Cardiac biomarkers in hyperthyroid cats

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ABSTRACT

Background: Hyperthyroidism has substantial effects on the circulatory system. The cardiac biomarkers NT-proBNP and troponin I (cTNI) have proven useful in identifying cats with myocardial disease but have not been as extensively investigated in hyperthyroidism.

Hypothesis: Plasma NT-proBNP and cTNI concentrations are higher in cats with primary cardiac disease than in cats with hyperthyroidism and higher in cats with hyperthyroidism than in healthy control cats.

Animals: Twenty-three hyperthyroid cats, 19 cats with HCM without congestive heart failure, and 19 euthyroid, normotensive healthy cats eight years of age or older. Fourteen of the hyperthyroid cats were re-evaluated three months after administration of ^{131}I.

Methods: A complete history, physical examination, complete blood count, serum biochemistries, urinalysis, blood pressure measurement, serum T4 concentration, plasma concentrations of NT-proBNP and cTNI, and echocardiogram was prospectively obtained from each cat.

Results: Hyperthyroid and HCM cats had plasma NT-proBNP and cTNI concentrations that were significantly greater than healthy older cats, but there was no significant difference between hyperthyroid and HCM cats with respect to concentration of either biomarker. In hyperthyroid cats that were re-evaluated three months after ^{131}I treatment, plasma NT-proBNP and cTNI concentrations as well as ventricular wall thickness decreased.

Conclusions and Clinical Relevance: Although there may be a role for NT-proBNP in monitoring the cardiac response to treatment of hyperthyroidism, neither NT-proBNP nor cTNI can be used to distinguish hyperthyroid cats from cats with HCM. Therefore, the thyroid status of older cats should be ascertained prior to interpreting results of cardiac biomarker testing.

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And last, but not least, this thesis is dedicated to Lump.

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ANP	atrial natriuretic peptide
BNP	B-type natriuretic peptide
cTNI	cardiac troponin I
FS	fractional shortening
HCM	hypertrophic cardiomyopathy
IVSd	thickness of interventricular septum during diastole
LA/Ao	ratio of left atrial to aortic diameter
LVPWd	thickness of left ventricular posterior wall during diastole
MHC	myosin heavy chain
NT-proBNP	NT-pro-B-type natriuretic peptide
RAAS	renin-angiotensin-aldosterone system
SERCA	sarco-endoplasmic calcium ATPase
T_3	3,5,3'-triiodothyronine
TSH	thyroid stimulating hormone

CHAPTER 1: LITERATURE REVIEW

A. Hyperthyroidism

Hyperthyroidism was first described in cats in the veterinary literature in 1980.[1] Since that time, it has become the most commonly diagnosed feline endocrinopathy, with a 2% prevalence across all ages of cats[2] in a referral population. Increased awareness of the condition and increased longevity of cats have likely contributed to this increase in frequency of diagnosis; however, hyperthyroidism is also thought to be a truly "new" disease which has emerged in recent decades.[3]

Functional adenomatous hyperplasia of both thyroid lobes accounts for approximately 70% of cases of feline hyperthyroidism. More rarely, the condition can be unilateral; fewer than 2% of cases are attributable to thyroid carcinoma.[4] Feline hyperthyroidism resembles toxic nodular goiter of humans in its histologic appearance.[5] This human condition can be caused by iodine deficiency[6] or exposure to goitrogens;[7] the underlying cause of feline hyperthyroidism is unknown. Multiple studies have identified consumption of a diet that consists of >50% canned food as a risk factor. Flame retardants, dust, certain compositions of canned food, litter box use, and exposure to flea and fly control products have also been discussed.[2,8-14] Speculation about the role of dietary iodine in feline hyperthyroidism exists[15] but a link has not been documented. Subclinical hyperthyroidism (normal free T4 with low TSH) may precede overt hyperthyroidism in cats.[16]

Other researchers have compared feline hyperthyroidism to Graves' disease in humans. In Graves' disease, circulating antibodies bind to and stimulate TSH receptors, leading to thyroid hormone production and secretion. However, multiple studies have failed to demonstrate specific thyroid stimulating immunoglobulins in hyperthyroid cats,[17-19] making a feline equivalent of Graves' disease unlikely.

Hyperthyroidism is seen almost exclusively in middle-aged to older cats.[20] Physiologic effects of elevated thyroid hormone concentrations lead to the common presenting signs of weight loss despite a good appetite, unkempt haircoat, and muscle weakness. Restlessness, vomiting and/or diarrhea, and polyuria and polydipsia are other common signs.[21] A palpable thyroid gland is present in most hyperthyroid cats.

Feline hyperthyroidism is a reversible condition. Options for treatment include thyroidectomy, administration of radioactive iodine, low-iodine diet, or long-term oral anti-thyroid medication. Methimazole, the most commonly used anti-thyroid medication in North America, acts by inhibition of thyroid peroxidase and thus prevents synthesis of thyroid hormones.[22] Side effects have been reported in up to 18% of cats treated with methimazole, including vomiting, blood dyscrasias, facial excoriations, and hepatotoxicity.[23] Radioactive iodine is concentrated in the hyperfunctional thyroid tissue and irradiates and destroys this tissue. Most cats are rendered euthyroid by a single treatment; adverse effects are rare but can include permanent hypothyroidism.[24] Euthyroidism can also unmask previously existing renal disease. Thyroidectomy is also curative; however, hyperthyroid cats can be poor candidates for general anesthesia. Potential complications of thyroidectomy include death, hypoparathyroidism, and laryngeal nerve damage.[25] Although only preliminary data collected by the manufacturer have been published to date, it appears that a new low-iodine feline diet is effective in establishing euthyroidism in up to 90% of cases if fed to hyperthyroid cats as their only food source.

B. Effects of thyroid hormones on the heart

Thyroid hormones alter cardiovascular function both directly, through effects on the heart, and also indirectly, through effects on the peripheral vasculature. Myocardial contractility and relaxation is enhanced by the genomic actions of 3,5,3'-triiodothyronine (T_3); specifically, T_3 increases the expression of genes that encode ion transporters, beta-1 adrenergic receptors, and contractile proteins. These effects increase both calcium release and reuptake from the sarcoplasmic reticulum. [26-28] The resultant increase in cytosolic calcium increases contractility, and the more rapid calcium reuptake enhances diastolic relaxation. T_3 increases the density of cardiac beta-1 adrenergic receptors in the dog,[29,30] pig, and rat.[28] Although not evaluated in the cat, it is likely that T_3 has a similar effect in this species. Cardiac myocyte contractility is determined by the velocity of myofibril movement that is in part influenced by the ATPase activity of myosin. Although T_3 increases expression of the high ATPase activity myosin heavy chain (MHC)α isoenzyme, only the MHCβ isoenzyme has been found in the myocardium of dogs and

cats, and therefore, increased expression of this gene probably plays an insignificant role in thyroid hormone effects on the myocardium of these species.[31]

Nongenomic effects of T_3 on cardiac myocytes include stimulation of sarco-endoplasmic calcium ATPase (SERCA) activity, an increase in the activity of the Na^+/K^+ pump, recruitment of slowly inactivating membrane sodium channels,[28] and an increase in membrane permeability to sodium ions.[32] In hyperthyroidism, these effects may enhance myocardial function but potentially predispose to cardiac arrhythmias.[33]

The direct cardiac effects of thyroid hormone excess include enhanced contractility and diastolic function that contribute to an increase in stroke volume. Cardiac output is further increased by positive chronotropic effects that result from heightened responsiveness to sympathetic stimulation conferred by an increase in the number of β_1-adrenergic receptors, enhanced rate of spontaneous depolarization of sinoatrial node cells[34] and reduced influence of the parasympathetic nervous system.[35-37]

In contrast, hypothyroidism has direct negative inotropic effects due to a shift from the predominant myosin isoenzyme to the low ATPase MHCβ[38] in some species, although this is likely of limited importance in the dog and cat. Diastolic relaxation is prolonged due to lower activity of SERCA, and heart rate is slowed because numbers of β_1-adrenergic receptors are reduced.[28] Cardiac function can further be impaired by fibrosis and accumulation of mucopolysaccharides in the myocardial interstitium,[39,40] increasing cardiac mass and wall stiffness. Severe, experimentally induced hypothyroidism has also been shown to result in impaired myocardial blood flow due to a loss of arterioles as well as progressive systolic dysfunction.[41]

In addition to direct effects on cardiac function, thyroid hormones induce important changes in the peripheral circulation. T_3 has been shown to rapidly and directly cause relaxation of vascular smooth muscle cells,[42] leading to a decrease in systemic vascular resistance. Generation of nitric oxide by endothelial cells is enhanced by thyroid hormones, contributing to arteriolar vasodilation in hyperthyroidism.[43] In addition, thyroid hormones increase metabolic rate and oxygen demands of the peripheral tissues,[28] resulting in locally mediated vasodilation. The renin-angiotensin-aldosterone system (RAAS) is activated in response to the decrease in resistance, thereby increasing plasma volume through sodium retention.[44] Thyroid hormone also stimulates erythropoietin

secretion,[45] resulting in increased red blood cell mass. These changes combine to increase preload and decrease afterload, resulting in increased stroke volume and cardiac output in hyperthyroid individuals. In contrast, thyroid hormone deficiency increases peripheral vascular resistance and thus contributes to increased cardiac afterload.[46] It is unclear if feline hyperthyroidism induces the aforementioned changes, given that systolic, diastolic, and mean systemic arterial pressures are increased in cats with experimental hyperthyroidism of 2 weeks duration.[56]

Cardiovascular Effects of Hyperthyroidism:

The reduced systemic vascular resistance and increases in diastolic relaxation, contractility, heart rate, blood volume, and oxygen requirements of peripheral tissue in hyperthyroidism lead to a marked increase in cardiac output. The hemodynamic burden and altered myocardial energetics that result can lead to cardiac dysfunction. Important clinical manifestations of hyperthyroidism-induced cardiac dysfunction in veterinary patients include arrhythmias, intolerance of aggressive fluid therapy or stress, hypertension, and congestive heart failure.[36,45]

Clinical findings

The cardiovascular effects of hyperthyroidism are responsible for some of the most prevalent clinical findings of hyperthyroidism. Heart murmurs or gallop sounds are auscultated in 35-50% of affected cats, and tachycardia is found in a slightly higher proportion.[47] Cardiomegaly is noted radiographically in approximately 26-40% of hyperthyroid cats.[48-50] The prevalence of electrocardiographic abnormalities has decreased in more recent reports compared with descriptions in the early 1980s. In addition to sinus tachycardia that is detected in 34% of hyperthyroid cats, electrocardiographic abnormalities include increased R wave amplitude consistent with left ventricular enlargement in 8%, and left anterior fascicle block pattern or right bundle branch block each in 6-10% of cases. In addition, arrhythmias including atrial and ventricular premature contractions, and less frequently atrioventricular block, atrial tachycardia, and ventricular tachycardia may be identified.[48,51-53] Although increased R-wave amplitude is a relatively common finding in hyperthyroid cats, it correlates poorly with echocardiographic evidence of left ventricular enlargement.[54] Although common in hyperthyroid humans,[35] atrial fibrillation occurs rarely in cats with this disease. In

veterinary patients, arrhythmias seem to generally resolve after treatment of hyperthyroidism, but this has not been well documented.

Abnormal echocardiographic findings are common, albeit usually mild, in hyperthyroid cats.[55] The most common echocardiographic abnormality in hyperthyroid cats is hypertrophy of the left ventricular posterior wall which is identified in approximately 72% of affected cats.[56] Other echocardiographic abnormalities of hyperthyroidism include left atrial enlargement in 50%, increased left ventricular end diastolic diameter in 47%, septal hypertrophy in 40%, and increased fractional shortening in 22%.[49,56] Enhanced left ventricular systolic function with normal diastolic function has been documented using Doppler tissue imaging in hyperthyroid cats.[57] Eccentric hypertrophy, that is, an increase in myocardial mass associated with an increase in ventricular volume, is expected secondary to the increase in preload and decrease in systemic vascular resistance. However, as has been reported in some hyperthyroid humans, concentric hypertrophy also occurs, which possibly reflects an increase in systolic wall stress.[58,59] The influence of concurrent disease such as renal dysfunction or systemic hypertension on myocardial remodeling is unknown, but could contribute to development of concentric hypertrophy. It is unclear from investigations of hyperthyroid cats if eccentric or concentric hypertrophy predominates. This likely reflects the paucity of reports and the complex pathogenesis of myocardial changes, both direct and indirect, in hyperthyroidism. Hyperthyroidism is known to be an independent risk factor for left ventricular hypertrophy in humans.[60] Thyroid hormone excess leads to increases in end-diastolic left ventricular diameter and volume but the magnitude of hypertrophy is modest and rarely associated with wall thicknesses outside the normal range.[61] There is echocardiographic evidence to suggest that hyperthyroid-induced cardiac changes resolve with treatment in many cats, but may persist in others.[55,56] However, published echocardiographic data obtained after resolution of hyperthyroidism are limited, and factors that might affect resolution of cardiac changes have received little attention.

There is considerable overlap in echocardiographic findings between cats with hyperthyroidism-related concentric hypertrophy and those with hypertrophic cardiomyopathy.[54] In addition, hypertension can cause left ventricular posterior wall and/or septal hypertrophy, similar to changes induced by hyperthyroidism.[62] Although

the prevalence of asymmetric hypertrophy and perhaps systolic anterior motion of the mitral valve may be greater in patients with primary myocardial disease, echocardiography cannot reliably differentiate these conditions; therefore, thyroid function and blood pressure should be evaluated in middle-aged and older cats with cardiac abnormalities.

In early studies of feline hyperthyroidism, congestive heart failure was present in 12-20% of affected cats.[48,49,52,53] However, the prevalence of this complication has decreased over time to 2% as the disease is diagnosed earlier.[20,47] Cats in congestive heart failure show clinical signs typical of that condition, such as dyspnea, anorexia, cyanosis, and weak femoral pulses. Pleural effusion secondary to congestive heart failure can obscure the cardiac silhouette on thoracic radiographs. Pulmonary venous engorgement and a patchy interstitial or alveolar pattern resulting from pulmonary edema may also be seen. As is the case in humans, heart failure in hyperthyroid cats would be expected to resolve after control of the thyroid hormone excess.[63,64] However, the response to treatment of heart failure in hyperthyroid cats with concurrent primary myocardial disease is less certain.

Heart failure occurs in 6% of humans with hyperthyroidism, usually associated with the development of atrial fibrillation.[65] Left ventricular dilation and decreased ejection fraction is found in about 50% of hyperthyroid humans with congestive heart failure. In humans with hyperthyroidism and preserved left ventricular ejection fraction, congestive heart failure almost always resolves after a euthyroid state is established. Resolution of heart failure and left ventricular dysfunction after treatment of hyperthyroidism is less predictable in patients with reduced ejection fraction, although indices of systolic myocardial function return to normal in most cases.[66,67]

The cardiac phenotype of hyperthyroid cats with congestive heart failure has been incompletely described. Jacobs et al[68] reported four cases of congestive heart failure characterized by pleural effusions in hyperthyroid cats, 2 of which died due to congestive heart failure. The echocardiographic abnormalities of these cats primarily reflected severe ventricular dilation with evidence of systolic myocardial dysfunction, rather than the ventricular hyperkinesis observed in many cases of hyperthyroid heart disease. While the poor outcome of these cases may be unusual, this case series demonstrates that

hyperthyroidism can contribute to, or directly cause, severe heart disease. Other less detailed reports of hyperthyroid cats with heart failure include those with systolic dysfunction[56] as well as normal contractility.[54] Because the cases with systolic dysfunction were reported when dilated cardiomyopathy secondary to taurine deficiency was common, it is possible that some of these cats had concurrent hyperthyroidism and dilated cardiomyopathy. However, dilated cardiomyopathy has also been reported in humans secondary to thyrotoxicosis.[69] In the absence of underlying primary cardiac disease, hyperthyroid cats with congestive heart failure are expected to recover with antithyroid therapy in conjunction with standard management of the cardiovascular abnormalities. However, factors that predict survival have not been established in this species.

It is likely that tachycardia and other effects of hyperthyroidism are detrimental in patients with underlying primary cardiomyopathy and could cause decompensation of a previously subclinical condition. As 16% of apparently healthy cats were found to have subclinical cardiomyopathy in a recent study,[70] hyperthyroidism and primary cardiomyopathy would be expected to co-exist in a substantial number of patients. Because cardiac changes in hyperthyroid heart disease may be similar to those resulting from primary hypertrophic cardiomyopathy, it is not currently known how frequently hyperthyroidism complicates preexisting heart disease. Serial echocardiograms after resolution of hyperthyroidism may be the most reliable method to determine if primary cardiomyopathy is present. However, diagnosis of primary cardiomyopathy prior to treatment of hyperthyroidism is problematic.

Hypertension is a common complication of hyperthyroidism in cats, although the prevalence varies considerably depending on the population studied.[71-76] The prevalence of hypertension in hyperthyroid cats is likely between 10 and 20%.[76] Blood pressure, vascular resistance, and cardiac output are related through Ohm's Law, and therefore systemic hypertension can result from an increase in vascular resistance, an increase in cardiac output, or from increases in both of these quantities. Thyroid hormone excess results in peripheral arteriolar vasodilation, inducing a decrease in systemic vascular resistance and activation of the RAAS with subsequent sodium and fluid retention. As expected, given this hemodynamic response, diastolic and mean arterial blood pressures

are decreased, while systolic pressure is increased in hyperthyroid humans. In contrast, systolic, diastolic, and mean systemic arterial pressures were all elevated in cats with experimentally-induced hyperthyroidism of two week duration;[77] these findings suggest an increase, rather than a decrease in vascular resistance but it is unclear if similar changes are present in cats with naturally-occurring hyperthyroidism. Hypertension likely results from the marked increase in cardiac output combined with expanded plasma volume due to activation of the RAAS and expanded red cell mass due to stimulation of erythropoietin release.[78,79] However, the role of the RAAS in in cats with hypertension associated with hyperthyroidism is unclear.[80] Hypertension has been shown to persist in at least the first four weeks of treatment with methimazole despite normalization of thyroid hormone concentrations[81] and some cats that are normotensive prior to treatment may become hypertensive when euthyroid.[71] Concurrent renal disease may play a role in the latter finding; approximately 15% of cats become azotemic after treatment of hyperthyroidism[76] as previously subclinical renal insufficiency is unmasked. However, in one study,[75] only 35% of cats that became hypertensive after treatment for hyperthyroidism were azotemic, so renal disease is likely not the sole cause of post-treatment hypertension. For these reasons, blood pressure should be measured at the time of diagnosis of hyperthyroidism and after the disease has been controlled.

The prognosis of hyperthyroid cats with congestive heart failure that do not have concurrent primary myocardial disease is probably good. However, some cats with hyperthyroidism and congestive heart failure respond poorly to treatment.[68] It is unclear if these cats have concurrent primary myocardial disease that has been exacerbated by hyperthyroidism or if the hyperthyroid-induced heart disease is irreversible for unknown reasons. Some hyperthyroid cats that have responded poorly to treatment for congestive heart failure had impaired contractility that was identified echocardiographically, but the significance of this finding is unclear.[39] Mere cardiac structural changes, tachycardia, and arrhythmias have not been shown to increase risk of death.[50,82]

C. Physiology of Biomarkers

A biomarker is a substance which can be objectively measured and serves as an indicator of normal biologic processes, pathologic processes, or pharmacologic responses

to a therapeutic intervention.[83] Potential clinical applications of biomarkers include early detection of subclinical disease states, diagnostic assessment of acute or chronic disease syndromes, risk stratification of patients, selection of appropriate therapeutic interventions, and monitoring response to therapy.[84] Cardiovascular disease is an area of research in which interest in biomarkers has grown exponentially in recent years.

The biomarker that has shown the most promise in providing general diagnostic and prognostic information regarding heart failure in human patients is NT-pro-B-type natriuretic peptide (NT-pro-BNP). Natriuretic peptides are a group of hormones which are synthesized by cardiomyocytes and contribute to the regulation of body fluid homeostasis and blood pressure.[85] The natriuretic peptide group is composed of at least six hormones,[85] but the best studied are atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP).

Although extracardiac tissues such as the brain, lungs, kidney, and adrenal glands have been found to express the BNP gene, only the heart has been shown to secrete BNP into circulation.[85,86] The human BNP gene codes for a 134 amino acid pre-pro hormone (132 amino acids in the cat)[87] which is rapidly processed to form the pro hormone proBNP by removal of a signal peptide. The proteolytic enzymes corin (a transmembrane protein found in the myocardium) and furin (expressed ubiquitously) cleave proBNP into the biologically inert NT-pro-BNP and the biologically active peptide BNP.[88,89] ProBNP is thought to be the predominant circulating form,[88] although studies of this question have been difficult since all current immunoassays for BNP also detect proBNP.[90] Since corin is present on the plasma membrane, it cleaves proBNP at the cell surface[90] resulting in co-secretion of NT-pro-BNP and BNP. Once in circulation, BNP is further truncated into a number of fragments with varying biologic activities.[91]

Physiologic actions of BNP are triggered via binding to natriuretic peptide receptor A (also known as GC-A)[92] on the surface of target cells, which increases the accumulation of intracellular cyclic GMP.[90] Natriuretic peptide receptors are located in diverse locations; vascular smooth muscle cells, endothelial cells, and medullary collecting duct cells have been specifically reported in addition to the adrenal gland and intestine.[93] In general, BNP acts to counteract the fluid-retaining and hypertensive actions of the renin-

angiotensin-aldosterone system. BNP induces a dose-dependent arterial relaxation[94] and strongly inhibits the vasoconstrictive effects of endothelin and vasopressin.[95] Systemic infusion of BNP has been demonstrated to increase glomerular filtration rate and induce natriuresis and diuresis.[94] BNP decreases cardiac output without causing systemic hypotension through exploitation of selective, sympathetic-independent vasoconstriction of the splanchnic circulation combined with sensitization of the heart to vagal input.[96] In the adrenal gland, BNP inhibits angiotensin-II induced steroidogenesis at multiple steps, including cholesterol biosynthesis, uptake, and transfer to the inner mitochondrial membrane.[97] In the colon, BNP upregulates expression and production of aquaporin 3 channels[98] and stimulates potassium- and bicarbonate-dependent chloride secretion.[99] The cumulative effects of most of these actions of BNP lead to fluid and sodium loss, counteracting the fluid- and sodium-retaining actions of the RAAS.

The effects of BNP on angiogenesis are not fully understood. BNP inhibits proliferation of vascular smooth muscle cells, apparently via inhibition of formation of intracellular reactive oxygen species.[100] However, BNP has also been demonstrated to stimulation proliferation, adhesion, and migration of endothelial progenitor cells.[101] Hypoxia induces extracardiac BNP expression[102] and may also modulate the pro- or anti-angiogenic effects of BNP. BNP-deficient mice are susceptible to cardiac fibrosis,[103] and BNP may act as a local paracrine factor modulating cellular proliferation and tissue remodeling.[92]

BNP is actively cleared from the circulation primarily through the actions of circulating neutral endopeptidases and by receptor-mediated binding and removal by the natriuretic peptide receptor-C; passive excretion also occurs in organs with generous blood flow, including the kidneys. Active clearance mechanisms do not seem to play a role in the breakdown of NT-pro-BNP, which is cleared passively by organ beds with large amounts of blood flow.[85] The kidneys appear to play an equal role in the clearance of NT-pro-BNP and BNP;[86,104] little is known about extrarenal clearance of NT-pro-BNP.

In healthy animals, BNP originates from storage granules subadjacent to the endocardium of the atria; plasma concentrations rapidly increase in response to sudden atrial wall stretch.[105] When BNP concentrations are consistently elevated, as in patients with chronic heart disease, the major site of BNP production switches from a localized to

a diffuse distribution within both the atria and ventricles.[106] Therefore, ventricular wall stress has a major influence on BNP concentrations in animals with heart disease.[107]

Heart failure is associated with activation of the sympathetic nervous system and renin-angiotensin-aldosterone axis, leading to sodium retention, systemic and renal vasoconstriction, and pathologic remodeling of the cardiac musculature.[108] The resulting volume expansion triggers natriuretic peptide secretion; ANP and BNP are released continuously from the heart, but the rate of the release is regulated by myocyte stretch.[93] The natriuretic peptides counteract the detrimental effects of chronic RAAS stimulation by promoting natriuresis, vasodilation, and diuresis.[109] Natriuretic peptides also suppress sympathetic outflow from the central nervous system, blunting the release of catecholamines at autonomic nerve endings[93] and inhibit cardiac fibrosis and remodeling.[110] However, the relationship of BNP and catecholamines is complex. While it was originally thought that natriuretic peptides decreased sympathetic cardiac stimulation,[96] it has more recently been demonstrated that in fact BNP promotes norepinephrine release from cardiac sympathetic nerves.[111] The resulting cardiac sympathetic overdrive detracts from the beneficial vascular effects of BNP in heart failure. These adrenergic effects of BNP are a possible reason that administration of a human recombinant BNP, nesiritide, failed to show a beneficial effect in patients with acute decompensated heart failure.[112] The increased sympathetic tone and altered calcium handling induced by chronically elevated BNP is pro-arrhythmogenic but can be ameliorated by administration of a β1-blocker.[113] BNP is also lipolytic and can contribute to excessive fatty acid mobilization in advanced heart failure.[114]

BNP and NT-proBNP are used in human medicine as effective markers for the diagnosis and prognosis of cardiac disease and failure. Although BNP is the biologically active form, NT-proBNP is clinically preferred as a biomarker due to its longer half-life (approximately two hours for NT-proBNP versus 22 minutes for BNP)[115] and stability in whole blood.[116] A recent consensus statement recommends evaluation of NT-proBNP concentrations for diagnosing and staging heart failure, making decisions regarding hospitalization or discharge, and identifying patients at risk for clinical events.[117] Even in asymptomatic individuals, elevated NT-proBNP concentrations are associated with increased risk of death, heart failure, atrial fibrillation, and transient ischemic attacks.[118]

In humans, NT-proBNP concentrations tend to be higher in females and increase significantly with age;[119] these results have not yet been demonstrated in a feline population.

In addition to cardiac disease, natriuretic peptide concentrations increase in septic shock, renal failure, and pulmonary hypertension. Several endocrine factors have also been demonstrated to stimulate natriuretic peptide release in vivo, including catecholamines, glucocorticoids, vasopressin, endothelin-1, and acetylcholine.[93] Endothelin, dobutamine, norepinephrine, epinephrine, and lipopolysaccharide induce BNP synthesis from isolated human atrial myocardiocytes in vitro without tissue stretch present,[120] likely explaining the increase in BNP in septic shock patients without evidence of myocardial dysfunction. Thyroid hormone concentration affects BNP in a myriad of ways, as discussed later.

Another biomarker of recent clinical and research interest is cardiac troponin I (cTNI). The troponin complex is made of three distinct proteins (I, T, and C); cardiac-specific isoforms exist for troponins I and T. The majority of cTNI is structurally bound in the contractile apparatus of skeletal and cardiac muscle and a small proportion is free in the cytosol. Cardiac troponin is released into the circulation upon damage to myocytes resulting in loss of membrane integrity but is not released when a myocyte undergoes apoptosis.[121] Cardiac troponins are detectable in serum within 3-4 hours of myocardial damage and remain detectable for up to 5 days.[122] cTNI has been recognized as the most sensitive and specific marker of myocardial cell necrosis in human patients[123] and normal concentrations are used to rule out acute myocardial infarction. cTNI is also elevated in humans with congestive heart failure; there is debate over whether these elevations represent irreversible necrosis of cardiomyocytes or transient reversible leakage secondary to myocyte stretch.[124] The specificity of cTNI for cardiac disease is not perfect; conditions such as end stage renal disease, acute respiratory disease, and some infectious diseases or toxins can also lead to mild to moderate increases in cTNI.[124] The exact mechanism of clearance of cTNI is unknown[125] but excretion through the kidney may be involved.[126]

D. Biomarkers in cats

Both NT-proBNP and cTNI have been studied in veterinary species. There is a lack of homology between human, canine, and feline BNP which precludes the use of human assays in veterinary medicine. However, the feline BNP gene was sequenced in 2002,[87] allowing the development of feline-specific NT-proBNP antibodies for assay analysis. In contrast, cTNI is highly conserved across species. The feline gene was sequenced in 2004 and found to be 92.4% homologous to the human gene.[127] Assays used to detect human cTNI have been validated in the cat.[128]

In humans, age and gender have been demonstrated to affect both cTNI[129] and NT-proBNP.[130] Age and gender were unrelated to NT-proBNP in a study of 114 normal cats and 113 cats with cardiomyopathy.[131] The effect of these variables on cTNI has not been evaluated. Obese humans with and without heart disease have lower NT-proBNP concentrations than their normal-weight counterparts.[132] To this point, no relationship of either biomarker with body condition score has been evaluated in cats, although body weight did not affect NT-proBNP.[131]

The majority of research in the cat has sought to define the usefulness of plasma NT-proBNP as a screening test for feline hypertrophic cardiomyopathy (HCM). In 2003, the first post-sequencing study of BNP in the cat demonstrated that expression of BNP was localized to the atria in cats free of cardiac disease but could be found diffusely throughout the ventricles of cats with HCM,[106] leading the authors to postulate that BNP could be used as a sensitive plasma marker of feline HCM. Further research into feline BNP was not published until 2008, when Connolly et al. demonstrated that plasma NT-proBNP could be used to differentiate healthy control cats from cats with heart disease (restrictive or hypertrophic cardiomyopathy) in the absence of heart failure.[133] A cutoff of 49 pmol/L demonstrated a sensitivity of 100% and a specificity of 89.3% for presence of cardiomyopathy. However, serum T4 was not measured in most of the cats in this study, and specific myocardial thicknesses of the cats in the heart disease group were not defined. Hsu et al.[134] followed this theme in 2009, finding that plasma NT-proBNP could differentiate Maine Coon cats with severe HCM (defined as a maximal left ventricular wall thickness of greater than 7mm) from Maine Coon cats with normal hearts or with equivocal (maximal wall thickness <6mm but papillary muscles subjectively enlarged) or moderate (maximal wall thickness 6-7mm) HCM. However, the normal, equivocal, and

moderate HCM groups were not significantly different from each other. Interestingly, Maine Coon cats with the A31P MYBPC mutation had significantly increased NT-proBNP when compared to cats without the mutation although their cardiac statuses were not different. The authors concluded that measurement of plasma NT-proBNP was inappropriate for use as a screening test for HCM, as many cats with moderate HCM would be falsely identified as being normal. Singh et al. were similarly unenthusiastic about the validity of plasma NT-proBNP as a screening test.[135] Using the same colony of Maine Coon cats as the Hsu study and an updated version of the NT-proBNP assay, this group also found that plasma NT-proBNP was significantly elevated in severe, but not in moderate or equivocal HCM. More recently, however, a multicenter study[131] in the United States with a diverse clinical population and studies based in Germany[136] and Japan[137] concluded that increased plasma NT-proBNP reliably discriminates normal cats from those with even mild HCM. In the Wess et al. study from Germany, a plasma NT-proBNP over 100 pmol/L demonstrated a sensitivity of 92.4% and a specificity of 93.9% for detection of HCM; this cutoff detected most cases of even mild HCM. Fox et al. proposed two cutoffs in the multicenter study: plasma NT-proBNP >46 pmol/L distinguished normal cats from those with occult cardiomyopathy with a sensitivity of 91.2% and a specificity of 85.8% whereas a cutoff of >100 pmol/L decreased sensitivity to 70.8% but increased specificity to 100%.

There are several possible reasons for these conflicting results. The Hsu and Singh studies were performed on a colony of Maine Coon cats, and this homogenous population may not represent the diverse population of cats seen in clinical practice. The interpretation of severity of HCM also varied between researchers. Subjective thickening of the papillary muscles without septal or ventricular wall thickening was interpreted as mild HCM in the Hsu and Singh studies, whereas Fox required end-diastolic thickness of the septum or left ventricular wall to exceed 6mm. Only 3/10 cats in the severe HCM group in the Hsu study had enlarged left atria while all 43 cats in the severe group in the Wess study had this abnormality. Although they found that plasma NT-proBNP was significantly higher in cats with enlarged left atria, the Fox group did not report the number of cats with this finding. In addition, not all of the cats investigated by the Fox group were screened for renal failure and/or hyperthyroidism. Although all of the cats in

the Fox study were asymptomatic, 44/61 of the cats with severe disease examined by Wess et al. were dyspneic secondary to congestive heart failure at the time of sampling. Further research is necessary to determine the clinical utility of NT-proBNP for detecting mild to moderate asymptomatic HCM; however, the larger numbers of cats evaluated by Fox and Wess and the diverse nature of their populations make it seem likely that their results will be more applicable to clinical practice.

In addition to screening for HCM, feline plasma NT-proBNP has been evaluated for other uses. Lalor et al.[138] demonstrated that plasma NT-proBNP concentrations are elevated in hypertensive cats with chronic kidney disease. Unfortunately, no hypertensive cats without kidney disease were evaluated, so it remains to be answered if hypertension leads to NT-proBNP elevations or if lack of clearance secondary to poor renal function is the culprit. Two studies[139,140] attempted to determine whether plasma NT-proBNP could distinguish between cardiac and non-cardiac etiologies in cats with respiratory distress. Both studies concluded that this test was indeed useful in this situation; one proposed an optimum cutoff of 220 pmol/L (with a sensitivity of 93.9% and a specificity of 87.8%) while the other suggested 265 pmol/L (with a sensitivity of 90.2% and specificity of 87.9%). The lack of a cageside test for NT-proBNP has to this point limited clinical interest in the test; however, preliminary results[141] indicate that a positive result on a new point of care ELISA correlates to a plasma NT-proBNP concentration >130 pmol/L and may increase its clinical utility.

ANP has also been briefly evaluated as a biomarker in feline cardiac disease, but appears to be less useful than BNP. A 2006 study found no significant difference between plasma NT-proANP concentrations in echocardiographically normal cats versus cats with subclinical HCM.[142] In a more severely affected population in 2008, C-terminal ANP was significantly higher in cats with various forms of cardiomyopathy when compared to normal cats.[143] Another 2008 study[133] demonstrated a significant difference in NT-pro-ANP between healthy cats, cats with heart disease but without failure, and cats in heart failure. However, in this study, NT-pro-BNP outperformed NT-pro-ANP in differentiating between these groups; when pairwise comparisons of the AUCs were performed, there was a significant difference between using NT-pro-ANP and NT-pro-

BNP to differentiate control from study cats. In the same study, NT-pro-BNP also demonstrated a greater correlation with left atrial to aorta ratio than did NT-pro-ANP.

The first research regarding cTNI in cats was in 2001, when a reference range of <0.16 ng/mL was established based on 21 asymptomatic cats.[123] In 2002, Herndon et al.[144] demonstrated that plasma cTNI was significantly higher in cats with HCM than in normal cats and that a cutoff of <0.157 ng/mL was 85% sensitive and 97% specific for detecting HCM. Unfortunately, the HCM group in this study included both asymptomatic cats and cats in congestive heart failure, limiting the clinical applicability of these findings in asymptomatic cats. Using a different assay with a detection limit of 0.2ng/mL but a similar population of cats, Connolly et al.[128] reported similar findings in 2003. Porciello et al.[145] demonstrated elevated plasma cTNI in cats in azotemic renal failure; however, subclinical cardiomyopathy was not ruled out in these cats as echocardiograms were not performed. As with NT-proBNP, two studies[146,147] have examined the utility of plasma cTNI to differentiate between cardiac and noncardiac causes of dyspnea in cats. cTNI appears less useful than NT-proBNP for this purpose; although cTNI concentrations were significantly higher in both studies in cats dyspneic as a result of congestive heart failure, there was significant overlap between the groups.

Both cTNI and NT-proBNP have become accepted as useful biomarkers in feline cardiac research. Jung and Kittleson[148] used both biomarkers to evaluate the effect of atenolol in asymptomatic cats with severe HCM; as plasma concentrations of neither decreased, they concluded that atenolol does not decrease myocardial ischemia and myocyte death in these cats.

E. Effect of hyperthyroidism on cardiac biomarkers

There are multiple potential physiologic mechanisms resulting from hyperthyroidism that could cause elevated plasma NT-proBNP concentrations both indirectly and directly. As previously discussed, excessive thyroid hormone concentrations lead to an increase in blood volume secondary to RAAS activation and excessive erythropoieitin secretion. This increased volume would be expected to cause myocardial stretch, triggering BNP secretion. Decreased systemic vascular resistance and RAAS activation in hyperthyroid states would be expected to trigger release of endothelin, which would stimulate BNP

release as well. The increased influence of the sympathetic nervous system secondary to increased numbers of cardiac β1-adrenergic receptors likely contributes to an increase in BNP secretion as well, due to the direct stimulation of BNP release induced by catecholamines as well as secondary to the tachycardia that results from increased sympathetic tone.

In addition to these indirect physiologic triggers of BNP release, there appear to be direct effect of thyroid hormones on BNP concentrations. In 1993, Kohno et al.[149] demonstrated that both T_4 and T_3 stimulate release of BNP from rat atrial and ventricular myocytes in primary culture. In 2003, Liang et al.[150] followed up on Kohno's work and demonstrated that the BNP gene itself is a target of T_3. Upon exposure to T_3, isolated neonatal rat ventricular myocytes demonstrated a 600% increase in BNP secretion, 300% increase in BNP mRNA expression, and 300-500% increase in BNP promoter activity. These studies showed a direct effect of thyroid hormones independent of the physiologically expected response to myocyte stretch stimuli secondary to the previously discussed increased plasma volume and red cell mass induced by a hyperthyroid state. Katz et al.[151] demonstrated that the c-GMP mediated effects of BNP on cardiac function were disrupted in iatrogenically hyperthyroid rabbits compared to euthyroid rabbits both in vitro and in vivo. As cardiac hypertrophy was present in the hyperthyroid rabbits, this study could not distinguish direct effects of thyroid hormone from those secondary to hypertrophy.

While it is known that excessive thyroid hormone concentrations contribute to tachyarrhythmias and increased myocardial oxygen demand, it is unclear if these effects would cause enough myocyte damage for detectable leakage of cTNI to occur. Research into the effects of thyroid status on cTNI in any species has been scant. In 1990, Dieckman and Solaro[152] found that iatrogenically hypothyroid rats developed a reversible decrease in cardiac TNI. In contrast, healthy horses iatrogenically made hyperthyroid by levothyroxine supplementation did not have a significant increase in cTNI during a 48 week study.[153] Twenty-three cats with naturally-occurring hyperthyroidism were studied by Connolly et al.[154] before and after treatment with radioactive iodine. Forty-eight percent of the cats had detectable (>0.2ng/mL) levels of cTNI prior to therapy compared to 17% of the cats examined six months after treatment. These results failed to achieve

statistical significance, but the study was hindered by use of an assay with a lower limit of detection of 0.2ng/mL. Diastolic septal thickness was significantly higher in cats with detectable cTNI compared to those without. There was no correlation between presence of a murmur and serum cTNI levels. The only echocardiographic measurements to significantly change following treatment were fractional shortening and systolic septal thickness. Direct effects of thyroid hormone on cTNI synthesis or plasma concentrations have not been demonstrated.

The effect of thyroid status on NT-proBNP in various species has been more extensively studied. In the Kohno et al. study previously discussed,[149] BNP was demonstrated to be elevated in iatrogenically hyperthyroid rats. Multiple studies have demonstrated elevated NT-proBNP[155-159] and BNP[149,160,161] in hyperthyroid humans. The single conflicting conclusion was drawn by Manucheri et al.[162] in 2006. This group examined a group of hypo- and hyperthyroid humans who all achieved euthyroidism with treatment. Although NT-proBNP levels rose in each treated hypothyroid individual and fell in each treated hyperthyroid individual, this group concluded that the changes in NT-proBNP were within expected variability and thus were not clinically significant. They also did not detect a significant difference in NT-proBNP between the hypothyroid and hyperthyroid individuals. There are several possible reasons for the different conclusions between the Manucheri study and all of the rest; the most likely is that the Manucheri paper suffered from small sample sizes (17 patients in the hypothyroid group and 21 in the hyperthyroid group) and larger numbers of patients were needed to draw an accurate conclusion.

In addition to mere elevation in hyperthyroid states, serum NT-proBNP has also been demonstrated to be correlated with thyroid hormone concentrations[158,159] and inversely correlated with TSH concentrations.[163] In humans, NT-proBNP concentrations fall after treatment of hyperthyroidism.[158] Wei et al.[164] found in 2005 that plasma BNP was elevated in hyperthyroid patients with echocardiographic evidence of left ventricular dysfunction but was similar to healthy control patients in hyperthyroid individuals with normal left ventricular function. The results of Arikan et al.[159] in 2007 demonstrated that NT-proBNP was positively correlated with interventricular septal thickness and negatively correlated with left ventricular ejection fraction. A conclusion cannot be

drawn, however, as to whether elevated BNP is strictly secondary to the myocardial thickening induced by hyperthyroidism or merely rising in parallel with the hypertrophy secondary to direct stimulation from thyroid hormones. In the single veterinary study of hyperthyroidism and NT-proBNP,[165] findings were consistent with those in humans: NT-proBNP is elevated in hyperthyroid cats and decreases after treatment of hyperthyroidism. Hyperthyroid humans would be expected to seek care at an earlier stage of their disease than would owners of hyperthyroid cats, so with further research the differences in NT-proBNP between hyperthyroid and healthy populations may prove to be more marked in cats than in humans.

F. Conclusion and Research Justification

As echocardiographic findings can be similar between cats with thyrotoxic cardiomyopathy and cats with primary hypertrophic cardiomyopathy and the prognosis of these two diseases may differ, it would be clinically useful to have a diagnostic test to assist in this distinction. In humans, hyperthyroid patients with left ventricular dysfunction have higher BNP concentrations than hyperthyroid patients with normal left ventricular function.[164] Although the pathogenesis of hyperthyroidism in cats and humans is likely different, the physiologic effects of elevated thyroid hormone concentrations are expected to be similar. It is plausible to suggest that elevated cTNI concentrations may occur in association with hyperthyroidism as a result of tachyarrhythmias and increased myocardial oxygen demand, and this elevation has been demonstrated in horses and cats. However, the utility of cardiac biomarkers to differentiate permanent from transient cardiomyopathy in hyperthyroid states in any species has not been evaluated. The hypothesis of the current study, that although both biomarkers will be elevated in hyperthyroid patients, they will not discriminate hyperthyroid cats from those with primary hypertrophic cardiomyopathy, is based on results from descriptive studies in the human and veterinary literature. In cats with transient thyrotoxic cardiomyopathy, biomarkers are expected to return to normal over time as the cardiovascular and direct effects of hyperthyroidism are removed.

In addition to expanding knowledge of hyperthyroidism and biomarkers in cats, there is a more practical justification for this study. Publications for general veterinary

practitioners are littered with advertisements promoting the use of NT-pro-BNP as a screening test for cardiomyopathy even in asymptomatic cats. Before the results of this test can be accurately interpreted, further research is needed in cardiac biomarkers in cats. It is important for practitioners to know if diseases other than primary cardiomyopathy can cause elevations in NT-pro-BNP in order to assist their diagnostic and prognostic recommendations if this test is used.

CHAPTER 2: CARDIAC BIOMARKERS IN HYPERTHYROID CATS

A. Introduction

Cardiac abnormalities, including murmurs and gallop sounds, are often detected during physical examination of hyperthyroid cats. Cardiovascular abnormalities in hyperthyroid cats are diverse and comprise subtle to severe myocardial changes that can be associated with the development of heart failure. There is evidence that myocardial abnormalities resolve after treatment in many cats, but persist in others.[55,56] It is relevant that subclinical echocardiographic abnormalities are common in the general feline population; 15.5 % of apparently healthy euthyroid cats were found to have primary cardiomyopathy in a recent study.[70] Therefore, it is uncertain whether cardiac abnormalities that persist after resolution of hyperthyroidism are the result of hyperthyroidism or concurrent primary cardiac disease. An inexpensive and readily available method that reliably differentiates thyrotoxic heart disease from primary hypertrophic cardiomyopathy prior to treatment of hyperthyroidism would provide prognostic information, guide treatment recommendations, and possibly avoid unnecessary and costly diagnostic evaluation. In addition, it would be useful to have a method to predict severity of cardiovascular changes caused by hyperthyroidism prior to definitive treatment.

Primary myocardial disease is a diagnosis of exclusion that is based on the results of blood pressure determination, metabolic assessment, and echocardiography. Specifically, hypertrophic cardiomyopathy (HCM) is defined as hypertrophy of a non-dilated ventricle in the absence of metabolic or hemodynamic stimuli such as hyperthyroidism or hypertension.[166] While echocardiography is instrumental in the diagnosis of HCM, it is not readily available to many general practitioners. In addition, thyrotoxic heart disease may result in echocardiographic changes similar to those of cats with primary myocardial disease, making results of echocardiography difficult to interpret. The bloodborne cardiac biomarkers N-terminal pro-brain natriuretic peptide (NT-proBNP), secreted in response to myocardial stretch, and cardiac troponin I (cTNI), released from the cytosol of damaged cardiomyocytes, may assist with the aforementioned diagnostic challenge.

Many studies of hyperthyroid humans have demonstrated increased plasma concentrations of NT-proBNP,[156,158,160] and T4 has been shown to stimulate BNP release from isolated rat cardiocytes.[149] Limited investigation of hyperthyroid cats has documented elevated NT-proBNP and cTNI.[154,165] However, it remains to be determined if cardiac biomarkers can be used to distinguish primary cardiac disease from cardiac changes due to hyperthyroidism in cats. Biomarkers can be used to evaluate cats with clinical signs suggestive of congestive heart failure, those with abnormal heart sounds in the absence of clinical signs of cardiovascular disease, and as a screening test in asymptomatic cats. Therefore, it is important to determine if hyperthyroidism affects plasma concentrations of NT-proBNP and cTNI independent of substantial heart disease.

We tested the hypothesis that plasma NT-proBNP and cTNI concentrations are higher in cats with hypertrophic cardiomyopathy than in cats with hyperthyroidism and higher in cats with hyperthyroidism than in healthy control cats. The primary objective was to describe plasma concentrations of these biomarkers in three groups of cats: cats with naturally occurring hyperthyroidism, cats with primary hypertrophic cardiomyopathy, and healthy older cats. Secondary objectives were to determine if biomarkers could be used to differentiate these populations and to describe changes in cardiac function and biomarker status after resolution of hyperthyroidism.

B. Materials and Methods

Animals

Sixty client-owned cats were prospectively evaluated. The cats were divided into three groups. Group 1 consisted of 23 cats presented to the Veterinary Teaching Hospital with hyperthyroidism based on compatible clinical findings and serum total T4 concentration above the upper reference limit. Group 2 consisted of 18 euthyroid, normotensive cats that were diagnosed with hypertrophic cardiomyopathy as echocardiographically defined below. Group 3 consisted of 19 euthyroid normotensive healthy cats (as determined by history, physical examination, systolic blood pressure, laboratory testing, and echocardiography) eight years of age or older which acted as controls. Exclusion criteria for all groups included current or previous congestive heart failure, current or previous treatment of cardiac disease, presence of any concurrent

systemic disease, or azotemia. Cats were excluded from group 1 if they had been treated with antithyroid medication unless they had been treated for <3 weeks and there was clinicopathologic evidence that treatment did not result in euthyroidism. If applicable, oral antithyroid medication was discontinued at least two weeks prior to evaluation. Fourteen cats in group 1 comprised the recheck group and were re-evaluated three months after administration of 4.5 mCi of ^{131}I. The study was approved by the Virginia Tech Animal Care and Use Committee. All owners provided informed consent.

Experimental Protocol:

The results of a complete history, physical examination, CBC, serum biochemistry panel, urinalysis, blood pressure measurement, serum T4 concentration, plasma NT-proBNP concentration, plasma cTNI concentration, and echocardiography were evaluated for all cats; exceptions are described below. Orthogonal thoracic radiographs were also obtained from all cats in groups 1 and 2. Results of auscultation by a board-certified cardiologist were used to determine the presence and intensity of a murmur.

Urine was collected by cystocentesis. Systemic systolic arterial blood pressure was estimated by use of a Doppler flow meter[a] method with a manometric cuff; five determinations were recorded and averaged. Clinically relevant hypertension was defined as systolic blood pressure >180 mmHg; cats were excluded from the groups 2 and 3 if systemic blood pressure exceeded this figure. Ten to twelve milliliters of blood were obtained via jugular venipuncture (seven milliliters for cats less than 3kg body weight) for CBC, biochemistries, T4, NT-proBNP, and cTNI. Blood for NT-proBNP was placed in an EDTA tube and immediately centrifuged for 10 minutes at 1417 x G. Plasma was immediately harvested, placed in duplicate spray-dried K_2EDTA tubes, and stored at -80C for up to four months before shipping to a reference laboratory for assay. Plasma NT-proBNP was measured using a commercially available, previously validated horseradish peroxidase, colorimetric end-point assay for the quantitative determination of feline NT-proBNP[b] with a lower limit of detection of 23 pmol/L.[140] Samples for cTNI were placed in lithium heparin tubes and immediately centrifuged for 10 minutes at 1417xG. Plasma was immediately harvested, stored in duplicate at -80C for up to four

months, and then shipped overnight to the New Bolton Center of the University of Pennsylvania Veterinary School for analysis using a previously validated[123] assay and flourometric analyzer[c] with an analytical sensitivity of 0.03ng/mL. All laboratory measurements other than NT-proBNP and cTNI were performed by the Virginia-Maryland Regional College of Veterinary Medicine Veterinary Teaching Hospital laboratory using standard procedures.

Echocardiograms were performed as described elsewhere[167] and thoracic radiographs were reviewed for evidence of cardiac failure (i.e., pulmonary edema or pleural effusion) by one of two board-certified cardiologists (J.A. or A.L). The cardiologist was unaware of pre-treatment measurements and results of biomarker testing. Hypertrophic cardiomyopathy was defined as an end-diastolic wall thickness ≥6mm affecting >50% of any region of the interventricular septum or the left ventricular posterior wall.[70] Cats in groups 1 and 2 were categorized according to echocardiographic results into the following groups:[136] no evidence of cardiomyopathy (thickness of left ventricular posterior wall in diastole [LVPWd] and thickness of interventricular septum during diastole [IVSd] <6.0mm), mild cardiomyopathy (focal or generalized hypertrophy with LVPWd and/or IVSd 6.0-6.5mm and ratio of left atrial diameter to aortic diameter [LA/Ao]< 1.5), moderate cardiomyopathy (focal of generalized hypertrophy with LVPWd and/or IVSd 6.5-7.0 and LA/Ao <1.8 or LVPWd and/or IVSd 6.0-6.5mm and LA/Ao 1.5-1.8), and severe cardiomyopathy (focal or generalized hypertrophy with LVPWd and/or IVSd > 7.0mm or LVPWd and/or IVSd >6.0mm and LA/Ao >1.8).

According to current guidelines from the reference laboratory for interpretation of NT-proBNP results in asymptomatic cats,[d] a concentration of <100pmol/L indicates that clinically significant heart disease is unlikely and ≥100 pmol/L indicates that heart disease is likely. Results were stratified into these two groups for interpretation.

Statistical Analysis:

Statistical analysis was performed with commercial software.[e] Normal probability plots demonstrated that age, weight, blood pressure, thyroid hormone concentration, heart rate, and echocardiographic variables followed a normal distribution while biomarker concentrations were skewed. Subsequently, one-way ANOVA followed

by Tukey-Kramer's procedure for multiple comparisons was used to compare normally distributed variables between groups. Residual plots from each of the ANOVA models were inspected to verify that the errors were normally distributed with a constant variance. Fisher's exact test was used to compare groups with respect to frequency of male sex, murmurs and supraphysiologic biomarker concentrations. Differences in biomarker concentrations between groups were evaluated by a Kruskal-Wallis one-way analysis of variance followed by Dunn's test for multiple comparisons. Change in biomarker concentrations after treatment with radioiodine was evaluated with a Wilcoxon signed rank test. Correlations were assessed using Spearman rank correlation coefficients. Statistical significance was set to $p<0.05$.

Samples with a T4 of less than 6.4 mmol/L were arbitrarily assigned a value of 6.3 mmol/L for data analysis; samples with a T4 of greater than 193 mmol/L were assigned a value of 194 mmol/L. Likewise, samples with an NT-proBNP concentration less than 24pmol/L were arbitrarily assigned a value of 23 pmol/L; the single sample with an NT-proBNP concentration of greater than 1500pmol/L was assigned a value of 1501 pmol/L.

C. Results

Group 1 included 11 castrated male and 12 spayed female cats. 20 of these cats were domestic shorthairs, and there was one cat each of domestic longhair, Norwegian Forest Cat, and Himalayan. Group 2 included 13 castrated males, four spayed females, and one intact female cat. Ten of these cats were domestic shorthairs, seven were domestic longhairs, and one was a Persian. Group 3 included 11 castrated male and 8 spayed female cats. Fifteen of these cats were domestic shorthairs, two were domestic longhairs, one was a Scottish Fold, and one was a Siamese. There were significantly more males in group 2 than in group 1 ($p=0.02$), although the sex distribution of neither group differed significantly from the control group ($p=0.20$ between groups 1 and 3 and $p=0.07$ between groups 2 and 3).

The mean+/-standard deviation age was 12.4 +/- 2.2 years for group 1 (range 9-17 years), 7.6 +/- 4.5 years for group 2 (range 1-15 years), and 10.3 +/- 2.2 years for group 3 (range 8-16 years). Group 2 was significantly younger than group 1 ($p<0.001$) and group

3 (p=0.028). There was no significant difference in age between groups 1 and 3 (p=0.08). The mean +/- standard deviation body weight was 4.3 +/- 1.1 kg for group 1, 5.2 +/- 1.5 kg for group 2, and 4.7 +/- 1.0 kg for group 3. Body weight was not significantly different between groups (p=0.06 between groups 1 and 2, p=0.52 between groups 1 and 3, and p=0.46 between groups 2 and 3).

The average systolic blood pressure of group 2 was lower than groups 1 or 3 (Table 1). Four of 22 of the group 1 cats for which blood pressure was recorded had average systolic pressures above 180 mmHg. Three of these cats returned for three-month rechecks; two of them were still hypertensive and the third had become normotensive. No cat developed hypertension after treatment with radioiodine.

A murmur was present in 14/23 (61%) of group 1 cats, 17/18 (94%) of group 2 cats, and 4/19 (21%) of group 3 cats. The single cat in group 2 without a murmur was evaluated because a full sibling had been diagnosed with HCM. Thyroid hormone concentrations and heart rate were significantly higher in group 1 than groups 2 or 3 (Table 1). Thoracic radiographs of cats in groups 1 and 2 revealed no evidence of congestive heart failure (pleural effusion, pulmonary edema, or pulmonary venous congestion)

The median NT-proBNP and cTNI concentrations were significantly higher in groups 1 and 2 than group 3 (Table 2; Figures 1 and 2). Ten of 23 (43%) of group 1 cats, 10/18 (55%) of group 2 cats, and 1/19 (5%) of group 3 cats would have been classified as "heart disease likely" based on an NT-proBNP concentration in excess of 100 pmol/L. There was no significant difference in the percentage of cats with a NT-proBNP greater than 100pmol/L (p=0.54) between groups 1 and 2, although percentage of each group was significantly higher than the control group (p=0.006 for group 1 and p=0.001 for group 2). Of the 13 group 1 cats classified as echocardiographically normal, six (46%) had cTNI concentrations above the reference range and five (38%) had NT-proBNP concentrations in the "heart disease likely" range.

Plasma cTNI was above the upper reference limit (>0.16 ng/mL)[123] in 12/23 (52%) of group 1 cats, 7/18 (39%) of group 2 cats, and 1/19 (5%) of group 3 cats. There was no significant difference between proportions of cats with elevated plasma cTNI in groups 1 and 2 (p=0.28); however, the percentage of the group with elevated plasma

cTNI (p=0.001 and p=0.02, respectively) for each of group 1 and group 2 was significantly greater than the control group.

The recheck group comprised six castrated males and eight spayed females. Twelve were domestic shorthairs, one was a domestic longhair, and one was a Norwegian Forest Cat. Myocardial thickness (p=0.02 for IVSd and p=0.001 for LVPWd) as well as percent fractional shortening (%FS) (p=0.006) significantly declined after radioiodine therapy (Table 3). Four of the seven cats in the recheck group that had a murmur on initial examination had a murmur ausculted after treatment. One cat initially without a murmur developed one after treatment. Median plasma NT-proBNP (p=0.002) and cTNI (p=0.001) concentrations significantly decreased three months after treatment with radioiodine. Plasma cTNI was within the normal range for all cats at the three-month recheck.

Neither biomarker was significantly correlated with end-diastolic internal dimension (LVIDd) of the left ventricle, %FS, or systolic blood pressure (Table 4). cTNI was significantly but weakly correlated with thyroid hormone concentration and heart rate whereas NT-proBNP was not. Concentrations of both biomarkers were correlated with IVSd and LVPWd (measured in both 2 dimensional and M-mode), left atrial diameter, LA/Ao, and intensity of murmur. When group 2 was excluded from the analysis, T4 was positively correlated with both biomarkers, IVSd, and LVPWd.

Two of the cats (14%) were biochemically hypothyroid (total T4 <6.4 mmol/L) at their three-month recheck after radioiodine therapy. Neither cat was azotemic or exhibiting clinical signs of hypothyroidism, and cTNI and NT-proBNP for both cats were normal. One cat was still biochemically hyperthyroid at the three-month recheck; however, his T4 had decreased from 149 mmol/L to 43.6 mmol/L (reference interval 16.0-37.7 nmol/L), clinical signs of hyperthyroidism had resolved, cTNI had decreased from 1.53 ng/mL to 0.08 ng/mL, NT-proBNP had decreased from 190 pmol/L to 46 pmol/L, and body weight had increased by 0.8kg.

Blood pressure was not recorded from four cats. Blood pressure was not measured in one cat in group 2 due to temperament; blood pressure was inadvertently not recorded for one cat in group 1 and in two cats at the three-month recheck. One cat in group 2 did not have sufficient urine in its bladder to collect a urine sample. A complete

blood count was not performed on one cat in group 2 as the sample clotted prior to analysis.

D. Discussion

The results of this study are consistent with others which show that the cardiac biomarkers NT-proBNP and cTNI are elevated in cats with hypertrophic cardiomyopathy and cats with hyperthyroidism.[154,165] However, the demonstration that neither of these biomarkers can be used to distinguish hyperthyroid cats from cats with primary cardiomyopathy is arguably the most important clinically relevant finding. Therefore, the thyroid status of cats, particularly older cats, should be ascertained prior to interpreting results of biomarker testing.

To the author's knowledge, previous descriptions of cTNI in cats with HCM have included cats currently or previously in congestive heart failure. We deliberately chose a study population without failure in order to more closely approximate the clinical dilemma of a hyperthyroid cat with or without abnormal cardiac auscultation in which biomarkers may be used as diagnostic aids. Thus, although the HCM population in this study had significantly higher cTNI concentrations than normal cats, our median plasma cTNI concentration of 0.14ng/mL is lower than median concentrations ranging from 0.66ng/mL to 1.59ng/mL which others have reported.[128,144,146,147] In addition to including cats in congestive heart failure, two of the previous studies[128,147] used a different assay than the current study, possibly accounting for some of the discrepancy.[168] Cardiac troponin I results for normal cats in the current study were consistent with those previously published.[123] As only 39% of our HCM cats had elevated cTNI concentrations, this biomarker in isolation is not a sensitive indicator of cardiomyopathy. Results of this study support and strengthen the findings of Connolly et al.[154] that cTNI concentrations are elevated in hyperthyroid cats and decrease after treatment of hyperthyroidism.

Forty-five percent of cats with hypertrophic cardiomyopathy in this study had plasma NT-proBNP concentrations in the "cardiac disease unlikely" reference range. Although concentrations in HCM cats were higher than in normal cats, the poor sensitivity of NT-proBNP for detecting HCM in the current study contrasts with the

findings of other studies[131,136] which concluded that NT-proBNP is a sensitive and specific screening test for cats with HCM. While one of these studies[136] included cats in congestive heart failure (in which myocardial stretch would be expected to markedly increase NT-proBNP concentrations), the other[131] included only asymptomatic cats. The latter study included cats with restrictive, dilated, and unclassified cardiomyopathy in contrast to the present study which only included cats with hypertrophic cardiomyopathy. The authors are not aware of comparisons of NT-proBNP concentrations among cats with different forms of cardiomyopathy. Results of the current study are more consistent with those that have demonstrated elevated NT-proBNP primarily in cats with severe HCM.[134,135] However, as both of these studies only included Maine Coon cats with hereditary HCM, it is difficult to make direct comparisons. Even in the severe cardiomyopathy group in the current study, 25% of cats would have been considered unaffected if plasma NT-proBNP were used as a screening test.

Inclusion of hypertensive cats is a limitation of the study as hypertension is associated with elevated concentrations of both measured biomarkers in humans.[169,170] In hypertensive cats with concurrent chronic kidney disease, NT-proBNP is markedly elevated.[138] However, since hypertension is often present in hyperthyroid cats, we judged it inappropriate to exclude clinical cases based on hypertension alone. When the data were analyzed with the hypertensive cats excluded, statistical conclusions remained unaltered (data not shown). In addition, in the present study, there was no correlation between blood pressure and either biomarker. Three of the four hypertensive hyperthyroid cats had LVPWd >6mm, so it is likely that their biomarkers were elevated at least in part secondary to cardiac changes. None of the hypertensive cats had retinal lesions suggestive of end-organ damage, indicating that persistent severe hypertension was unlikely in these cats. "White-coat" hypertension is another possible, although unlikely, explanation for the hypertension.

Two different board certified cardiologists performed the echocardiograms, a factor that may have introduced variability. However, serial exams were performed by the same cardiologist in all but two cats, where the images from the first study were re-measured by the cardiologist who performed the three month recheck. Images were obtained in a standardized fashion. As many of the cats had asymmetrical hypertrophy,

our data may not reflect the thickest part of the myocardium for each cat; some cats may therefore have been underclassified in the severity of their cardiomyopathy.

Changes to echocardiographic parameters after treatment of hyperthyroidism have been previously reported.[55,56,154] The results of this study support previous findings that thyrotoxic cardiomyopathy is largely reversible. Significant decreases were found in myocardial thickness and fractional shortening in cats examined three months after radioiodine treatment. T4 stimulates BNP release from cultured rat atrial and ventricular myocytes,[149] indicating a direct effect independent of myocardial stretch and other cardiovascular factors. Interestingly, in the current study, 62% of the hyperthyroid cats echocardiographically classified as "normal" had elevated NT-proBNP, cTNI, or both. While no firm conclusion can be drawn, this finding may serve as further support of a direct effect of hyperthyroidism on biomarker concentrations. However, as T4, echocardiographic measurements, and plasma biomarker concentrations all improved after treatment with radioiodine in the present study, it is difficult to separate direct effects of T4 on plasma biomarker concentrations from changes in these biomarkers induced by improvements in cardiac structure and function.

Two of the hyperthyroid cats that were evaluated three months after radioiodine fell into the "severe cardiomyopathy" classification at their initial visit (LVPWd of 8.5mm in one cat and 9.9mm in the other). In the former cat, echocardiographic parameters returned to normal at the recheck, with all ventricular wall measurements less than 6mm. Both biomarkers also returned to normal in this cat. However, in the latter cat, LVPWd remained increased (8.2mm) at the recheck, and the murmur did not decrease in intensity. Although this cat's plasma cTNI concentration returned to normal, plasma NT-proBNP remained elevated (255 pmol/L, which was more than double any other post-treatment plasma NT-proBNP concentration). This cat was re-examined 11 months after treatment with ^{131}I and diagnosed with hypertrophic cardiomyopathy as wall thickness remained abnormal (LVPWd 8.51mm) despite documented euthyroidism. The length of time required for resolution of cardiac changes induced by hyperthyroidism is unknown, and this cat's myocardial thickness could return to normal following a more prolonged period of euthyroidism. However, based on the responses of the other thirteen cats and persistence of myocardial thickening for almost one year after resolution of

hyperthyroidism, it is likely that this cat has co-existing HCM in addition to resolving thyrotoxic cardiomyopathy. These results indicate a potential use for NT-proBNP to monitor the cardiac response to treatment of hyperthyroidism; a NT-proBNP that remains elevated three months after resolution of hyperthyroidism may indicate underlying cardiomyopathy and an echocardiogram should be recommended in this subset of cats. Further prospective evaluation of hyperthyroid cats with severe myocardial thickening is needed to explore this recommendation.

In summary, the cardiac biomarkers NT-pro BNP and cTNI are elevated in hyperthyroid cats; in the majority of cases, they returned to the normal range 3 months after treatment with ^{131}I. These biomarkers did not assist in differentiation between cats with primary HCM and cats with thyrotoxic cardiomyopathy.

CHAPTER 3: CONCLUSIONS AND FURTHER RESEARCH

This study demonstrates that the cardiac biomarkers NT-proBNP and cTNI are elevated in hyperthyroid cats and decrease with resolution of hyperthyroidism. As cats were not rechecked until three months after administration of ^{131}I, this study cannot determine whether the biomarker concentrations fell in tandem with T_4 concentrations, which are within the normal range at a one-month checkup in most cats, or with echocardiographic measurements of myocardial thickening. The former finding would indicate a direct effect of T_4 concentration on these biomarkers whereas the latter would suggest an indirect effect secondary to resolution of the cardiovascular changes induced by a hyperthyroid state. Further research in this area should include more frequent rechecks to answer this question.

In addition, further research should focus on the hyperthyroid cats with abnormal echocardiographic findings. In this study, 13 out of 23 hyperthyroid cats had normal myocardial measurements. The cats with thyrotoxic cardiomyopathy leading to a thickened myocardium represent a more interesting study population which better represents the clinical dilemma which we would like to ultimately solve: is there a way to differentiate thyrotoxic cardiomyopathy from primary HCM in a hyperthyroid cat? Results of this study indicate that these biomarkers cannot be used for this purpose.

This study demonstrates that if general practitioners use NT-proBNP as a screening test in older cats (as it is currently marketed), the thyroid status of these cats must be known prior to interpretation of elevated NT-proBNP concentrations. However, NT-proBNP may have a role in monitoring the cardiac response to treatment of hyperthyroidism, and this area also deserves further research.

FOOTNOTES

[a] Park's Doppler Flow Detector, Aloha, OR

[b] Cardiopet proBNP, IDEXX Laboratories, Westbrook ME

[c] Stratus CS stat fluorometric analyzer, Dade Behring Inc., Newark DE

[d] Service Update, January 2012, IDEXX reference laboratories, Westbrook ME

[e] SAS version 9.2, Cary NC

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APPENDIX A: FIGURES

Figure 1. Scatter plot of plasma NT-proBNP concentrations for each group. Asterisk represents one cat in group 2 with a concentration of 1501 pmol/L.

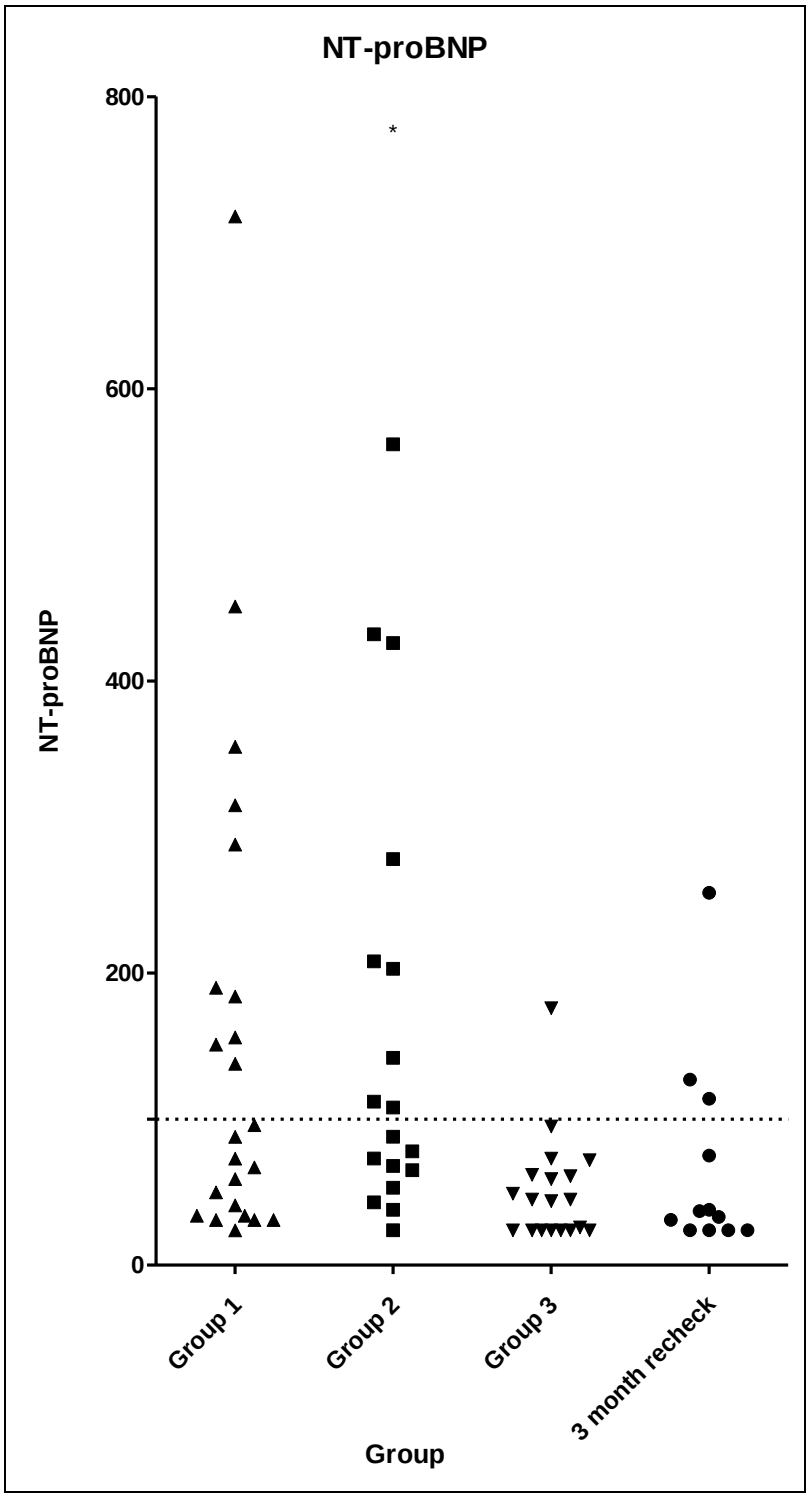
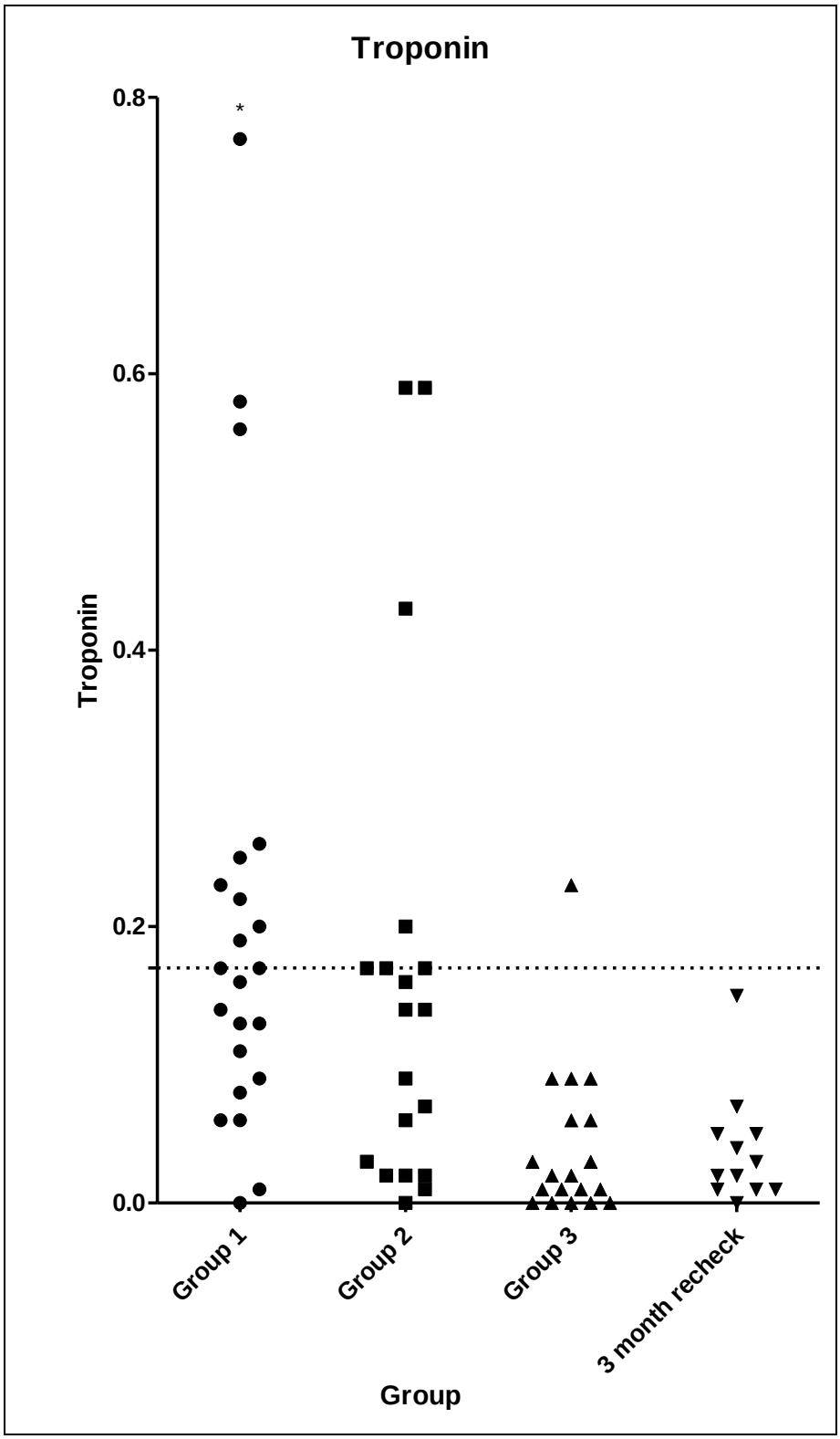


Figure 2. Scatter plot of plasma cTNI concentrations for each group. Asterisk represents one cat in group 1 with a cTNI of 1.53 ng/mL.



APPENDIX B: TABLES

Table 1. Descriptive statistics of groups

	T4 (nmol/L)		**Blood Pressure (mmHg)**		**Heart Rate (bpm)**		**% of group with murmur**	**M-mode IVSd (mm)**		**M-mode LVPWd (mm)**		**%FS**		**M-mode LA:Ao**	
	Mean	SD	Mean	SD	Mean	SD		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Group 1 (n=23)	107.6[a]	53.2	157[a]	22	224[a]	30	61[a]	5.3[a]	0.8	6.0[a]	1.6	60[a]	10	1.4[a]	0.3
Group 2 (n=18)	25.6[b]	6.8	125[b]	26	177[b]	24	94[b]	6.0[b]	0.8	6.4[a]	1.4	61[a]	7	1.3[a]	0.2
Group 3 (n=19)	26.1[b]	5.5	144[a]	21	195[b]	29	21[c]	4.4[c]	0.5	4.6[b]	0.6	60[a]	7	1.3[a]	0.2

Different superscript letters within columns designate significant differences ($p<0.05$).

Table 2. Cardiac biomarker concentrations within groups

Group	Median (range) NT-proBNP	Median (range) cTNI	Echocardiographic Classification	NT-proBNP	
				<100 pmol/L	>100 pmol/L
1	92 pmol/L[a] (<24-718)	0.17 ng/mL[a] (0.0-0.153)	Normal	8	5
			Mild	1	1
			Moderate	2	2
			Severe	2	2
			Total	13	10
2	100 pmol/L[a] (<24->1500)	0.14 ng/mL[a] (0.0-0.59)	Mild	1	2
			Moderate	5	2
			Severe	2	6
			Total	8	10
3	60 pmol/L[b] (<24-176)	0.02ng/mL[b] (0.0-0.23)	Normal	18	1

Different superscript letters within columns designate significant differences ($p<0.05$).

Table 3. Changes to selected variables before and three months after treatment of hyperthyroidism with ^{131}I (n=14 cats)

	Prior to radioiodine		**3 month recheck**		
	Mean	**SD**	**Mean**	**SD**	**p value**
T4 (nmol/L)	112.7	49.3	23.2	11.7	<0.001
M-mode IVSd (mm)	5.3	0.9	4.6	0.8	0.019
M-mode LVPWd (mm)	6.0	1.6	5.1	1.2	0.001
%FS	56	9	50	9	0.006
M-mode LA:Ao	1.4	0.2	1.3	0.2	0.51
	Median	**Range**	**Median**	**Range**	
NT-proBNP (pmol/L)	70	31-718	37.5	<24-255	0.002
cTNI (ng/mL)	0.14	0.0-1.53	0.04	0.0-0.15	0.001

Table 4. Correlations of biomarkers to selected variables

	cTNI		NT-proBNP			T4		
	Spearman's correlation coefficient	p value	Spearman's correlation coefficient	p value	# of observations cTNI/NT-proBNP	Spearman's correlation coefficient	p value	# of observations
M-mode IVSd	0.296	0.02	0.447	<0.001	60	0.507	<0.001	42
M-mode LVPWd	0.483	<0.001	0.417	<0.001	60	0.500	<0.001	42
M-mode LA:Ao	0.283	0.03	0.411	0.001	60	0.281	0.07	42
%FS	-0.040	0.76	-0.035	0.79	60	-0.051	0.74	42
HR	0.265	0.04	-0.286	0.026	60			
Intensity of murmur	0.373	0.003	0.473	<0.001	60			
BP	0.078	0.56	-0.080	0.55	58			
T4	0.635	<0.001	0.461	0.002	60			