VPC and ECHO group" (no clinical signs, >100 VPCs/24h and abnormal M-mode measurements) and a "Clinical group" (dogs showing clinical signs with abnormal ECG and echo).

Troponin I was significantly higher in all disease groups compared with the control group. Moreover the "clinical group", animals with the most advanced disease, had the highest cTnI levels. No significant association between the number of VPCs and cTnI levels was found.

Animals with initial normal echo and Holter results that developed DCM within 1.5 years had increased troponin levels at the time of examination. Therefore the ability to detect the "incipient stage", which the currently "gold standard" methods were unable to detect, suggests that cTnI is a useful additional screening test for cardiomyopathy in Doberman Pinschers. It was recommended that dogs with increased cTnI levels should be re-examined more frequently **[Wess et al. 2010]**.

B.2) PORTUGUESE WATER DOGS

Juvenile DCM (JDCM) in Portuguese Water Dogs (PWD) is an early onset disease with a poor prognosis. The usefulness of cardiac biomarkers to detect JDCM was evaluated in one study of 4 affected and 4 unaffected age-matched puppies. There was no significant difference in results and cTnI was not considered useful in the diagnosis of JDCM of PWD **[Sleeper et al. 2002]**.

B.3) BOXERS

Myocardial disease in boxers is often characterized by ventricular arrhythmias, rather than the ventricular dilation and systolic dysfunction seen in DCM of other breeds. Due to similarities with arrhythmogenic right ventricular cardiomyopathy (ARVC) in humans, boxer cardiomyopathy is often referred to as ARVC **[Meurs, 2004]**. Three different stages are commonly recognized: asymptomatic dogs with ventricular tachyarrhythmias; dogs with syncope; dogs with systolic dysfunction and heart failure. Sudden cardiac death may occur without previous clinical signs **[Hariu and Carpenter, 2010].**

In a study evaluating the use of non-invasive techniques for the early diagnosis of ARVC in Boxers, serum cTnI concentrations from 30 dogs, 10 boxers with early ARVC (>1000 VPCs/24h and echocardiographic variables within the normal range), 10

control boxers (<5 VPCs/24h and normal echo) and 10 control non-boxer dogs, were compared **[Baumwart et al. 2007]**.

A significant (P=0.01) difference in cTnI concentrations was found between Boxers with ARVC and control boxers. Mean serum cTnI concentration was 0.142 ± 0.05ng/ml for boxers with early ARVC, 0.079 ±0.03 for control boxers and 0.023 ± 0.01ng/ml for non-boxers. A significant difference was observed for cTnI concentration between groups. Because of the difference between the two control groups (Boxers and non-Boxer dogs), it was suggested that Boxers, as a breed, may have higher reference range values for serum cTnI concentrations.

A significant correlation (P <0.001) was found between the serum cTnI concentration and number of VPCs/24h for the combined Boxer population. Furthermore a significant (P <0.001) correlation was also identified between the concentration of cTnI and the grade of arrhythmia, suggesting that cTnI could be an indicator of severity of the disease. However there was an overlap between the apparently unaffected (control) and ARVC Boxers.

B.4) SURVIVAL, CHF AND ARRHYTHMIAS IN CARDIOMYOPATHIES

Another study, published in 2004, evaluated plasma cTnI concentrations in 269 canine patients with naturally occurring heart disease: 176 were determined to be healthy, 26 were diagnosed with CM, 37 with mitral valve disease (MVD) and 30 with congenital subaortic stenosis (SAS) **[Oyama and Sisson, 2004]**.

The median cTnI concentrations in dogs with CM were significantly greater than in healthy dogs and those with SAS. Within the healthy population a weak but significant correlation between age and cTnI was found. In dogs with CM there was no difference in median cTnI between dogs with and without congestive heart failure (CHF) and between those with and without arrhythmias. The median survival time of dogs with CM and cTnI >0.20ng/ml (11/26) was 112 days versus 357 days in 15 dogs with cTnI <0.20ng/ml.

B.5) OCCULT CARDIOMYOPATHY

A study evaluating alternative methods for the early detection of CM in Boxers, Doberman Pinschers and Great Danes looked at the ability of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and cTnI to detect occult CM in a high-risk population **[Oyama et al. 2007]**.

One hundred and eighteen dogs were prospectively enrolled; occult DCM was diagnosed in 21 dogs and the remaining 97 were considered healthy based on ECG and echocardiographic findings. Dogs with occult DCM had significantly greater plasma concentrations of ANP, BNP and cTnI compared with clinically normal dogs. Analysis of the area under the curve (AUC) of the receiver operator characteristic curve (ROC) analysis revealed, however, that only BNP had sufficient sensitivity and specificity to be of diagnostic use.

C. Mitral valve disease

Myxomatous degeneration leading to mitral valve regurgitation is the most common acquired cardiac disease in the dog. Mitral valve disease (MVD) may affect any breed of dog, but the clinical consequences are observed primarily in elderly and small-breed dogs **[Abbott, 2008].**

C.1) CRP AND cTnI IN DOGS WITH MVD

CRP and cTnI were measured in 81 dogs of which 11 were healthy with unremarkable echocardiograms, 39 were dogs with mild MVD, 13 had moderate MVD and 18 had severe MVD (based on left atrial and mitral jet size **[Ljungwall et al. 2010]**.

Plasma cTnI was found to increase with increasing severity of MVD. Significantly higher concentrations of cTnI were measured in dogs with moderate ($P=0.0011$) and severe ($P=0.0001$) MVD, compared with healthy dogs. Dogs with severe MVD had significantly higher concentrations of cTnI than dogs with mild ($P<0.0001$) and moderate ($P=0.0019$) MVD. There was also a strong correlation between age and cTnI. Therefore the age of the patient needs consideration when interpreting results of cTnI in canine patients.

C.2) cTnI IN DOGS WITH MVD & CLASS IV CONGESTIVE HEART FAILURE:

In this prospective study cTnI and cTnT were measured in dogs with MVD and class IV CHF (*New York Heart Association classification system*) **[Linklater et al. 2007]**. Only 6/15 dogs had a detectable level of cTnI and just 1/15 had cTnT above the detection limit. Long-term survival data was available for 14 of 15 dogs (1 dog was still alive 2.5yrs after presentation). The dogs with detectable concentrations of cTnI had a median survival time of 67.5 days whereas patients with non-detectable troponins survived for a median of 390 days. This difference was statistically significant.

C.3) cTnI AND SURVIVAL IN DOGS WITH MVD

The prognostic potential of high-sensitivity troponin I (hscTnI) and NT-proBNP was assessed in a prospective study of 202 dogs with MVD and 30 controls **[Hezzell et al. 2012]**. The relationship between hscTnI and survival was found to be non-linear, as dogs with hscTnI concentrations >0.025 had shorter survival times regardless of the exact concentration. A ROC curve was constructed and the AUC was 0.629.

Multivariable analysis indicated that left ventricular end-diastolic diameter (LVEDDN), age, heart rate and hscTnI were significantly and independently associated with decreased survival time for all-cause mortality in dogs with naturally occurring MVD. Importantly, hscTnI was not found to be independently associated with survival time in dogs that died or were euthanized because of cardiac disease. Combining serum hscTnI and NT-pro BNP measurements more accurately identified those dogs with a shorter survival time than did either measurement alone. However, when other variables (including echocardiographic variables) were known, the independent prognostic value of including both serum values was lost.

Repeated measures models indicated that the rate of increase of both NT-pro BNP and hscTnI was more rapid in dogs that died because of cardiac disease than in those that died because of non-cardiac conditions. The increase in cTnI occurred late in the course of cardiac disease whereas NT-proBNP increased earlier.

C.4) MYOCARDIAL ARTERIOSCLEROSIS IN DOGS WITH MVD AND CHF

The degree of arteriosclerotic and fibrotic changes was studied in the myocardium at necropsy of 50 dogs with MVD and CHF **[Falk et al. 2013]**.

cTnI concentrations increased with increasing grade of both global fibrosis and fibrosis in the papillary muscles. The type of fibrosis seen in this study was of “replacement type” where myocardial tissue is replaced by fibrous tissue in larger or smaller areas. This type of fibrosis is almost invariably caused by ischaemia and pathologists refer to it as “infarction” **[Jönsson, 1972]**. Serum cTnI concentrations seem to reflect the presence of both intramyocardial fibrosis and arteriosclerosis. This lends support to the hypothesis that arteriosclerosis may cause myocardial damage and may play a role in the pathogenesis of heart failure in MVD in dogs.

D. Congenital cardiac disease

Two published studies **[Spratt et al. 2005, Fonfara et al. 2010]** have shown that congenital disease cases tend to have lower cTnI values than dogs with acquired disease. However, a study of 30 dogs with congenital SAS (Ao velocity >2.1 m/s) showed a median cTnI of 0.08ng/ml (interquartile range 0.01-0.94ng/ml), which was significantly higher than the controls **[Oyama et al. 2004]**.

E. Survival in canine cardiac disease

A retrospective study by **Fonfara et al. [2010]** focused on the use of cTnI concentrations to assess the severity of disease, survival and prognosis in 120 dogs with different cardiac diseases (MVD, CM, congenital disease and arrhythmias)

Survival analysis, using Kaplan Meier curves, showed that dogs with cTnI between 0.151 and 1ng/ml had a median survival time of 24 months whereas dogs with a cTnI concentration higher than 1ng/ml had a median survival time of 3 months. Longer survival times with lower cTnI concentrations were confirmed in dogs with median concentrations of 0.18, 0.07 and 0.05ng/ml that survived more than 1 year, 2 years and 3 years, respectively.

Subsequently, cTnI assays were repeated in 30 animals. Initial cTnI concentrations in dogs with the highest cTnI values that died were not statistically different to those that survived. However, in dogs that survived cTnI concentrations at follow-up were significantly reduced compared to the initial value (P=0.015). Repeat cTnI analysis during follow-up investigations may be useful to assess progression and response to treatment in dogs with cardiac disease. Cardiac conditions were increasingly severe in animals with higher cTnI results and this was accompanied by significantly reduced survival. Interestingly, most dogs with bradyarrhythmias had higher cTnI values, but the underlying cause for this is not known. (*See also B.4 and C.4 above*).

1.2.1.2 NON-CARDIAC CAUSES OF INCREASED cTnI

A. Breed and exercise

Greyhounds, whether racing or non-racing, have increased left ventricular dimensions and wall thicknesses compared to other breeds of the same weight **[Boon, 2011]**. In man the effects of exercise on the heart regress after several weeks of exercise cessation but increased dimensions persist in retired racing

greyhounds. This cardiac enlargement in greyhounds is thought to have both congenital and exercise-induced components [Pape et al. 1984].

One study reported that cTnI concentrations in healthy retired greyhounds were significantly higher than in non-greyhound control dogs [LaVecchio et al. 2009]. The authors warned that a variable proportion of normal/healthy greyhounds could be erroneously diagnosed with having myocardial disease based on the presence of a functional heart murmur, higher vertebral heart score (VHS) on radiographs and increased cTnI concentrations.

In another study cTnI and CK-MB were measured in racing greyhounds [Tharwat et al. 2013]. The median cTnI concentration in the racing dogs was not significantly different from that of the non-racing controls. However, within 2 hours of racing 7km, cTnI concentrations were significantly higher than pre-race values in 97% of the animals. The cTnI levels had dropped back to the pre-race values 24hrs later. The causes of race-induced cTnI elevations are unclear, may be explained by mild hypoxia during exercise and may indicate that racing cause reversible cardiac injury in healthy greyhounds.

Finally, another research group collected blood samples from ten Alaskan sled dogs exercised for 160 km for 5 successive days (day plan: 80 km in 5h – 7-8 h resting – 80 km return journey). For all dogs, serum cTnI was below the level of detection of the immunoassay prior to exercise. Using 0.11ng/ml as a cutoff (analyzer: Immulite troponin I), exercise resulted in high cTnI in all dogs, peaking after the first day of exercise but persisting throughout the 5 days of training [McKenzie et al. 2007].

B. Infectious diseases

B.1) LEPTOSPIROSIS

Acute renal failure remains the most common clinical finding in 90% of reported cases of leptospirosis in domestic small animals. However, other organs may be involved and hepatic disease occurs concurrently with acute renal failure in 10-20% of cases [Harkin, 2009]. Epicarditis and endocarditis have been described in dogs with experimentally induced leptospiral infection [Greenlee et al. 2005].

One retrospective case study assessed the relationship of the concentrations of cTnI and the acute phase proteins C-reactive protein (CRP) and haptoglobin (Hp) with outcome in dogs naturally infected with *Leptospira interrogans* [Mastrorilli et al. 2007]. Twenty dogs met the inclusion criteria (a positive leptospiral microscopic

agglutination test), of which 16 were symptomatic; three dogs had ventricular arrhythmias. Eleven of the 16 symptomatic dogs had increased cTnI concentrations (0.4 to 60.1ng). The cTnI concentration was significantly correlated with CK (P=0.005) and AST (P=0.001) activities, but not with urea (P=0.118). Furthermore, cTnI, CRP/Hp ratio, urinary albumin to creatinine ratio (UAC) and urinary total protein to creatinine ratio (UPC) were significantly higher in non-survivors than in survivors.

The lack of correlation between urea and cTnI in this study could suggest that myocardial damage was due to direct myocyte injury rather than being secondary to azotaemia ("uraemic syndrome"), although urea will increase for many reasons. As cTnI concentrations were higher in non-survivors, cTnI was proposed as a potential prognostic marker in leptospirosis infections. It was also noted that coagulation abnormalities and DIC were common findings in the dogs of this study.

B.2) DIROFILARIASIS (Heartworm)

cTnI, cTnT, myoglobin and D-dimer were measured in dogs with and without adult heartworm infection **[Carretón et al. 2011]**. Levels of cTnT in all dogs were below the limit detection. cTnI concentrations were significantly lower in dogs without clinical signs (group 1: microfilaraemic, negative for *D. immitis* antigen) compared to group 2 (positive for *D. immitis* antigen but not microfilaraemic) and group 3 (positive for *D. immitis* antigens and microfilaraemic). No statistically significant difference was found between group 2 and 3.

The hearts of 24 naturally infected dogs were examined by the same group using routine histology and immunohistochemistry with anti-myoglobin and anti-cTnI antibodies **[Carretón et al. 2012]**. There was no apparent association between worm burden and the presence of myocardial lesions and there was no correlation between the plasma levels of cardiac biomarkers and the presence of myocardial lesions

This group also measured cTnI, myoglobin, CK-MB and AST in dogs infected with *D. immitis* during adulticide treatment with ivermectin, doxycycline and melarsomine. In this study dogs with a high worm burden (n=6) showed increased cTnI concentrations; dogs with a low burden (n=9) had similar levels to that of healthy dogs. After 60 days of treatment concentrations of cTnI decreased in all dogs, but

in those animals with high burdens the concentrations of cTnI, although decreased, remained above the reference range [Carretón et al., 2013].

Increased cTnI concentrations have been also demonstrated in dogs with **other vector-borne diseases** such as: Babesiosis [Moore and Williams, 1979, Lobetti et al. 2002 and Taboada and Lobetti, 2006], Ehrlichiosis [Lakkawar et al. 2003, McQuiston et al. 2003, Diniz et al. 2008, Mylonakis et al. 2010, Koutinas et al. 2012 and Champion et al. 2013] and Chagas disease, also known as "American trypanosomiasis", [Barr et al. 2005, Kjos et al. 2008 and Spikler, 2009].

C. Anaemia

Mortality of up to 70% has been reported in dogs diagnosed with immune-mediated haemolytic anaemia (IMHA) and often is associated with thromboembolism [Tircoveanu and Van Der Linden, 2008]. Lesions due to coagulopathy (macrothrombi, microthrombi, fibrin deposition and haemorrhage) and cholestasis were the most common abnormalities at postmortem examination of 34 dogs with IMHA, 10 having lesions in the heart: myocardial necrosis (n=6) and myocarditis (n=4) [McManus et al. 2001].

cTnI concentrations have been determined in dogs with IMHA to analyze the incidence of myocardial damage in this population [Gow et al. 2011]. Eleven healthy, 27 IMHA and 49 hospitalized dogs without cardiac, renal or haematological disease were included in this study. A significant difference was found between the serum cTnI concentrations of dogs with primary IMHA and the hospitalized dogs ($P<0.005$) and healthy dogs ($P<0.01$). There was no significant difference in the cTnI concentrations between dogs with marked anaemia compared to those with moderate anaemia. The median serum cTnI concentration was higher in non-survivors compared to dogs that survived for at least 4 weeks, although this was not significantly different (only 4 dogs died in the follow up period thus the study was severely underpowered).

D. Renal failure

cTnI concentrations have been analyzed in dogs with renal failure in the absence of concurrent cardiac disease. In the first study, 39 dogs with renal failure were included and 28 animals (71%) had cTnI concentrations above the reference interval

[Porciello et al. 2008]. The median cTnI concentration in dogs with azotaemia (creatinine >152µmol/L and isosthenuria) was 0.43ng/ml (range: 0.02-396ng/ml) and no correlation was found between the degree of azotaemia and cTnI. Canine patients with other systemic illnesses (diabetes, peritonitis, lymphoma, leishmaniasis, hepatitis, hyperadrenocorticism, neoplasia and intestinal foreign bodies), without evidence of cardiac disease, were also evaluated in this study. cTnI was also increased in 12/17 (70%) of these patients, confirming that increased cTnI levels are not uncommon in many diseases without obvious antemortem evidence of cardiac involvement.

Another study compared cTnI concentrations in dogs with renal failure (and without evidence of cardiac disease) with those in healthy dogs [Sharkey et al. 2009]. Of the dogs with renal failure, 66% had cTnI concentrations above the reference range and cTnI levels were significantly higher in dogs with renal failure when compared to healthy dogs. The systolic blood pressure in dogs with renal failure was also significantly higher when compared with control dogs ($P<0.001$), but there was no significant correlation between serum cTnI concentration and systolic arterial blood pressure. The necropsy performed on 3 out of 4 dogs that died revealed cardiac lesions in all 3 (multifocal fibrosis with lymphocytic infiltration, endocardiosis and a focal haemorrhagic infarct with necrotizing myocarditis).

E. Gastric dilatation-volvulus

The severe distension of the stomach results in hypovolemic shock, and endotoxaemia secondary to portal vein occlusion contributes to the shock syndrome. Altered microvascular perfusion with hypoxemia and endotoxaemia also favours the development of disseminated intravascular coagulation (DIC) [Gibson, 2015]. Myocardial ischaemia may cause cardiac arrhythmias, which are frequently seen in dogs with GDV (40-70%) and may contribute to death [Schober et al. 2002].

In this study by Schober et al. 85 dogs with GDV were prospectively recruited to assess the usefulness of cTnI and cTnT as outcome predictors and to evaluate the correlation with the presence of arrhythmias. cTnI was detected in 74 patients (87%) whereas cTnT was measurable in 43 (51%). ECG abnormalities were identified in 68 (80%) dogs, ventricular arrhythmias being most prevalent. For both cTnI and cTnT, serum concentrations in dogs with no or mild ECG changes were significantly lower

than values for dogs with moderate or severe ECG abnormalities, values for dogs with moderate ECG abnormalities being significantly lower than for dogs with severe ECG changes. Sixteen (19%) dogs died and 10 of those had ventricular arrhythmias. Serum cTnI and cTnT concentrations were significantly higher in dogs that died than in dogs that survived ($P < 0.05$). Serum cTnI and ALT activity 48 hours after surgery were independent predictors of outcome with an accuracy of 91% **[Schober et al. 2002]**.

F. Pyometra

A serious consequence of the uterine infection in pyometra is endotoxaemia and possible progression into a systemic inflammatory response syndrome (SIRS). Myocardial injury and failure secondary to endotoxaemia, inflammation, disseminated bacterial infection and infarction are suspected to be contributing factors to unexpected deaths in bitches with pyometra **[Maretta et al. 1989]**.

cTnI concentrations have been measured in bitches with pyometra in two studies. The first compared cTnI results between 58 bitches with pyometra and 9 controls **[Hagman et al. 2007]**. Detectable levels of cTnI were found in 7 (12%) of the 58 cases. One of the two dogs with the highest cTnI levels had an arrhythmia on the first day post-surgery and died later during that day. Myocarditis and disseminated bacterial infection was demonstrated on post-mortem examination. There was a trend for increased mortality in the pyometra patients with detectable cTnI levels.

A second study evaluated the occurrence of myocardial damage in dogs with pyometra pre- and post-operatively, as well as its relation to the presence of SIRS **[Pelander et al. 2008]**. Fifteen bitches undergoing surgery for neutering (n=12) and mammary tumour removal (n=3) were used as controls. All controls had undetectable pre-operative cTnI concentrations, whereas 13/46 (28%) bitches with pyometra had increased cTnI values. After surgery, 2 controls and 18 pyometra cases had increased serum cTnI levels. Thirty seven out of 46 pyometra cases met two or more criteria for SIRS. There was no statistically significant difference between SIRS positive and SIRS negative animals.

G. Blunt chest trauma

Concentrations of cTnI, cTnT, α-hydroxybutyrate dehydrogenase and CK-MB were determined in 33 dogs with severe blunt chest trauma **[Schober et al. 1999]**. These were compared with values from healthy controls (n=40) and correlated with the presence of ECG abnormalities. cTnI was detected in 21/33 (64%), above the reference range in 18 (55%) and above the cut off of 2ng/ml (used to separate minor and major cardiac lesions in humans) in 16 dogs (48%). In contrast, cTnT was increased in 9/33 dogs (27%) and was above 0.1ng/ml in four dogs (12%). Relevant arrhythmias were found in only 5 dogs (15%) although ECG records were taken 12 to 24 hours after injury and some abnormalities may have disappeared. Nevertheless, no correlation was found between the occurrence of ECG abnormalities and biochemically detectable myocardial cell damage.

H. Systemic inflammation

Langhorn and colleagues have assessed myocardial injury and survival in critically ill dogs admitted to an intensive care unit (ICU) with evidence of systemic inflammation (CRP concentrations greater than 35 mg/L), and two studies have been published (2013, 2014) involving the same population of dogs **[Langhorn et al. 2013, 2014 (c)]**. Animals with evidence of structural cardiac disease or those receiving cardiotoxic drugs before admission were excluded. cTnI and cTnT were compared in 8 healthy controls and the 42 dogs with trauma, neoplasia, respiratory disease, neural conditions, haematological disorders, splenic haematoma, peritonitis, GDV and mucocele that met the inclusion criteria.

The first study focused on short-term survival (28 days) **[Langhorn et al. 2013]**. Forty of the 42 dogs (95.2%) had cTnI concentrations above the reference range at the time of ICU admission. Non-survivors had significantly higher cTnI concentrations than survivors and control dogs ($P < 0.001$). Twenty one dogs had cTnT concentrations above the detection limit of the assay. Non-survivors had significantly higher cTnT than survivors. Admission cTnI and cTnT concentrations were highly correlated. Both cardiac troponins were found to be good prognostic markers, however, cTnI was the best short-term marker.

Their second study aimed to determine the long-term (1-12mths) prognostic potential of cTnI and cTnT and also evaluated the value of daily troponin

measurements in the ICU [Langhorn et al. 2014 (c)]. One year outcome was available for 38/42 dogs. The 1-year mortality rate was 47% (18/38); 11 died within the first 28 days post-admission. Non-survivors had significantly higher cTnI and cTnT concentrations than survivors. Peak cTnI concentrations were not significantly higher than admission concentrations (serial determination of cTnT was not possible due to ethical regulation and serum volume restrictions). The median day of ICU hospitalization that cTnI peaked was day 1.5. There was no significant difference between admission cTnI, peak cTnI and admission cTnT between short and long-term non-survivors.

It was concluded that daily measurements of troponins in the ICU were of value as peak cTnI concentration, but not admission cTnI, was related to 1yr case mortality. In addition, changes in circulating cTnI did not distinguish non-survivors from survivors. In contrast to the previous study, admission cTnT was the best long-term prognostic marker.

J. Cardiotoxicity

J.1) SNAKE ENVENOMATION

Cardiac arrhythmias are commonly seen in humans and animals bitten by venomous snakes, and myocardial damage, including haemorrhage and necrosis, is suspected to have a negative impact in their outcome [Blum et al. 2004]. The presence of myocardial damage as a contributing factor to morbidity and mortality in dogs bitten by the European viper (*Vipera berus*) has been evaluated. Blood samples and 2-minute ECG recordings were collected at presentation, and at 12, 24 and 36 hours in 24 envenomed dogs. Eight dogs had an increased serum cTnI concentration at one or more of the four sampling time points after the snake bite. Six dogs had abnormal ECGs but only 3 of these 6 dogs had increased cTnI concentrations [Pelander et al. 2010].

A collaborative study between the University of Pretoria (South Africa) and the University of Copenhagen (Denmark) documented myocardial injury and systemic inflammation in dogs envenomed by 3 different snake species (V. berus, Bitis arietans and Naja annulifera). cTnI concentrations were correlated with CRP. These results suggest that myocardial damage is secondary to the inflammatory reaction rather than a direct cardiotoxic effect of the venom. Interestingly, a higher degree

of cardiac injury (higher cTnI concentration) was not present in dogs that presented with or developed arrhythmias **[Langhorn et al. 2014a].**

J.2) CHEMOTHERAPY

Anthracyclines (doxorubicin and epirubicin) are commonly used in oncology as they are among the most potent chemotherapeutic agents. Unfortunately, their cardiac toxicity is well characterized in human, laboratory and small animals limiting their use in both human and veterinary medicine **[Adamcova et al. 2005].** Anthracycline cardiotoxicity is cumulative, irreversible, acute or delayed, and in a small percentage of the patients fatal. In humans, the prognosis of patients who develop CHF is poor (50% mortality in 1 year) **[Von Hoff et al. 1979]**. Prevention is, therefore, the best approach and monitoring for early detection of cardiomyopathy is of paramount importance in the management of patients undergoing anthracyline chemotherapy. The percentage of cardiac complications in dogs at standard doses is approximately 3-18%. **[Selting et al. 2004, Takemura et al. 2007 and Chatterjee et al. 2010].**

cTnT has been measured in dogs undergoing doxorubicin treatment. The study included 64 dogs with untreated neoplasia, asymptomatic DCM, advanced CHF, muscoskeletal trauma and dogs receiving doxorubicin **[DeFrancesco et al. 2002]**. Two dogs with lymphosarcoma on doxorubicin were studied. Both dogs had detectable concentrations of cTnT. In this study, normal dogs and dogs with untreated neoplasia had cTnT concentrations below the detection limits. After a cumulative doxorubicin dose of 150mg/m^2 the highest cTnT concentration ocurred after 3.5 weeks, whereas after a cumulative dose of 180 mg/m^2, the highest concentrations were seen after 2 weeks. One of the dogs died from suspected doxorubicin-induced cardiotoxicity (ventricular arrhythmia)

Another report analyzed the usefulness of cTnI to predict cardiac complications in dogs with lymphoma and osteosarcoma undergoing doxorubicin chemotherapy **[Selting et al. 2004]**. Serum cTnI increased in some dogs treated with doxorubicin. Several dogs did not show evidence of heart disease following doxorubicin administration during the monitoring period but had increased cTnI concentrations. cTnI failed to predict cardiac adverse events in dogs treated with doxorubicin.

K. Non-cardiac dyspnoea

NT-proANP, BNP and endothelin-1 have been demonstrated to be useful for distinguishing between dogs with cardiac (CHF) and non-cardiac causes of dyspnoea, whereas cTnI failed to distinguish these causes **[Prosek et al. 2007]**. Twenty six dogs with non-cardiac diseases such as pneumonia (n=11), pulmonary neoplasia (n=8), neoplastic pleural effusion (n=3), laryngeal paralysis (n=2) and chronic bronchitis (n=2) were included in that study. The authors hypothesized that severe hypoxia due to respiratory disease may result in secondary cardiac injury or may be a consequence of endothelial damage in the pulmonary vasculature or a result of the activation of angiotensin-converting enzyme.

A diagrammatic representation of the non-cardiac causes of increased cTnI in the dog is shown in Figure 1.4.

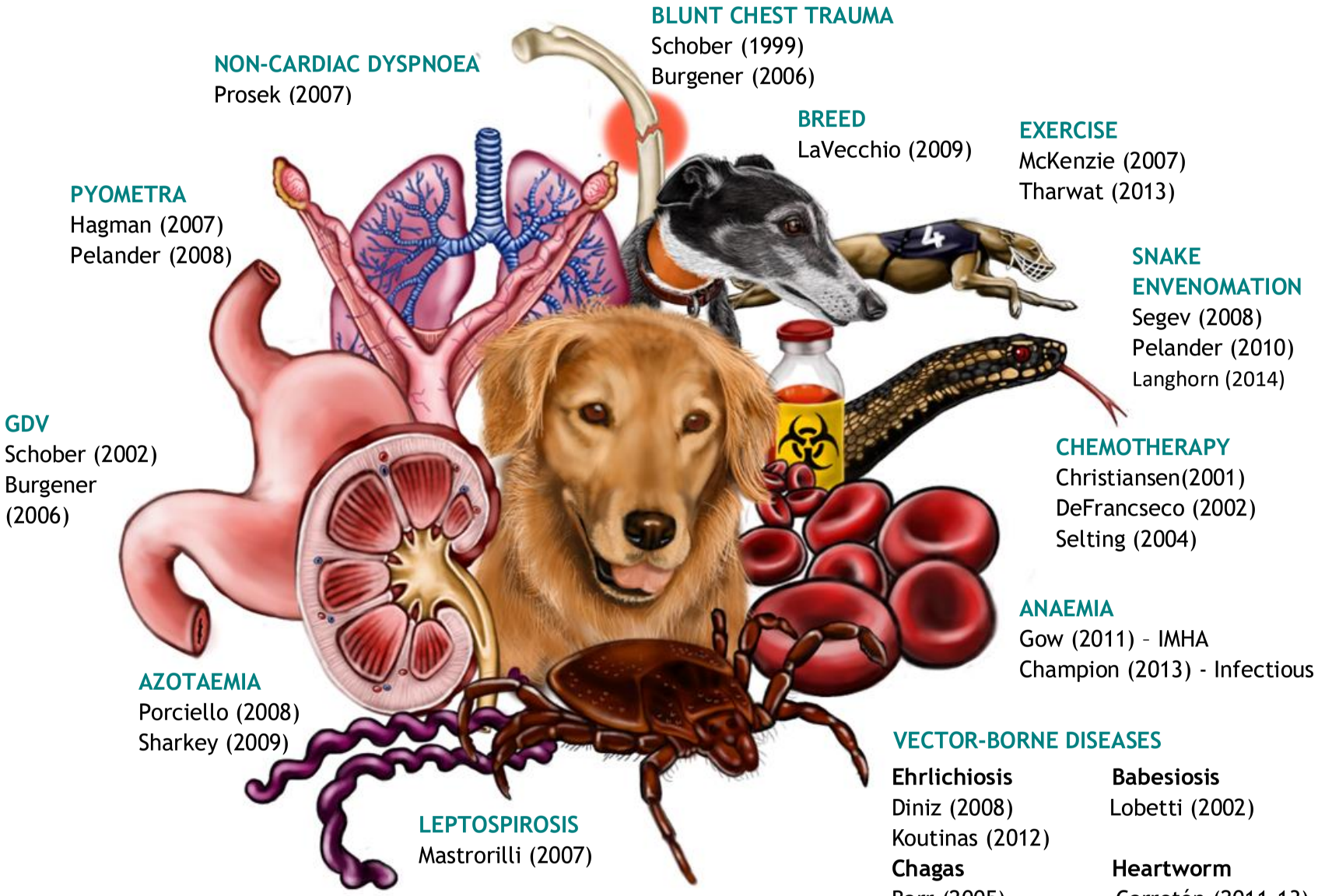

FIGURE 1.4. Non-cardiac causes of increased cTnI in the dog.

1.2.2 TROPONINS IN FELINE MEDICINE

1.2.2.1 CARDIAC DISEASE

A. CARDIOMYOPATHIES AND TROPONIN IN CATS

Cardiomyopathies are highly prevalent in the feline population and represent a heterogeneous group of disorders that result in structural and functional impairment of the heart muscle **[Fox,2003].**

Hypertrophic cardiomyopathy (HCM) is the most common cardiomyopathy in cats **[Schober and Todd, 2010]**. The left ventricle is hypertrophied and develops excessive scar tissue in the absence of secondary causes (hyperthyroidism, systemic hypertension, aortic stenosis or acromegaly). As a result, there is poor relaxation leading to diastolic dysfunction with resulting CHF, arterial thromboembolism (ATE) or sudden death **[Fox, 2003 and Factor et al.1991]** .

The aetiology of HCM remains unknown in many cats. Mutations in the myosin binding protein C gene have been described in families of certain breeds such as Maine Coon and Ragdoll. Therefore, HCM is a primary and likely dominant heritable myocardial disorder. It is a developmental disease that occurs as the cat ages, at any time during its life span, from adolescent to geriatric **[Kienle, 2008 and Côté et al. 2011 (a)].**

Several studies have reported increased troponin values in cats with HCM. One study, included 33 healthy cats free of cardiac disease and 20 animals with moderate to severe HCM. The median cTnI in the control group was < 0.03 ng/ml whereas it was 0.66 ng/ml in cats with HCM. Two animals with ATE showed the highest cTnI. In addition, a weak correlation between cTnI and the thickness of the left ventricular posterior wall in diastole (LVPWd) was seen, suggesting a possible association with disease severity **[Herndon et al. 2002].** Another study, published in 2003, recruited 16 HCM/hypertrophic obstructive cardiomyopathy (HOCM) affected and 18 healthy cats. The median cTnI in the controls was < 0.20 ng/ml (below the detection limit), 0.91 ng/ml for the HCM group and 1.30 ng/ml for the HOCM group. There was a weak correlation between cTnI level and LVPWd **[Connolly et al. 2003].**

Discordant results were obtained regarding CHF when the two studies are compared. cTnI concentrations were significantly higher in cats in CHF in the former study whereas there was not significant association in the latter study.

B. MYOCARDIAL DAMAGE IN FELINE HCM

In none of the studies mentioned were histopathological examinations performed on the hearts from cats included, thus correlation of high cTnI concentrations with myocardial damage was not definitively made.

There are several pathophysiologic mechanisms in cats with HCM that could explain high troponin values. Firstly, microvessel or intramural coronary artery disease is commonly seen in feline, canine and human hypertrophic hearts. Cats with HCM have abnormally thick intramural/ small coronary arteries with enlarged media and intima layers and markedly narrowed lumen. The arterial wall thickening results from cellular hyperplasia of connective/fibrous tissue. The abnormal intramural coronary arteries are commonly associated with areas of fibrosis, suggesting that these changes are significant and may produce myocardial ischaemia, necrosis and fibrotic replacement. A majority of cats with HCM (74%) have abnormal coronary arteries **[Liu et al. 1993, Herndon et al. 2002, Connolly et al. 2003 and Ferasin, 2012].**

The hallmarks of HCM in cats are myofibre disorientation, hypertrophy of the cardiomyocytes and increased matrix or replacement fibrosis. The presence of myocardial hypertrophy in the absence of adequate increases in myocardial capillary density could contribute to ischaemia, myocyte death and subsequent cTnI release. Abnormal coronary flow dynamics due to compression applied by thickened muscle and abundant connective tissue could decrease the coronary reserve **[Krams et al. 1998]** . Finally, neurohormonal activation may induce further myocyte damage due to the cardiac remodeling effects (angiotensin II, aldosterone and endothelin) **[Krum 2000, Weber 2001 and Connolly et al. 2003]**.

C. DYSPNOEA AND TROPONIN IN CATS

Cats with cardiomyopathies can be presented to the clinician as respiratory emergencies due to pulmonary oedema or pleural effusion, the final consequence of left-sided CHF. Dyspnoea in cats may be attributable to many other causes such as airway diseases, neoplasia, infection and pleural space diseases **[Starybrat and Tappin, 2016]**.

In order to clarify if cTnI could help practitioners in the management of dyspnoeic cats, 3 different studies have been carried out and all reached the same conclusion

that cTnI is not useful to distinguish between cardiac and non-cardiac dyspnoea in cats.

In the first study, all cats with cardiogenic dyspnoea had cTnI concentrations > 0.2 ng/ml. Conversely, of the 12 cats with non-cardiogenic dyspnoea, 6 had plasma cTnI concentrations < 0.2 ng/ml but 6 values exceeded >0.2ng/ml, the reference limit for healthy cats. Dyspnoeic cats with plasma cTnI concentrations that exceeded 1.42 ng/ml most likely had CHF as the cause of respiratory distress. In dyspnoeic cats with plasma cTnI concentrations between 0.2-1.42 ng/ml, the cause of dyspnoea remained uncertain and thoracic radiographs or echocardiography were indicated **[Herndon et al. 2008].**

In 2009, a second study included 30 animals in respiratory distress due to non-cardiac diseases and 23 in CHF. Significantly higher cTnI concentrations were found in animals in CHF when compared with dyspnoeic animals without cardiac disease. Significant overlap between the 2 groups was present thereby reducing the clinical value of the assay **[Connolly et al. 2009 (a)]**.

Finally, a third study evaluated the use of a point-of-care multiuse analyser for cTnI, employing from 16 to 22 µL of blood and with a turnaround time of 10 minutes. Thirty seven healthy cats and 39 dyspnoeic cats were included. Similar findings to the two previous studies were reported. Cats with cardiac disease had significantly higher values of cTnI than did healthy cats and cats with a non-cardiac cause of dyspnoea. However, there was overlap of cTnI concentrations between the 2 groups of cats with dyspnoea **[Wells et al. 2014]**.

1.2.2.2 NON-CARDIAC DISEASE

Increased cTn levels indicate cardiac cell injury but do not define the cause of the injury. Elevations are not uncommon in patients with conditions other than primary cardiac disease. In humans, cTnI has been proven to be elevated in renal insufficiency, septic shock, gastrointestinal bleeding, cirrhosis, diabetic ketoacidosis, hypothyroidism, chronic obstructive pulmonary disease, stroke, seizure, strenuous exercise, chemotherapy, rhabdomyolysis, pregnancy, rheumatological disease and non-cardiac surgery **[De Gennaro et al. 2008]**.

In consequence, many recent investigations on cTnI in cats have focused their attention on non-cardiac disease as a source of increased troponin.

A. Hyperthyroidism

In 2005, a study with 23 hyperthyroid cats showed that 50% of them had cTnI concentrations within the detectable level of the assay at the first visit. In contrast, only 3 out of 18 cats that completed 4 weeks of treatment using radioactive iodine had detectable concentrations **[Connolly et al. 2005]**

Another publication concluded that both cardiac biomarkers, NT-proBNP and cTnI, are increased in cats with HCM and cats with hyperthyroidism. Importantly, neither NT-proBNP nor cTnI appeared to distinguish hypertrophy associated with hyperthyroidism from primary HCM. Increased cTnI levels in hyperthyroid patients decreased with treatment, supporting Connolly´s findings **[Sangster et al. 2014]**.

B. Azotaemia

Following previous findings in humans, a retrospective observational study in 2008 demonstrated that dogs and cats with acute or chronic azotaemic renal failure often have elevated cTnI levels **[Porciello et al. 2008]**. Approximately 72% of cats with chronic kidney disease had cTnI levels above published reference intervals. Interestingly, the severity of renal failure did not correlate with the cTnI concentration.

The underlying mechanism that correlates cTnI and renal disease remains unknown. Secondary cardiac toxicity due to uraemia, decreased renal excretion of troponin or myocardial dysfunction caused by severe systemic disease are some of the hypotheses suggested **.**

C. Anaemia

A recent paper, published in 2014, revealed that anaemic cats had significantly higher cTnI concentrations compared with cats with non-anaemic, non-cardiac and non-renal illnesses **[Lalor et al. 2014]**.

It was impossible to establish a causal association between anaemia and myocyte damage because of the cross-sectional study design. Two hypotheses were proposed for the higher cTnI: left sided cardiac enlargement as a result of haemodynamic compensatory mechanisms or direct cardiac myocyte damage.

D. Hypertension

Although nothing is known about the effect of hypertension on cTnI in veterinary patients, individuals with elevated blood pressure are routinely excluded in veterinary cTnI studies **[Borgeat et al. 2014, Langhorn et al. 2014(b)]**

In December 2012 a paper published in the Journal of Hypertension demonstrated that cTnI was elevated in 32.7% of human patients with hypertensive crises. Importantly, a significant majority (76%) of the patients with elevated troponin levels had underlying obstructive coronary artery disease **[Pattanshetty et al. 2012]**.

E. Blunt chest trauma

Cats with blunt chest trauma (BCT) have been shown to have increased cTnI concentrations **[Kirbach et al. 2000]**. One study included 31 cats with BCT, which were evaluated at 12-24h and 60-72h after the event. Alpha-hydroxybutyrate deshydrogenase (HBDH), CK-MB, cTnT and cTnI were measured and all were significantly increased 12-24h after trauma.

F. Chemotherapy

Anthracycline induced cardiomyopathy (AICM) is well characterized in dogs and people in contrast to few reports of doxorubicin toxicity in cats. A difference in susceptibility to doxorubicin exists between species and dogs appear to be more prone to suffer from AICM than cats or people. Cardiac arrhythmias do not seem to be a major concern in cats whereas atrial and ventricular arrhythmias are well recognized in dogs and people receiving doxorubicin **[O´Keefe et al. 1993]**. Unsurprisingly, cTnI levels have been proven to be elevated in dogs and people treated with anthracyclines **[Selting et al. 2004 and Granger, 2006]**. To the author's knowledge no results have been published for cats.

A diagrammatic representation of the non-cardiac causes of increased cTnI in the cat is shown in Figure 1.5.

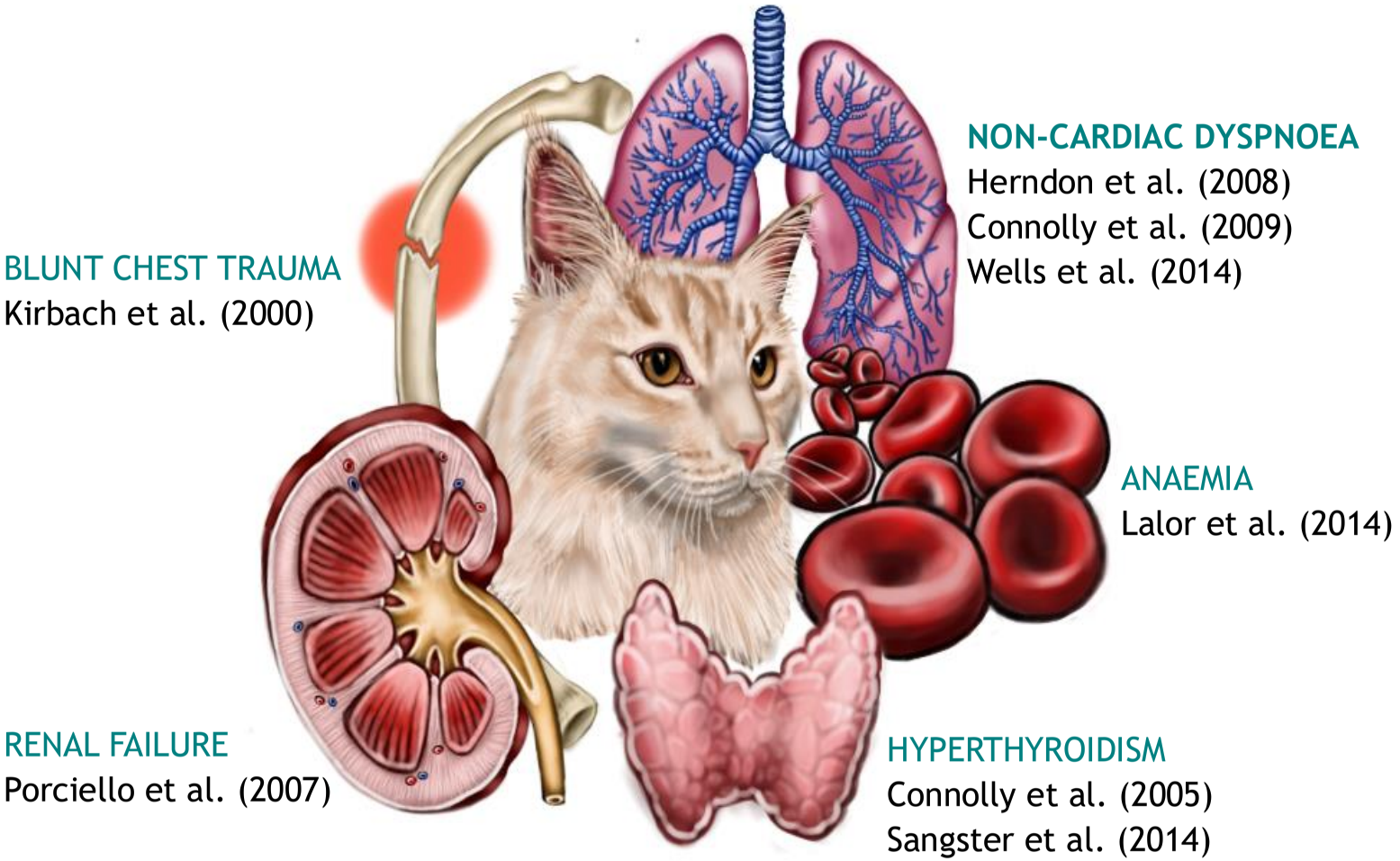

FIGURE 1.5. Non-cardiac causes of increased cTnI in the cat.

1.3 INFLAMMATION, ACUTE PHASE PROTEINS AND CARDIAC DISEASE IN CATS.

Acute phase response in feline medicine

The acute phase reaction (APR) is considered a dynamic process, which involves systemic and metabolic changes providing an early non-specific defence mechanism in animals exposed to a pathogenic insult **[Petersen et al. 2004]**.

APR begins as a local reaction induced by cytokines synthesised by activated macrophages. Monocytes activate in the presence of bacterial toxins or local tissue injury and synthetise and release the pro-inflammatory cytokines (IL-1, IL-6 and TNF-α). These cytokines are soluble mediators which function as "messengers" between the local site of injury and organs involved in homeostasis (central nervous system, autonomic nervous system and adrenal glands) **[Paltrinieri, 2008]**.Therefore, cytokines induce both local and systemic effects to establish a rapid and intense protective response **[Paltrinieri, 2007]**.

These mediators have multiple sources, multiple targets and act together through overlapping pathways **[Gabay et al. 1999]** finally producing the hallmarks of the APR:

1. Fever
2. Leucocytosis
3. Behavioural changes
4. Changes in the concentration of APP

i. FEVER

Pro-inflammatory cytokines are unable to pass through the blood-brain barrier thus they induce the synthesis of leptin (adipocytes) and prostaglandins (all nucleated cells) which act as intermediate molecules and modulate the activity of the thermo-regulation center in the hypothalamus **[Sirko et al. 1989 and Paltrinieri, 2008]**.

ii. LEUCOCYTOSIS

The APR-induced leukocytosis is biphasic. The first wave of leucocytes is released from the marginal pool due to the increased levels of cortisol. Cytokines induce the release of corticotropin releasing factor (CRF) at the hypothalamus, CRF activates the hypophysis that synthetizes adrenocorticotrophic hormone (ACTH) which

stimulate the adrenal glands to produce cortisol. Cortisol reduces the adhesiveness of the white blood cells to endothelium. The marginal pool is remarkable in the feline species as the circulating/marginal leucocytes ratio is 1:3 **[Smith, 2000; Cowell and Decker, 2000]**. The second and long-lasting wave is the consequence of the direct stimulation of IL-1 and TNF-α on the bone marrow. They increase the replication and differentiation of myeloid precursors **[Paltrinieri, 2008]**.

Only about 50% of cats with subacute or chronic inflammation actually have increased numbers of neutrophils **[Segev et al. 2006]**. The degree of neutrophilia is usually higher when inflammation is localized rather than in generalized infections where neutropenia frequently occurs **[Cowell and Decker 2000]**.

Hyperacute leukocytosis is a common event in cats. Leukocytosis can also be seen as a consequence of excitement, fear or stress. A rapid increase in leucocytes is not absolutely specific for inflammation **[Cowell and Decker 2000]**.

iii. BEHAVIOURAL CHANGES

The cytokine-induced stimulation of the central nervous system results in the activation of the hypothalamo-pituitary-adrenal and the hypothalamo-pituitary-gonadal axes resulting in lethargy, anorexia, adipsia and disinterest in social and sexual activities **[Karrow,2006].**

iv. ACUTE PHASE PROTEINS

Cytokines modulate protein synthesis by the hepatocytes **[Cerón et al. 2005].** During the APR, the blood concentration of certain proteins changes dramatically as a result of a complex pattern of induction involving cytokines and glucocorticoids. These proteins are known as “acute phase proteins” (APP). Some will increase, called “positive” whereas others decrease, “negative APP” **[Paltrinieri, 2008].** The main positive and negative feline APP are summarised in table 1.1.

TABLE 1.1. Acute phase proteins in Feline Medicine

1. NEGATIVE APP:
- Albumin (?)
- Transferrin
- Apolipoprotein A1
- Retinol binding protein
- Cortisol binding protein
- Transthyretin

2. POSITIVE APP:
- C-Reactive protein (?)
- α-acid glycoprotein
- Haptoglobin
- Serum Amyloid A
- Complement fractions (C3,C4)
- LPS binding protein
- Ceruloplasmin
- α-globulins with antiprot. activity

iv.a) NEGATIVE APP

No data about the possible role of albumin as a negative APP are available in cats. It has been reported to decrease in many feline inflammatory conditions although it is unknown if this decrease is the result of extravasation of the albumin from the vessels to inflamed tissues or true decreased hepatic production **[Paltrinieri, 2008]**.

iv.b) POSITIVE APP

The magnitude of the increase is highly variable between the different proteins and among different animal species (See Figure 1.6). Depending on the fold increase from the base line (they are usually unmeasurable in blood) the APP are further classified in "major", "moderate" and "minor".

1) **MAJOR** – 10 to 100-fold increase
2) **MODERATE** – 2 to 10-fold increase
3) **MINOR** – only a slight increase

Major proteins are often observed to increase within the first 48 hours and show a rapid decline whereas moderate and minor both increase more slowly but are more prolonged in duration. Moderate and minor APP are more often observed during chronic inflammatory processes **[Cray et al. 2009]**.

Measuring the changes in the APP concentration is used as a "molecular thermometer". The serial quantification of APP can provide an assessment of the response to a pathogenic insult. To optimize their use, it is recommended that APP profiles should include at least one positive major and one positive moderate APP. A basic profile for a cat should include SAA (as a major APP) and Hp (as moderate) **[Cerón et al. 2008]**.

Data regarding APP levels in cats are scarce and mostly focused on general aspects of feline APP biology. Most of the studies have been focused on AGP, SAA and, to a lesser extent, Hp. These three molecules are considered most important APP in the cat. CRP does not seem to be involved in the acute response in this species [Cerón et al. 2005, Paltrinieri, 2008, Eckersall and Bell. 2010 and Eckersall and Schmidt, 2014].

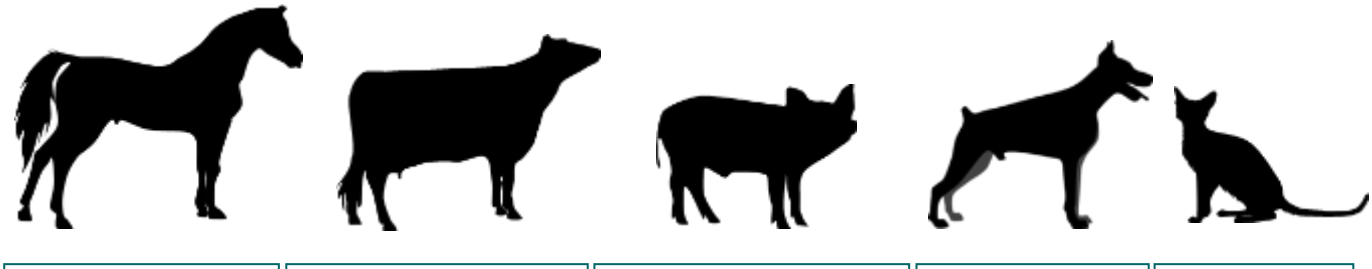

FIGURE 1.6. Major and minor positive APP in domestic animal species. The APP pattern may vary from one species to another. Species differences in classification of major/minor positive APP are apparent.

1.3.1 SERUM AMYLOID A

Serum amyloid A proteins are a family of closely related apolipoproteins. SAA proteins range in size from 104 to 112 amino acids and are considered the precursors of amyloid A, the main component of the proteinaceous plaques deposited in major organs as occasional consequence of chronic inflammatory diseases ("Amyloidosis") [Husby et al. 1994]. The liver is the primary source of circulating SAA although extrahepatic production has been described in several species [Upragarin et al. 2005]. Expression and production have been detected in atherosclerotic lesions [Meek et al. 1994; Yamada et al. 1996]; brain of patients with Alzheimer´s disease [Liang et al. 1997; Chung et al. 2000] and epithelial cells from several normal tissues such as breast lobule, colon mucosa, prostate, kidney and lung in human patients [Urieli-Shoval et al. 1998]. The functions of SAA are thought to involve lipid metabolism, lipid transport, chemotaxis and regulation of the inflammatory process [Upragarin et al. 2005].

a) FELINE AMYLOIDOSIS

Amyloidosis results from extracellular deposition of insoluble, fibrillar and proteinaceous amyloid in multiple organs **[Jimenez et al. 2011]**. Reactive systemic amyloidosis is the most common in the cat **[Van der Linde-Sipman et al. 1997 and Gruys, 2004]**. Deposition of amyloid causes pressure atrophy of adjacent cells. Usually, the kidney is the organ most commonly involved **[Grant Maxie and Newman 2007]**. In Abyssinian cats, the high degree of deposition in the kidney leads to renal failure at a young age **[Van der Linde-Sipman et al. 1997]**. In contrast, systemic amyloidosis in Siamese occurs in the liver leading to hepatic rupture and intra-abdominal hemorrhage **[Beatty et al. 2002, Khoshnegah and Movassaghi, 2010 and Chew et al. 2011]**.

DiBartola et al. evidenced in 1989 that Abyssinian cats with amyloidosis had significantly higher (P=0.05) SAA concentrations than healthy Abyssinian cats and hospitalized Abyssinian cats. In addition, healthy non-Abyssinians had significantly (P=0.05) lower SAA concentrations than healthy Abyssinians and hospitalized Abyssinians. However, affected and healthy Abyssinian cats could not reliably be distinguished on the basis of SAA concentration because of the wide range of SAA values in these 2 groups of cats **[DiBartola et al, 1989]**.

b) INFECTION

b.1) *Mycoplasma*

Haemotropic mycoplasmas are small, unculturable, pleomorphic and gram-negative bacteria which parasitise red blood cells to survive. Haemotropic mycoplasmosis causes what is commonly known as “Feline Infectious Anaemia”. *Mycoplasma haemofelis* (Mhf), previously known as “Ohio strain”, is the most pathogenic organism and causes anaemia in immunocompetent animals. On the other hand, *Candidatus Mycoplasma haemominutum* (CMhm), known as “California strain” before, and *Candidatus Mycoplasma turicensis* do not cause anaemia unless concurrent disease or immunosuppression is present **[Hagiwara, 2009 and Korman et al. 2012]**.

The APR to experimental infection with haemotropic mycoplasmas (Mhf and CMhm) was characterized in a collaborative study in 2012. Three acute phase proteins were measured in feline serum: SAA, AGP and Hp **[Korman et al. 2012]**.

SAA was significantly elevated during both Mhf and CMhm infections. Peak concentrations were reached on days 16-23 post infection (pi). Mhf was associated with significantly higher SAA concentrations than CMhm infection, consistent with Mhf being more pathogenic. SAA levels usually returned close to pre-infection values by day 44 pi consistent with a self-limiting APR, despite sustained circulating haemoplasma copy numbers. This finding indicates than an elevated SAA concentration suggests acute (less than 44 days post-exposure) rather than chronic infection. Increases in SAA mirrored the development of anaemia.

b.2) *Bordetella bronchiseptica*

A Japanese group inoculated *Bordetella bronchiseptica* into the bronchi of 6 healthy cats and measured both AGP and SAA. Although no signs of bronchopneumonia were observed on physical examination, both APP were slightly increased after inoculation. SAA peak serum concentrations were observed sooner when compared to AGP in infected animals **[Shida et al. 2012].**

b.3) Feline infectious peritonitis

Concentration of SAA, AGP and Hp were studied in cats exposed to Feline Coronavirus (FCoV) and cats that developed Feline Infectious Peritonitis (FIP). Cats were recruited (n=67) and allocated in 3 groups: 24 cats were classified as controls or "specific pathogen free", 11 were FCoV-exposed (without clinical signs) and 32 suffered from FIP (25 "wet" and 7 "dry" form). SAA was measured in 64 samples from 50 animals. Mean SAA concentration in controls was 10.21 ± 8.32 µg/ml, 7.92 ± 7.12 µg/ml for FCoV-exposed and 82.22 ± 50.23 µg/ml for FIP cats. The highest SAA concentration was found in FIP cats. Results from cats with wet FIP did not differ from those with dry FIP patients. Compared to controls, a 10-fold increase of SAA was detectable in affected cats. Repeated samplings from FIP and FCoV-exposed cats showed that when FIP appeared in the group, all cats had increased SAA, AGP and Hp levels. This increase was persistent in FIP cats whilst it was transient in the FCoV-exposed cats. These results concluded that SAA might also be a marker of FIP and highlighted its possible role in the diagnosis and monitoring of the disease **[Giordano et al. 2004].**

b.4*) Chlamydiae*

Both SAA and AGP have been determined in cats subclinically infected with *Chlamydiae*. Sixty two cats were recruited and SAA concentrations were assessed in

54 individuals. SAA concentrations were above the reference range in 16 cats (30%). SAA concentrations were higher in subclinical shedders than they were in cats that did not shed *Chlamydiae* **[Holst et al. 2011]**.

b.5) Retrovirus and interferon- ω therapy

A collaborative study, involving researchers from Portugal, United Kingdom and France assessed the ability of recombinant feline interferon-ω to produce a stimulation of innate host defence system in cats. To quantify this response, changes in the concentrations of SAA, AGP and CRP in naturally infected retroviral positive cats were determined. Sixteen cats were recruited and divided in 3 groups: FIV positive cats (n=7), FeLV positive cats (n=6) and co-infected animals (n=3). All the animals received 3 cycles of injections. For SAA, all the cats behaved similarly. A significant increase of SAA concentration was observed after therapy. On day 65, mean SAA concentration was 1.6 times higher than day 0, proving the effectiveness of this immune therapy **[Leal et al. 2014].**

c) PANCREATITIS

SAA concentrations were monitored over an 831-day period in a cat with pancreatitis. SAA levels were increased at the onset of the disease. Long term monitoring from days 68 to 831 revealed a good correlation between SAA concentrations and the relapse of clinical signs in the cat. It was suggested that SAA might be a useful tool for monitoring the disease and its response to treatment **[Tamamoto et al. 2009]**.

d) INFLAMMATORY MARKER: SURGERY & VARIOUS DISEASES

A Japanese team analyzed concentrations of SAA in sera from clinically normal cats, hospitalized cats, cats with experimentally induced inflammation and cats that underwent urinary surgery. Concentrations of SAA were significantly higher in hospitalized cats than in normal cats. Cats with experimentally induced inflammation received an intramuscular injection of turpentine oil or lipopolysaccharide of *Escherichia coli* (LPS). Concentrations of SAA began to increase 8h after injection and reached peak values 36-48h after administration. In view of these results, SAA was considered a responsive protein in the early stage of the acute phase reaction in cats **[Kajikawa et al. 1999]**.

In 2003, another Japanese group determined SAA concentrations in 3 healthy cats undergoing ovariohysterectomy before and after surgery. The concentrations of SAA were found to increase at approximately 3-6hr and reached peak values at 21-24hr after surgery. These results suggested that SAA reacts rapidly to inflammatory stimuli and can be a useful marker of inflammation. This group also compared SAA concentrations from normal healthy cats (n=45) and cats with various diseases (n=312). The concentrations from normal cats were statistically different from the diseased cats ($P<0.001$). It was concluded that SAA could be a useful marker for some, but not for all diseases **[Sasaki et al. 2003]**.

SAA has been also measured in cats having experimental surgeries. That study included 11 healthy subjects that had ovariohysterectomy (n=5) or gastrotomy (n=6). Peak concentrations of SAA after the procedures were 4.8 to 26.8 times higher for neutering and 11.5 to 23.4 times higher for gastrotomy in comparison with pre-treatment concentrations. These peak serum concentrations were observed at 1 or 2 days after surgical treatment and SAA was undetectable after 5 days post-surgery in most of the cats. **[Shida et al. 2012]**.

Finally, in a recent report APP concentrations (SAA, AGP and Hp) were compared between "sick" and "healthy" cats (based on clinical history and clinical signs). APP were measured in 67 cats (33 were sick and 34 were healthy) and haematology and biochemistry results were available from 15 sick cats. Log transformed serum amyloid A was found to be higher in sick, older and female cats. Median SAA concentration in healthy individuals (n=34) was 1.2µg/mL (range: 0.1-12.7) whereas median SAA concentration in sick cats (n=32) was 2.1µg/mL (range: 0.4-124.8) **[Kann et al. 2012]**.

e) PROGNOSTIC MARKER

The relationship between SAA concentration and prognosis in cats has been recently evaluated. 175 Cats were recruited and divided into 2 groups according to their SAA concentration. Median survival time of the non-elevated SAA group was 571 days compared with 72 days for the elevated SAA group. The animals were further subclassified according to diagnostic category: neoplasia (n=65), inflammatory (n=64) and other diseases (n=46). There was no difference in the distribution of disease type between non-elevated SAA and elevated SAA groups (P=0.106).

According to these results, SAA was considered as a significant and independent prognostic marker in cats with a wide variety of diseases [Tamamoto et al. 2013].

1.3.2 α_1-ACID GLYCOPROTEIN (AGP)

Human AGP was first described in 1950. AGP is mainly secreted by hepatocytes although extra-hepatic synthesis exists in several tissues such as breast epithelial cells [Gendler, 1982], placenta [Thomas, 1989], endothelial cells [Sörensson, 1999], granulocytes and monocytes [Shibata, 1984; Fournier, 1999]. Although its exact biological function remains obscure, AGP has been shown to inhibit several activities of the neutrophils, down-regulating the local inflammatory response. It also seems to inhibit platelet aggregation, enhance the cytokine secretion from monocytes and interacts with endothelium leading to changes in its permeability [Hochepied, 2003].

Most of the studies on feline AGP have focused on infectious diseases, with a particular interest in FIP.

a) **INFECTIOUS DISEASES**

a.1) Feline infectious peritonitis

FIP is mainly a disease of domestic cats although it has been recognized in several wild feline species as lion, leopard, cheetah, jaguar, lynx, serval, caracal, European wild cat, Sand cat and Pallas cat [Colby and Low, 1970; Colly, 1973; Poelma et al. 1974; Theobald, 1978; Tuch et al. 1974; Pedersen, 1983; Pfeifer et al. 1983; Watt et al. 1993 and Juan-Sallés et al. 1998].

Currently, there is no effective treatment for FIP and mortality is extremely high once clinical signs appear. Some cats live with the disease for weeks, months or, rarely, years [Pedersen, 2014].

A definitive ante-mortem diagnosis of FIP can be challenging [Pedersen, 2014]. The presence of effusion in the abdomen or, less frequently, in the pleural space is one of the most diagnostic features of the effusive (wet) form of FIP [Pedersen, 2014]. Measuring coronavirus antibody titers in serum or plasma is still controversial. FIP virus (FIPV) is a mutation in feline enteric coronavirus (FECV). FECV and FIPV are virtually identical and thus evoke the same antibody responses. Titers higher than 1:3200 are highly suggestive of FIP [Hartmann et al. 2003].

AGP has been extensively used as a test for FIP. High levels of AGP in cats with experimentally induced FIP were first described in 1988 **[Stoddart, 1988]**. Later on, AGP and Hp were studied in view to distinguish between healthy cats, cats with naturally occurring FIP, cats with similar signs to FIP but negative diagnosis on histopathological examination and FIV cats. Although both were significantly higher in FIP animals, only AGP was useful to distinguish between groups. FIV cats had significantly elevated AGP and Hp levels too, illustrating they are not pathognomonic for FIP. AGP was thought to be of considerable value in the diagnosis of FIP, particularly in conjunction with the clinical signs, antibody titer and albumin:globulin ratio **[Duthie et al. 1997]**. More recently, it has been demonstrated that three different APP (SAA, AGP and Hp) are increased in cats with FIP. Importantly, those cats exposed to FCoV but asymptomatic had a transient increase in APP concentration in contrast to FIP subjects, which experienced persistent elevation **[Giordano et al. 2004]**.

A recent study has focused on the value of AGP to differentiate symptomatic FIP from non-FIP cats. The non FIP cats were subdivided into 5 categories: inflammatory processes other than FIP (n=26), asymptomatic FCoV infection (n=49), injection site sarcoma (n=19), post-vaccination (n=7) and clinically healthy cats (n=3). AGP was higher in animals with symptomatic FIP than in the whole non-FIP group. In addition AGP was higher in FIP animals than in each subcategory of the non-FIP group (P=0.01) **[Paltrinieri et al, 2007a]**.

a.2) Chlamydia

Controversial information has been published regarding AGP concentrations in cats infected with *Chlamydiae*. In one experimental infection study all the cats with conjunctivitis demonstrated a febrile response accompanied by elevations of AGP **[TerWee et al. 1998]**. In another study of infected cats only 9/57 cats had AGP concentrations above the reference range **[Holst et al. 2011]**.

a.3) Retrovirus and interferon- ω therapy

The effect of recombinant interferon-ω therapy in naturally retroviral infected cats has been recently investigated. Along with SAA and CRP, AGP levels were measured at day 0, 20, 30 and day 65 of treatment. There was an overall increase of AGP concentrations from day 0 to day 65. As APP increased during interferon therapy,

this treatment may actually modulate pro-inflammatory innate mechanisms in cats [Leal et al. 2014].

b) NEOPLASTIC DISEASE

b.1) Lymphoma

AGP concentrations were measured in 25 healthy cats and 9 cats with lymphoma prior to induction of chemotherapy, one week after induction, at complete response, and at monthly intervals. The cats with lymphoma had increased AGP concentrations however, in contrast to the human literature, AGP was not correlated with either remission duration or survival time in cats [Correa et al. 2001].

b.2) Carcinomas, sarcomas and round cell tumours

In a further study that included 97 tumor-bearing and 51 healthy cats, mean AGP concentrations were significantly higher (*P=0.0051*) for tumor-bearing cats than for healthy controls. Importantly, the range of serum AGP concentrations for tumour-bearing cats completely included that of the healthy population. The overlap between AGP values from patients with neoplasia and those from healthy individuals makes AGP a limited tool for monitoring the presence and progression of malignant disease in the cat [Selting et al. 2000].

c) ANAEMIA SECONDARY TO INFLAMMATORY DISEASE

A German group characterized the possible pathogenic mechanisms causing anaemia secondary to inflammatory disease (AID) in 21 cats with naturally occurring inflammatory diseases in 2006. The study included 12 cats with abscesses, 6 with pyothorax and 3 with fat necrosis (secondary to trauma or surgery). The concentrations of AGP were increased in 14 out of 14 cats examined (range: 0.6-2.3 g/L, median: 1.2 g/L and normal reference range: 0.1-0.48 g/L). Concentrations of AGP were increased up to 12 days after draining the abscesses. Increased concentrations of APP in conjunction with leukocytosis and clinical signs were indicative of the inflammatory processes in this population of cats [Ottenjann et al. 2006].

d) SURGERY, INDUCED INFLAMMATION AND HOSPITALIZATION

In Kajikawa´s study there was no significant difference in APP concentrations between male or female cats. Concentrations of AGP in sera from hospitalized cats were much higher than in clinically normal cats. AGP levels increased in cats with experimentally induced inflammation (turpentine oil or *E. coli* LPS injection) and

those that underwent urinary surgery. The mean concentration of the APP reached a maximum 48 hours after injection. A correlation between AGP and SAA was detected although SAA was found to increase earliest [Kajikawa et al. 1999].

e) OTHER CONDITIONS

Kann et al. measured AGP in 67 cats. Sick cats (animals with a wide and unknown spectrum of clinical disorders) had higher AGP concentrations than healthy individuals. An interaction between health status (sick/healthy) and gender was significant at $P<0.05$. AGP was correlated with SAA and Hp. The largest correlation was found between Hp and AGP, followed by SAA and AGP [Kann et al. 2012].

1.3.3 Haptoglobin

Hp is a 54 kDa protein, which was firstly discovered, by Polonovski and Jayle in 1938 [Quaye, 2008 and Adams et al. 2013]. Hp gene is expressed primarily in hepatocytes, however, it has been recently described in other locations such as adipose tissue, ovary, lungs, testis, arteries and placenta [Kalmovarin et al. 1991, Friedrichs et al. 1995 and Yang et al. 1995]. Hp has a pronounced antioxidant and anti-inflammatory action, preventing the deleterious effects associated with circulating free haemoglobin (Hb). In addition, Hp actively participates in polymorphonuclear leukocytes recruitment, free radical quenching, tissue repair and regeneration. Reduced expression (hypohaptoglobinaemia) or absence of the Hp protein (ahaptoglobinaemia) is associated with allergic (skin and lungs) and anaphylactic transfusion reactions [Shimada et al. 2002, Gilstad, 2003 and Larsen et al. 2006].

a) ANAEMIA SECONDARY TO INFLAMMATORY DISEASE

Hp levels have been determined in cats with AID. Haptoglobin was increased in 13 out of 14 cats examined (range: 5.3-13 g/L, median: 8.8 g/L and reference range: 0.04-3.84 g/L). The Hp concentration showed an inverse correlation to the number of aggregated reticulocytes [Ottenjann et al. 2006].

b) SURGERY, INDUCED INFLAMMATION AND HOSPITALIZATION

Similar to what was found with SAA and AGP, Kajikawa et al could not identify differences between males and females in both healthy and diseased cats regarding Hp concentrations. Hospitalized cats had higher Hp concentrations than clinically normal cats. Hp was also elevated before surgery or turpentine oil administrations

in those cats that were kept in small boxes. Hp levels increased after surgery and turpentine oil injection. SAA, AGP and Hp were considered acute phase reactants in feline patients [Kajikawa et al. 1999].

c) **FIP AND FIV**

AGP and Hp have been analyzed in healthy cats, cats with naturally occurring FIP, cats with similar signs to FIP but negative diagnosis on histopathological examination and FIV cats. Both APP were significantly higher in FIP animals however, only AGP was useful to distinguish between groups. FIV cats had significantly elevated AGP and Hp levels too, illustrating that these APP are not specific for FIP [Duthie et al, 1997].

SAA, AGP and Hp were evaluated in cats exposed to FCoV and cats that developed FIP. The study included 24 controls, 11 FCoV-exposed and 32 FIP cats. FIP cats had the highest Hp concentrations [Giordano et al. 2004].

d) **OTHER CONDITIONS**

Haptoglobin concentrations were measured in 40 cats, comparing levels from healthy individuals to cats with a wide variety of diseases ("sick group"). Similar to SAA and AGP results, higher Hp concentrations were detected in sick cats compared to healthy subjects. An association between Hp levels and gender was present. Finally, correlation between different APP was studied and Hp and AGP showed the strongest correlation [Kann et al. 2012].

1.3.4 C-Reactive protein

CRP was the first acute phase protein described. William S. Tillet and Thomas Francis discovered this reactant in 1930, studying the serum of patients with pneumococcal pneumonia [Tillet and Francis, 1930]. Plasma CRP is mainly produced and degraded by hepatocytes and its plasma half-life is about 19 hours [Ablij and Meinders, 2002; Pepys and Hirschfield, 2003]. Alternative generation sites have been found such as brain neurons in patients with Alzheimer´s disease [Yasojima et al. 2000], peripheral lymphocytes [Kuta et al. 1986] and within atherosclerotic plaques [Torzewski et al. 1998]. The main biologic function of CRP is determined by its ability to recognize pathogens and damaged cells of the host and to mediate their elimination by recruiting the complement system and phagocytic cells [Volanakis, 2001]. CRP has been shown to be an independent predictor of future cardiovascular

events (myocardial infarction, stroke, peripheral arterial disease and sudden cardiac death) in multiple prospective epidemiological studies in human patients [Ridker, 2003]. Prospective data also demonstrated that CRP is a stronger predictor of risk of cardiovascular events than is low-density lipoprotein cholesterol and it is also known as a marker of atherothrombotic disease [Ridker et al. 2002]. The excellent correlation of circulating CRP concentrations with the severity, extent, and progression of many different pathologies, and the prognostic significance of these associations, are consistent with CRP not just being a marker of disease but also contributing to pathogenesis [Pepys and Hirschfield, 2003]. CRP is considered a major acute phase protein in humans, dogs and non-human primates [Cray et al. 2009].

a) SURGERY, INDUCED INFLAMMATION AND HOSPITALIZATION

Kajikawa et al. introduced the concept that CRP is not a highly responsive protein in the acute response in the cat. In that study, CRP concentrations were increased in cats kept in small boxes before surgery or turpentine oil/LPS injection (as were SAA, AGP and Hp). CRP started to increase 8h after injection, similar to SAA and AGP but earlier than Hp, which increased from 24h after administration. Most of the APP reached the maximum level at 48 hours but when the magnitude of increase was compared, it was greater in SAA, AGP and Hp than CRP. Median SAA concentration before injection was 29.1 ±15.1 μg/ml whereas SAA levels 48h after injection were 118.4 ±3.5. Similarly, AGP before administering the oil was 556.7 ±77.4 μg/ml and 3179.4 ±227.6 48h after. In contrast, median CRP concentrations before the procedure was 204.1 ±35.9 μg/ml and slightly increased to 269.6 ± 81.6 μg/ml 48 hours later. Comparable findings were obtained for cats undergoing urinary surgical procedures. As a result, CRP has been considered an insensitive protein in the acute phase reaction in the cat since 1999 [Kajikawa et al. 1999].

b) RETROVIRUS INFECTION AND INTERFERON-ω THERAPY

Recently, a new report has been published assessing the effect of interferon-ω therapy in the immune response of cats naturally infected with retrovirus. All the acute phase proteins significantly increased in cats undergoing this therapy. From the beginning (day 0) until the end of treatment (day 65), a significant increase of CRP concentrations was noted. Despite not being considered a useful biomarker of

inflammation in cats for years, this study showed that this APP behaves similarly to SAA and AGP [Leal et al. 2014].

1.3.5. Inflammation in feline cardiomyopathies

Replacement fibrosis is a common histopathological hallmark of feline CM. The gross and histologic changes associated with end-stage hypertrophic cardiomyopathy (ES-HCM) in cats have been described previously [Cesta el al, 2005]. The most common findings were myofibre disarray, extensive multifocal areas of fibrosis and thickened coronary arteries. The most severely affected cat had a markedly dilated left atrium with intra-auricular thrombi on gross inspection of the heart. Histologic examination showed areas of oedema and fibrosis surrounding islands of necrotic myocytes (myocardial infarctions). Interestingly, scattered lymphocytes, plasma cells and macrophages infiltrated the areas of fibrosis around the infarcts

The underlying cause of restrictive cardiomyopathy (RCM) in cats remains unknown. It has been hypothesized that it could be secondary to hypereosinophilic syndrome [Fox,2004]. However there is not an adequate body of evidence in veterinary medicine and eosinophils are rarely seen in feline myocardium.

A second hypothesis considers viral induced or an immune mediated pathway [Fox, 2004]. Feline Panleukopenia (Feline Parvovirus) virus genome has been isolated in cats with cardiomyopathy [Meurs et al. 2000]. Myocardial inflammatory infiltrates including neutrophils and lymphocytes were detected in 55% of the cats with HCM, and both myocarditis and panleukopenia virus genome were identified in 11% of the hearts in that study. It is suggested that, as the virus affects cells undergoing rapid mitosis, myocardial infections might be expected to occur soon after birth. Cats that survive the viral myocarditis might later develop cardiomyopathy. Myocarditis might contribute somehow to development and progression of feline cardiomyopathies.

Cats with endomyocarditis (ECM) show varying degrees of inflammation. Histologic findings were compared between 37 cats with ECM and 35 with RCM. In the ECM cats, 26 of the 37 exhibited predominantly neutrophil and macrophage infiltrates. Lymphocytes and plasma cells were present only in two cats and another two individuals had mixed inflammatory infiltrates. In contrast, RCM cases had marked left ventricular endocardial fibrosis without inflammation. However, small numbers

of lymphocytes were seen in 2 cats and neutrophils were visualized in one subject **[Stalis et al. 1995]**.

Two forms of RCM have been identified: myocardial RCM and endomyocardial RCM. The latter is known as endomyocardial fibrosis (EMF). Although the main feature identified is extreme endocardial thickening, in some cases there is distinct endomyocarditis. Endomyocardial infiltrates are usually mononuclear cells and macrophages with occasional neutrophils **[Fox, 2004]**.

1.3.6. Inflammation in human HCM

Cardiac histopathology, inflammatory cytokines, high sensitivity C-reactive protein (hsCRP) and cardiac phenotype using MRI have been employed to investigate the role of inflammation in the phenotypic expression of myocardial fibrosis in human HCM **[Kuusisto et al, 2012].**

In that study, endomyocardial biopsies were taken by fluoroscopic guidance from the right ventricle and from the interventricular septum. Interstitial and perivascular fibrosis was found in about 90% of cases. Inflammatory cell infiltration, including mononuclear inflammatory cells and eosinophilic granulocytes, was found in 37% of the patients. The degree of histopathological myocardial fibrosis significantly correlated with late gadolinium enhancement (LGE) in cardiac magnetic resonance (cMRI). A high intensity signal is seen when extracellular matrix is expanded because it retains the gadolinium. The grade of myocardial inflammatory cell infiltration correlated with fibrosis in histopathological samples ($P=0.034$) and with LGE in cMRI. Furthermore, circulating levels of hsCRP, IL-1β, IL-1RA, IL-6 and IL-10 were significantly higher in patients with HCM than in control subjects. Importantly, levels of hsCRP were significantly associated with histopathological myocardial fibrosis ($p<0.05$). All the other cytokine levels tended to be higher in patients with moderate or marked histopathological findings compared with those with no or mild findings.

This study demonstrated that a low-grade myocardial and general inflammation response is present in human HCM. It is suggested that myocardial fibrosis in HCM may be an active process modified by an inflammatory response.

The following pathogenic mechanism for myocardial fibrosis formation in HCM was proposed: the primary injury (mechanical stress due to disorganized cellular

architecture, myocardial ischaemia or neuroendocrinological activation) induces NF-κß up-regulation in the myocardium. NF-κß, in turn, activates production of pro-inflammatory cytokines, inflammatory cell invasion into the myocardium and activation of fibroblasts, finally leading to myocardial fibrosis.
Feline and canine models of HCM share the same histopathological findings with human patients with HCM **[Liu et al. 1993]**. The 4 morphologic hallmarks (asymmetric patterns of wall thickening, disorganization of cardiac muscle cells, abnormal intramural coronary arteries and myocardial fibrosis) are present in human, canine and feline hearts although the frequency of these differs substantially among species (e.g. myocyte disorganization or asymmetric patterns are more common in humans than in dogs or cats).

The CTnI test was initially designed for early diagnosis of MI in human patients. However, several factors influence cTnI release and measurements. Despite the increasing number of publications studying cTnI in small animals, many conflicting results have been reported. Consequently, veterinary clinicians struggle to interpret the test correctly. After a critical approach to literature review, the main goal of the two retrospectives studies was to investigate the relationship between cTnI and survival in dogs and cats taking into account every limiting and confounding factor described in previous publicatons (e.g. lack of standardisation of cTnI assay systems or increases of cTnI due to primary non-cardiac conditions). Furthermore, it has been proven that humans who have HCM, experience both systemic and myocardial inflammation. Histopathological similarities among human and cats with HCM exist and myocardial inflammatory infiltrates are commonly found in both. To the author´s knowledge, no previous reports addressing the presence of systemic inflammatory response in cats with cardiac disease have been published. As a result, the main purpose of the prospective study was to determine the characteristics of the acute phase response in cats with a particular interest in the subset of cardiac patients.

CHAPTER 2

Retrospective study in dogs

Serum cTnI and survival in canine cardiac diseases.

2.1 Aims and objectives

- to investigate the relationship between cTnI concentrations and survival in dogs with cardiac disease.
- to clarify if cTnI concentrations were associated with cardiac death in the individual patient.
- to assess the impact of age, gender, cardiac disease, presence of CHF and arrhythmia on cTnI concentrations and survival in dogs with cardiac disease.

2.2 Material and methods

This was a retrospective study that included dogs admitted to the Small Animal Hospital of the University of Glasgow between August 2009 and September 2012 which had cTnI measured. These animals were referred for further evaluation of suspected cardiac abnormalities detected on thorough clinical examination or ECG. Repeated samples from the same individual collected on different visits were excluded for statistical purposes. If several measurements were available, only the highest result was included in the analysis. After reviewing the clinical histories, only dogs with cardiac disease were included. No controls were recruited for this study.

2.2.1 ANIMALS

Dogs were eligible for inclusion where there was evidence of cardiac disease on physical examination and/or echocardiographic examination. All subjects were examined and scanned by a single experienced cardiologist (Dr. Paul Wotton). Dogs with a haematocrit below 37%, creatinine >155µmol/L in the absence of diuretic therapy, persistent systolic arterial blood pressure >180mmHg or concurrent disease (e.g. neoplasia or trauma) were excluded.

2.2.2 CARDIAC BIOMARKER

Samples were collected by jugular venipuncture and placed in plain serum tubes, which were allowed to clot and sent immediately for analysis on the same day. Cardiac troponin I was tested with an "Immulite® 2000 Troponin I" (Siemens) analyzer. The lower detection limit of the assay is 0.1ng/ml and the normal reference range for dogs is cTnI <0.16ng/ml.

2.2.3 CLINICAL OUTCOME

Survival times were calculated from the date of sampling to the date of death or euthanasia. The outcome was obtained either from the electronic records of the

primary clinicians or by contacting owners or referring practitioners by telephone. Animals that were euthanized or died from non-cardiac conditions were excluded from the study.

2.2.4 CHF, ARRHYTHMIAS AND TYPE OF CARDIAC DISEASE.

Dogs were further divided into subgroups based on the absence or presence of CHF. CHF was determined by evaluation of clinical signs, thoracic radiographs and ultrasonography (pulmonary oedema, pleural effusion or ascites). Dogs were also divided into subgroups based on the presence or absence of arrhythmia on ECG. The heart rhythm was further characterized using a subjective severity scale. Finally, dogs were divided into six categories dependent on their final diagnosis considering clinical records, echocardiographic examinations, radiographs, ECG and fluid analysis. These categories included valvular disease, cardiomyopathy, cardiac neoplasia, congenital disease, idiopathic pericardial effusion, and rhythm disturbances in the absence of structural cardiac disease.

2.2.5 STATISTICAL ANALYSIS

Statistically analyses were performed using commercially available software. Data were non-normally distributed and results were compared using the Mann-Whitney U-test and graphically assessed with box-plots graphs. Median and ranges were used to provide descriptive statistics.Survival was analyzed using Kaplan Meier curves. ROC curves were created to assess the prognostic capability of cTnI and to identify the most useful cut-offs to predict cardiac death. Regression analysis was performed to identify if the cTnI results could have been influenced by confounding factors such as age, breed or type of disease. Statistical significance was defined as $P < 0.05$.

2.3 Results

2.3.1 STUDY POPULATION

After the analysis of clinical histories, ninety-four dogs with a wide variety of cardiac diseases were finally included. Of these, 39 were intact males, 23 male neutered, 22 female neutered and 10 were intact females. The median age of the population was 8 years old (range: 3 months-14 years). Retrievers were overrepresented, with Labrador the most common breed (Labrador n=16, Golden Retriever n=2, Retriever n=2). Other common breeds included in this study were Boxer (n=7), Spaniels (Cavalier King Charles n=6, Cocker n=5, Springer n=2), Terriers (West Highland White

n=2, Border n=1, Bull n=1, Terrier n=1), Newfoundland (n=5), Doberman (n=4), Great Dane (n=4), German Shepherd (n=3) and Dogue de Bordeaux (n=3). There were 2 dogs each of the following breeds: Deerhound, Rhodesian Ridgeback, Weimaraner, Bulldog and Poodle; there was 1 dog each of the following breeds: Vizla, Saint Bernard, Jack Russell, Husky, Alaskan Malamute, Pointer and Collie. Finally, 9 cross-breed dogs were also recruited. Arrhythmia alone was the most common finding on cardiac auscultation of this population of dogs (n=32) whilst murmurs were audible in 26 dogs, combinations of murmur and arrhythmias were detected in 24 subjects, four dogs presented with muffled heart sounds and eight individuals had normal cardiac auscultations. Forty two dogs presented with respiratory signs (cough, dyspnoea or tachypnea) and 23 had suffered collapsing episodes. Of these 23, 15 had arrhythmias whereas 8 had sinus rhythm. CHF was diagnosed in 32 dogs of the study group.

Fifty-six dogs were presented with arrhythmias; auscultation in 32 dogs revealed an arrhythmia alone whereas 24 dogs were presented with a combination of arrhythmia and a murmur. Of these, there were 52 dogs with tachyarrhythmias and 4 with bradyarrhythmias. Supraventricular arrhythmias were more prevalent and atrial fibrillation was the most commonly diagnosed. Tables 2.1 and 2.2 summarize the rhythm alterations identified in dogs of this study population. There were 38 dogs that had no rhythm disturbances detected.

TABLE 2.1. Tachyarrhythmias in dogs with cardiac

SUPRAVENTRICULAR	Number of dogs
Sinus tachycardia	1
Sinus tachy. + ectopics	3
SPVCs	4
SPV tachycardia	4
SPV tach + block	1
Atrial Fibrillation	12
Atrial Fib + ectopics	3

VENTRICULAR	Number of dogs
VPCs	11
AIVR	1
V. tachycardia	8
VPCs+ SPVCs	4

TABLE 2.2. Bradyarrhythmias in dogs with cardiac

SUPRAVENTRICULAR	Number of dogs
3rd degree AV block	2
Sick Sinus Syndrome	2

****SPVCs**, Supraventricular premature complexes; **AIVR**, accelerated idioventricular rhythm; **VPCs**, ventricular premature complexes

The heart rhythm was further characterized using a subjective severity scale. In this classification system 0 represented the absence of rhythm disturbances, 1 was used for mild, 2 for moderate and 3 was used for severe arrhythmias. In those animals with multiple rhythm disturbances (e.g. atrial fibrillation and ventricular premature complexes), the final severity score was obtained by adding 1 point to the score given to most severe arrhythmia present (e.g. slow atrial fibrillation (score =2) with premature ventricular complexes: final score = 3). There were 38 dogs in group 0 (no rhythm disturbances), 17 in group 1, 21 in group 2 and 18 dogs in group 3. The arrhythmia severity score system is included in table 2.3.

TABLE 2.3. Arrhythmia Score System.

ARRHYTHMIA SEVERITY SCORE SYSTEM				
SCORE	**Rhythm**			**Number of dogs (n)**
0 = None	Sinus /Sinus arrhythmia			38
1 = Mild (n=17)	Bradyarrhythmia	SUPRAVENTRICULAR	Sick sinus rhythm	2
	Tachyarrhythmia	SUPRAVENTRICULAR	Sinus tachycardia	1
			Slow SPV tachycardia (<160 bpm)	3
			SPVCs	4
		VENTRICULAR	AIVR	1
			Single/Sporadic/Uniform VPCs	6
2= Moderate (n=21)	Bradyarrhythmia	SUPRAVENTRICULAR	3rd AV BLOCK	2
	Tachyarrhythmia	SUPRAVENTRICULAR	Fast SPV tachy (>160 bpm)	1
			Slow Atrial Fibrillation (<160 bpm)	7
		VENTRICULAR	Frequent/Runs/Multiform VPCs	5
		COMBINATIONS	VPCs+SPVCs	3
			Sinus tachycardia + SPVCs	2
			Sinus tachycardia + VPCs	1
3= Severe (n=18)	Tachyarrhythmia	SUPRAVENTRICULAR	Fast Atrial Fibrillation (>160 bpm)	5
		VENTRICULAR	Ventricular tachycardia	8
		COMBINATIONS	Pairs/triplets VPCs + SVPCs	1
			Atrial fib + VPCs	3
			Fast SPV tachy + AV block	1

****SPV tachycardia**, supraventricular tachycardia; **SPVCs**, Supraventricular premature complexes; **AIVR**, accelerated idioventricular rhythm; **VPCs**, ventricular premature complexes; **AV block,** atrio-ventricular block.

Finally, dogs were classified depending on their final diagnosis in 6 different groups of disease. There were 29 dogs with MVD, 32 with cardiomyopathies, 8 with cardiac

tumors, 12 with congenital cardiac diseases, 8 with IPE and 5 dogs with rhythm disturbances in absence of evident underlying structural cardiac disease.

2.3.2 BIOMARKER CONCENTRATION

a) cTnI AND CONGESTIVE HEART FAILURE

Those dogs that were in CHF had significantly (P=0.021) higher cTnI concentrations than patients with compensated cardiac diseases. Although both groups had median cTnI concentrations above the reference range, dogs in failure had median cTnI concentrations of 0.52ng/ml whereas it was 0.33ng/ml in compensated canine patients. In the latter, there were 18 out of 62 dogs (29%) with cTnI concentrations below the reference range (<0.16ng/ml). In contrast, normal cTnI concentrations were detected in only 2 dogs in failure whilst 30 of 32 had increased cTnI.

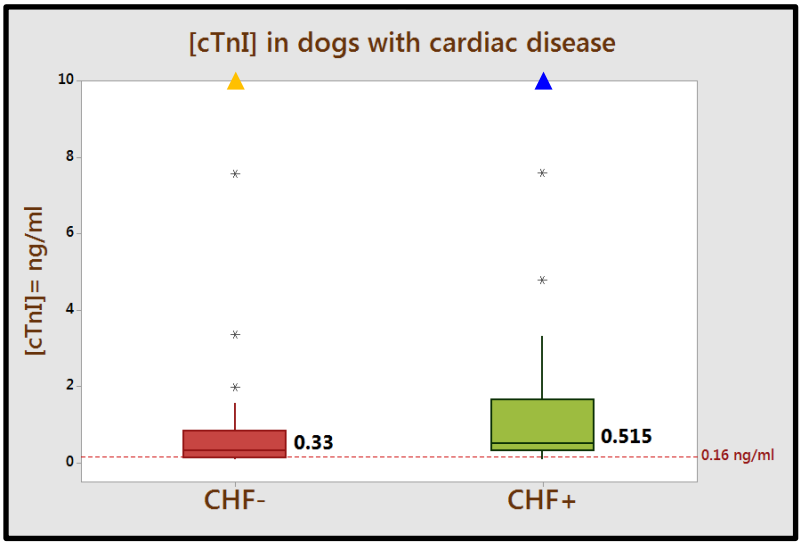

FIGURE 2.1. Boxplot of cTnI concentrations in dogs with and without CHF. cTnI concentrations were significantly higher in dogs with CHF (P=0.0021). 32 dogs in failure (CHF+) were included in the study. This group had a median cTnI concentration of 0.52 ng/ml (range:0.1-180). On the other hand, there were 62 dogs without CHF (CHF-). Median cTnI concentration of this group was 0.33 ng/ml (range:0.1-173 ng/ml). The yellow triangle (▲) represents 5 values above 10 ng/ml whilst the blue (▲) represents 4 values higher than 10 ng/ml.

b) cTnI AND ARRHYTHMIA

cTnI concentrations were significantly (P<0.0001) higher in dogs with arrhythmias than in those with sinus rhythm. Dogs with arrhythmias had median cTnI concentrations of 0.73ng/ml whereas dogs with sinus rhythm had median cTnI concentrations of 0.18ng/ml. There were 53 dogs (95%) in the arrhythmic group with

cTnI concentrations above the reference range and 3 individuals (5%) with cTnI below 0.16ng/ml. These three patients with normal cTnI concentrations had single atrial ectopics, regular junctional tachycardia (160 bpm) and sick sinus syndrome. In contrast, 17 patients with sinus rhythm (45%) had cTnI concentrations <0.16ng/ml whilst 21 (55%) had increased cTnI concentrations.

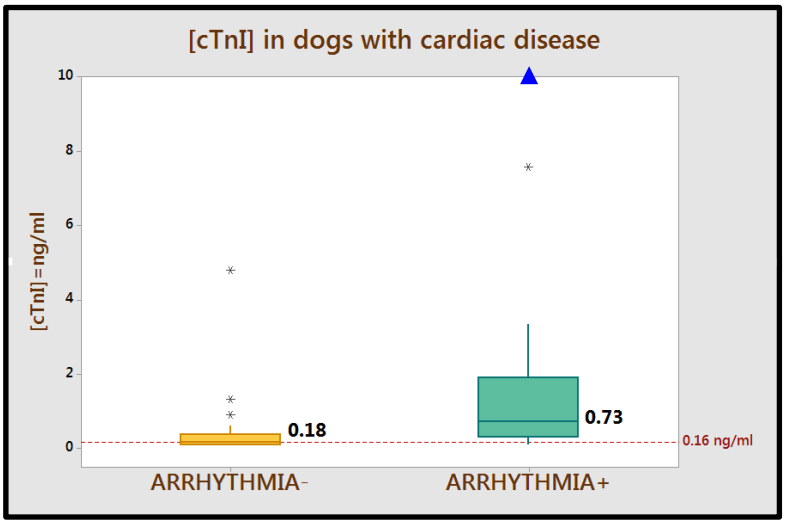

FIGURE 2.2. Boxplot of cTnI concentrations in dogs with and without arrhythmias. Dogs with arrhythmias had significantly higher cTnI concentratios than dogs with sinus rhythm (P=0.0000). There were 38 dogs which presented without arrhythmias. Those had a median cTnI of 0.18 ng/ml (range: 0.1-4.8 ng/ml).Whereas 56 dogs presented with rhythm disturbances. Those had median cTnI of 0.73 ng/ml (range: 0.1-180 ng/ml). The blue triangle ( ) represents 9 values above 10 ng/ml

Dogs with severe arrhythmias (e.g. ventricular tachycardia and fast atrial fibrillation) had significantly higher cTnI concentrations than dogs with sinus rhythm (P=0.000) and those with mild (P=0.002) and moderate (P=0.012) arrhythmias. Dogs with mild and moderate arrhythmias had significantly higher cTnI levels (P=0.015; P=0.000) than subjects with sinus rhythm. However, no difference in cTnI concentrations between dogs with mild and moderate arrhythmias was found (P=0.218). Although all groups had median cTnI concentrations above the reference range, animals with severe arrhythmias had median cTnI significantly high (median cTnI=2.43ng/ml) in comparison with animals with moderate (median cTnI= 0.61ng/ml) and mild (median cTnI =0.42ng/ml)

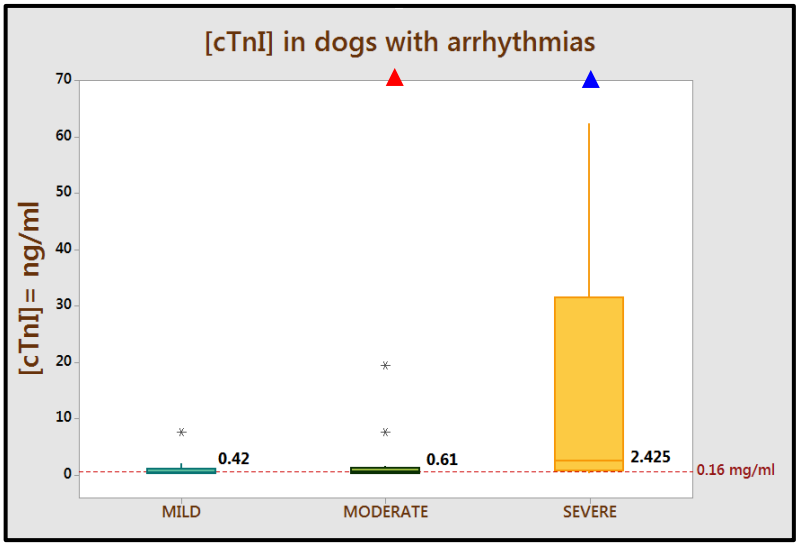

FIGURE 2.3. Boxplot of cTnI concentrations in dogs with mild, moderate and severe arrhythmias. Dogs with mild arrhythmias (n=17) had a median cTnI concentration of 0.42 ng/ml (range: 0.1-7.6 ng/ml) ; dogs with moderate arrhythmias (n=21) has a median cTnI of 0.61 ng/ml and those with severe arrhythmia (n=18) had a median cTnI of 2.43 ng/ml. Dogs with severe arrhythmias (e.g. ventricular tachycardia and fast atrial fibrillation) had significantly higher cTnI concentrations than dogs with sinus rhythm (P=0.000) and those with mild (P=0.002) and moderate (P=0.012) arrhythmias. The blue triangle ( ) represents 2 values above 70 ng/ml and the red triangle ( ) represents 1 value above 70 ng/ml.

a) cTnI AND TYPE OF CARDIAC DISEASE

Dogs whose final diagnosis was cardiomyopathy had significantly higher cTnI concentrations than dogs with acquired valvular disease (P=0.012), congenital cardiac disease (P=0.002) and rhythm disturbances (P=0.028). There were no differences between dogs with cardiomyopathy and those with cardiac tumours (P =0.087) or those with idiopathic pericardial effusion (P=0.09). Moreover, those patients with cardiac tumors had significantly (P=0.018) higher cTnI concentrations than dogs with congenital cardiac disease. No differences between the other groups were found.

Median cTnI concentrations were below the reference range only in those dogs with congenital cardiac disease whereas it was 1.92ng/ml in dogs with cardiac tumors, 0.61ng/ml in those with cardiomyopathies, 0.37ng/ml in individuals with acquired valvular disease, 0.25ng/ml in dogs with rhythm disturbances and 0.183ng/ml in subjects with idiopathic pericardial effusion.

c) cTnI AND SURVIVAL

There were 39 dogs that were alive when survival information collection finished (27th February 2014) whilst 55 dogs died before this date due to their cardiac disease. Non-survivors had significantly (P=0.003) higher cTnI concentrations than those that survived. Median cTnI concentration for non-survivors was 0.49ng/ml whereas it was 0.25ng/ml for survivors.

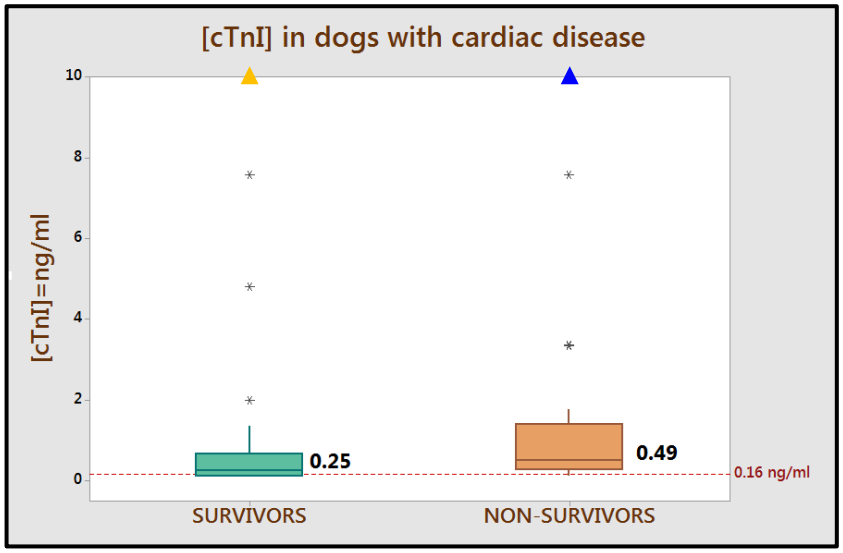

FIGURE 2.4. Boxplot of cTnI concentrations in survivors and non-survivors. Non survivors (n=55) had a median cTnI of 0.49 ng/ml (range: 0.10-180 ng/ml) whereas survivors (n=39) had median cTnI of 0.25 ng/ml (range: 0.10-127 ng/ml). Non-survivors had significantly higher cTnI concentrations than survivors (P = 0.003). The yellow triangle ( ) represents 2 values over 10 ng/ml and the blue triangle ( ) represents 7 values above 10 ng/ml

The correlation between cTnI levels and survival was further studied with Kaplan Meier curves. In order to create the curve, dogs were divided in 2 groups: dogs with increased cTnI concentrations (>0.16ng/ml) and dogs with non-elevated cTnI concentrations (<0.16ng/ml). There were 74 and 20 dogs included in each category, respectively. Dogs with increased cTnI concentrations had significantly shorter survival times than those with cTnI within the reference range. Dog with increased cTnI survived a median of 29 months whereas dogs with non-elevated cTnI concentrations survived a median of 44 months.

The difference between the curves was quantified to assess statistical significance. As the comparative analysis depends upon the whole curve and not upon isolated points, Log-Rank test was the system of analysis of choice. Log-Rank test for the

data in this retrospective study was P=0.006, thus these two curves were significantly different. Consequently, the probability of survival of dogs with non-elevated and elevated cTnI concentrations was significantly different. Survival times were significantly shorter in those dogs with elevated cTnI concentrations.

The ability of the troponin test to predict cardiac mortality in dogs with primary cardiac diseases was assessed with ROC curves. The AUC of the ROC curve was 0.69, demonstrating the potential of cTnI testing to predict outcome in these patients. However, the best cut-off for predicting cardiac death was cTnI >0.33ng/ml which produced a sensitivity of 76% and a specificity of 65%, revealing that the test produces considerable numbers of false positives and negatives.

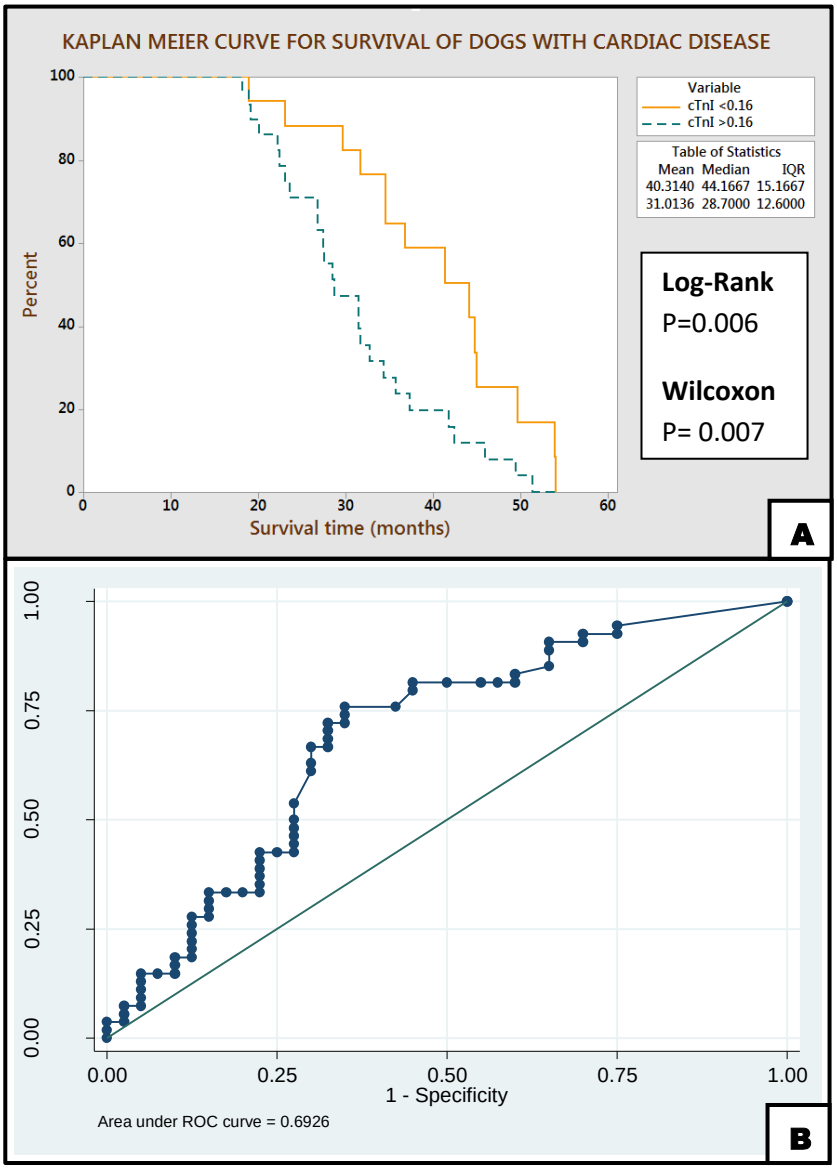

FIGURE 2.5. Kaplan Meier curve (A) and ROC curve (B) for cTnI and survival in dogs with cardiac disease

There were 39 dogs that were alive in the end of study (February 2014). Of these, 13 had non-elevated cTnI concentrations whereas 26 survivors had elevated cTnI (range: 127-0.18ng/ml). Last blood sample was collected in September 2012 thus these patients survived 1.5 years despite having increased cTnI concentrations.
Further analysis of this subset of patients, revealed that median age was 8 years old, median cTnI concentration was 0.38ng/ml (range: 127-0.18ng/ml), 15 dogs had arrhythmias (4 dogs had 3-score arrhythmias, 5 had 2-score arrhythmias and 6 had 1 score-arrhythmias) and 5 had CHF. Regarding their final diagnosis, 12 dogs were diagnosed with CM, 5 with acquired valvular disease, 4 with congenital disease, 3 had IPE and 2 had primary rhythm disturbances. cTnI concentrations and clinical information from this group of patients are summarized in table 2.4.
Arrhythmias might play an important role in the release of cardiac troponin. The review of data collected from dogs with non-cardiac diseases but increased cTnI concentrations (higher than 1ng/ml) in the same study period, revealed that a high proportion of these patients presented with arrhythmias. There were 16 out of 29 dogs with non-cardiac disease and cTnI >1ng/ml that presented with rhythm disturbances. Tachyarrhythmias were also the most prevalent in this group of dogs. cTnI concentrations and clinical data from this subset of patients are summarized in table 2.5.

e) REGRESSION ANALYISIS

Univariable and multivariable regression analysis revealed that cTnI was not associated with age or gender (P= 0.21, P=0.13). However, cTnI was associated with the presence of arrhythmia (P=0.05) and CHF.

[cTnI]	Age	Breed	Final diagnosis	CHF	score	Arrhythmia	Survival (years)
127	3	Weimaraner	Occult DCM	N	3	V.TACH	3
31.3	8	Doberman	DCM	N	3	bursts of VPCs (300 bpm)	2
7.58	10	Siberian Husky	Tachycardia induced CM	N	2	SUPRAVENTRICULAR TACHYCARDIA 300 bpm (atrial flutter?)	2
4.8	8	Labrador	IPE	Y	0	N (120 bpm)	4
1.98	1	Newfoundland	Occult DCM	N	1	VPCs	4
1.34	10	Crossbreed	IPE	N	0	N (100 bpm)	3
0.94	10	Labrador	MVD	N	1	VPCs	4
0.92	5	Boxer	ARVC	N	3	VPCs (pairs-triplets) - APCs	3
0.92	11	Labrador	DCM	N	0	Sinus rhythm ECG (arrhythmia on auscultation)	2
0.65	8	Dalmatian	MVD	N	1	VPCs	2
0.64	8	Doberman	DCM	N	3	V. TACH	2
0.47	7	Doberman	Occult DCM	N	0	N	3
0.41	12	Crossbreed	MVD	N	1	SVPCs	3
0.35	1	Alaskan Malamute	VSD	N	0	N	1
0.31	5	Dogue de Burdeaux	DCM	N	2	Atrial fibrillation (100-110 bpm)	3
0.31	9	Cocker Spaniel	DCM	Y	2	VPCs and SPV tachy.	2
0.31	6	Dogue de Burdeaux	DCM	N	2	Atrial fibrillation (140-180 bpm)	2
0.27	10	Toy Poodle	MVD, PH	Y	0	N	3
0.25	10	Crossbreed	SPV Tachy.	N	1	SPV tachycardia and AV disassociation (100 bpm)	2
0.25	11	Labrador	MVD	N	2	Atrial fibrillation (130 bpm)	2
0.24	4	Boxer	Aortic stenosis	N	0	N	2
0.24	8	Springer Spaniel	Sick sinus syndrome	N	1	Sick sinus syndrome	2
0.23	7	Cocker Spaniel	DCM	Y	0	N	3
0.216	2	Bulldog	IPE	Y	0	N	2
0.18	6	Boxer	Aortic stenosis	N	0	N	3
0.18	6	Newfoundland	Subaortic stenosis	N	0	N	2

TABLE 2.4. Dogs with cardiac disease, increased cTnI and prolonged survival.

****DCM,** dilated cardiomyopathy; **CM,** cardiomyopathy; **IPE,** idiopathic pericardial effusion; **MVD,** mitral valve disease; **ARVC,** arrhytyhmogenic right ventricular cardiomyopathy, **VSD**, ventricular septal defect; **PH**, pulmonary hypertension; **SPV tachycardia**, supraventricular tachycardia; **SPVCs**, Supraventricular premature complexes; **AIVR**, accelerated idioventricular rhythm; **VPCs**, ventricular premature complexes; **AV block,** atrio-ventricular block; **AV-disassociation**, atrio-ventricular disassociation.

[cTnI]	Age	Final diagnosis	Arrhythmia	Dox.	Hct	Creat	Survival (days)
588	3	Pancreatitis/ peritonitis/Acute renal/ liver failure	V.TACH and RT phenomenon	NO	63.7	/	1
180	10	HSA - Spleen	V.TACH	NO	33.9	103	197
115	11	Anaemia Thrombocytopaenia	VPCs (R-T phenom.)	NO	14.7	163	0
68.6	7	HSA - splenic	AIVR	NO	16.9	78	888
63.5	8	Splenic mass	Sinus tachy and multiform VPCs	NO	12.7	93	1055
24.2	9	Iliac thrombi - Hypertension- renal disease	SPV tachy (250 bpm)	NO	64.2	228	5
16.2	1	SRMA	NO	NO	47	99	1246
14.2	9	HSA - skin	NO	YES	38.9	92	724
8.49	12	HSA- skin	RBBB	YES	38	127	54
8.21	6	Mediastinal lymphoma	NO	NO	55	120	1
7.9	11	Insulinoma (Susp)	NO	NO	51.6	64	58
6.8	6	Cardiac contusion	NO	NO	20.5	/	372
6.4	12	Lung mass	SPCVs (VPCs in previous visits)	NO	61.4	92	138
6.27	7	Lymphoma	NO	NO	53.7	/	383
6.04	11	Splenic sarcoma	VPCs	NO	64.5	72	24
5.21	6	Lymphoma	NO	YES	35.9	64	139
4.35	1	Megaoesophagus/ Pneumonia / IM myositis	VPCs	NO	47.4	111	16
3.8	7	Open (Lungs)	NO	NO	38.6	99	0
3.13	8	Haemoptysis and dyspnoea UO	1st AV block - VPCs	NO	42.7	79	1
2.41	8	cruciate ligament disease	Sinus tachycardia	NO	/	84	638
2.39	9	splenic HSA	NO	NO	38.1	123	24
1.77	8	IMHA	NO	NO	/	/	299
1.72	8	neutropenia Borrelia+	NO	NO	39.3	/	35
1.5	6	Multicentric lymphoma	NO	YES	43.9	86	490
1.34	9	Brain neoplasm	SPV tachycardia (260 bpm)	NO	46	93	0
1.3	11	Hypothyroidism and CRF	SPV tachy (300 bpm)	NO	34.3	/	552
1.22	8	Lymphoma	VPCs	YES	44.6	/	85
1.22	8	Lymphoma	NO	YES	36.3	126	27
1.2	10	Abdominal neoplasia	Sinus tachy and VPCs	NO	30.1	102	0

TABLE 2.5. Unpublished data compiling clinical information from dogs with non-cardiac diseases and cTnI concentrations higher than 1ng/ml.

2.4 Discussion

The results from this retrospective study confirmed there is an association between cTnI and outcome in dogs with cardiac conditions. Patients with increased cTnI (cTnI >0.16ng/ml) had a median survival time of 29 months whereas those dogs with normal cTnI concentrations (cTnI <0.16ng/ml) had a median survival time of 44 months. Median cTnI concentration was 0.49ng/ml for non-survivors whilst it was 0.25ng/ml for survivors and this difference was statistically significant. However, when the efficacy of the test was assessed with ROC curves (AUC = 0.69), low specificity (65%) and sensitivity (76%) were obtained. Similar results were observed in a report that included 202 dogs with MVD. The AUC of the ROC curve in that study was 0.63 and, employing a cut-off of 0.025ng/ml, a sensitivity of 82% and a specificity of 43% were produced **[Hezzell et al. 2012].** The overlap of cTnI concentrations between survivors and non-survivors and the resultant low prognostic sensitivities and specificities have been also described in short- and long-term survival studies in critically ill dogs with systemic inflammation **[Langhorn et al. 2013 and 2014c].** Therefore, caution should be exercised as some animals escape the rule of "higher cTnI concentrations = shorter survival times". Cut-off concentrations obtained with outcome predictive systems should not be used to predict cardiac death in individual patients and decisions of euthanasia should not be made based on cTnI results.

The association between cTnI concentrations and survival in dogs with cardiac disease has barely been explored before. One study reported shorter survival times with increased cTnI concentrations in dogs with cardiac disease. Patients with cTnI concentrations between 0.151 and 1ng/ml had a median survival time of 24 months whereas dogs with cTnI greater than 1ng/ml survived no longer than 3 months. Dogs that survived more than 1 year, 2 years and 3 years had a median cTnI concentration of 0.18ng/ml, 0.07ng/ml and 0.05ng/ml, respectively **[Fonfara et al. 2010].** This association between cTnI levels and outcome has been demonstrated in dogs with cardiomyopathy and mitral valve disease in 3 additional studies. In the first, dogs with cardiomyopathy and cTnI <0.20ng/ml survived 357 days whereas those patients with cardiomyopathy and cTnI >0.20ng/ml had a median survival time of 112 days **[Oyama and Sisson, 2004].** In another report, cTnI was detectable in 6 out of 15 dogs with mitral valve disease (MVD) and congestive heart failure. In those, median

survival time was 68 days whereas those with undetectable cTnI levels (9/15) survived for 390 days **[Linklater et al. 2007].** Finally, cTnI was also associated with decreased survival times for all-cause mortality in 202 dogs with naturally MVD. However, cTnI was not found to be independently associated with survival in those dogs that died or were euthanized because of cardiac disease **[Hezzell et al. 2012]**. In the present study, the presence of an arrhythmia was significantly associated with higher cTnI concentrations. In dogs with arrhythmias (n=56) the median cTnI concentration was 0.73ng/ml whereas in dogs in sinus rhythm this was 0.18ng/ml. Moreover cTnI concentrations correlated with the severity of the arrhythmia. Dogs with severe arrhythmias had significantly higher cTnI concentrations than those with moderate or mild rhythm disturbances. In dogs with severe arrhythmias the median cTnI concentration was 2.43ng/ml compared with 0.61ng/ml and 0.42ng/ml in animals with moderate or mild rhythm abnormalities, respectively. Controversial results have been published regarding the association between cTnI and arrhythmias in dogs with cardiac conditions. cTnI concentrations correlated with VPC/24h and the severity of arrhythmia in 20 boxers with and without ARVC **[Baumwart et al. 2007]**. In another study, which included 336 Doberman Pinschers, cTnI was able to predict VPCs (AUC = 0.79/ SY = 71%/ SPY = 87%) but no significant linear association between number of VPCs/24 hour and cTnI levels was found **[Wess et al. 2010].** Arrhythmias were detected in 16 of 23 dogs (70%) with cTnI concentrations >1.01ng/ml (the group with highest cTnI values in that study population) in another report. Of these 16 dogs, seven were diagnosed with atrio-ventricular block, one with sick sinus syndrome, four with ventricular tachycardia and 4 with ARVC **[Fonfara et al. 2010]**. cTnI concentrations were significantly increased in 21 Doberman Pinchers, Great Danes and Boxers with ECG (1 or more ventricular premature complexes during performance of 4 min ECG, n = 11) or echocardiographic (n=20) evidence of occult cardiomyopathy **[Oyama et al. 2007]**. In contrast, no difference in median cTnI concentrations was found between 26 dogs with cardiomyopathy with and without arrhythmias **[Oyama and Sisson, 2004].**

cTnI concentrations have been demonstrated to be elevated in dogs with several non-cardiac conditions as anaemia **[Gow et al. 2011]**, renal failure **[Porciello et al. 2008 and Sharkey et al. 2009]**, GDV **[Schober et al. 2002 and Burgener et al. 2006]**, pyometra **[Hagman et al. 2007 and Pelander et al. 2008]**, blunt chest

trauma [Schober et al. 1999], snake envenomation [Segev et al. 2008, Pelander et al. 2010,Langhorn et al. 2014a], chemotherapy [Christiansen et al. 2002, DeFrancesco et al. 2002 and Selting et al. 2004], exercise [McKenzie et al. 2007 and Tharwat et al. 2013] and infectious diseases [Mastrorilli et al. 2007, Lobetti et al. 2002 and Diniz et al. 2008]. Arrhythmias are not uncommon in these clinical scenarios. Rhythm alterations were frequently detected in dogs with non-cardiac diseases included in the studies mentioned above. In 85 dogs with GDV, electrocardiographic abnormalities were detected in 68 (80%) and 36 out of 68 had ventricular arrhythmias. cTnI concentrations for dogs with no or mild ECG changes were significantly lower than values for dogs with moderate and severe ECG abnormalities [Schober et al. 2002]. Electrocardiographic abnormalities were detected in 33 out of 150 of *Erhlichia canis* infected dogs in another study. Dogs with severe ECG changes had higher cTnI than dogs with mild or moderate ECG abnormalities. High concentrations were detected in dogs with VPCs (median cTnI 2.15ng/dl) and supraventricular tachycardia (0.22ng/dl) [Diniz et al. 2008]. In 48 dogs suffering from snake envenomation, arrhythmias were found in 14 of them (29%) and sinus tachycardia was the most prevalent (57%). Cardiac arrhythmias were correlated with cTnT but not cTnI concentrations [Segev et al. 2008]. In another study with 24 envenomed dogs, 6 dogs had ECG abnormalities and 3/6 had increased cTnI levels [Pelander et al. 2010]. Finally, arrhythmias were identified in 9 out 34 dogs with Babesiosis. Those showing VPCs had high cTnI levels. There was a significant correlation between the presence of VPCs and high cTnI concentrations [Lobetti et al. 2002].

Importantly, cTnI has been demonstrated to be elevated in human patients with angiographycally normal coronary arteries and benign arrhythmias as paroxysmal supraventricular tachycardia (PSVT). This dysrhythmia is usually managed without difficulty and rarely results in adverse clinical outcomes. As a result, the prognostic significance of elevated troponins in patients with PSVT has been questioned [Redfearn et al. 2005, Chow et al. 2010, Carlberg et al. 2011 and Xue et al. 2014].

The role of arrhythmias in the release of cardiac troponin in small animals remains obscure. We hypothesized that arrhythmias might be a contributing factor to cardiac troponin release in small animals too (“tachycardia induced elevation of cTnI”).

Tachyarrhythmias were a common finding in dogs in this study with high cTnI.Some of these patients with marked elevation of cTnI concentrations and severe arrhythmias presented prolonged survival. Myocardial ischemia secondary to the presence of arrhythmia may contribute to the magnitude of cTnI release in these patients.

Regression analysis failed to show correlation between cTnI and gender or age. However, cTnI increased significantly with increasing age in 176 healthy dogs and 81 dogs with MVD in previous reports. Age is thought to cause myocardial changes leading to cTnI leakage as the aged heart, even in the absence of demonstrable cardiovascular disease, loses up to 35% of its total myocyte number **[Oyama and Sisson, 2004 and Ljungwall et al. 2010]**.

Although our results demonstrated that cTnI was significantly associated with the presence of CHF, conflicting results have been published before. No difference in median cTnI concentration was found in 37 dogs MVD and 26 dogs with CM with and without CHF **[Oyama and Sisson, 2004]**. In another prospective study, only six out of 15 dogs with MVD and class IV CHF had detectable levels of cTnI, nevertheless these patients had shorter survival times than those with cTnI concentrations within the reference range **[Linklater et al. 2007].** However, in 35 dogs with acquired and/or congenital cardiac conditions serum cTnI varied with severity of congestive heart failure. In that study, dogs displaying clinical signs of congestive heart failure had the highest cTnI levels. Dogs in CHF with clinical signs (class II and class IIIA of the ISACHC – International Small Animal Cardiac Health Council - classification) had significantly higher cTnI values than controls and dogs with CHF without clinical signs (class IA and IB) **[Spratt et al. 2005]**. cTnI concentrations are thought to increase late in the course of the cardiac disease (higher values are expected in advanced stages) and correlate with the severity of the cardiac condition **[Fonfara et al. 2010, Ljungwall et al. 2010 and Hezzell et al. 2012].**

The retrospective nature of the current study resulted in a number of limitations. Animals that were euthanized were categorized as non-survivors. Dogs could have been misclassified regarding their cause of death (cardiac, non-cardiac) as many of them died at home or were put to sleep and concurrent diseases cannot be ruled out completely. Haematology and biochemistry were not available for every patient thus anaemic and azotaemic dogs could have been inadvertently included.

Furthermore, the ideal scenario would have been utilization of age-matched controls in survival studies.

One of the major limitations of this study is that, when multiple cTnI measurements were available, only the highest was taken into account. Little is known about the fluctuations in cTnI concentration in the progression of the different canine cardiac diseases. Therefore, this analysis makes statistical results difficult to interpret. Results employing CTnI concentrations at admission would have been more useful for clinical purposes.

The results of this study confirm that high cTnI concentrations are associated with shorter survival times in dogs with cardiac diseases. However, due to the overlap of cTnI concentration between survivors and non-survivors, and the low specificities and sensitivities produced, caution should be exercised when interpreting individual results. The role of arrhythmias in cTnI leakage remains unknown in small animals and it requires further investigation. Arrhythmias are commonly detected in dogs with cardiac or non-cardiac disease and increased cTnI levels. Clinical decisions should not be made on the basis of cTnI results alone. Nevertheless, dogs with increased troponin values require medical attention and a full cardiac examination. Meticulous cardiac auscultation and continuous ECG monitoring should be recommended in dogs with increased cTnI concentrations.

CHAPTER 3

Retrospective study in cats:

Serum cTnI and survival in feline cardiac and non-cardiac disease.

3.1 Aims and objectives

- To study cTnI concentrations in cats with a wide variety of cardiac diseases.
- To test the ability of cTnI to distinguish between cats with different types of cardiomyopathy.
- To evaluate the ability of cTnI to discriminate between cats with cardiac, thyroid and systemic diseases.
- To investigate the relationship between cTnI concentrations and survival in cats with cardiac disease.
- To clarify if cTnI concentrations could accurately predict cardiac death in the individual patient.
- To assess the impact of age, gender, cardiac disease, presence of CHF and arrhythmia on cTnI concentrations and survival in cats with cardiac disease.

3.2 Material and methods

This was a retrospective study that included cats admitted to the Small Animal Hospital of the University of Glasgow between 2009 and September 2012, which had cTnI measured in blood samples collected as part of a normal clinical investigation. Repeated samples from the same individual collected on different visits were excluded for statistical purposes. If several measurements were available, only the highest result was included in the analysis. The clinical histories were reviewed and they were classified in 3 different categories regarding their main disease: cats with cardiac disease, cats with hyperthyroidism and cats with systemic diseases. No controls were recruited for the present study.

3.2.1 ANIMALS

a) Cats with cardiac disease

Cats were eligible for inclusion where there was evidence of cardiac disease on physical examination. The presence of arrhythmias, gallop sounds and murmurs or combinations of these were considered valid inclusion criteria.

Cats with a haematocrit below 23%, creatinine >180 µmol/L in the absence of diuretic therapy, thyroxine >50 nmol /L, persistent systolic arterial blood pressure >180 mmHg or concurrent disease (e.g. neoplasia or trauma) were excluded from the cardiac disease group.

b) Hyperthyroid cats

The inclusion criteria for this group was a thyroid hormone level >50 nmol/L. Cats with haematocrit below 23%, creatinine >180 µmol/L, persistent systolic arterial blood pressure >180 mmHg or concurrent disease (e.g. neoplasia or trauma) were excluded from the hyperthyroid group. Cats with signs of cardiac disease on physical examination and increased thyroxine levels were included in this group.

c) Cats with systemic diseases

Cats were included in this group if they were diagnosed with a systemic non-cardiac disease and they did not show evidence of cardiac disease on physical examination. Animals with haematocrit below 23%, creatinine >180 µmol/L, thyroxine >50 nmol /L or persistent systolic arterial blood pressure >180 mmHg were excluded.

3.2.2 CARDIAC BIOMARKER

Samples were collected by jugular venipuncture and placed in plain serum tubes, which were allowed to clot and submitted for analysis on the same day. Cardiac troponin I was tested with an "Immulite® 2000 Troponin I" (Siemens) analyzer. This is a chemiluminescent immunometric assay. The bead is coated with monoclonal murine anti-troponin I antibody and the liquid phase consists of alkaline phosphatase (bovine calf intestine) conjugated to polyclonal anti-troponin I antibody. This assay has a lower detection limit of 0.1ng/ml. The normal reference range for cats is <0.16ng/ml.

3.2.3 CLINICAL OUTCOME

Survival analysis was performed for cardiac cases only, due to the low number of cats in the other groups. Survival times were calculated from the date of sampling to the date of death or euthanasia. The outcome was obtained either from electronic records of the primary clinicians or by contacting owners or referring practitioners by telephone. Animals that were euthanized or died from non-cardiac conditions were excluded from the study.

3.2.4 STATISTICAL ANALYSIS

Statistical analyses were performed using Minitab statistical software. Data were non-normally distributed and results were compared using the Mann-Whitney U test

and graphically assessed with box-plots graphs. Median and interquartile ranges were used to provide descriptive statistics.

Survival was analyzed using Kaplan-Meier curves. Cats were censored if they were alive at the end of the study period. ROC curves were created to assess the prognostic capability of cTnI and to identify the most useful cut-offs to predict cardiac death and cardiac death within a year.

Regression analysis was performed to identify if the cTnI results could have been influenced by confounding factors such as age, breed or type of disease. Statistical significance was defined as $P < 0.05$.

3.3 Results

3.3.1 STUDY POPULATION CHARACTERISTICS:

a) Total population

In total 141 samples were available for analysis. 53 were repeat samples and were excluded. The remaining samples (n=68) were placed in the three categories: cardiac disease (n=51), hyperthyroid disease (n=9), systemic disease (n=8).

b) Cardiac disease

Fifty one cats were included in the cardiac disease group. Of those, 16 were female (4 entire and 12 neutered) and 35 were male (3 entire and 32 neutered). The most common breed was domestic shorthair (n=30), followed by exotic shorthair (n=11), British shorthair (n=3), Maine Coon (n=3), Domestic Longhair (n=3) and Sphynx (n=1). The median age was 6 years old (range: 1-15 years).

Murmurs were the most common finding on physical auscultation (n=38), however gallop sounds were detected in almost half of the population studied (n=24) and 15 animals presented with audible arrhythmias.

Electrocardiograms were available for these 15 animals. The most frequent rhythm disturbances in this population were VPCs (n=9). Other arrhythmias were SPVCs (n=2), atrial fibrillation with VPCs (n=2), VPCs and SPVCs combined (n=1) and 1 cat presented with second degree A-V block.

Fifty out of the total 51 cats were scanned by the same experienced cardiologist (Dr. Paul Wotton). The most prevalent disease was HCM (n=29) followed by unclassified cardiomyopathy (UCM) (n=10) and RCM (n=9). A diagnosis of HCM was made based on maximal end-diastolic thickness of the left ventricular posterior wall

(LVPWd) or interventricular septum (IVSd) greater than 6 mm. For RCM, the inclusion criteria were normal thickness of the left ventricular walls and a restrictive left ventricular filling pattern (high E:A ratio on transmitral flow). The UCM category included those with normal thickness of the walls, normal ventricular dimensions, reduced systolic function and absence of a restrictive pattern on transmitral flow. One cat had pulmonic stenosis and one cat had second degree AV block. One cat had cardiomegaly on thoracic radiographs, an arrhythmia and gallop sounds, but the disease was not characterized as echocardiography was not undertaken.

CHF was defined as presence of pleural or pericardial effusion seen on echocardiographic examination as well as presence of pulmonary oedema or pleural effusion on thoracic radiographs. Using these criteria, 28 animals were considered to be in CHF whereas 23 animals were not.

c) Hyperthyroid disease

Nine cats were included in this category. Six cats were female neutered and the other 3 male neutered. Most were Domestic Shorthairs (n=8) and there was one Siamese. The median age was 14 years old (range: 7-15 years).

Three animals presented with audible murmurs, 2 had gallop sounds and 2 showed arrhythmias (SPVCs and VPCs). Five experienced some degree of respiratory distress (tachypnoea or dyspnoea). Six out of 9 animals underwent echocardiographic examination and all of them had cardiac abnormalities.

d) Systemic disease

Eight cats met the inclusion criteria for this group. The group comprised 1 intact male, 6 neutered males and 1 neutered female. There were 6 Domestic Shorthairs, 1 Domestic Longhair and 1 Persian. The median age was 3.5 years old (range: 1-14 years). These patients were finally diagnosed with idiopathic epilepsy, bronchopneumonia, pulmonary neoplasia, asthma and collapsing episodes of unknown origin.

None of these animals presented with cardiac abnormalities on physical examination. Two animals were scanned and no abnormalities were detected on echocardiography.

3.3.2 BIOMARKER CONCENTRATIONS

a) Cats with cardiac disease

There were 49 cats (96%) with cardiac disease with increased cTnI concentration. Only two cats (4%) included in his category had cTnI concentrations below the detection limit of the assay (<0.1ng/ml).

The range of cTnI in cats with cardiac disease was between 0.1 and 35ng/ml and the median cTnI concentration was 1.03ng/ml.

cTnI concentrations from cats with HCM, UCM, and RCM were compared using the Mann-Whitney U test. cTnI concentrations were not significantly different between disease types.

Cats with HCM (n=29) had a median cTnI concentration of 0.81ng/ml (range: 0.1-35ng/ml), whereas it was 1.03ng/ml for cats with RCM (range: 0.26-9.53ng/ml) and 2.47ng/ml for UCM cats (range: 0.16-21.2ng/ml).

Three cats with HCM had cTnI values below the detection limit (0.1ng/ml), whereas all UCM cats (n=10) and RCM cats (n=9) had cTnI above the reference range.

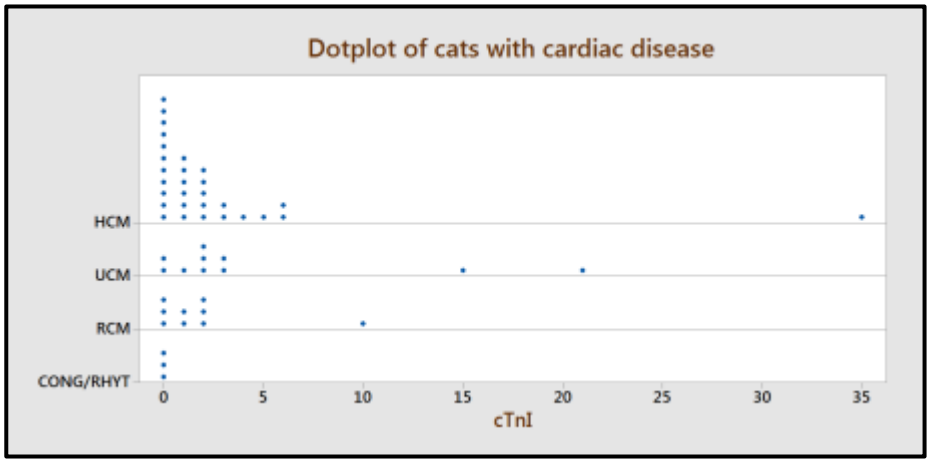

FIGURE 3.1 Dotplot of cTnI concentrations in cats with cardiac disease. Note that most of the cats had cTnI between 0 and 3ng/ml, demonstrating why no statistically significant difference was found between types of cardiomyopathy.

Cats that were in CHF had significantly higher cTnI concentrations than cats without CHF (*P*<0.0001). Median cTnI concentration from animals in failure was 2.185ng/ml (range of 0.27-35ng/ml), whereas cats without failure had a median cTnI concentration of 0.41ng/ml (range of 0.1-4.76ng/ml).

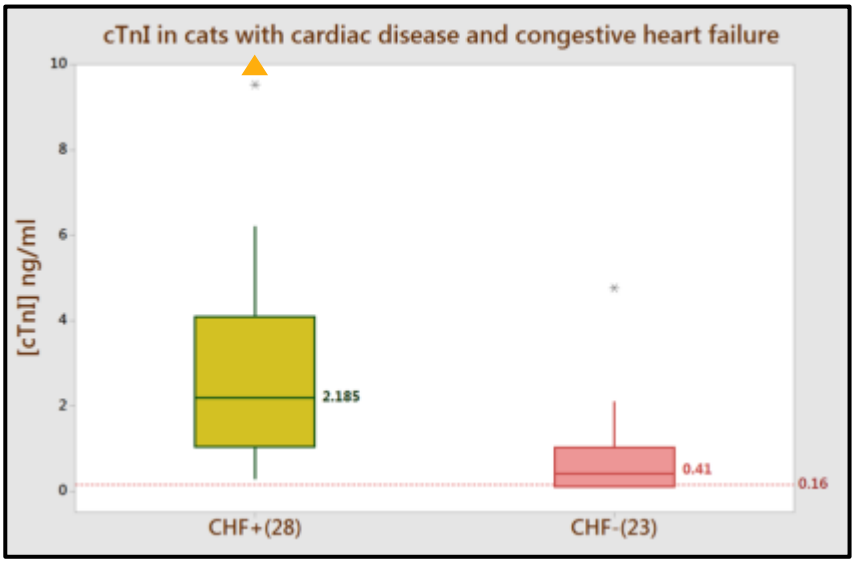

FIGURE 3.2 Boxplot of cTnI concentrations in cats with (+) and without (-) CHF. CHF was diagnosed in 28 cats, with median cTnI of 2.185ng/ml (range: 0.27-3ng/ml). Twenty-three cats had compensated cardiac disease (CHF-). These cats had a median cTnI concentration of 0.41ng/ml (range 0.1-4.76ng/ml). Cats in CHF had significantly higher cTnI concentrations than cats without CHF (P <0.0000). The yellow triangle (▲) represents 3 values over 10ng/ml.

b) Cats with hyperthyroidism

All the cTnI measurements were above the reference range in the cats with hyperthyroidism. The median concentration of cTnI in this group was 0.757ng/ml (ranging from 0.16 to 2.14ng/ml).

c) Cats with non-cardiac systemic diseases

Four cats with systemic disease (50%) had increased cTnI concentrations while the other four had cTnI levels below the detection limit. Two out of the four animals with elevated cTnI were presented with collapsing episodes of unknown origin and the other two were finally diagnosed with primary respiratory diseases. The median cTnI concentration for this group was 0.165ng/ml (range 0.1ng/ml to 0.23ng/ml).

3.3.3 COMPARISON BETWEEN GROUPS

The cTnI concentrations in cats with cardiac disease were significantly higher than those from cats with systemic disease (P =0.0076). In contrast, there was no significant difference between cTnI levels in cats with cardiac disease and those with hyperthyroid disease (P=0.48). The difference between hyperthyroid and systemic disease cats did not reach statistical significance either (P=0.06).

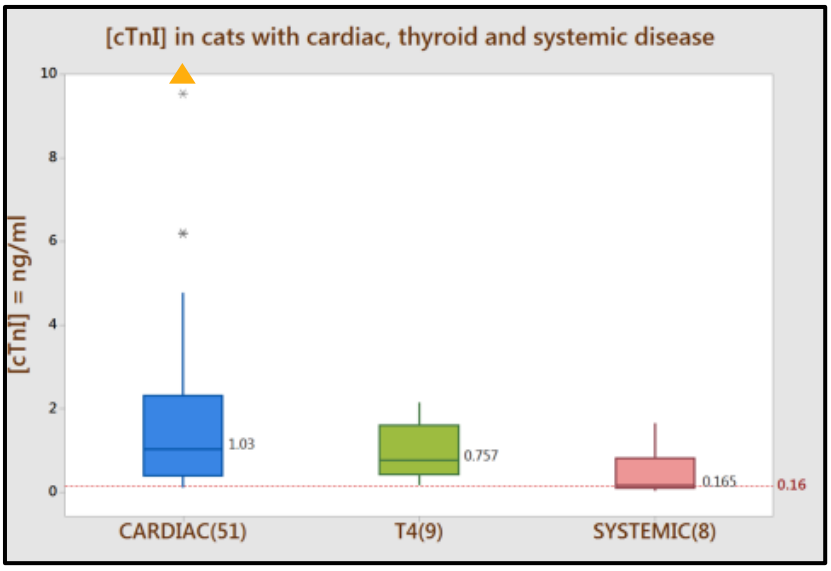

FIGURE 3.3 Boxplot of cTnI concentrations in cats with cardiac, hyperthyroid and systemic diseases. There were 51 cats with cardiac disease, with median cTnI of 1.03ng/ml (range: 0.1-35ng/ml); 9 cats with hyperthyroid disease, with median cTnI of 0.757ng/ml (range 0.16-2.14ng/ml) and 8 cats with systemic disease, with median cTnI of 0.165ng/ml (range: 0.1-0.23ng/ml). The yellow triangle (▲) represents 3 values over 10ng/ml.

3.3.4 SURVIVAL ANALYSIS IN CATS WITH CARDIAC DISEASE

Twenty eight (55%) cats died or were euthanized because of the progression of their cardiac disease during the study period.

Non-survivors had significantly higher cTnI concentrations than survivors (*P*=0.0001). The median cTnI concentration in non-survivors was 2.11ng/ml (range: 0.27-35ng/ml) whereas the median cTnI concentration in survivors was 0.41ng/ml (range: 0.1-4.76ng/ml).

No cats with aortic thromboembolism (ATE) at the time of sampling were included in the present study. However, two animals died from ATE. The first one was an 8 years old, male neutered, Sphynx diagnosed with RCM and a cTnI concentration of 1.73ng/ml. This patient died 8 days from the date of sampling. The other cat was a 5 year old, male neutered, Maine Coon with HCM and a cTnI value of 2.68ng/ml who survived for 7 months before suffering an ATE episode.

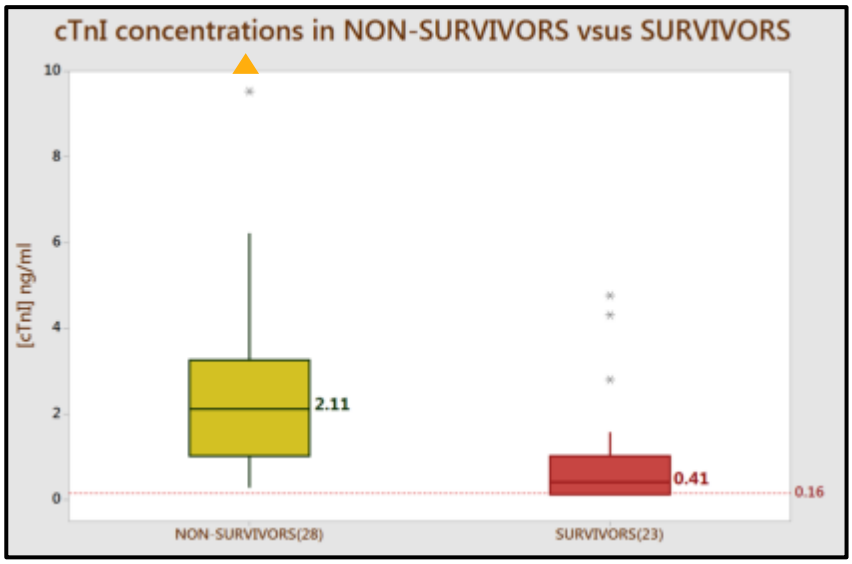

FIGURE 3.4. Boxplot of cTnI concentrations in survivors and non-survivors cats with cardiac disease. 28 cats died during the study period, non-survivors had a median cTnI of 2.11ng/ml (range: 0.27-35ng/ml). On the other hand, 23 cats remained alive; survivors had a median cTnI of 0.41ng/ml (range: 0.1-4.76ng/ml). Non-survivors had significantly higher cTnI concentrations than survivors (P=0.0001). The yellow triangle (▲) represents 3 values over 10ng/ml

In order to produce Kaplan-Meier (K-M) curves, cats with cardiac disease were categorized in 4 different subgroups depending on their cTnI concentrations. Group 1 included cats with cTnI concentrations above 5ng/ml, group 2 cats with cTnI levels between 0.5-5ng/ml, group 3 cats with cTnI between 0.15-0.5ng/ml and finally group 4 consisted of cats with cTnI values lower than 0.15ng/ml.

All cats in group 1 (n=6) were dead at the end of the study. In group 2 (n=26) 79% were dead at the end of the study. In group 3 (n=12) 25% were dead at the end of the study. All of group 4 (n=6) were alive at the end of the study.

The non-continuous nature of the K-M curves emphasizes that they are not smooth functions but rather step-wise estimates; thus, calculating survival can be difficult [Rich et al. 2010], although a "rough estimation" of survival can be done. For example, if a cat is included in group 4 (coloured in blue), its probability of surviving 12 months is 100 percent. Conversely, if a cat is categorized in group 3 (green), its probability of surviving a year is around 50%.

The difference between these curves was quantified in order to assess statistical significance. The 4 curves were found to be significantly different using a log rank test. Consequently, the probability of survival of cats with cTnI concentrations of

>5ng/ml, 0.5-5ng/ml, 0.15-0.5ng/ml and <0.1 was significantly different. Survival times were significantly shorter in cats with higher cTnI levels.

Animals with cTnI concentrations above 5ng/ml (group 1) had significantly (*P*=0.0001) lower survival times (median 0.933 months) than cats with cTnI between 0.5 and 5ng/ml (group 2) which survived a median of 14.9 months.

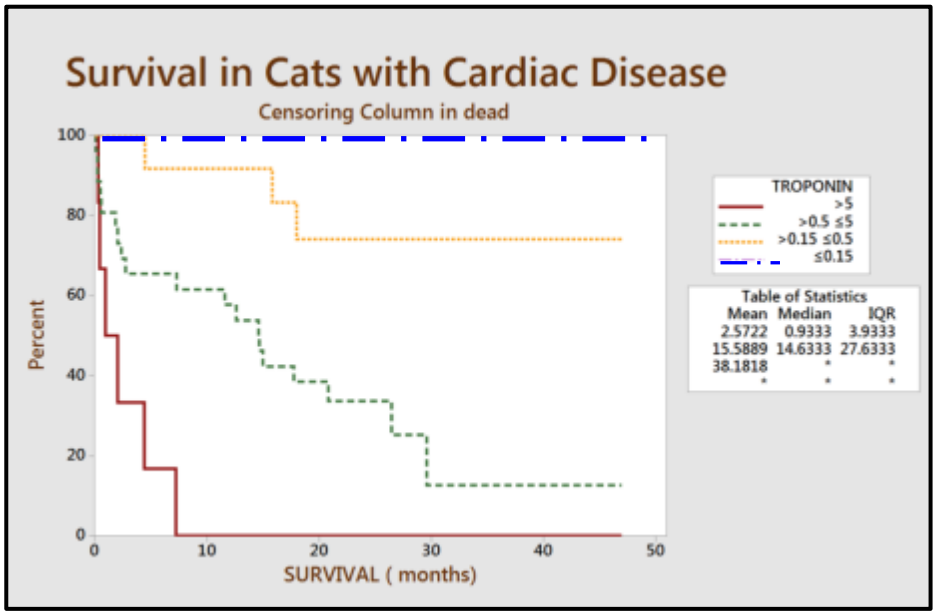

FIGURE 3.5. Kaplan-Meier curve for survival analysis in cats with cardiac disease. Cats were allocated in 4 groups regarding their cTnI concentrations. Cats with cTnI <0.15 are represented in blue (n=6); cats with cTnI between 0.15 and 0.5 appear in yellow (n=12), cats with cTnI levels between 0.5 and 5 are seen in green (n=26) and cats with cTnI above 5 are represented in red (n=6).

The ability of the troponin test to predict cardiac mortality in cats with primary cardiac diseases was assessed with ROC curves. Two different ROC curves were plotted, one for cTnI as a predictor of cardiac death and another for cTnI as predictor of cardiac death within one year. The area under the curve (AUC) was used to compare them. The AUC of cTnI as a predictor of cardiac death was 0.82 and the AUC of cTnI as a predictor of cardiac death within one year was 0.778. Both were close to 0.8, which is considered to be good accuracy for a diagnostic test.

The best cut-off for predicting cardiac death was 0.64ng/ml, which produced a sensitivity of 80.4% and a specificity of 70%. The best cut-off to predict cardiac death within a year was 1.73ng/ml, corresponding to a sensitivity of 72.22% and specificity of 72.73%.

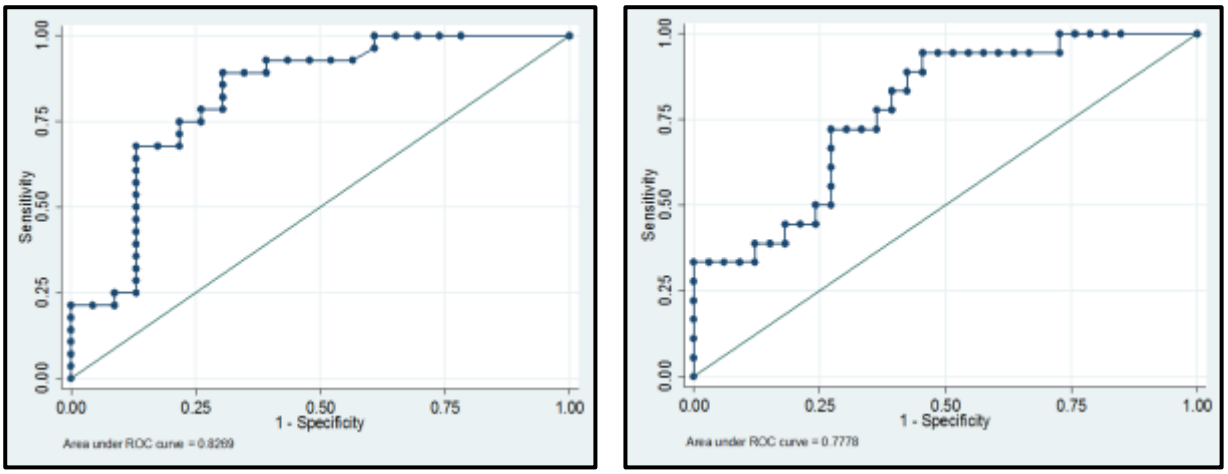

FIGURE 3.6. ROC curves for cTnI and survival in cats with cardiac disease. A, cTnI as predictor of cardiac death (AUC=0.8269). B, cTnI as predictor of cardiac death within a year (AUC =0.778)

3.3.5 REGRESSION ANALYSIS AND ANOVA

Univariable and multivariable analysis revealed that age, gender or type of cardiomyopathy were not statistically associated with cTnI. In contrast, cTnI was significantly correlated with CHF. Higher troponin values were found in animals in CHF. Using a cTnI concentration of 1.13ng/ml as a cut-off, 83% of the animals in CHF were correctly classified (sensitivity 73% and specificity 91%).

3.4 Discussion

To the author´s knowledge, this is the first study that has demonstrated that cTnI is increased in several feline cardiac disease phenotypes. Some authors have previously reported that cats with HCM have increased cTnI values, however there are no previous reports regarding cTnI in UCM and RCM [Herdon et al. 2002, Connolly et al. 2003, Borgeat el al. 2014 and Langhorn et al. 2014b]. However, cTnI seems not to be a useful test to distinguish between HCM, RCM and UCM as the difference between concentrations in these groups was not statistically significant.

No histopathological examinations were performed in the cases included in this study; thus it was impossible to establish a causal relationship for cTnI release. However, abnormal coronary arteries with thickened walls and narrowed lumens are a common finding in cats with HCM [Fox, 2003]. These abnormal vessels are

commonly surrounded by areas of "replacement fibrosis" caused by ischaemia. End-stage HCM, the final phase of the disease, is seen in some cats. The hallmark histological feature of end stage HCM is multifocal myocardial scarring (large regions of replacement fibrosis) for which myocardial infarction is considered the most likely cause. The small vessel disease present in HCM may lead to alterations of blood flow, causing ischaemia, subsequent myocardial cell death and replacement fibrosis. Moreover, intracardiac thrombi and thromboembolism appear to be very common in this subset of cats, thus coronary arterial thromboembolism is likely to occur **[Cesta et al. 2005]**. Some authors have suggested that the myocardiocyte disarray seen in human patients with HCM can contribute to ischaemia **[Varnava et al. 2001]**. As with HCM, RCM also shows a broad spectrum of pathological phenotypes. The endomyocardial form, known as endomyocardial fibrosis (EMF), is associated with marked replacement fibrosis involving the endocardium or endomyocardium **[Fox, 2004]**. Commonly both atria are enlarged in RCM; in some cases atrial hypertrophy is evident, whereas in others the atrial walls are severely thinned. This is thought to be as a consequence of marked myocyte loss and collagen replacement. Similar to HCM cats, intracardiac thrombi and arterial thromboembolism are frequently observed. The term UCM is used when there are structural markers of RCM without proof of impaired diastolic ventricular filling **[Côté et al, 2011b]**.

Replacement fibrosis and ischaemia are, thus, common in feline cardiomyopathies and are the most likely causes of myocardial damage and cTnI release in these patients. Some similar histopathological findings are common to these 3 clinical types of feline cardiac disease and could be the explanation for the lack of difference in cTnI concentrations between them.

Only one cat was presented with pulmonic stenosis. This patient had a troponin value of 0.11ng/ml. No reports describing cTnI values in cats with congenital diseases have been published. Dogs with congenital disease have lower cTnI values that those with acquired heart diseases and further studies are required to see if a similar finding may be present in cats **[Oyama and Sisson 2004, Spratt et al. 2005 and Fonfara et al. 2010]**.

No cats with ATE at the time of sampling were included in this study. However, two animals later died from ATE; one of them had a cTnI concentration of 1.73ng/ml and died 8 days after sampling. The second cat had a cTnI value of 2.68ng/ml and died

7 months after being sampled. A previous study included 2 animals with ATE and these cats had the highest troponin concentrations of the population examined **[Herndon et al. 2002]**. Several different factors may have influenced the progression to ATE in our two cases, such as therapeutic protocol or type of disease. Further studies are needed to see if cTnI is a good predictor of ATE in cats.

In the present study cTnI has been shown to be a good predictor of survival regardless of type of cardiomyopathy in cats. Cats with cardiac disease and cTnI levels above 5ng/ml survived for a median time of 0.933 months while cats with cardiac disease and cTnI concentrations between 0.5 and 5ng/ml survived a median time of 14.9 months. The best cut-off for predicting cardiac death was 0.64ng/ml, with a sensitivity of 80.4% and a specificity of 70%.

Two recent papers have focused on the usefulness of cTnI as a predictor of mortality in cats with cardiac diseases. The first one specifically recruited purebred cats (Maine Coon, British Shorthair, Norwegian Forest and Exotic Shorthairs) with suspected familial HCM and demonstrated that both cTnI and cTnT concentrations at admission were prognostic for survival **[Langhorn et al. 2014b].** The second study showed that a circulating concentration of cTnI greater than 0.7ng/ml was a significant predictor of cardiac death in cats of any breed with HCM. Importantly, cTnI remained significant even if the size of the left atrium or the presence of CHF remained uncertain **[Borgeat et al. 2014]**.

In the present study, the regression analysis showed that cTnI was correlated with CHF. Those animals that were in CHF had significantly higher cTnI values and significantly lower survival times. Controversial information has been published about CHF and cTnI in cats. One study also showed that cats with CHF at the time of cTnI measurement had significantly higher (P=0.0095) concentrations than did cats with no CHF **[Herndon et al. 2002].** However, in another study the authors were not able to demonstrate a statistically significant difference in cTnI levels between cats with CHF due to cardiomyopathy and those without (P=0.16) **[Connolly et al. 2003]**. Any discrepancy between studies may be related to low numbers. This study has shown that, in a well characterized population of cats, cTnI may differentiate cats with CHF from cats without CHF, but it is important to be aware that three previous studies have all shown that whilst cats with dyspnoea due to congestive heart failure tend to have higher cTnI concentrations than cats with non-

cardiac dyspnoea, there was a high degree of overlap between the groups. cTnI is considered to be unsuitable to distinguish between causes of dyspnoea in cats **[Herndon et al. 2008, Connolly et al. 2009a and Wells et al. 2014]**

Cardiac biomarkers have previously been shown to be elevated in cats with hyperthyroidism **[Connolly et al. 2005 and Sangster et al. 2014]** and this study confirms this finding. This study has also shown that cTnI cannot distinguish between cardiomyopathy secondary to thyroid disease and any of the primary cardiomyopathies HCM, RCM and UCM. Structural cardiac changes due to elevated circulating thyroid hormone levels in cats are well known. Tachycardia, hypertension, increased erythropoietin release, activated renin-angiotensin-aldosterone system and chronic sympathetic stimulation are common features and can lead to further cardiac remodeling **[Côté et al. 2011c]**

cTnI concentrations were significantly higher in cats with cardiac disease in comparison with cTnI from patients with systemic disease where cardiac disease, renal disease, anaemia and systemic hypertension had been excluded (P= 0.0076). However 50% of the animals included in the latter group had cTnI concentrations above the reference range. The cause of the increased cTnI in this group is uncertain. cTnI has been shown to increase in many non-cardiac conditions in both human and veterinary patients. Anaemia **[Lalor et al. 2014]**, azotaemia **[Porciello et al. 2008]**, cardiac contusion **[Kirbach et al. 2000],** non-cardiac causes of dyspnoea **[Herndon et al. 2008, Connolly et al. 2009a and Wells et al. 2014]** and hyperthyroidism **[Connolly et al. 2005 and Sangster et al. 2014]** have been shown to cause increased troponin I in cats. Exercise **[McKenzie et al. 2007 and Tharwat et al. 2013]**, snake envenomation **[Segev et al. 2008, Pelander et al. 2010 and Langhorn et al. 2014a]**, chemotherapy **[Christiansen et al. 2002, Defrancesco et al. 2002 and Selting et al. 2004]**, vector-borne diseases as Ehrlichiosis **[Diniz et al. 2008 and Koutinas et al. 2012]**, Babesiosis **[Lobetti et al. 2002]**, Dirofilariasis **[Carretón et al. 2011, 2012 and 2013]** and Chagas disease **[Barr et al. 2005]**, Leptospirosis **[Mastrorilli et al. 2007]** gastric volvulus and dilatation **[Schober et al. 2002 and Burgener et al. 2006]**, pyometra **[Hagman et al. 2007 and Pelander et al. 2008]** have all been shown to result in increased cTnI concentrations in dogs. cTnI is not a specific clinical marker; it indicates the presence of myocardial injury but does not define the underlying cause. Of the four cats with systemic disease that

had elevated cTnI in this study, two had respiratory disease and two were presented with non–cardiogenic causes of collapse. In these cases elevation of cTnI may have been caused by hypoxia.

The regression analysis failed to show a significant relationship between cTnI and age, sex or type of cardiac disease. This supports the findings of Borgeat et al. (2014), as no correlation was found between age and cTnI in that study. However, some studies found a significant interaction between age and cTnI levels in dogs **[Oyama and Sisson, 2004 and Ljungwall et al. 2010]**. They theorized about the aged heart and myocyte loss in the absence of cardiac disease. These findings, may suggest a difference between cats and dogs and needs further investigation.

The retrospective nature of the current study resulted in a number of limitations. Animals that were euthanized were categorized as non-survivors. Cats could have been misclassified regarding their cause of death (cardiac, non-cardiac) as many of them died at home or were euthanized and concurrent diseases cannot be ruled out completely. Haematology and biochemistry were not available for every patient, thus anaemic and azotaemic cats could have been inadvertently included. The final number of patients included in the study was small reducing the power of the statistical calculations. In an ideal scenario age-matched controls should have been recruited for the survival studies. Finally, animals included in the systemic disease group did not undergo echocardiographic examination and therefore subclinical cardiac disease could not be ruled out.

In summary, this study has shown that increased cTnI is associated with long-term mortality in cats of any breed with a wide variety of cardiac diseases, not only HCM. Despite the fact that higher troponin values are associated with shorter survival times, decisions on euthanasia should not be made based on cTnI results alone as a significant overlap exist between survivors and non-survivors. Clinicians should consider cTnI as an additional tool in their routine diagnostic investigations, but always being aware of the limitations of this biomarker and the numerous non-cardiac diseases that can cause elevation.

CHAPTER 4

Prospective study in cats

APP and cardiac biomarkers in feline cardiac and non-cardiac diseases.

4.1 Aims and objectives

- To determine the concentration of four different APP in a well characterized population of cats.
- To assess the usefulness of APP to distinguish cats with cardiac, hyperthyroid or non-cardiac systemic diseases.
- To investigate the elevation of cardiac biomarkers in cats with non-cardiac diseases.
- To analyze the relationship between cardiac biomarkers and markers of inflammation in cats with a wide variety of diseases.
- To identify possible confounding factors for both cardiac biomarkers and markers of inflammation in cats.

4.2 Material and methods

The present prospective study protocol was approved by the Research Ethics Committee of the School of Veterinary Medicine, University of Glasgow. Every animal included in the present study received humane care with strict ethical and professional standards. Cats were eligible for inclusion if they were admitted to the Small Animal Hospital from January to June 2014 and collecting blood was part of the clinical investigation of their primary clinical condition. Full echocardiographic examination was intended in all cats, however in animals with non-cardiac conditions only brief echocardiography was done as time limitations were established by the Ethic and Welfare Committee. Informed owner consent to participate was obtained for all cats. Individuals were assigned to four different categories depending on their final diagnosis: cats with cardiac disease, cats with hyperthyroidism, cats with systemic diseases other than cardiac or hyperthyroidism and cats with comorbidities (animals with combinations of diseases included in the previous categories). Furthermore, cats with cardiac disease were further classified depending on the presence or abscence of CHF. CHF was diagnosed combining clinical signs, echocardiography, abdominal ultrasonography and thoracic radiographs (pleural effusion, pulmonary edema and peritoneal effusion). No healthy animals were recruited for the present study.

4.2.1. ANIMALS

a) GROUP I. CATS WITH CARDIAC DISEASE

Cats were eligible for inclusion if evidence of cardiac disease was present on physical examination and confirmed on ultrasonography. The presence of arrhythmias, gallop

sounds and murmurs or combinations of these were considered valid inclusion criteria. All the individuals were scanned by experienced echocardiographers, confirming the presence of structural cardiac disease.

Hypertension, hyperthyroidism (defined as thyroxine higher than 50 nmol/L) or evidence of concurrent diseases were criteria of exclusion.

b) GROUP II. CATS WITH SYSTEMIC DISEASE

Cats diagnosed with systemic non-cardiac diseases were included in this group. Animals with concurrent hyperthyroidism (thyroxine levels >50nmol/L) or cardiac disease were excluded. However, animals were included in this group if abnormalities were found on cardiac auscultation but echocardiographic findings were normal. Cats with abnormal thoracic auscultation that were not scanned were excluded, as significant underlying cardiac disease could not be ruled out.

c) GROUP III. CATS WITH HYPERTHYROIDISM

Cats with serum thyroxine levels above 50 nmol/L were assigned to this group. Both untreated cats and those undergoing treatment for hyperthyroidism were included in this category. The presence of a concurrent systemic disease was a criterion of exclusion.

d) GROUP IV. CATS WITH COMOBIRDITIES

This group included cats with heart disease or hyperthyroidism and simultaneous extracardiac disorders. The diagnosis of hyperthyroidism was made if cats had thyroxine levels above 50 mol/L. Animals were considered to have underlying cardiac conditions if they had abnormalities on cardiac auscultation and abnormal echocardiographic findings. Animals with systemic disease and abnormalities on cardiac auscultation, which were not scanned, were included in this category.

4.2.2. STUDY PROTOCOL

a) Sample collection and storage

Blood was obtained by venipuncture and placed in plain serum tubes. Blood was allowed to clot for 15-20 minutes at room temperature, centrifuged and then serum was carefully separated and stored at -20 degrees C until the moment of analysis. Samples were aliquoted into 4 vials and were stored for a maximum of 6 months before analysis. When possible, 100 µL were used for SAA and Hp measurements, 50 µl for AGP, 50 µL for CRP at Acute Phase Protein Laboratory (Glasgow University,

UK) and 300 µl were sent on dry ice to IDEXX Laboratories (West Yorkshire, UK) for hscTnI and NT-pro-BNP analysis immediately.

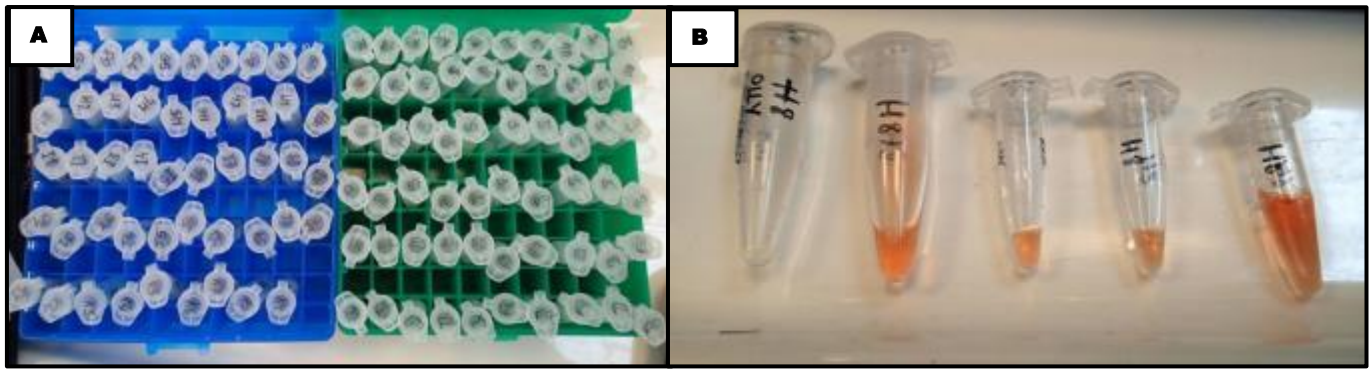

FIGURE 4.1. Transport and aliquoting serum samples. A, Polypropylene storage boxes with 109 serum samples were sent on ice to Reactiv Lab for analysis. B, Serum samples were divided in 4 aliquots to measure 6 different biomarkers of inflammation (SAA, Hp, AGP and CRP) and myocardial damage (cTnI and NTproBNP).

b) Echocardiography

A full echocardiographic study was performed in all cats apart from those animals with non-cardiac conditions in which echocardiography was not part of the investigations for their primary conditions. In these cats (group II – patients with systemic disease), only a brief echocardiographic study was done as time limitations were established by the Ethics and Welfare Committee. Echocardiographic examinations were performed in non-sedated cats in right lateral recumbency. The examination included two dimensional, M-mode and Color-Doppler images. Animals which underwent brief echos were considered to have structural cardiac disease if the ventricular septum and/or left ventricular free wall measured at the end of diastole was greater than 6 mm, left atrium - aorta ratio was greater than 1.5, abnormalities were observed on Color Doppler and/or systolic function was reduced.

c) **Biomarker analysis**

c.1. SAA

SAA was determined using an Automated Latex Agglutination Turbidimetric Immunoassay (Eiken SAA TIA, MAST®). This turbidimetric immunoassay was developed for use with human serum and employs anti-human SAA rabbit and mouse monoclonal antibodies. However, it has been previously validated in canine, feline and equine serum with acceptable reliability **[Christensen et al. 2012].** This assay range is 5-500 µg/ml and normal reference value is less than 8µg/ml.

c.2. Hp

Hp was determined by measuring the peroxidase activity of haptoglobin/haemoglobin complex (Hp-Hb).The reaction mixture is prepared with haemoglobin derived from sheep. The level of peroxidase activity of Hp-Hb is determined spectrophotometrically **[Eckersall,2002].** The test has been previously used in cats with urinary tract obstruction **[Schmidt et al. 2015].** Normal reference range for cats is 0.27-3.79 g/L and the detection limit of the assay is 0.05 g/L.

c.3. AGP:

Agar gel Immuno-diffusion was the test of choice for AGP determinations. Sheep anti-AGP antibodies were incorporated into melted 1% agarose gel. The detection limit of the assay is 200 µg/ml and concentrations above 500 µg/ml were considered significant.

c.4.CRP

Quantitative determination of CRP was performed using a highly sensitive two-site enzyme-linked immunoassay (Cat CRP ELISA®, Kamiya Biomedical Company). The company has previously validated the test in cats and it has been also employed to assess the acute phase response in cats receiving interferon-ω therapy **[Leal et al. 2014].** However, this kit is available for research use only.

The lower detection limit of the assay is 0.0 mg/ml and the reference range for feline patients is 0.1-0.2 mg/ml.

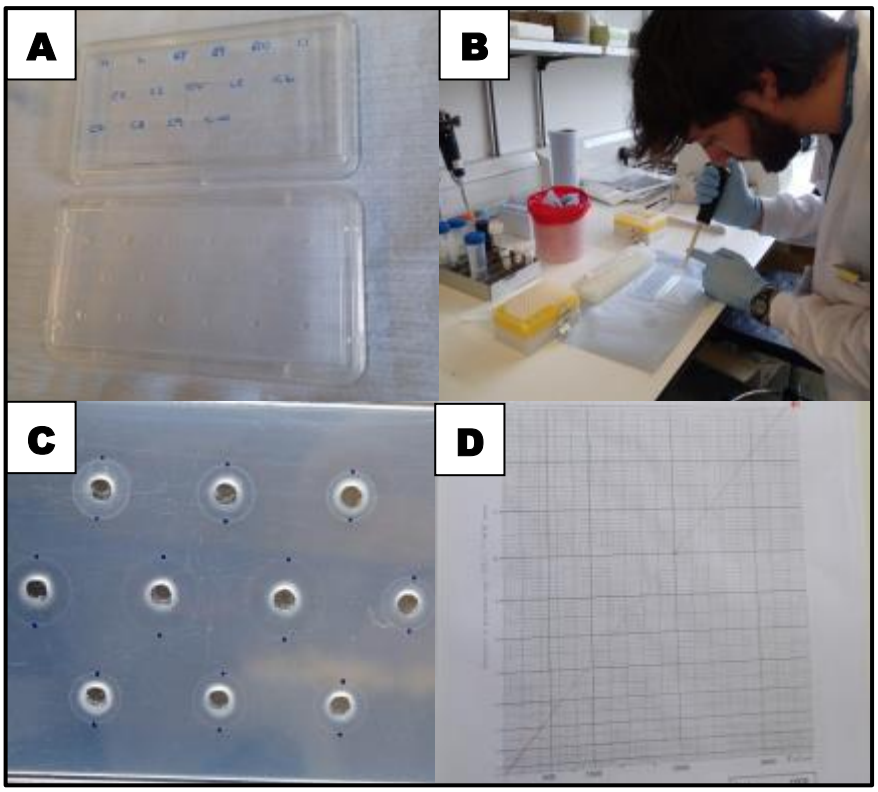

FIGURE 4.2. Immuno-diffusion test for AGP determination. A, Sheep anti-AGP antibodies were fixed in 1% agarose gel. The cover which protects the plate against external contamination was marked to identify the position of the samples. B, Serum samples were added into wells with a micropipette. C, After 20 hours of incubation, white precipitin rings were visible around the wells at the equivalence zone. D, Standard curve.

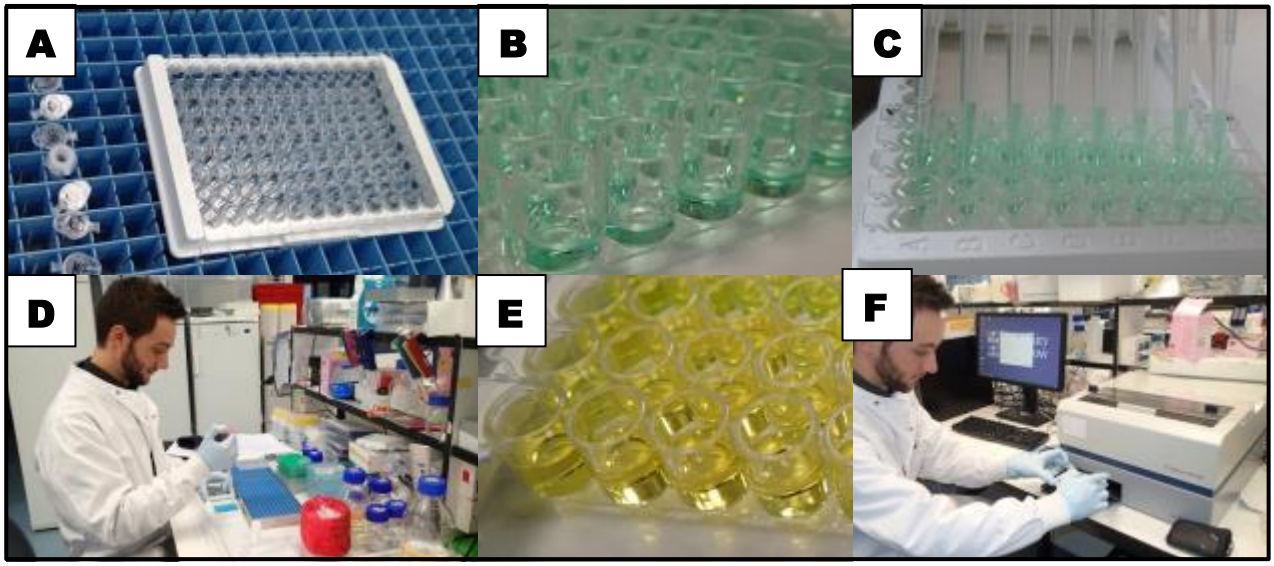

FIGURE 4.3. Double antibody sandwich ELISA test for CRP determination. A, Microtitre plates are coated with anti-CRP. B, 1:8000 dilution of the sample is added to each well. C, Unbound proteins are removed by washing. D, Addition of antibodies conjugated with HRP. E, After washing and removing the unbound conjugated antibodies, Tetramethylbenzidine was pipetted into each well .F, Spectrophotometer.

c.5. HscTnI

HscTnI was tested using a chemiluminescent immunoassay (Access AccuTnI®, Beckman Coulter, Inc). This cTnI test employs paramagnetic particles as solid phase. Materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. The detection antibody is conjugated with alkaline phosphatase and when its chemiluminescent substrate is added, light is produced. The light is measured by spectrophotometry. The detection limit of the assay is <0.01 ng/ml and the normal reference range for cats is <0.04 ng/ml.

c.6. NT-proBNP

A two-site enzyme-linked immunoassay (Feline Cardiopet Plus ELISA®, IDEXX) was the test of choice in the present study. This sandwich assay method incorporates sheep antibodies and one monoclonal antibody specific for feline NT-proBNP.

The lower detection limit of the assay is 24 pmol/L whereas the higher is 1500 pmol/L. The algorithm supplied by IDEXX for interpretation of measured NT-proBNP in cats states that in those animal whose NT-proBNP concentrations are lower than 100 pmol/L, clinically significant cardiomyopathy is highly unlikely. For those whose NT-proBNP levels are between 100-270 pmol/L, clinically significant cardiomyopathy is unlikely but early disease may be present. Finally, in animals with concentrations higher than 270 pmol/L, clinically significant cardiomyopathy is highly likely.

4.2.3. STATISTICAL ANALYSIS

Statistical analysis was performed using commercially available software (Minitab, version 17.1.0). Variables were tested for normality and 2-sample T-tests were employed to compare normally distributed data whilst Mann-Whitney U-tests were used for non-normally distributed variables. The data were graphically assessed with box-plots and median and ranges were used to provide descriptive statistics. Univariable and multivariable regression analysis were performed to identify variables (e.g. age, breed, anaemia, azotemia or type of disease) that were potential confounders of biomarker concentrations. Finally, ROC curves were plotted to assess the usefulness of biomarkers as predictors of CHF and determine optimal cut-off points. Statistical significance was defined as $P < 0.05$.

Biomarker	*Method*	*Detection limit*	*Reference range*	*Laboratory*
SAA	Automated Latex Agglutination Turbidimetric Immunoassay (Eiken SAA TIA, MAST®)	5-500 µg/ml	<8 µg/ml	Acute Phase Laboratory, University of Glasgow, UK
Hp	Haemoglobin Binding Assay	0.05 g/L	0.27-3.79 g/L	
CRP	Two-site enzyme linked immunoassay (CAT CRP ELISA®, Kamiya Biomedical Company)	0.0 mg/ml	0.1-0.2 mg/ml	
AGP	Agar Gel Immunodiffusion	200 µg/ml	<500 µg/ml	Virology Laboratory, Veterinary Diagnostic Services, University of Glasgow
hscTnI	Chemiluminescent immunoassay (Access AccuTnI®, Beckman Coulter, Inc)	0.01 ng/ml	<0.04 ng/ml	IDEXX Laboratories (West Yorkshire)
NT-proBNP	Two-site enzyme-linked immunoassay (Feline Cardiopet Plus ELISA®, IDEXX)	24 -1500 pmol/L	<100 pmol/L	

TABLE 4.1.Methods of analysis of cardiac and inflammatory biomarkers used in this study.

4.3 Results

4.3.1. STUDY POPULATION

In total 109 serum samples were collected, but nine were repeat samples and excluded from further analysis. In addition, the serum of one cat that was receiving Oxyglobin at the moment of sampling was also discarded. This product may produce alterations in chemistry values determined by colorimetric methods **[Adamantos et al. 2005]**. In consequence, 99 subjects were finally included in this prospective study.

Of the 99 cats, 54 were neutered males, 31 neutered females, 10 intact males and four intact females. Regarding breeds recruited, 76 were Domestic Shorthair (DSH), six Ragdoll, three Siamese, three Bengal, two Maine Coon, two Domestic Longhair

and one each of Burmese, Birman, Persian, Russian Blue, Tonkinese, Korat and British Shorthair. The median age of this population was 9.7 years (range: 5 months - 19 years) and the median weight was 4.3 kg (range: 1.40 - 7.20 Kg). Biomarker results in the study population are summarized in Table 4.2.

	hscTnI *(ng/ml)*	***NT-proBNP*** *(pmol/L)*	***SAA*** *(µg/ml)*	***Hp*** *(g/L)*	***AGP*** *(µg/ml)*	***CRP*** *(mg/ml)*
n	97/99	97/99	95/99	96/99	96/99	86/99
Median	0.07	115	5	1.06	560	0.19
Range	0.01-4.96	24-1500	5-240	0.05-20.03	200-2420	0.02-0.48
Undetectable	8	12	62	0	2	0
Non-elevated	26	34	2	87	39	52
Elevated (n)	63	51	31	9	55	34
Elevated (%)	65%	53%	33%	9%	57%	40%

TABLE 4.2. Panel of cardiac and inflammatory biomarkers in the study population. It includes the number of animals which were tested for each substance (n), median concentration of the biomarker in the population (Median), the range of concentration, number of cats with biomarker concentrations below the detection limit (Undetectable), those with biomarker concentrations within the reference range (non-elevated) and number (n) and proportion (%) of cats with increased biomarker concentrations.

a) GROUP I. Cats with cardiac disease

After six months of recruitment, 22 cats met the inclusion criteria for this category. Group I included 10 neutered males, six neutered females, five intact males and one intact female. Sixteen of these were DSH and there was one cat each of Maine Coon, Ragdoll, Russian Blue, Bengal, Burmese and Domestic Longhair. The median age was 8.5 years (range: 7 months - 17 years) and median weight was 4.6 Kg (range: 2.6 - 6.9 Kg). The most common finding on physical examination was an audible murmur (13/22), generally grade III/VI or louder. Sporadic premature beats were detected in one cat and isolated gallop sounds in another. Combinations of a murmur and gallop sounds were detected in two animals whilst the combination of a murmur and arrhythmia was audible in one cat only. Arrhythmias were shown to be VPCs and SPVCs on electrocardiographic recordings, respectively. No abnormalities on cardiac auscultation were recorded in four cats. However, these four patients were presented in significant respiratory distress secondary to CHF, confirmed by transthoracic ultrasonography.

All the individuals had structural cardiac disease confirmed by echocardiography. The most prevalent disease was HCM (11/21), followed by UCM (6/21) and RCM

(1/21). Two animals presented with congenital cardiac disease (VSD and mitral valve dysplasia) and one showed dynamic left ventricular outflow tract obstruction. One cat admitted to the Out of Hours Service underwent incomplete echocardiographic examination, a dilated left atrium and pleural effusion was confirmed on echo however a definitive diagnosis was not made.

CHF was defined as presence of pleural/pericardial effusion seen on echocardiographic examination and/or presence of pulmonary oedema or pleural effusion on thoracic radiographs. Following these criteria, 11 cats were in CHF in the present study. Biomarker results in group I are summarized in Table 4.3.

a.1. Influence of congestive heart failure

Animals in CHF had significantly higher concentration of hscTnI (P=0.0086), NT-proBNP (P=0.0001) and AGP (P=0.002) than cats with stable cardiac diseases. However, no differences in SAA (P=0.0554), Hp (P=0.5114) or CRP (P=0.08) concentrations were found between these two categories.

Median hscTnI concentration in cats without CHF was 0.15 ng/ml (range: 0.02 - 0.74 ng/ml) whereas from cats in CHF it was 0.43 ng/ml (range: 0.12 - 3.82 ng/ml). Median NT-proBNP was 320 pmol/L in cats with no CHF (range: 24 - 1095 pmol/L) whereas cats in CHF had a median NT-proBNP concentration of 1434 pmol/L (range: 594 - 1500 pmol/L). One cat had NT-proBNP levels below the detection limit of the assay and two had concentrations within the reference range. These three individuals were all included in the no-failure category. All the animals in CHF had NT-proBNP higher than 270 pmol/L (reference value for clinically significant cardiomyopathy given by IDEXX Laboratories) and seven cats with fully compensated cardiac disease had NT-proBNP above this reference value. Finally, cats in CHF had a median AGP concentration of 780 µg/ml (range: 360 - 1680) whereas it was 320 µg/ml (range: 220 - 840 µg/ml) for cats with no CHF. Median SAA concentration in cats with CHF was 29.20 µg/ml whereas in cats with compensated cardiac disease it was <5 µg/ml. Six cats in failure had elevated SAA concentrations whereas only 2 compensated cats had SAA above the reference range. However, there was no statistically significant difference between these two groups (P=0.0554).

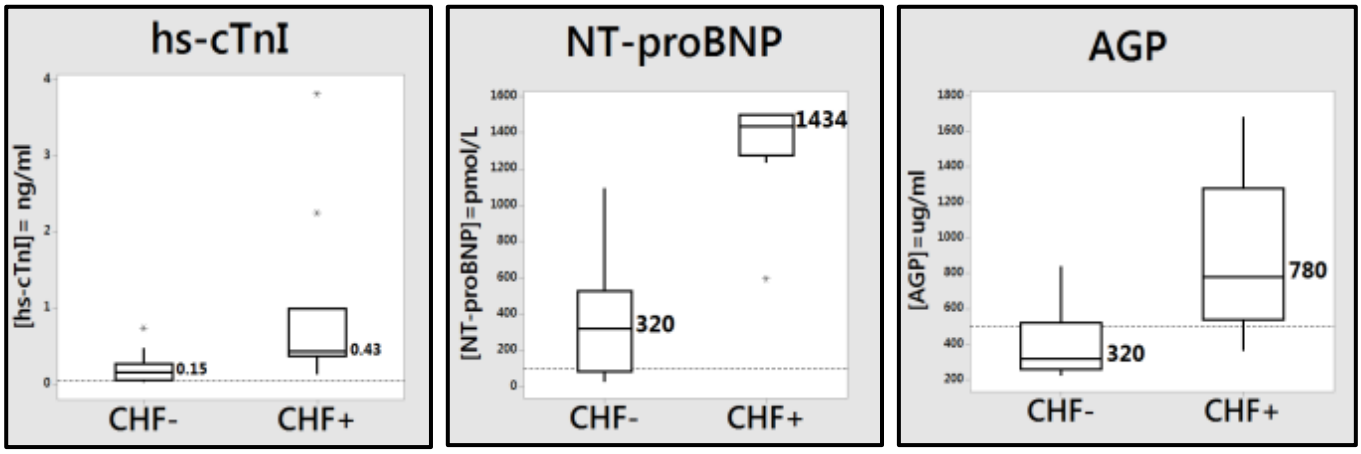

FIGURE 4.4. Boxplots illustrating hscTnI, NT-proBNP and AGP concentrations in cats with cardiac disease. Cats in CHF had significantly* higher hscTnI, NT-proBNP and AGP concentrations in comparison to cats with compensated cardiac disease.

The performance of hscTnI, NT-proBNP and AGP as predictors of CHF in cats was examined with ROC curves. The greatest AUC and test with the best predictive ability for CHF in feline patients was NT-pro BNP. The AUC for this marker was 0.98, revealing an excellent behavior of NT-proBNP to diagnose CHF in cats. The AUC for hscTnI and AGP were 0.84 and 0.89 respectively.

An NT-proBNP concentration higher than 1234 pmol/L was the optimal cut off point for prediction of CHF in cats with cardiac disease, with a sensitivity of 90.91% and a specificity of 100%. Employing this cut off point, 95.45% of cats in CHF included in group I would have been correctly diagnosed.

The logistic regression analysis found no benefit in combining these three biomarkers (as a “panel of cardiac biomarkers”) to predict CHF in cats with primary cardiac disease.

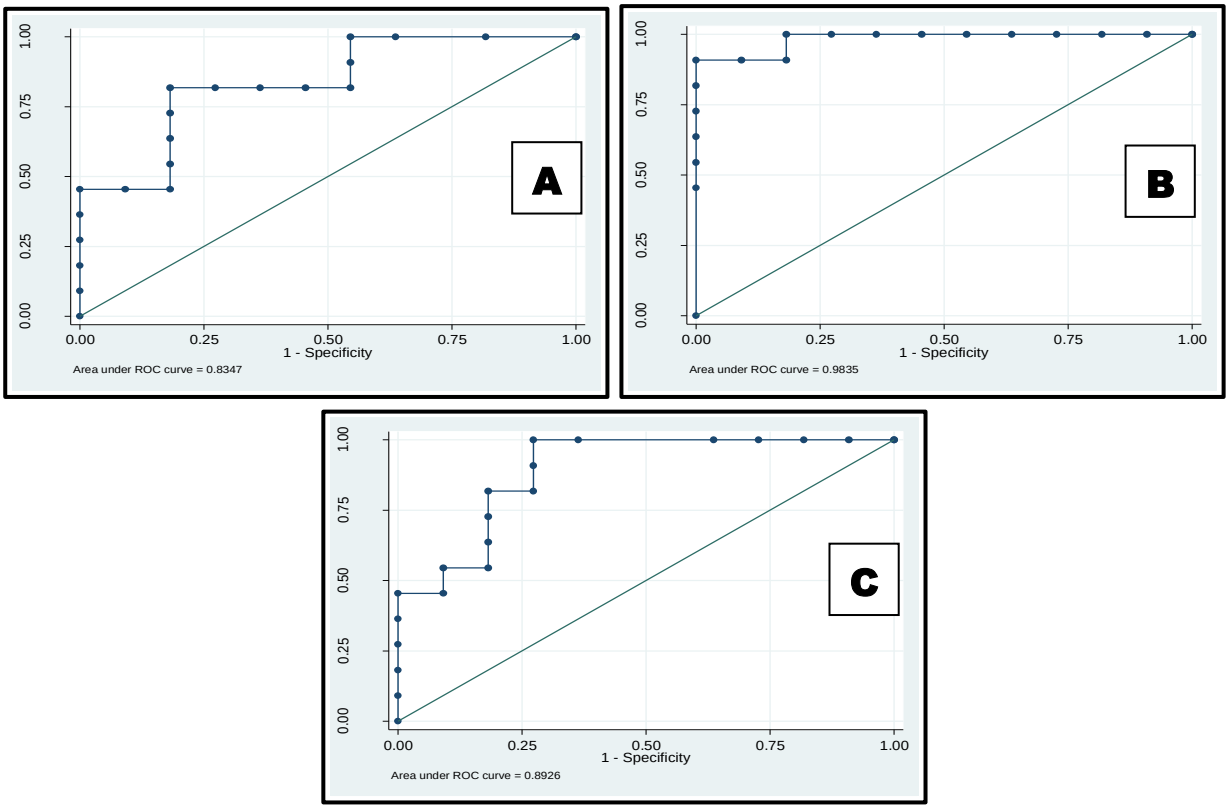

FIGURE 4.5 . ROC curves for NT-proBNP, hscTnI and AGP as predictors of CHF in cats with cardiac disease. A, ROC curve for hscTnI, this test had an AUC of 0.8347. B, ROC curve for NT-proBNP, its AUC was 0.9835. C, ROC curve for AGP whose AUC was 0.8926.

b) GROUP II. CATS WITH SYSTEMIC DISEASES

There were 43 cats that met the requirements for inclusion in this group. Twenty three of these cats were neutered males, 15 were neutered females, 4 intact males and 1 intact female. This group included 32 DSH, two Ragdolls, two Siamese and there was one cat each of Bengal, Birman, British Shorthair, Domestic Longhair, Korat, Persian and Tonkinese. The median weight of this group of cats was 3.73 Kg (range: 1.4 - 7.2 kg) and the median age was 9 years (range: 5 months - 19 years).

Brief echocardiographic examinations were performed on 35 of these patients (81%) and no structural abnormalities were identified. Five of the 35 scanned cats had soft murmurs (grade II/VI or less) on auscultation. These five cats had normal echocardiographic studies and three of them were anaemic. Eight cats from group II did not undergo echocardiographic examination due to their fractious nature or poor health; all of these had normal cardiac auscultations. Biomarker concentrations obtained from cats included in group II are summarized in table 4.3.

		hscTnI *(ng/ml)*	*NT-proBNP* *(pmol/L)*	*SAA* *(µg/ml)*	*Hp* *(g/L)*	*AGP* *(µg/ml)*	*CRP* *(mg/ml)*
Cardiac	***n***	22/22	22/22	22/22	22/22	22/22	21/22
	Median	0.32	985	5	1.05	530	0.16
	Range	0.02-3.82	24-1500	5-191.30	0.76-10.94	220-1680	0.04-0.33
	Undetectable	0	1	13	0	0	0
	Non-elevated	2	2	1	21	10	15
	Elevated (n)	20	19	8	1	12	6
	Elevated (%)	95%	83%	38%	4%	54%	29%
Systemic	***n***	43/43	42/43	41/43	41/43	41/43	42/43
	Median	0.04	49.50	5	1.10	680	0.21
	Range	0.01-1.43	24-945	5-168.50	0.05-20.03	200-2420	0.09-0.40
	Undetectable	7	8	27	1	1	0
	Non-elevated	17	22	1	34	13	21
	Elevated (n)	19	12	13	6	27	21
	Elevated (%)	44%	29%	32%	15%	66%	50%
T4	***n***	10/11	10/11	9/11	10/11	10/11	11/11
	Median	0.18	458	5	0.99	380	0.17
	Range	0.04-0.29	24-1500	5-66.5	0.68-3	300-600	0.13-0.25
	Undetectable	0	1	6	0	0	0
	Non-elevated	1	0	0	10	7	9
	Elevated (n)	9	9	3	0	3	2
	Elevated (%)	90%	90%	33%	0%	30%	18%
Comorbidities	***n***	22/23	23/23	23/23	23/23	23/23	12/23
	Median	0.075	87	5	1.03	560	0.20
	Range	0.01-4.96	24-1097	5-123	0.54-14.80	200-2040	0.02-0.48
	Undetectable	1	2	16	0	1	0
	Non-elevated	6	10	0	21	9	7
	Elevated (n)	15	11	7	2	13	5
	Elevated (%)	68%	48%	30%	9%	57%	42%

TABLE 4.3. Panel of cardiac and inflammatory biomarkers in 4 groups of disease: cardiac, systemic, hyperthyroidism (T4) and comorbidities. n, animals which were tested for each substance; Median, median concentration of the biomarker in the population; Range, range of concentration; Undetectable, number of cats with biomarker concentrations below the detection limit; Non-elevated, those with normal biomarker concentrations /within the reference range; Elevated (n) number and proportion (%) of cats with increased biomarker concentrations.

c) GROUP III. Cats with hyperthyroidism

After six months of recruitment, eleven hyperthyroid cats were included in the present study. Nine of these eleven patients were neutered male, one was a neutered female and the other one was an intact female. All recruited hyperthyroid cats were DSH. The median age of group III was 13 years (range: 6 - 18 years) and median weight was 4.13 kg (range:2.5-5.5 kg). Of the eleven cats, thoracic auscultation abnormalities were detected in six individuals. Four cats had audible murmurs and 2 had gallop sounds. Echocardiographic examination was performed on nine hyperthyroid cats and abnormalities were detected in five of them. Three cats were in CHF. Biomarker concentrations from hyperthyroid cats are included in table 4.3.

d) GROUP IV. Cats with comorbidities

Group IV population comprised 23 cats with two or more concurrent conditions included in the previous groups hence, cardiac, systemic or hyperthyroid diseases (e.g HCM and inflammatory bowel disease or hyperthyroidism and mediastinal neoplasia). Of these, twelve were neutered males, nine neutered females and one intact female and one intact male. There were 17 DSH, three Ragdolls, one Siamese, one Bengal and one Maine Coon which fullfilled the inclusion criteria. The median age of the group was 8 years (range: 10 months to 17 years) and median weight was 4.1 kg (range: 2 - 7 kg). Nineteen of the twenty-three cats had abnormal thoracic auscultations. Murmurs were audible in fourteen cats, gallops sounds were detected in one cat, three individuals had an audible arrhythmia and one cat had a murmur and gallop sounds combined. Electrocardiography showed VPCs in three cases. Nineteen cats underwent echocardiographic examination. The four cats that were not scanned had abnormal cardiac auscultation and the following concurrent conditions: pulmonary neoplasia, gastric lymphoma and hepatopathy (n=2). As these cats had systemic diseases but the absence of concurrent cardiac disease could not be ruled out, they were included in group IV instead of group II. Of the nineteen cats that were scanned, 16 had some kind of abnormality on their echocardiographic studies, with compensated HCM the most common finding. Two cats had pleural effusion and one of these also had a small volume of pericardial effusion. The first was a two-year old, male neutered ragdoll with biatrial enlargement whose final diagnosis was feline infectious peritonitis (Feline Coronavirus antibody titre greater

than 1280, hyperproteinemia, hyperglobulinemia, anaemia and abnormal appearance of the right kidney) and concurrent hypertrophic cardiomyopathy and CHF. The second case was a cat finally diagnosed with feline systemic reactive angioendotheliomatosis after a post-mortem examination, as vascular proliferative lesions were present in the myocardium, liver and kidneys. The echocardiographic examination of this patient revealed normal atrial size, good systolic function, mild pericardial effusion, moderate pleural effusion and hypertrophy of the left ventricular free wall and interventricular septum. The results of this group are compiled in table 4.3.

4.3.2. COMPARISON BETWEEN 4 STUDY GROUPS

Cats with cardiac disease had significantly higher hscTnI and NT-proBNP concentrations than cats with systemic diseases ($P<0.001$, $P<0.001$) and comorbidities ($P=0.010$, $P<0.001$). No differences regarding cardiac biomarker levels were found between cats with cardiac disease and hyperthyroidism ($P=0.13$; $P=0.30$). Hyperthyroid cats had significantly higher NT-proBNP concentrations than cats with systemic disease ($P<0.001$) and comorbidities ($P=0.03$). Hyperthyroid cats had significantly higher hscTnI concentrations than cats with systemic diseases ($P=0.02$) but not when compared with those with comorbidities ($P=0.25$). Finally, cats with comorbidities showed significantly higher hscTnI and NT-proBNP levels than cats with systemic disease ($P= 0.04$; $P =0.03$).

Regarding the APP results, no significant differences in either SAA or Hp concentrations were detected between the four groups of disease. Cats with systemic diseases had significantly higher AGP and CRP concentrations than cats with hyperthyroidism ($P=0.008$; $P=0.01$) but no differences were identified when compared with cats with cardiac diseases ($P=0.14$; $P =0.08$) or those with comorbidities ($P=0.34$; $P=0.55$).

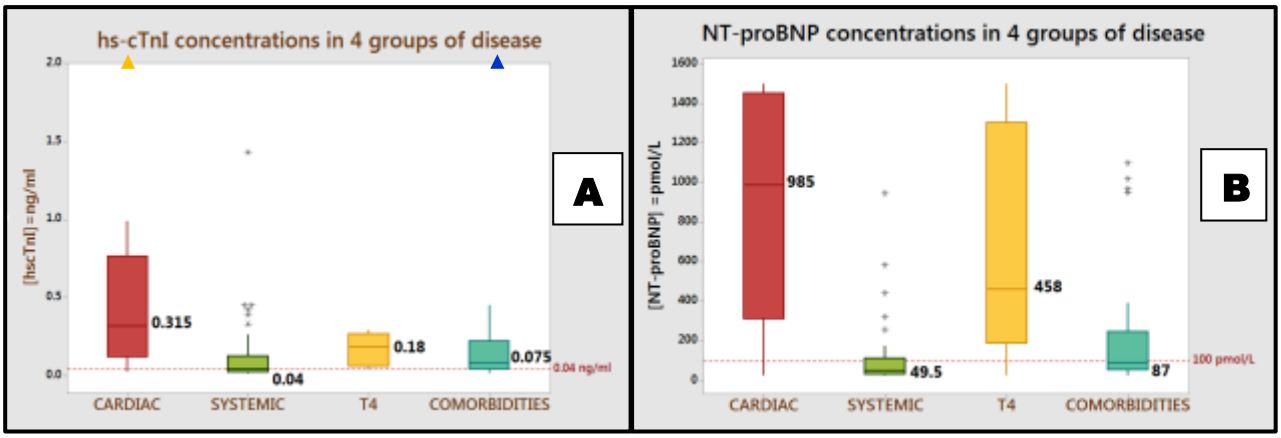

FIGURE 4.6. Boxplot of hscTnI (A) and NTproBNP (B) concentrations in the 4 study groups: cardiac, systemic, hyperthyroid and comorbidities. ▲ 2 values above 2ng/ml ; ▲ 1 value above 2 ng/ml.

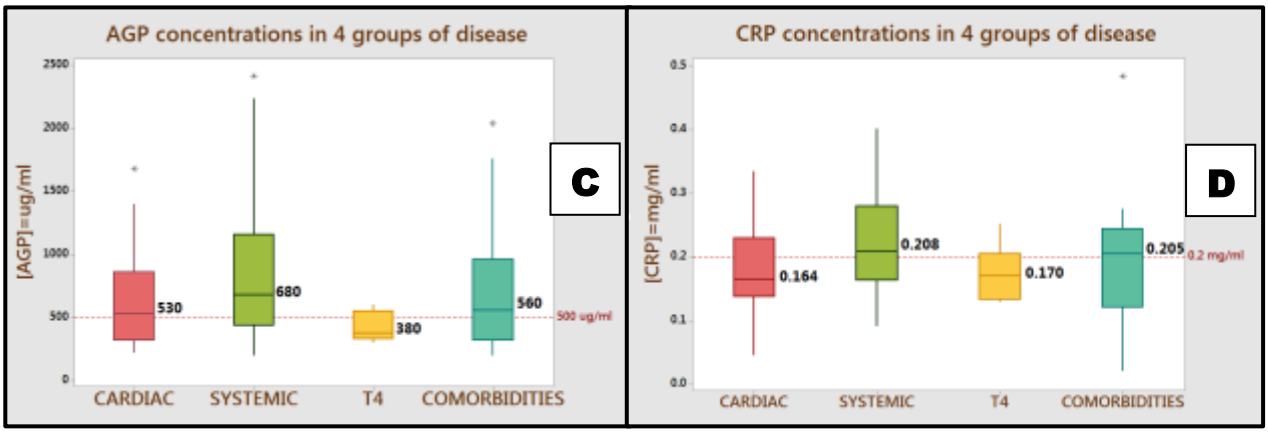

FIGURE 4.7. Boxplot of AGP(C) and CRP (D) concentrations in the 4 study groups : cardiac, systemic, hyperthyroid and comorbidities.

4.3.3. IDENTIFYING POSSIBLE CONFOUNDING FACTORS

Regression analysis was performed to identify potential associations between biomarker concentrations and characteristic of the population of cats included in this study. Biomarker concentration was adjusted for age, gender, breed, weight, absolute haematocrit value, anaemia (haematocrit <30%), albumin concentration, absolute creatinine concentration, azotemia (creatinine >180 µmol/L) and treatment (antibiotics and/ or anti-inflammatory drugs).

Univariable regression analysis provided evidence of dependence of hscTnI and NT-proBNP on circulating creatinine concentration (P=0.012; P=0.002) and the presence of azotemia (P=0.005; P<0.001).

Creatinine was measured in 90 out of 99 cats in the present study. Median creatinine concentration in the population was 132.5 µmol/L, 75 patients had non-elevated

levels and 15 were azotaemic (>180 µmol/L). Increased hscTnI concentrations were found in 63 cats of the total population and 51 individuals had increased NT-proBNP values. There were 19 cats with systemic disease with increased hscTnI concentrations. Of these, 6 cats had normal plasma creatinine concentrations, haematocrit and echocardiographic examination. On the other hand, there were 12 patients with systemic disease that had increased NT-proBNP. Four of these, had normal creatinine, haematocrit and echocardiographic examinations.

SAA concentrations were associated with body weight (P=0.03), haematocrit (P=0.004) and the presence of anaemia (P=0.03). However, after adjusting for weight, anaemia and haematocrit using multivariate analysis the associations were no longer statistically significant (P=0.063). There was an inverse relationship between SAA and haematocrit. AGP concentrations were associated with weight (P=0.005), albumin levels (0.008), haematocrit (P<0.001) and anaemia (P<0.001). When AGP concentrations were adjusted for haematocrit, weight and albumin levels using multivariable analysis, the association between AGP and albumin was no longer significant (P=0.44). Similarly to SAA, one could expect higher AGP concentrations in individuals with lower haematocrit values.

Haematocrit values were available from 72 cats out of the 99 included in the study. The median haematocrit was 34.4%. There were 22 cats with haematocrit values below 30% whereas 50 were above this reference range. SAA was increased in 31 cats whereas AGP was elevated in 55.

No associations were found between Hp or CRP with the variables age, weight, gender, anaemia, haematocrit, albumin, creatinine, azotaemia or treatment.

4.4. Discussion

4.4.1. APP

Each animal species has its own major APP that must be considered the marker of choice for diagnostic purposes **[Paltrinieri, 2008]**. While CRP and SAA are considered major APP in the dog, SAA and AGP are thought to play a major role in the APR in the cat. However, data regarding APP levels in cats are scarce and mostly focused on general aspects of feline APP biology **[Ceron et al. 2005; Paltrinieri, 2008]**.

The concentrations of four positive acute phase reactants were determined in this well-characterized population of 99 cats. AGP was the most frequently increased acute phase protein in the study population as 57% of the patients had elevated concentrations. Interestingly, CRP was the second most commonly elevated, being above the reference range in 40% of the patients whereas SAA was increased in 33% and Hp in 9% of the cats.

Most of the studies on feline AGP have focused on infectious diseases, with particular interest in FIP. Many studies illustrated that AGP is not exclusive or pathognomonic for FIP as cats with FIV or exposed to FCoV but asymptomatic had increases in AGP concentrations **[Duthie et al. 1997; Giordano et al. 2004; Paltrinieri et al. 2007a, Paltrinieri et al. 2007b].** Indeed, cats with lymphoma **[Correa et al. 2001]**, tumor bearing cats **[Selting et al. 2000]**, cats with anaemia secondary to abscesses, pyothorax and fat necrosis **[Ottenjann et al. 2006]**, cats infected with Chlamydiae **[TerWee et al. 1998]**, and cats with a wide variety of conditions **[Kann et al. 2012]** have been shown to have increased AGP concentrations.

One of the most interesting findings of the present study is the possible usefulness of AGP as a predictor of CHF in cats with cardiac conditions. Cats with CHF had significantly higher concentrations of hscTnI, NT-proBNP and AGP. In addition, APP concentrations in cats with cardiac disease were not significantly different compared to those obtained from cats with systemic diseases. ROC curves revealed that AGP is as good as hscTnI and NT-proBNP as a predictor of CHF in cats with cardiac conditions. However combining these three biomarkers did not provide additional information.

Importantly, mean AGP was not significantly different in cats with systemic disease and those with cardiac disease. Given that AGP was significantly higher in cats with cardiac disease and CHF when compared with cats with stable cardiac disease, hence concurrent systemic disease in the former cannot be completely rule out. However, these patients underwent several physical examinations and clinical tests, making this scenario unlikely.

There is a growing body of evidence in both human and veterinary medicine to suggest that inflammation plays an important role in the aetiopathogenesis of CHF ("The cytokine hypothesis of CHF"). It is thought that CHF progresses, at least in part, as a result of the local and systemic effects exerted by these cytokines **[Araujo**

et al. 2009]. CRP is elevated in human patients with CHF [Pye et al. 1990; Huang et al. 2004] and it has been proven to predict short and long-term CHF development in patients with stable coronary heart disease and patients with myocardial infarction [Berton et al. 2003; Suleiman et al. 2003; Sabatine et al. 2007; Williams et al. 2008]. CRP has been also associated with a significant improvement in the prediction of adverse events in cardiovascular disease, beyond that observed with conventional risk factors [Kaptoge et al. 2012]. Controversial data have been published regarding CRP concentrations in dogs with cardiac disease and CHF. One study showed that dogs in CHF had significantly higher CRP concentrations compared to the healthy controls [Cunningham et al. 2012] whereas there was no association with CHF in three other studies [Rush et al. 2006; Ljungvall et al. 2010; Cunningham et al. 2013]. To the authors' knowledge, this is the first study to evidence increased AGP concentrations in cats with cardiac disease in CHF.

AGP has been shown to be present in several extra-hepatic tissues including the myocardium and active synthesis has been proven in endothelial cells [Siegel et al. 1985; Sörensson et al. 1999]. This study was not designed to find causal relationships and further investigations will be required to clarify the association between CHF and increased AGP concentrations in cats.

Notably, SAA was undetectable in a significant number of patients despite being considered a major APP in the cat. There were 62 out of 95 cats with SAA concentrations below the reference range (<5µg/ml). Two large studies on SAA concentrations in cats have been published. The first compared SAA concentrations in healthy cats (n=45) and cats with a wide variety of conditions (n=312). SAA concentrations from controls were significantly lower in comparison with those obtained from diseased cats. However, among different disease groups, those cats with enteritis (n=9), oral disorders (n=14) and liver disorders (n=21) had SAA concentrations not significantly different than controls [Sasaki et al. 2003]. The second study examined a population of 175 cats. Of these 110 had normal SAA concentrations whereas 65 (37%) had increased SAA concentrations [Tamamoto et al. 2013]. Similarly, in the present study only 33% of the subjects had increased SAA concentrations. No differences between cats with cardiac disease, hyperthyroidism, comorbidities or systemic disease in terms of SAA concentrations were found. Thus, SAA might be a useful marker for some, but not for all feline inflammatory

conditions. Furthermore, SAA is believed to be one of the APPs most rapidly responsive in the cat **[Eckersall and Bell, 2010]**. Major proteins are often observed to increase markedly within the first 48 hours after the triggering event and often have a rapid decline due to their short half-life. Moderate and minor proteins increase more slowly and may be observed more often during chronic inflammatory processes **[Cray et al. 2009]**. Hence, these results might mirror the presence of chronic conditions or animals with treated (well-controlled) diseases in our study population.

Multiple regression analysis demonstrated an inverse relationship between AGP, SAA and haematocrit. Increased SAA values have been described in cats with experimentally induced feline infectious anaemia **[Korman et al. 2012]** and increased AGP concentrations have been described in cats with anaemia secondary to inflammatory diseases (abscesses, pyothorax and fat necrosis) **[Ottenjann et al. 2006]**. In view of these results, hematology analysis should be performed when interpreting SAA and AGP measurements.

CRP is considered a major acute phase protein in dogs, humans and non-human primates while it is thought to be an insensitive protein in the feline acute phase reaction **[Kajikawa et al. 1999; Ceron et al. 2005; Cray et al. 2009; Eckersall and Bell 2010; Jain et al. 2011]**. As a consequence, data regarding CRP concentrations are scarce and it has not been well studied in the cat. Conflicting results have been published regarding the role of CRP in the feline APR. One study revealed that, in contrast to the considerable elevations of SAA, Hp and AGP after turpentine oil administration or urinary surgical procedures only small increases were observed in CRP concentrations in cats **[Kajikawa et al. 1999]**. Another study assessed the usefulness of interferon-ω therapy in cats naturally infected with retrovirus. In that study, CRP behaved similarly to SAA and AGP, increasing significantly after 65 days of treatment **[Leal et al. 2014]**. CRP was the second most commonly increased APP in the present study. Group II (systemic disease) and Group IV (comorbidities) had median CRP concentrations slightly above the reference range. Thus, CRP may also have a value as a biomarker in some conditions and further investigations are required to determine its role in feline inflammation.

Hp is considered a minor APP in the cat. Increased Hp concentrations have been described in cats with anaemia secondary to inflammatory diseases **[Ottenjann et**

al. 2006], cats which were given subcutaneous turpentine oil or LPS and cats that underwent urinary surgery [Kajikawa et al. 1999], cats with FIP and FIV infections [Duthie et al. 1997; Giordano et al. 2004] and cats with a wide variety of unspecified conditions [Kann et al. 2012]. In contrast to previous publications, Hp was only elevated in 9 out of 96 cats in the present prospective study. Regression analysis did not show any association between anaemia and Hp. Thus the usefulness of Hp as an inflammatory biomarker in feline medical conditions seems to be less promising than expected.

4.4.2. Cardiac biomarkers

The two cardiac biomarkers behaved similarly. ROC curve analysis revealed that both markers were useful to distinguish cats with CHF from the group of cats with compensated cardiac disease although NT-proBNP was superior. Indeed, NT-proBNP has been shown to provide a reliable means of discriminating cats with CHF from those with primary respiratory causes of dyspnea in several studies [Collins, 2013; Wess et al. 2008; Fox et al. 2009; Connolly et al. 2009b] whereas cTnI seems to be unsuitable for the same purpose [Herndon et al. 2008; Connolly et al. 2009a; Wells et al. 2014]. Nevertheless, recent publications have demonstrated the potential of cTnI as a prognostic biomarker in cats with cardiomyopathy [Langhorn et al. 2014b; Borgeat et al. 2014]. Even more, cTnI has been proven to be superior over NT-proBNP as a prognostic marker in cats with HCM [Borgeat et al. 2014]. Clinicians should then know that these biomarkers seem to offer different information; while NT-proBNP is better as a CHF predictor, cTnI appears to be superior at predicting poor outcomes in cats with cardiac disease.

Hyperthyroid cats had increased NT-proBNP and hscTnI concentrations in the present study. These findings were predictable, as cardiac biomarkers have been previously demonstrated to be elevated in cats with hyperthyroidism [Connolly et al. 2005; Menaut et al. 2012; Sangster et al. 2014]. Our prospective study not only supports these findings but also confirms that cardiac and hyperthyroid cats cannot be distinguished from their APP concentrations.

One of the strengths of this prospective study is that most of the animals underwent echocardiographic examination to prevent misclassification of the patients with subclinical cardiac disease. Heart scans were performed in 84 out of 99 cats, including 35 out of 43 cats with systemic diseases. Despite this, many cats in group

II had increased hscTnI (44%) and NT-proBNP (29%) concentrations. Cardiac biomarker elevations are not uncommon in patients with conditions other than primary cardiac diseases. In cats, cTnI has been proven to be elevated in hyperthyroidism **[Connolly et al. 2005; Sangster et al. 2014]**, azotemia **[Porciello et al. 2008]**, anaemia **[Lalor et al. 2014],** blunt chest trauma **[Kirbach et al. 2000],** and non-cardiogenic causes of dyspnea **[Herndon et al. 2008; Connolly et al. 2009a; Wells et al. 2014].** On the other hand, NT-proBNP has been demonstrated to be elevated in cats with hyperthyroidism **[Menaut et al. 2012; Sangster et al. 2014]** and hypertension **[Lalor et al. 2009]**. The latter identified a significant positive correlation between NT-proANP and plasma creatinine concentration but this correlation was not present with NT-proBNP. In contrast, the presence of azotemia was a significant confounding factor in the evaluation of NT-proBNP in the present study. In fact, an NT-proBNP and plasma creatinine association has also previously been described in human and canine patients **[McCullough and Sandberg, 2003; Luchner et al. 2005; Boswood et al. 2008; Schmidt et al. 2009]** No associations between these markers and anaemia were found in the present study.

LIMITATIONS

An important limitation of this study was the storage of the samples. Serum samples were kept at -20°C for a maximum of 6 months. Recently, a 4-month stability study was carried out revealing that cTnI recovery decreased below acceptable limits (down to 72%) when samples were stored for 4 months at -20°C **[Langhorn et al. 2014b]**. Previous studies have shown apparently normal NTproBNP bioreactivity in feline samples stored for up to ten years at -80°C however, slight but significant reductions have been observed in human samples stored at -20°C for two years **[Cauliez et al. 2008; Lalor et al. 2009]**. This could have resulted in a minor degradation of cardiac biomarkers in the samples. Secondly, biochemistry and hematology were not available for every cat included in this study. Thirdly, many recruited cats were already on treatment (antibiotic or anti-inflammatory drugs) at the moment of sampling and APP are known to fluctuate in response to medication **[Tamamoto et al. 2009].** Fourthly, samples were collected from cats with a wide variety of conditions and no controls were recruited. This may have an influence on interpretation as it has been shown that magnitude of increase of some APP differs

between illnesses [Sasaki et al. 2003]. Fifthly, echocardiography was not performed in all cats.

CONCLUSION

In conclusion, in this well characterized population of cats the most commonly increased APP was AGP followed by CRP, SAA and Hp, respectively. This is opposite to the recommendation of measuring SAA and Hp as inflammatory panel in cats. Further investigations, including these 4 APP, are required to understand their real role in the feline APR.

APPs were shown to be unsuitable to distinguish between cats with cardiac, hyperthyroid and non-cardiac diseases.

AGP was as good as NT-proBNP or hscTnI to distinguish between cats with cardiac disease with and without CHF. This possible role of AGP as a marker of CHF has not been described before and further research should be done.

NT-proBNP and hscTnI concentrations were significantly higher in cats with cardiac disease and hyperthyroidism than in cats with systemic disease. However, both cardiac biomarkers were elevated in a substantial number of cats with non-cardiac non-hyperthyroid systemic diseases.

No correlation was found between cardiac and inflammatory markers making the theory of myocardial damage/troponin release induced by inflammation less likely in patients with non-cardiac diseases.

A positive association was detected between cardiac biomarkers and plasma creatinine concentrations and between anaemia and SAA and AGP concentration. Haematology and biochemistry analysis are required in order to interpret biomarker results properly.

FINAL CONCLUSIONS AND FUTURE RESEARCH

The number of publications studying cTnI in small animal veterinary medicine has significantly increased in the last decade. Several factors may influence cTnI release and measurement. In consequence, many conflicting results have been reported challenging the ability of clinicians to interpret the test.

Two essential recommendations to read cTnI results are :

It is imperative to consider cTnI as a non-specific biomarker of myocardial injury. An elevation of cTnI indicates the presence of myocardial injury but does not define the underlying cause. cTnI is a highly specific biochemical marker (we are certain about its cardiac origin) but non-specific clinical marker (we are uncertain the cause). Elevations are not uncommon in veterinary patients with conditions other than primary cardiac disease. Therefore, raised cardiac troponin alone will never allow practitioners to make a clinical diagnosis. Full blood work and cardiac examination should be done in patients with elevated cTnI.

Secondly, the lack of standardization of cTnI assay systems has meant an inability to compare absolute values between methods. Reference ranges and cut-off values obtained in previous studies with different analyzers are not interchangeable and should not be used in practice to make clinical decisions. Practitioners should use reference ranges given by their laboratory.

Results of this study confirm that high cTnI concentrations are associated with shorter survival times in dogs and cats with cardiac disease. Animals with higher cTnI concentrations had shorter survival times. However, cTnI was superior to predict cardiac death in cats than in dogs. Several factors may have affected these results. There was a wide clinical and morphologic heterogeneity of canine cardiac diseases included in the study. Pathophysiological mechanisms may vary notably between cardiomyopathies, valvular, congenital and/or pericardial diseases and this could directly impact on the magnitude and velocity of cTnI release. In contrast, the feline population was more homogenous as most of the cats suffer from cardiomyopathy. Furthermore, a high incidence of arrhythmias was present in the canine population. The role of arrhythmias in cTnI release remains unknown. Significantly higher cTnI levels were found in those dogs with more severe arrhythmias but some of them experienced fairly good outcomes. The author hypothesizes that the presence of an arrhythmia (especially tachyarrhythmias) may cause a significant transient increase

of cTnI that would cease once the rhythm is controlled. Therefore, the author believes the prognostic value of cTnI in dogs which present with arrhythmias should be interpreted with caution. Finally, due to the overlap of cTnI concentration between survivors and non-survivors in both canine and feline patients, clinical decisions should not be done relying merely on cTnI results.

Assessing the relationship between arrhythmias, cTnI and survival is an enthralling area of research. Comparative studies with serial cTnI measurements in dogs with well controlled arrhythmias versus animals with uncontrolled rhythm disturbances will be of particular interest.

Results of the prospective study demonstrated that AGP was the most commonly elevated APP in our well-characterized population of cats. AGP was followed by CRP, SAA and Hp concentrations respectively.

The feline APR is still uncharted territory, the information available is scarce and conflicting. CRP is considered a non-responsive protein in the APR in this species and measurement of SAA and Hp is recommended to assess inflammation in cats. Clarifying the real role of CRP, AGP, SAA and Hp in feline APR represents an intriguing line of future research.

A considerable number of cats with non-cardiac diseases had increased hscTnI and NT-proBNP. Cardiac biomarkers were associated with plasma creatinine levels. Nevertheless, there was still a group of patients with normal echo, normal haematocrit and non-azotaemic with increased cardiac markers. Several authors have proposed inflammation as a source of troponin release in dogs with non-cardiac diseases. In the present study, the relationship between cardiac and inflammatory markers did not reach statistical significance. However, in cats with cardiac disease AGP was significantly higher in animals in CHF and turned out to be as good predictor of CHF in cats as NT-proBNP and hscTnI. Thus, inflammation may be involved in the release of cardiac biomarkers in some but not all feline conditions. Further investigations regarding the usefulness of AGP as a predictor of CHF and cardiac death in cats would also be an attractive future work.

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