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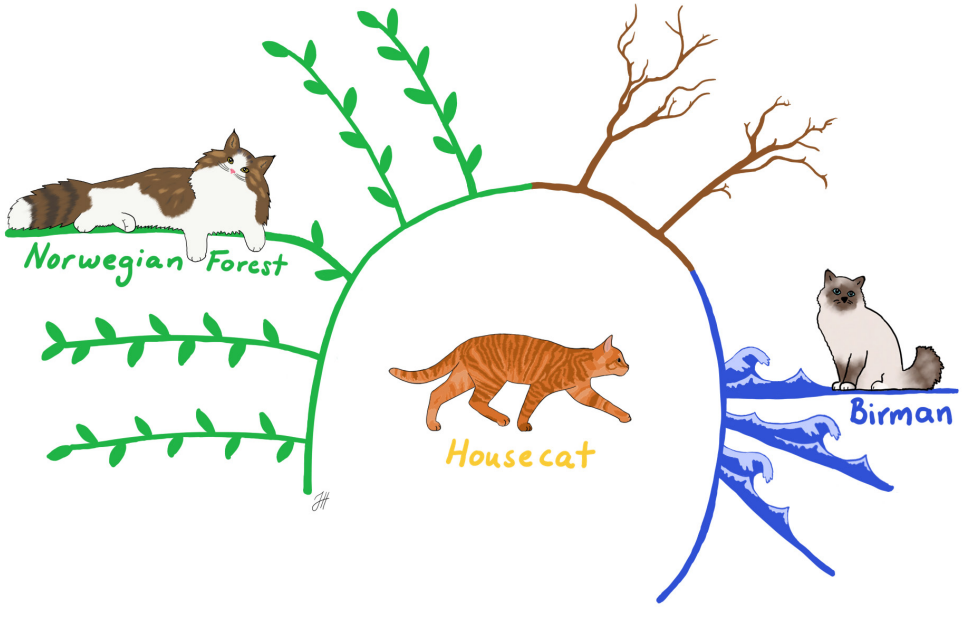
Cardiac biomarkers in cats

Associations with feline characteristics and hypertrophic cardiomyopathy

Sofia Hanås



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Associations with feline characteristics and hypertrophic cardiomyopathy

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Cover: An illustration of the phylogenetic tree of cat breeds, freely interpreted by the artist.
(Illustration: J. Hanås)

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Abstract

Cardiac biomarkers may be used to support diagnosis of diseases such as hypertrophic cardiomyopathy (HCM), a common cardiac disease in cats. However, the impact of feline characteristics on these biomarkers is relatively unexplored.

The biomarkers N-terminal-prohormone-B-type natriuretic peptide (NT-proBNP), cardiac troponin I (cTnI), microRNA (miRNA), blood pressure (BP) and pulse rate (PR) were studied in healthy Birman, Norwegian Forest (NF) and Domestic Shorthair (DSH) cats. Major aims of the thesis were to assess potential associations between these cardiac biomarkers and feline characteristics in healthy cats, and to compare measured values of the circulating biomarkers in healthy cats with cats with HCM.

Blood pressure and PR increased with age, and NF and DSH cats had higher BP than Birman cats. For plasma NT-proBNP, male cats had higher concentrations than female cats. Regarding cTnI, neutered male cats had higher serum concentrations than intact female cats, and Birman cats had higher cTnI concentrations than NF cats. In healthy cats, breed had an effect on miRNA-profiles in whole blood when NF cats were compared to DSH cats. In cats with HCM, NT-proBNP and cTnI concentrations were higher in cats with HCM and left atrial enlargement (LAE) than in cats with HCM without LAE and in healthy cats.

In conclusion, in healthy cats, *breed* was associated with BP, cTnI and miRNA, *sex* was associated with NT-proBNP and cTnI, and *age* was associated with BP and PR. For NT-proBNP and cTnI, cats with HCM and LAE had higher concentrations than cats with HCM without LAE, and than in healthy cats.

Keywords: troponin, blood pressure, NT-proBNP, miRNA, breed, Birman, Norwegian Forest.

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Hjärtbiomarkörer hos katt

Sammanfattning

Hjärtbiomarkörer används ofta i diagnostiskt syfte för att stödja diagnos vid sjukdom såsom, hypertrofisk kardiomyopati (HCM), en vanlig hjärtsjukdom hos katt. Men hur kattens egenskaper påverkar dessa biomarkörer är relativt outforskade.

Hjärtbiomarkörerna N-terminal-prohormon-B-typ natriuretisk peptid (NT-proBNP), kardiellt troponin I (cTnI), mikroRNA (miRNA), blodtryck (BP) och pulsfrekvens (PR) studerades hos friska birmor, norska skogkatter (NF) och huskatter (DSH). Huvudsyften med denna avhandling var att utvärdera potentiella samband mellan hjärtbiomarkörer och egenskaper hos friska katter, och att jämföra uppmätta värden av de cirkulerande biomarkörerna hos friska katter med katter med HCM.

Blodtryck och PR ökade med stigande ålder, och NF- och DSH-katter hade högre BP än birmor. För plasma NT-proBNP hade hankatter högre koncentrationer än honkatter. Vad gäller cTnI hade kastrerade hankatter högre serumkoncentrationer än intakta honkatter, och birmor hade högre cTnI koncentrationer än NF katter. Hos friska katter, påverkade ras miRNA-profiler i hel-blod när NF-katter jämfördes med huskatter. Hos katter med HCM var NT-proBNP och cTnI högre hos katter med HCM och vänster förmaksförstoring (LAE) än hos katter med HCM utan LAE och hos friska katter.

Sammanfattningsvis, hos friska katter, var *ras* associerad med BP, cTnI och miRNA, *kön* var associerat med NT-proBNP och cTnI, och *ålder* var associerat med BP och PR. För NT-proBNP- och cTnI-koncentrationer hade katter med HCM och LAE högre koncentrationer än katter med HCM utan LAE och än friska katter.

Nyckelord: troponin, blodtryck, NT-proBNP, miRNA, ras, Birma, norsk skogkatt.

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Dedication

To my family, to our happy dogs, and to our amiable horses.
You all enrich and inspire me every day.
This thesis is mainly about cats!

“It´s all about cats!”

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Hanås S*, Holst BS, Ljungvall I, Tidholm A, Olsson U, Häggström J, and Höglund K (2021). Influence of clinical setting and cat characteristics on indirectly measured blood pressure and pulse rate in healthy Birman, Norwegian Forest and Domestic Shorthair Cats. *Journal of Veterinary Internal Medicine*, 35 (2), pp. 801-811

II. Hanås S*, Holst BS, Höglund K, Häggström J, Tidholm A and Ljungvall I (2020). Effect of feline characteristics on plasma N-terminal-prohormone B-type natriuretic peptide concentration and comparison of a point-of-care test and an ELISA test. *Journal of Veterinary Internal Medicine*, 34 (3), pp 1187-1197.

III. Hanås S*, Larsson A, Rydén J, Lilliehöök I, Häggström J, Tidholm A, Höglund K, Ljungvall I, and Holst BS. Cardiac troponin I in healthy Norwegian Forest, Birman, and Domestic Shorthair cats and in cats with hypertrophic cardiomyopathy. (submitted)

IV. Hanås S*, Ohlsson Å, Holst BS, Laurent J, Andersson G, Höglund K, Tidholm A, Ljungvall I, Häggström J. A study of the feline microRNA transcriptome in whole-blood in healthy cats and in cats with preclinical hypertrophic cardiomyopathy. (manuscript)

*Corresponding author

The contribution of Sofia Hanås to the papers included in this thesis was as follows:

I. Contributed to the design of the study, measured blood pressure in all cats, participated in the statistical analysis and drafted the manuscript. Contributed to the interpretation of results and the revision of the final manuscript.

II. Participated in the planning of the study, examined and sampled all cats, participated in the statistical analysis, performed the laboratory analyses of the point-of-care test, and drafted the manuscript. Contributed to the design of the study, interpretation of results and revision of the final manuscript.

III. Contributed to the design of the study, examined and sampled all cats, performed the assay validation, participated and performed the statistical analysis, drafted the manuscript. Contributed to the interpretation of results and to manuscript revision.

IV. Contributed to the design of the study, examined and sampled all cats, participated in sample analysis and statistical analysis, participated in drafting the manuscript. Contributed to interpretation of the results and manuscript revision.

Abbreviations

ANP	Atrial natriuretic peptide
APPC	Arterial pulse pressure curve
APPW	Arterial pulse pressure wave form
BCS	Body condition score
BH-A	Benjamini-Hochberg adjustment
Biomarker	Biological marker
BNP	Brain or B-type natriuretic peptide
BP	Blood pressure
BW	Body weight
Carrier-O	Carrier-owner
Carrier-VO	Carrier-veterinarian-owner
CHF	Congestive heart failure
CM	Cardiomyopathy
CMIA	Chemiluminescent microparticle immunoassay
CV_B	Between-run repeatability
CV_R	Within-run repeatability
CV_{WL}	Within-laboratory repeatability
cTn	Cardiac troponin
cTnI	Cardiac troponin I
CV	Coefficient of variation
DBP	Diastolic blood pressure
DCM	Dilated CM
DLH	Domestic longhair cat
DSH	Domestic Shorthair
EDTA	Ethylenediaminetetraacetic acid

ELISA	Enzyme linked immunosorbent assay
FS	Fractional shortening
HCM	Hypertrophic cardiomyopathy
HDO	High-definition oscillometry
HR	Heart rate
hs-cTnI	High-sensitivity cTnI assay
IVSd	Interventricular septum in diastole
$IVSd_{inc\%}$	Percentage increase interventricular septum in diastole
IQR	Interquartile range
LA	Left atrium
LAE	Left atrial enlargement
LA/Ao	Left atrial-to-aortic root diameter ratio
LV	Left ventricular
LVFWd	Left ventricular free wall in diastole
$LVFWd_{inc\%}$	Percentage increase left ventricular free wall in diastole
LVIDd	Left ventricular internal diameter in diastole
$LVIDd_{inc\%}$	Percentage increase left ventricular internal diameter in diastole
LVIDs	Left ventricular internal diameter in systole
NF	Norwegian Forest cat
NP	Natriuretic peptides
NT-proBNP	N-terminal-prohormone-B-type natriuretic peptide
MAP	Mean arterial blood pressure
mRNA	Messenger ribonucleic acid
miRNA	Microribonucleic acid
miRNAome	Total miRNA transcriptome
MYBPC3	Myosin binding protein-C3 gene
MYH7	Myosin heavy chain gene 7 gene
NGS	Next-generation sequencing
POCT	Point-of-care test
Pre-miRNA	Precursor microRNA
Pri-miRNA	Primary transcripts microRNA
proBNP	prohormone-BNP
PR	Pulse rate
RAAS	Renin-angiotensin-aldosterone system
RNA	Ribonucleic acids
ROC	Receiver operator characteristic

SBP	Systolic blood pressure
SD	Standard deviation
SE	Sensitivity
SP	Specificity
SV	Stroke volume
SVR	Systemic vascular resistance
Table-VO	Table-veterinarian-owner
TT4	Total thyroxine

1. Introduction

Biological markers (biomarkers) may be used in diseases such as hypertrophic cardiomyopathy (HCM). A biomarker, according to the Biomarkers Definitions Working Group, 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." (Group 2001; Califf 2018) There are many types of biomarkers. Molecular biomarkers, which have biophysical properties allowing their measurement in biological samples from plasma, serum, or via biopsy; and physiologic biomarkers, which measure a process in the body, like blood pressure (BP) measurement (Group 2001; Califf 2018). Accurate and precise biomarkers are essential in research (Califf 2018), as well as in animal health care. Cardiac biomarkers, such as BP, N-terminal-prohormone-B-type natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI) may be used to support the HCM diagnosis in cats. The impact of feline characteristics on these biomarkers is relatively unexplored. Of these biomarkers, BP measurement —a measurable physiologic biomarker— differs from other circulating biomarkers. In cats, hypertension has been reported to increase left ventricular (LV) wall thickness due to increased systemic vascular resistance regardless of the underlying cause (Snyder *et al.* 2001; Nelson *et al.* 2002; Brown *et al.* 2007; Taylor *et al.* 2017; Acierno *et al.* 2018). Many cats with hypertension have been reported to have underlying diseases, like kidney disease (Syme et al. 2002; Bijsmans et al. 2015) and hyperthyroidism (Kobayashi *et al.* 1990). Blood pressure measurements are performed routinely at veterinary clinics (Bodey & Sansom 1998; Sparkes *et al.* 1999; Bijsmans *et al.* 2015; Hori *et al.* 2019). In healthy cats, only a few reports on how different clinical settings are associated with BP and pulse rate (PR) have been published (Quimby *et al.*

2011; Nibblett *et al.* 2015). In this thesis, BP was included as a cardiac biomarker because of the importance of excluding hypertension, as a cause of LV hypertrophy to be able to diagnose HCM.

Natriuretic peptides such as NT-proBNP and troponins such as cTnI are the most commonly used circulating cardiac biomarkers in cats and dogs (Borgeat *et al.* 2015a; Langhorn & Willesen 2016; de Lima & Ferreira 2017). The active B-type natriuretic peptide (BNP) is rapidly produced as prohormone-BNP (proBNP) by cardiomyocytes in response to myocardial wall stretch (Weber & Hamm 2006). Intracellularly, inactive proBNP is cleaved to inactive stable NT-proBNP, which has a longer plasma half-life than the active more labile BNP; NT-proBNP is therefore analysed in clinical settings (Daniels & Maisel 2007). Cardiac troponins consist of calcium-modulated protein complexes, which are involved in regulating actin-myosin cross-bridges responsible for myocardial contraction. Circulating cTnI is released in response to myocardial injury (Apple & Collinson 2012). Circulating microRNAs (miRNAs) are small endogenous non-coding ribonucleic acids (RNAs) that play an important role in gene regulation (Bartel 2004). MicroRNAs may be potentially useful in diagnosing cardiovascular diseases in cats.

In cats, HCM is the most common cardiac disease, with a reported prevalence of approximately 15% of all domestic cats and 25% of all cats over nine years of age (Paige et al. 2009; Wagner et al. 2010; Payne et al. 2015b). Currently, echocardiography is the best method of diagnosing HCM. Important prognostic indicators for survival in cats with HCM include left atrial enlargement (LAE) and LV hypertrophy (Payne *et al.* 2013). Diagnosis of feline HCM is difficult if echocardiography and cardiac expertise are unavailable. Analysis of circulating cardiac biomarkers in a blood sample is widely available, as it does not require specialized training and can be measured as part of the clinical assessment. Circulating cardiac biomarkers may thus aid non-specialists in identifying cats with suspected cardiac diseases, such as HCM (Borgeat *et al.* 2015a; Luis Fuentes & Wilkie 2017).

The information concerning feline characteristics such as breed, sex, age, body weight (BW), and body condition score (BCS) is scarce or contradictory regarding potential associations with BP, NT-proBNP, and cTnI concentrations, and expression of miRNAs. This thesis therefore aimed at evaluating the association between these cardiac biomarkers and feline characteristics in healthy cats. Furthermore, concentrations of circulating

NT-proBNP and cTnI in healthy cats were compared to concentrations in cats with HCM, with and without LAE. MicroRNA transcriptome in feline whole blood in healthy cats was compared in NF and DSH cats, and between healthy cats and cats with preclinical HCM.

2. The ideal biomarker

An ideal biomarker has both high sensitivity and high specificity for the target organ of measurement, which in this thesis is the heart. High sensitivity for an ideal cardiac circulatory biomarker includes high concentration in serum/plasma/blood after an event, rapid release for early detection and diagnosis, and long half-life in circulation. High specificity for an ideal cardiac biomarker includes characteristics such as absence in non-myocardial tissues; i.e. the biomarker is organ-specific, quantifiable, released in proportion to the severity of the disease or lesion studied, and preferably has no overlapping values between diseased and healthy cats. Furthermore, the biomarker should have a high specificity, being greatly upregulated or downregulated specifically in samples from cats with the cardiac disease in question, and unaffected by comorbidities (Group 2001; de Lima & Ferreira 2017). Another example of an ideal circulatory biomarker are that is it easy and inexpensive to measure, and its measurement produces rapid results (de Lima & Ferreira 2017), reflecting the disease's pathophysiology and allowing for better diagnosis, prognosis and management of the disease (Dolci & Panteghini 2006; Castiglione *et al.* 2021). In veterinary medicine, several biomarkers are routinely used, but cardiac biomarkers are utilized to a comparatively lesser extent (de Lima & Ferreira 2017).

3. The history of the domestic cat

3.1 The ancestor of the house cat

To identify which subspecies of the wildcat *Felis silvestris* had been the direct ancestor of the house cat (domestic cat), researchers examined DNA in 979 cats, including wildcats from Africa, Europe, Asia, Afro-Asia, and domestic cats from southern Africa, Azerbaijan, Kazakhstan, Mongolia and the Middle East. DNA from the wild cats clustered into five groups, and within each group the wild cats came from the same region of the world (Table 1). House cats only clustered to the Middle Eastern wildcat, *Felis silvestris lybica*, which was therefore reported to be the ancestor of the domestic cat, *Felis silvestris catus*, (Table 1) (Driscoll *et al.* 2007; Driscoll *et al.* 2009). Archaeological discoveries of a cat (*Felis silvestris lybica*) buried beside a human, presumably the cat's owner, were found in Cyprus in 2004. This finding was dated to ~9500 years before the present time. Cats were not native to the island of Cyprus at that time, suggesting that people must have brought cats to Cyprus by boat. This finding suggests that cats were already being kept as pets in the Middle East 9500 years ago (Vigne *et al.* 2004). Before this archaeological finding, experts believed the Egyptians first domesticated the cat approximately 3600 years ago (Driscoll *et al.* 2009).

Table 1. *Felis silvestris* subspecies

Wildcat	Subspecies	Geographic origin
Middle Eastern wildcat	*Felis silvestris lybica**	Africa, Near East
Central Asian wildcat	*Felis silvestris ornata*	Middle East, central Asia
Southern African wildcat	*Felis silvestris cafra*	Southern Africa
European wildcat	*Felis silvestris silvestris*	Europe
Chinese mountain cat	*Felis silvestris bieti*	China
Domestic cats	*Felis silvestris catus*	Middle East or Egypt

* The ancestor to the housecat (domestic cat) *Felis silvestris catus*

3.2 The cats domesticated themselves

Based on historic records, researchers believe that the domestication of the Middle Eastern wildcat, *Felis silvestris lybica*, to a domesticated pet took thousands of years (Driscoll *et al.* 2009). Early agricultural settlements with wild grain stores constituted an attractive food source for the house mouse. House mice and human food waste attracted wild cats. Experts have proposed that features of wild cats, such as large eyes and round foreheads made people feel affection toward them. These features may have enabled cats to develop relationships with people because they elicited nurturing feelings. People likely took kittens home as pets because they found them lovable. Over time and by natural selection for tameness, wild cats that adapted to live and proliferate around human settlements were domesticated (Driscoll *et al.* 2009). These cat populations grew isolated from their wild ancestors and evolved into the domesticated cat, *Felis silvestris catus* (Gentry *et al.* 2004). When the farmers migrated from the Middle East and spread to the rest of the ancient world the cat, *Felis silvestris catus*, followed (Driscoll et al. 2009). Due to domestic cats' social skills, adaptability, and rodent-hunting, humans have distributed domestic cats throughout the world (Belton & Schmieder 2021).

Cats have historically been bred by selecting individuals by behaviour or phenotype from locally-adapted cat populations, therefore cat breeds from the same geographic region often cluster together in genetic analyses. The NF cat was developed in Northern Europe and the Birman cat was developed in Asia. Both these breeds genetically resemble the randomly-bred populations of their respective geographic origins (Lipinski *et al.* 2008). The majority of cat breeds can be traced to four regional ancestral cat populations:

1) Western derived breeds (for example NF, Maine Coon, Persian), 2) Eastern derived breeds (for example Birman, Burmese, Siamese), 3) Mediterranean breeds (Turkish Van and Turkish Angora), and Arabian Sea breed (Sokoke), (Figure 1) (Alhaddad *et al.* 2013; Kurushima *et al.* 2013). Therefore, many cat breeds have been reported to be genetically close to landrace cats from their regions of origin (Lipinski *et al.* 2008).

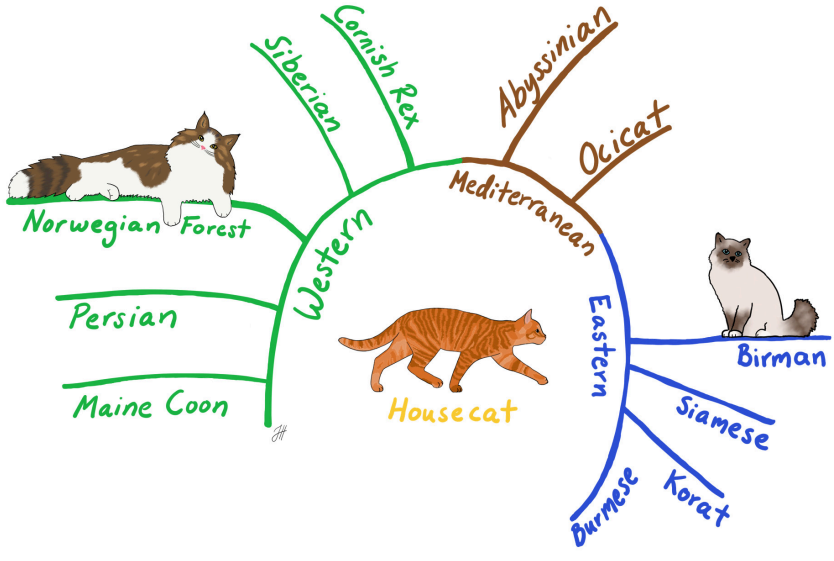


Figure 1. Schematic simplified illustration of domestic cat populations that have been reported to be genetically different; Norwegian Forest cats descend from western-derived breeds; Birman cats are descended from eastern-derived breeds. The Domestic Shorthair cat is a mixed breed cat. Inspired by (Lipinski *et al.* 2008; Alhaddad *et al.* 2013). Illustration by Jenny Hanås.

3.3 Modern cat breeds

Different cat breeds are relatively homogenous in body size and shape with the main difference being the characteristics of their coats. Purebred cats have been bred for grace, beauty and for people-friendly characteristics (Driscoll *et al.* 2009). The first cat show in Europe was held in London's Crystal Palace in 1871, which displayed only a few cat breeds (Siamese, Manx, French Persian (Angora) longhaired cats, English shorthaired cats,

Polydactyl cats, and a Scottish Wild cat). The first cat show in America was held in New York's Madison Square Garden in 1881 (Kurushima *et al.* 2013). Currently 45–73 different cat breeds are recognised by cat fancy organizations (Cat Fanciers' Association, The International Cat Association, Governing Council of the Cat Fancy, and Federation Internationale Feline).

Pedigree cats are descendants of landrace cats from discrete parts of the world which have been selected for one or more distinct traits. Cat breeding associations have tried to diversify their breed populations with randomly bred cats from the presumed ancestral origin of their breed. Therefore, most cat registries use the term pedigreed and not purebred. (Kurushima *et al.* 2013)

3.3.1 Domestic Shorthair cat

Figure 2. Domestic Shorthair cat.
Photo Majsan Stääv.

Domestic Shorthair cats are mixed-breed cats without a pedigree. These cats are commonly medium-sized and have a short coat with a wide array of colours. According to the World Cat Federation, DSH cats have a balanced, solid body structure, with proportionate head, nose and ears, (Figure 2). If a mixed-breed cat has a semi-long to long coat they are called Domestic Long-hair cat (DLH). DSH/DLH cats are randomly bred domestic crossbreeds and these mixed breed cats comprise the majority of the world's pet cats (Gandolfi & Alhaddad 2015). The genetic diversity of these randomly bred mixed breed cats has been reported to be higher than that of specific breeds that have been more highly structured and thus often exhibit lower heterozygosity (Menotti-Raymond *et al.* 2008; Gandolfi *et al.* 2018).

3.3.2 The Sacred Birman cat

Figure 3. Birman cat.
Photo Berenike Ström.

The Birman official cat breed is also known as ‘Sacred Birman’ or ‘Sacred cat of Burma’. The origin of the sacred Birman cat is obscure and this breed is surrounded by a legend. One story about the Birman is that this breed originally came from Burma where these cats were sacred companions to the priests of the temple—‘The sacred cats of Burma’. There are several stories of how the first Birman cats came to France. These cats then became the foundation of the Birman breed. The name of the breed comes from ‘Birmanie’, a French word for Burma. In the 1920s the Birman breed was recognized in France as an official breed. Breeding of Sacred Birmans was threatened during World War II, but some Birmans survived the war in France. The Birman breed was recognized in England by the Governing Council of Cat Fancy in 1966, in United States in 1967, and by The International Cat Association in 1979.

According to the breed standard, Birmans are of medium size and have a rectangular body with semi-longhair coat, round face and deep sapphire blue eyes, (Figure 3). The genetic diversity of the Birmans is very low, which is in accordance with the history of this cat breed (Lipinski *et al.* 2008; Menotti-Raymond *et al.* 2008; Gandolfi *et al.* 2018).

3.3.3 Norwegian Forest cat

Figure 4. Norwegian Forest cats. Photo Anna Eklund.

As the name indicates, the NF cat originates from Norway where it is an old breed appearing in folk tales and mythology. The NF cat probably followed the Vikings from Norway on their ships to keep the ships clear of rodents. The breed was almost lost due to hybridization with free-roaming DSH in Norway, when 1930s Norwegian cat fanciers became determined to save the breed. The NF cat was accepted as a breed in 1987.

According to the breed standard, the NF cat is a large semi-longhaired cat with a long body with a distinguishing double coat (dense undercoat covered by long smooth hairs), and almond shaped eyes with eye colours in shades of green, gold, green-gold or copper, (Figure 4). Norwegian Forest cats have been reported to have higher genetic variation than the Birman breed but lower genetic variation than DSH/DLH cats (Gandolfi *et al.* 2018).

3.4 Cats today

Cats are popular pets worldwide. Studies show that being a pet owner is associated with a lower risk of social isolation (i.e. feelings of loneliness), depression (Stanley *et al.* 2014), and decreased risk of hypertension than not owning a pet (Krittanawong *et al.* 2020). Cats in our society are valuable because cats promote well-being for humans by providing companionship, emotional support, entertainment, happiness and relaxation (Wells 2009). In our modern society, cats and dogs are highly valued, and in a study of how people conceptualize cats, cats' personalities, along with love, were found to

be the most important (Hoffmann *et al.* 2018). During the Covid-19 pandemic, a study reported that animal ownership seemed to mitigate some of the detrimental psychological effects during lockdown in the context of the social distancing and isolation. Companion animals were, furthermore, important for emotional support to their owners during the Covid-19 lockdown, regardless of species (Ratschen *et al.* 2020).

3.5 Biological variation and feline characteristics

In healthy cats (Campora *et al.* 2018), and people (Fraser & Harris 1989), biomarker variations may be affected by biological variation between and within individuals. Descriptions of biologic variation of biomarkers have been reported in people (Harris 1974; Fraser & Harris 1989), and in cats (Baral *et al.* 2014; Falkenö *et al.* 2016; Trumel *et al.* 2016), which is important for establishing normal reference intervals. For example, in healthy cats, considerable interindividual biological variation has been found in thyroid hormones, but there is considerably less intraindividual variability in healthy cats (Prieto *et al.* 2020).

The impact of feline characteristics (such as breed, sex, age, BW and BCS) on different biomarkers is relatively unexplored. There are reports of breed differences, such as high serum creatinine in Birman cats (Gunn-Moore *et al.* 2002; Paltrinieri *et al.* 2014; Öhlund *et al.* 2021), high serum alkaline phosphatase activity and calcium phosphate concertation in NF cats, and low globulin concentrations in NF cats and Siberian cats (Paltrinieri *et al.* 2014; Öhlund *et al.* 2021). One study have reported that age, sex and BW had breed-related effects on several plasma biochemical variables (creatinine, glucose and total protein) (Reynolds *et al.* 2010). Breed, sex and BW have also been reported to affect haematological and biochemical variables in Maine Coon cats (Spada *et al.* 2015). These studies have reported that interbreed differences could be important in interpreting the results of several specific biomarkers.

4. Feline cardiomyopathy

In cats, recent guidelines for the definition and classification of cardiomyopathies (CM) have been published (Luis Fuentes *et al.* 2020). These guidelines for cats with CM propose that the classification in cats should be based on structural and functional characteristics, or phenotype, (Figure 5)(Luis Fuentes *et al.* 2020). The CM phenotypes include cats with CM of known aetiology such as secondary to hypertension, hyperthyroidism,or a sarcomeric gene mutation, as well as cats with unknown aetiology (i.e. most cats with the CM phenotype). During the time when an underlying aetiology is being investigated for the CM, the cat might be diagnosed to have, for example, a 'hypertrophic cardiomyopathy phenotype' according to cardiac morphology and function. When, and if, an underlying cause is found then this information is added, like for example HCM phenotype in conjunction with hypertension (Luis Fuentes *et al.* 2020).

In cats, the HCM phenotype is the most commonly diagnosed CM phenotype (Ferasin *et al.* 2003; Paige *et al.* 2009; Payne *et al.* 2015b). Cats with the HCM phenotype have diffuse or regionally-increased LV thickness with a non-dilated left chamber (Luis Fuentes *et al.* 2020). In this thesis, the cats included in the population with cardiac disease were diagnosed with the HCM phenotype, thus other feline CM phenotypes are not further described. However, more detailed information about feline HCM phenotype is described later in this chapter. In this thesis, the HCM phenotype is referred to as HCM.

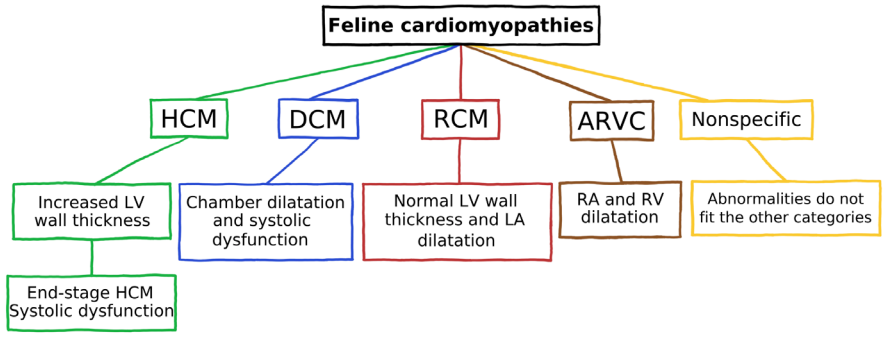


Figure 5. Proposed classification system of feline cardiomyopathies after echocardiographic phenotype. Hypertrophic cardiomyopathy can progress to end-stage HCM, which is characterised by systolic dysfunction.
HCM, hypertrophic cardiomyopathy; DCM dilated cardiomyopathy; RCM, restrictive cardiomyopathy; ARVC, arrhythmogenic right ventricular cardiomyopathy; LV, left ventricle; LA, left atrium; RA, right atrium; RV, right ventricle. Inspired by (Luis Fuentes *et al.* 2020). Illustration by Jenny Hanås.

4.1 Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy has been called a disease of the sarcomere, because HCM is primarily caused by mutations in genes that encode for sarcomeric proteins in several species, including in people. Although only a few genetic variants have hitherto been suggested associated with HCM in cats, a genetic background is also widely suspected in cats.

A sarcomere is the basic contractile unit of the cardiomyocyte. Each sarcomere consists of two main protein filaments, actin and myosin, which are responsible for muscle contraction. Damage to the structure or function of the sarcomeres cause myocardial disorders called cardiomyopathies. In cats and in people, cardiomyopathy is a disorder of the myocardium in which the heart muscle is structurally and functionally abnormal in the absence of other cardiovascular disease that could have caused this myocardial abnormality (Elliott *et al.* 2008; Luis Fuentes *et al.* 2020).

Hypertrophic cardiomyopathy is a heart muscle disease characterized by LV hypertrophy in the absence of other explanations for wall thickening (such as systemic hypertension, aortic stenosis, dehydration, and hyperthyroidism) (Campbell & Kittleson 2007; Elliott *et al.* 2008; Sugimoto *et al.* 2019; Luis Fuentes *et al.* 2020) It is a common primary cardiovascular disease in cats (Payne *et al.* 2015b) and people (Maron *et al.* 2012). The

disease was first described in people in 1958 (Teare 1958) and in cats in the 1970s (Tilley *et al.* 1977). The prevalence of HCM has been reported to be approximately 0.2% in people (McKenna *et al.* 2017). In cats, studies from the United Kingdom, have reported a prevalence of approximately 15% in certain cat populations (Paige *et al.* 2009; Wagner *et al.* 2010; Payne *et al.* 2015b), compared to approximately 3% in other cat populations (Haggstrom *et al.* 2016).

4.1.1 Characteristics of cats with HCM

Hypertrophic cardiomyopathy affects many breeds including Maine Coon, Ragdoll, British shorthair, Sphynx, Persian, and NF cats, as well as DSH/DLH cats (Kittleson *et al.* 1999; Meurs *et al.* 2005; Meurs *et al.* 2007; Gundler *et al.* 2008; Granstrom *et al.* 2011; Chetboul *et al.* 2012; Silverman *et al.* 2012; Marz *et al.* 2015). Males are overrepresented (Atkins *et al.* 1992; Rush *et al.* 2002; Ferasin *et al.* 2003; Payne *et al.* 2010; Granstrom *et al.* 2011; Trehiou-Sechi *et al.* 2012; Fox *et al.* 2018). The prevalence of HCM in cats has been reported to increase with age and the reported mean age of diagnosis is approximately 5–7 years (range: 3 months to 17 years) (Atkins *et al.* 1992; Rush *et al.* 2002; Ferasin *et al.* 2003; Abbott 2010).

4.1.2 Clinical signs

Cats affected by HCM may develop dyspnoea due to congestive heart failure (CHF), arterial thromboembolism, and may experience sudden cardiac death. In many cats with HCM the disease may remain preclinical for years (Atkins *et al.* 1992; Fox *et al.* 1995; Rush *et al.* 2002; Payne *et al.* 2010; Fox *et al.* 2018).

4.1.3 Echocardiographic diagnosis of HCM

The principal test for diagnosing LV hypertrophy and HCM in cats is echocardiography (Fox *et al.* 1995; Klues *et al.* 1995; Maron *et al.* 2003; Luis Fuentes *et al.* 2020), based on subjective impression of LV hypertrophy supported by measurement of maximal end-diastolic wall thicknesses via two-dimensional or M-mode echocardiography (Wagner *et al.* 2010; Haggstrom *et al.* 2015). In cats, LV hypertrophy is usually considered to be caused by HCM, provided that conditions such as hypertension, hyperthyroidism, and pseudohypertrophy have been excluded (Liu *et al.*

1984; Bond *et al.* 1988; Snyder *et al.* 2001; Nelson *et al.* 2002; Campbell & Kittleson 2007; Sugimoto *et al.* 2019; Luis Fuentes *et al.* 2020). Other variables, such as the presence of papillary muscle hypertrophy, end-systolic LV cavity obliteration, systolic anterior motion of the mitral valve (Schober & Todd 2010), spontaneous echo-contrast or thrombus (Schober & Maerz 2006) and left atrial size (Hansson *et al.* 2002; Abbott & MacLean 2006) are assessed during echocardiographic examination (Luis Fuentes *et al.* 2020).

4.1.4 Pathologic findings in cats with HCM

Feline HCM is characterised macroscopically by LV hypertrophy and often moderate to severe papillary muscle hypertrophy, (Figure 6B) (Fox *et al.* 1995; Maron *et al.* 2009). Left ventricular wall thickness depends on the number of myocytes, myocyte size and volume of the interstitial space. In HCM hypertrophy is caused by an increase in individual cardiomyocyte and the fibrous connective tissue mass (Unverferth *et al.* 1987).

Histopathological findings include myocardial fiber disarray, cardiomyocyte enlargement, and deformation of intramural coronary arteries with thickened media and narrowed lumen, and areas of myocardial fibrosis, (Figure 6D). These histopathological changes lead to increased LV chamber stiffness and decreased LV end diastolic volume (Maron *et al.* 1981; Maron *et al.* 1986; Liu *et al.* 1993; Fox 2003). End-stage HCM have reported pathologically remodelled LV with changes including dilatation of the chamber, wall thinning and fibrosis (Factor *et al.* 1991; Cesta *et al.* 2005; He *et al.* 2018). Figures 6A–D show a normal feline heart macroscopically and histologically compared to a feline heart affected with HCM.

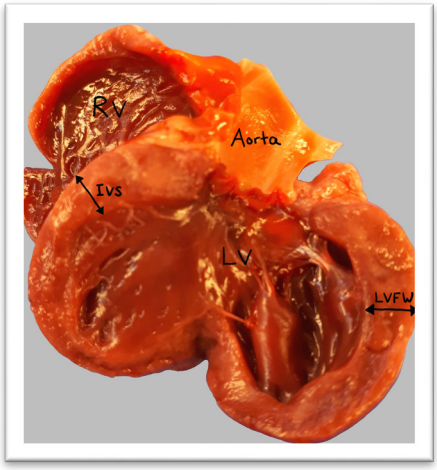


Figure 6A. Macroscopic features of a normal feline heart.

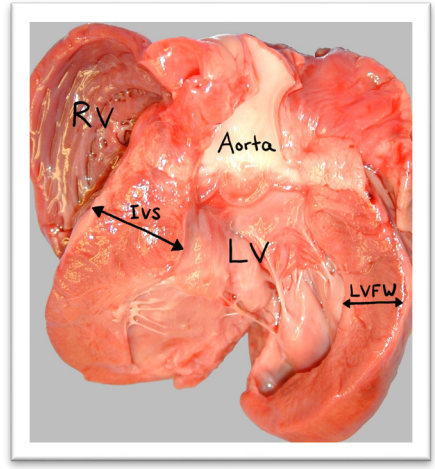


Figure 6B. Macroscopic specimen of a feline heart with concentric hypertrophic cardiomyopathy.

Figure 6A-B. Black arrows indicate the increased thicknesses of left ventricular free wall (LVFW) and interventricular septum (IVS). RV, right ventricle; LV, left ventricle. Photo Erika Karlstam.

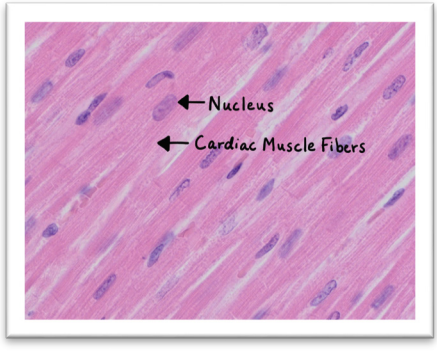


Figure 6C. Microscopic features of normal feline cardiac muscle cells (pink) showing branching cardiac muscle fibres and central nucleus (oval purple) within the cells.

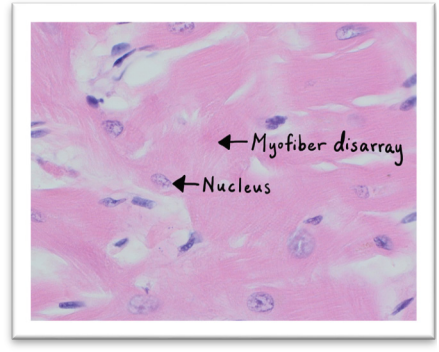


Figure 6D. Microscopic features of feline hypertrophic cardiomyopathy muscle cells (pink) showing myofiber disarray with myofiber disorientation appearing as bizarre and disorganized cellular structure.

Figure 6C-D. Hematoxylin and eosin staining and 400 times magnification. Photo Erika Karlstam.

4.1.5 HCM genetics

The first mutation associated with HCM in people, in the cardiac β-myosin heavy chain (MYH7), was sequenced in 1990 (Jaenicke *et al.* 1990). More than 1400 mutations associated with HCM have been found in people (Maron *et al.* 2012). In cats with HCM, only a few mutations have been found (Meurs *et al.* 2005; Meurs *et al.* 2007; Schipper *et al.* 2019).

The most common cause for HCM in people is mutation in a gene that encodes for a sarcomere protein (Yotti *et al.* 2019). Different mutations have been linked to HCM in domestic cats. Two of these occur in the MYBPC3 gene, one in the Maine Coon breed and another in the Ragdoll breed (Meurs *et al.* 2005; Meurs *et al.* 2007), and one the in MYH7 gene, in DSH cats (Table 2) (Schipper *et al.* 2019). There is one recent report of a mutation in the thin filament of the sarcomere, in the TNNT2 gene, found in a young male Maine Coon cat associated with HCM/CM and early CHF (McNamara *et al.* 2020). The mode of inheritance in feline HCM is considered to be autosomal dominant with incomplete penetrance and variable expressivity (Meurs *et al.* 2005; Godiksen *et al.* 2011; Longeri *et al.* 2013).

Table 2. Mutations associated with feline cardiomyopathy

Gene	Mutation	Breed	Cardiac disease	Described (year)	Authors
MYBPC3	A31P	Maine Coon	HCM	2005	Meurs et al.
MYBPC3	R820W	Ragdoll	HCM	2007	Meurs et al.
MYH7	E1883K	DSH	HCM	2019	Schipper et al.
TNNT2		Maine Coon	HCM/CM	2020	McNamara et al.
ALMS1		Sphynx	CM	2021	Meurs et al.

MYBPC, myosin binding protein-C; MYH, myosin heavy chain; DSH, domestic shorthair. Inspired by (McNamara et al. 2020)

Genetic testing for these specific mutations is recommended for Maine Coon and Ragdoll cats intended for breeding (Luis Fuentes *et al.* 2020). The gene test will indicate if the tested cat is heterozygous or homozygous. Both Maine Coon cats and Ragdoll cats that are homozygous for the mutations described within their breed, as well as first-degree relatives of cats affected by genetic HCM, have been described to have a higher risk for developing HCM (Mary *et al.* 2010; Borgeat *et al.* 2015b).

4.1.6 Prognosis for cats with HCM

Echocardiographic variables have been associated with prognosis in cats with HCM. Studies have shown that cats with HCM and LAE have decreased survival times compared to cats with HCM without LAE. (Fox *et al.* 1995; Rush *et al.* 2002; Payne *et al.* 2010; Payne *et al.* 2013; Schober *et al.* 2013). Presence of extreme LV hypertrophy have been reported to be a predictor of cardiac death (Fox *et al.* 1995; Payne *et al.* 2013). The cardiac biomarkers NT-proBNP and cTnI have been reported to be of prognostic value in cats with HCM. High concentrations of cTnI are associated with worse outcomes (Borgeat *et al.* 2014; Langhorn *et al.* 2014). A high concentration of NT-proBNP upon initial examination in cats with preclinical HCM has been reported to increase the risk for developing CHF, arterial thromboembolism or sudden cardiac death (Ironside *et al.* 2021).

4.1.7 Similarities between cats and people

There are several clinical, phenotypical (morphological and histopathological) and genetic similarities between cats and people with HCM. Spontaneously occurring feline HCM has, therefore, been suggested as a suitable animal model for HCM in people (Maron & Fox 2015; Freeman *et al.* 2017; Ueda & Stern 2017). For both cats and people, HCM clinical presentation varies from preclinical presentation of the disease (without any clinical signs), to severe signs of CHF, atrial fibrillation and sudden cardiac death (Elliott *et al.* 2014; O'Mahony *et al.* 2014), with males predisposed to acquiring the disease (Freeman *et al.* 2017).

5. Blood pressure

Blood pressure is a measurable physiologic cardiac biomarker. In cats, SBP increases with increasing age (Bodey & Sansom 1998; Bijsmans et al. 2015). The risk for developing systolic hypertension also increases with increasing age in cats (Jepson 2011). Many cats with hypertension have been reported to have underlying diseases such as kidney disease (Syme et al. 2002; Bijsmans et al. 2015), and hyperthyroidism (Kobayashi *et al.* 1990). Systemic hypertension can lead to LV hypertrophy due to increased systemic vascular resistance (Snyder *et al.* 2001; Nelson *et al.* 2002; Brown *et al.* 2007; Taylor *et al.* 2017; Acierno *et al.* 2018). In cats with LV hypertrophy, it is important to exclude hypertension and other non-cardiac diseases such as hyperthyroidism and hypersomatotropism (acromegaly) (Myers *et al.* 2014) when diagnosing HCM. In healthy cats, only few reports about the potential effect of how different clinical settings are associated with BP and pulse rate (PR) have been published (Quimby *et al.* 2011; Nibblett *et al.* 2015). In healthy dogs, breed differences have been identified for BP and PR (Bodey & Michell 1996; Hoglund *et al.* 2012), whereas in healthy cats studies specifically designed to investigate differences between breeds for these variables are lacking.

Systemic arterial BP is the force exerted from the pressure of blood flow on arterial walls. Systemic BP is divided into three categories: systolic arterial BP (SBP), mean arterial BP (MAP), and diastolic arterial BP (DBP). Systolic BP is the maximum pressure within the artery of each cardiac cycle, whereas DBP is the minimum pressure within the artery of each cardiac cycle (Skelding & Valverde 2020). Mean arterial BP is the average arterial pressure during a single cardiac cycle, comprising both systolic and diastolic pressures.

Regulation of BP is complex and involves cardiovascular, renal, nervous and endocrine systems, in combination with local tissue factors, to maintain BP within a narrow range. Blood pressure is influenced by cardiac output, and systemic vascular resistance (SVR). Cardiac output is influenced by HR and stroke volume (SV). The formula for MAP = (HR*SV)*SVR (Taylor *et al.* 2017). Arterial BP is a continuous variable that is characterised by significant variability deriving from complex interaction among hemodynamic, neuronal, humoral, behavioural, and environmental factors (Parati *et al.* 2013).

In cats, BP measurement is indicated in conditions associated with suspected systemic hypertension or hypotension, echocardiographic evidence of hypertrophy of the heart, renal disease, endocrine disease, or as an assessment of clinical status in an older cat. Blood pressure is also used for monitoring cardiovascular status of an animal during anaesthesia (Brown *et al.* 2007; Acierno *et al.* 2018). Increased BP may cause injury to eyes, the central nervous system, heart, and kidneys. In cats with systemic hypertension clinical manifestations include clinical signs such as acute blindness (haemorrhagic retinopathy), ataxia or disorientation (cerebral haemorrhage), LV hypertrophy and deteriorating renal function. An injury caused by systemic hypertension is referred to as 'end organ damage', i.e. target organ damage (Brown *et al.* 2007; Acierno *et al.* 2018).

5.1 Blood pressure measurements

In 1733, Stephen Hales performed the first direct BP measurement in a horse. Hales documented the rise and fall of blood with each pulse (pulse pressure) (Roguin 2006). In the beginning of the 1900s, the first simple indirect technique to measure arterial pressure was developed by the Russian surgeon Nikolai Korotkoff, using the maximum and minimum measurements of the arterial pulse. He incidentally invented the auscultatory measurement technique during the Russo-Japanese war, when he was ascertaining whether injured limbs would sustain circulation after ligature. In 1905, Dr. Nikolai Korotkoff presented his method "On the issue of the methods for measuring BP" at the Imperial Military Medical Academy in St Petersburg, Russia (Paskalev et al. 2005). In 1939, Korotkoff's auscultatory method for determining BP was accepted by cardiac societies in America and Great Britain as a standard technique for arterial BP measurement (Bramwell *et al.*

1939). The Korotkoff measurement technique for BP measurement is still used in people all over the world without any substantial improvements to the technique. However, automated techniques for BP measurement have been gradually introduced, including oscillometric BP measurement techniques in the 1970s and 1980s (Pickering *et al.* 2005).

5.2 Blood pressure measurement methods

As indicated above, BP can be measured as *direct BP* (invasive, requires a direct intra-arterial access for measurement) or *indirect BP* (non-invasive, by devices incorporating a cuff for measurement). Direct BP is more accurate and is considered the gold standard technique for assessing BP. However, direct BP is not practical for hypertension screening or for monitoring treatment for hypertension (Brown et al. 2007; Acierno et al. 2018). Telemetric BP measurement offers the possibility of automatically recording BP and HR via an implanted monitor; an invasive but direct BP measurement method. The telemetry system, used for research purposes, can record BP measurements in freely moving cats (Table 3) (Belew *et al.* 1999; Mishina *et al.* 2006).

All indirect BP methods use an inflatable cuff around an extremity (usually the base of either the tail or front leg in cats). The pressure in this cuff is measured by either a manometer or a transducer. To inflate the cuff, a squeeze bulb is used or the oscillometric machine automatically increases the pressure in the cuff so that it exceeds SBP, thereby occluding the artery. The cuff is then gradually deflated, either manually or automatically, by the machine, and changes in arterial flow are detected, which then are related to SBP, MAP, and DBP. The method of detection of the different BP variables varies between different indirect BP methods (Brown & Henik 1998).

For BP measurement, both the radial artery (front leg) and the coccygeal artery (tail) have been recommended in cats (Figure 7) (Brown et al. 2007). Slightly higher SBP has been obtained from the tail than from the front leg (Cannon & Brett 2012), but another report found no significant difference between these sites (Mishina et al. 1998). In cats that are awake, measurements from the tail were better tolerated and resulted in fewer failures than those from the front leg (Cannon & Brett 2012). According to current American College of Veterinary Internal Medicine guidelines, BP should be measured using a device validated for the species of interest, under

the circumstances in which the animal is being tested (Acierno *et al.* 2018). According to these standards, neither the Doppler ultrasonic sphygmomanometric nor the HDO technique has been fully validated for the use in cats (Brown *et al.* 2007; Burkitt Creedon 2013).

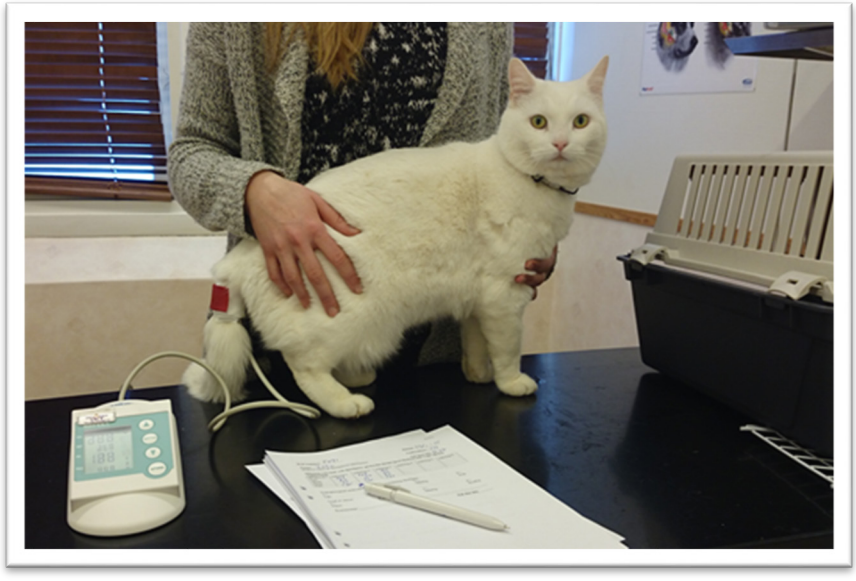


Figure 7. Blood pressure cuff on base of tail. Photo Sofia Hanås with permission from the owner.

5.2.1 Korotkoff's indirect auscultatory method

When using Korotkoff's auscultation method, developed in the early 1900s, for BP measurement in people, a stethoscope is placed over the brachial artery (major artery in the upper arm), making sounds from turbulent blood flow that can be heard when the pressure applied by the cuff is less than SBP and greater than DBP. The cuff is initially inflated to a level higher than SBP, thus completely occluding the artery, and no blood flow and no sound is auscultated. Cuff pressure is then slowly decreased and when the cuff pressure is less than the SBP, Korotkoff's sounds are first heard, due to turbulent blood flow through the partially occluded artery. Cuff pressure is slowly decreased further, and when cuff pressure reaches DBP pressure, the sound disappears (Figure 8) (Pickering *et al.* 2005).

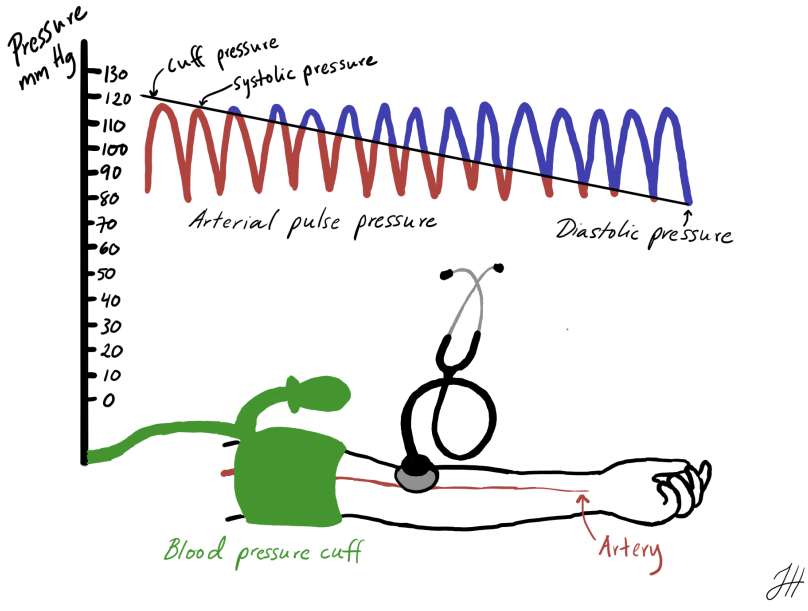


Figure 8. Auscultatory blood pressure (BP) method, Korotkoff's method, for indirect systolic (SBP) and diastolic blood pressure (DBP) measurement over the brachial artery. The arterial pulse pressure curve (APPC) on the top illustrates the points when the SBP and DBP are recorded. When the APPC is coloured red, the blood flow is occluded by the cuff, when the APPC is coloured blue, the blood flow is returning to the brachial artery as the cuff gradually deflates during the BP measurement. Inspired by a picture in (Nichols *et al.* 2011).
Illustration by Jenny Hanås.

5.2.2 Indirect Doppler ultrasonic sphygmomanometry

Doppler ultrasonic flowmeters detect a change in blood flow by using the Doppler shift, i.e. the change in frequency of the reflected sound caused by the motion of red blood cells inside the artery where BP is being measured. The operator listens to sounds from the flow detector and then reads the actual BP from a manometer connected to the occluding cuff proximal to the Doppler transducer (Brown & Henik 1998).

5.2.3 Indirect oscillometry

Traditional oscillometry

Oscillometric BP devices detect pressure fluctuations that are produced in the occluding cuff from arterial pulse pressure. A pressure sensor detects

these pressure oscillations in the cuff. The maximum oscillations are approximately the MAP, provided that the cuff size is correct (Mauck *et al.* 1980). Systolic BP and DBP can then be estimated indirectly, using empirically-derived algorithms. A regular pulse is a prerequisite to correctly calculate SBP and DBP. The advantages of this method are that no transducer needs to be placed over the artery, facilitating cuff placement, and the fact that the technique is less sensitive to external noise. Disadvantages are that the oscillations depend on factors other than BP; such as movement artefacts, (they are not tolerant of physical activity), and algorithms for calculating SBP and DBP differ between manufacturers. The oscillometric technique is used in ambulatory BP monitors, home monitors and for multiple measurements in the clinic (Pickering *et al.* 2005).

High definition oscillometry

The high definition oscillometric method (HDO) is a further development of the traditional oscillometric method. It has been reported to be a real-time analysis of arterial oscillations, performed to measure the pulse amplitudes for SBP, MAP, and DBP separately (Schmelting *et al.* 2009). In cats that are awake, HDO has been reported to overestimate SBP by approximately 10 mmHg and mildly underestimate DBP (Burkitt Creedon 2013; Martel *et al.* 2013). Reports indicate that the HDO method has fewer failures in obtaining BP readings in awake cats, compared to traditional oscillometry (Cannon & Brett 2012; Martel *et al.* 2013).

5.3 Challenges in the clinical setting

Increased BP in otherwise normotensive animals as a sequel to excitement, stress, and the anxiety of being at a veterinary clinic is termed *situational hypertension* (Acierno *et al.* 2018). Situational hypertension (previously called the 'white-coat effect') is caused by physiological stimuli that trigger the autonomous nervous system (Belew *et al.* 1999; Brown *et al.* 2007; Acierno *et al.* 2018). In cats, the average reported increases of BP and HR during a simulated veterinary visit were SBP 17±6 mmHg, MAP 14±6 mmHg, DBP 13±5 mmHg, and HR 27±8 beats/minute (Belew *et al.* 1999). To reduce stress during veterinary visits, American Animal Hospital Association and International Society of Feline Medicine have established feline-friendly handling guidelines (Rodan *et al.* 2011). To minimize

situational hypertension, BP measurement is preferably performed in a quiet room, before other procedures and after the cat has been acclimated to the environment for 5–10 minutes. Preferably, minimal restraint should be used during the measurement procedure. Current guidelines have recommended that the first BP measurement should be discarded and that 5–7 consecutive consistent BP values should be recorded (Acierno et al. 2018). Blood pressure measurement results are also influenced by external variables such as the operator in people (Mancia *et al.* 1987), dogs (Lyberg *et al.* 2021) and cats (Gouni *et al.* 2015), as well as by the presence of the owner during BP measurement in dogs (Hoglund *et al.* 2012). A minor diurnal effect <3 mmHg on BP in cats has been reported, with higher mean BP values during the day than during the night, although variations in BP were mostly associated with the presence of laboratory personnel during measurement (Brown *et al.* 1997). Blood pressure values and HR for normal awake cats are presented in Table 3.

Table 3. Arterial blood pressure values (mmHg) and heart rate (HR) obtained from awake healthy cats. Data are presented as mean ± standard deviation (SD)

Blood pressure method	**N**	**SBP (mmHg)**	**MAP (mmHg)**	**DBP (mmHg)**	**HR (bpm)**	**References**
Telemetric direct intra-arterial	20	118 ± 11	95 ± 10	78 ± 9	141 ± 31	(Mishina et al. 2006)
	6	126 ± 4	106 ± 5	91 ± 6	181 ± 4	(Belew et al. 1999)
Direct intra-arterial	21	132 ± 9	115 ± 8	96 ± 8	NA	(Slingerland et al. 2008)
	6	136 ± 13	117 ± 12	101 ± 9	196 ± 39	(Pypendop et al. 2017)
Oscillo-metry	104	139 ± 27	99 ± 27	77 ± 25	178 ± 26	(Bodey & Sansom 1998)
	60	115 ± 10	96 ± 12	74 ± 11	154 ± 27	(Mishina et al. 1998)

N, number of cats in the study; SBP, systolic blood pressure, MAP, mean arterial blood pressure, DBP, diastolic blood pressure; HR, heart rate; bpm, beats/minute.

5.4 Associations with feline characteristics

Feline characteristics have been reported to be associated with BP measurement results (Table 4).

Table 4. Feline characteristics reported to be associated with blood pressure

Variable	Blood pressure	References
Sex	Increased in males than in females. Increased in neutered cats than in intact cats.	(Lin *et al.* 2006; Payne *et al.* 2017)
Age	Increased with increasing age	(Bodey & Sansom 1998; Sansom et al. 2004; Lin et al. 2006; Bijsmans et al. 2015; Payne et al. 2017)
BW	Increased with increasing BW	(Payne et al. 2017)
BCS	Decreased with low BCS	(Payne et al. 2017)

BW, body weight; BCS, body condition score.

6. Natriuretic peptides - markers of hemodynamic stress

Natriuretic peptides are biomarkers that reflect myocardial wall stretch. These peptides serve a physiological purpose by maintaining homeostasis in the cardiovascular system by serving as counter-regulatory hormones for volume and pressure overload (Maisel *et al.* 2018). The natriuretic peptides include atrial natriuretic peptide (ANP), brain or B-type natriuretic peptide (BNP), and C-type natriuretic peptide. Both ANP and BNP are produced by cardiomyocytes and have hormonal activities (Nakagawa *et al.* 2019).

In 1981, it was reported that atrial tissue extract from rats was capable of inducing diuresis, natriuresis, and lowering BP (de Bold *et al.* 1981). Soon after, ANP was purified from human atria (Kangawa & Matsuo 1984), as the first natriuretic peptide (NP) (de Bold & Flynn 1983; Flynn *et al.* 1983). In 1988, brain natriuretic peptide, later renamed and replaced by B-type natriuretic peptide (BNP), was isolated from the porcine brain (Sudoh *et al.* 1988). The discovery of NPs showed that the heart has endocrine functions (Ogawa & de Bold 2014). The physiological effects of ANP and BNP include functions such as diuresis, natriuresis, vasodilation, and inhibition of aldosterone synthesis and renin secretion, enabling regulation of BP and fluid volume (Nakagawa *et al.* 2019). In healthy people, and in healthy dogs, BNP is produced in the atria, but ventricles and atria contribute to production of BNP in dogs and people with CHF in response to myocardial stretch, volume, and pressure overload (Mukoyama *et al.* 1991; Nakao *et al.* 1991; Luchner *et al.* 1998). In people and rats, BNP has been reported to be stored in small amounts in atrial granules with ANP granules (Nakamura *et al.* 1991; Ogawa *et al.* 1999). The increase in ANP and BNP secretion is proportional to cardiac dysfunction severity. These findings suggest that ANP and BNP secretion may be regulated by LV and LA wall tension (Yasue

et al. 1994; Ogawa *et al.* 1996; Bruneau *et al.* 1997). In people, a short half-life has been reported for ANP of approximately 2 minutes (Nakao *et al.* 1986), whereas BNP has been reported to have a half-life of approximately 22 minutes (Holmes *et al.* 1993).

6.1 N-terminal segment of prohormone BNP

In 1995, the cardiac biomarker N-terminal segment of prohormone BNP (NT-proBNP) was identified in human plasma (Hunt *et al.* 1995). In people, BNP is synthesized in the cardiomyocytes as a precursor protein, pre-proBNP. Pre-proBNP is subsequently processed to form the peptide, prohormone BNP (proBNP) formed by 108 amino acids. The proteolytic enzyme furin cleaves proBNP into the biologically active BNP (with a short half-life), formed by 32 amino acids, and the inactive, and more stable, NT-proBNP, formed by 76 amino acids present in plasma (Figure 9). The biomarker NT-proBNP can be measured using an immunoassay in people (Weber & Hamm 2006; Daniels & Maisel 2007), as well as in dogs and cats (de Lima & Ferreira 2017). N-terminal segment of prohormone BNP concentration has been reported to be increased in cats, dogs, (de Lima & Ferreira 2017) and people with cardiac diseases (Goetze 2012).

The reported ratio of production of BNP and NT-proBNP is 1:1. However, the plasma concentration of NT-proBNP is reported to be several times higher than the concentration of BNP, which may be explained by a slower elimination of NT-proBNP from the blood. In people, the half-life of NT-proBNP in the circulation is approximately 70 minutes (Pemberton *et al.* 2000; Yang *et al.* 2020).

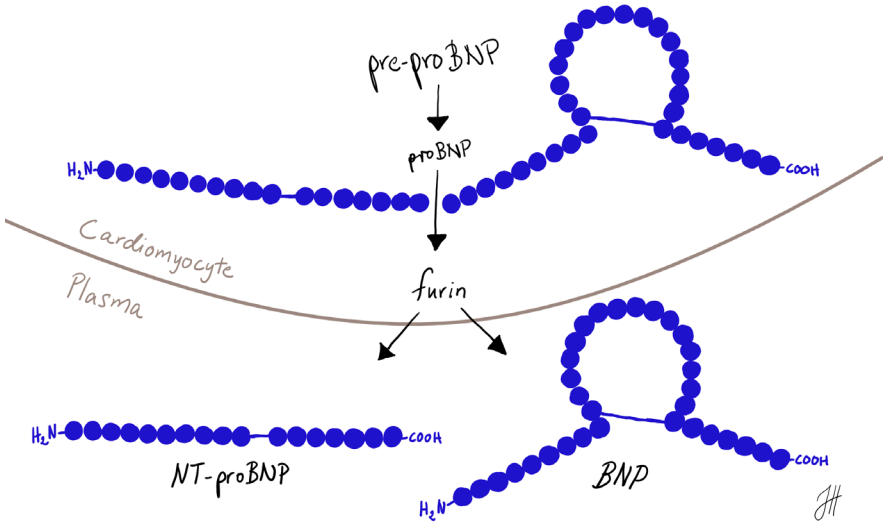


Figure 9. Synthesis of biologically active B-type natriuretic peptide (BNP) and N-terminal prohormone B-type natriuretic peptide (NT-proBNP) by enzymatic cleavage of prohormone-BNP (proBNP) by the proteolytic enzyme furin. Note that this is a simplified schematic figure and the illustration does not correspond to exact molecules. Illustration by Jenny Hanås.

6.2 Congestive heart failure and natriuretic peptides

Congestive heart failure is characterized by activation of several neurohormonal systems such as the renin-angiotensin-aldosterone system (RAAS), the sympathetic nervous system, and NPs. The RAAS increases sodium and water reabsorption and increases vascular tone to elevate BP and blood volume, thereby regulating BP on a long-term basis (Figure 10). The sympathetic nervous system has several cardiovascular effects including HR acceleration, increased contractility, reduced venous capacitance, and peripheral vasoconstriction. The hormone BNP has also been reported to have anti-fibrotic and anti-hypertrophic effects on the heart (Calvieri *et al.* 2012). In cats with CHF and respiratory distress, both quantitative ELISA and point-of-care tests (POCT) for NT-proBNP analysis have been useful in diagnosing CHF (Connolly *et al.* 2009; Fox *et al.* 2009; Wurtinger *et al.* 2017; Ward *et al.* 2018).

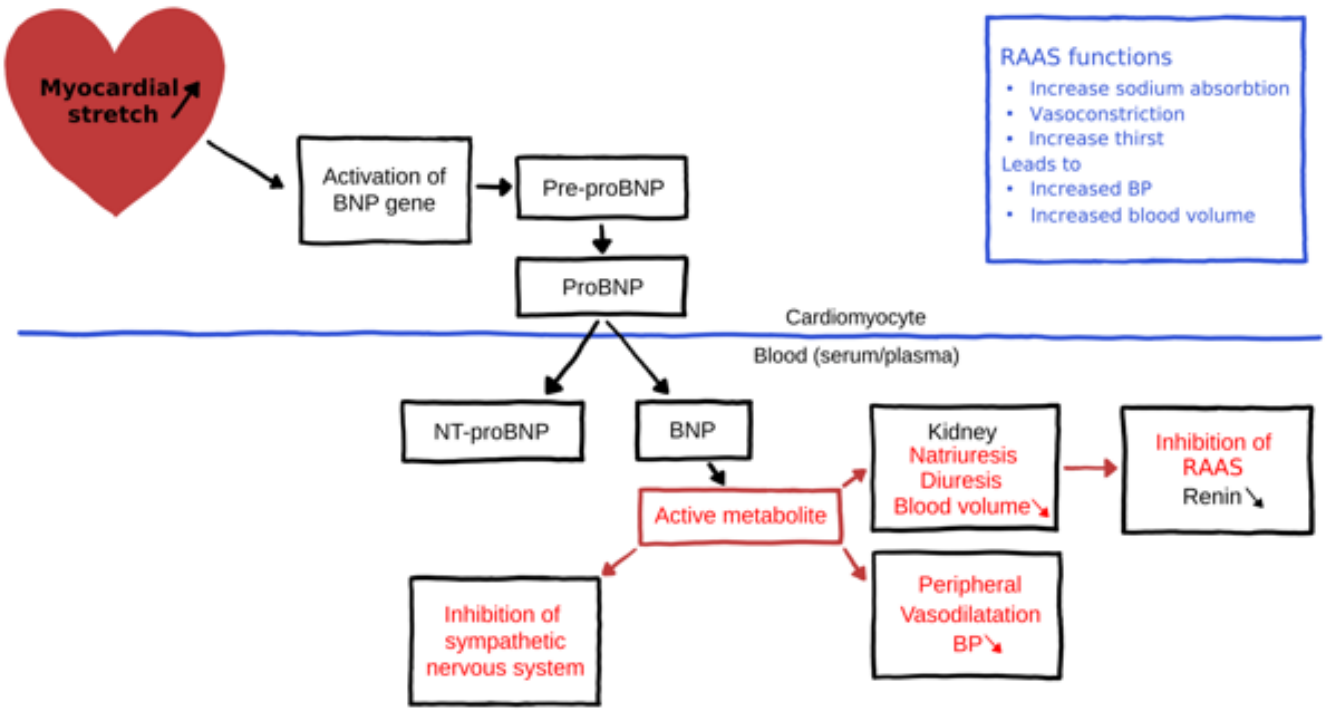


Figure 10. Myocardial stretch initiates the production of B-type natriuretic peptide (BNP). The active BNP metabolite causes a decrease in blood volume and blood pressure (BP), and inhibits the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS). The functions of RAAS are noted in the blue box to the right in the figure. Illustration by Jenny Hanås.

6.3 Immunoassays for natriuretic peptides

Structural differences for mammalian BNP have been reported in specific species, and BNP is therefore considered species-specific (Sudoh *et al.* 1989). The entire feline BNP gene has been sequenced and characterized, enabling production of feline ELISA tests for measuring NT-proBNP (Liu *et al.* 2002). Two NT-proBNP ELISAs have been validated for cats (Fox *et al.* 2009; Mainville *et al.* 2015).

6.3.1 First- and second-generation immunoassays

The first-generation of feline NT-proBNP immunoassays measured NT-proBNP in plasma samples with the reported intra- and inter-assay coefficients of variation (CV) < 15% (Fox et al. 2009; Fox et al. 2011). N-terminal-prohormone-B-type natriuretic peptide was reported to be unstable at room temperature and specialized protease inhibitor tubes for EDTA plasma samples were therefore required (Connolly et al. 2011). This assay used purified sheep antibodies for both capture and detection (Fox *et al.* 2009). To overcome the issue of room temperature instability, a second-generation feline NT-proBNP immunoassay was developed and validated for serum and plasma with maintained CV. The second-generation assay contained anti-feline NT-proBNP capture and detection antibodies which target more stable epitopes on the N-terminal portion of NT-proBNP fragment than the first-generation assay (Machen *et al.* 2014; Hezzell *et al.* 2016). The antibodies directed to this portion of the NT-proBNP fragment facilitate analyte detection without necessitating specialized protease inhibitor tubes. This ELISA is performed at a commercial laboratory; limiting the usefulness of this assay during an emergency situation (Mainville *et al.* 2015).

6.3.2 Point-of-care test

A semi-quantitative POCT that measures feline NT-proBNP concentration and provides results at the clinic is available. The POCT is a colorimetric ELISA which provides results based on the colour of the patient sample spot compared to the reference spot (Figure 11) (Machen *et al.* 2014; Harris *et al.* 2017a). The POCT assay results have been reported to be normal or abnormal based on a cut-off concentration of approximately 100 pmol/l according to the manufacturer (SNAP Feline proBNP, IDEXX Laboratories

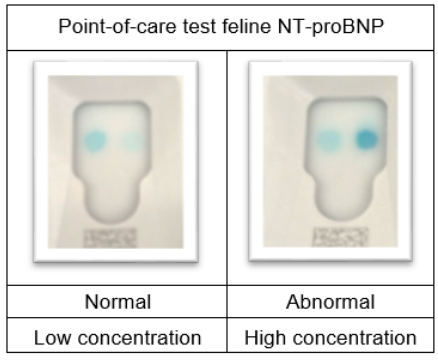


Figure 11. Point-of-care test for feline N-terminal-prohormone-B-type natriuretic peptide (NT-proBNP). The sample spot is on the right and the reference spot is on the left (should be light blue). Weak colour on sample spot implies low concentration and strong colour implies high concentration of NT-proBNP. Photo Sofia Hanås.

Inc). The POCT has been reported to give positive results in a transition interval between 108 and 200 pmol/l (Machen *et al.* 2014; Harris *et al.* 2017a). The POCT uses the same second-generation anti-NT-proBNP antibodies as the second-generation ELISA (Machen *et al.* 2014; Hezzell *et al.* 2016).

6.4 Storage, stability and biological variation

Using the first-generation feline NT-proBNP ELISA, feline NT-proBNP concentration was stable when measured in plasma samples stored at −80 C for up to 10 years (Lalor *et al.* 2009). The stability using the second-generation feline NT-proBNP assay measured by percent baseline recovery has been reported to be >94% for samples held for 7 days at 4°C, and >80% for samples held at 25°C for 3 days (Mainville *et al.* 2015). In people, the *in vitro* stability of EDTA plasma concentrations of NT-proBNP in room temperature has been reported to be at least three days (Yeo *et al.* 2003), and at least 2 years at −20°C (Cauliez *et al.* 2008). It is stable for more than one year at −80°, and after at least five freeze−thaw cycles (Nowatzke & Cole 2003).

There are reports indicating that there is high biological variation for NT-proBNP concentrations in cats (Harris *et al.* 2017b), dogs (Kellihan *et al.* 2009), and people (Melzi d'Eril *et al.* 2003). No diurnal variation was reported in plasma samples from people (Ludka *et al.* 2010; Crnko *et al.* 2020).

6.5 Natriuretic peptides in cats with cardiac disease

Studies have shown that plasma NT-proBNP analysed with both ELISA and POCT could discriminate cats with cardiac disease from healthy cats (Table 5). Cats with various cardiac diseases were included in these studies.

Table 5. Studies of the plasma concentration of NT-proBNP in cats.

Indication	N	Assay	Cut-off	References
Quantitative ELISA				
Differentiate cardiac vs. respiratory disease	21	CP	258 pmol/l	(Hassdenteufel et al. 2013)
Differentiate cardiac vs. respiratory disease	167	CP	265 pmol/l	(Fox et al. 2009)
Differentiate cardiac vs. respiratory disease	40	CP	214 pmol/l	(Humm et al. 2013)
Detect severe HCM	41	FC	44pmol/l	(Hsu et al. 2009)
HCM screening and assessment of disease severity	201		100 pmol/l	(Wess et al. 2011)
HCM screening and assessment of disease severity	227	CP	99 pmol/l	(Fox et al. 2011)
Detect moderate to severe heart disease	146	CP	100 pmol/l	(Machen et al. 2014)
HCM screening	88	CP	95 pmol/l	(Tominaga et al. 2011)
Detect cardiac disease	78	CS	49 fmol/ml	(Connolly et al. 2008)
Semi-quantitative ELISA (point-of-care test)				
Detect moderate to severe heart disease	146	SNAP	100 pmol/l	(Machen et al. 2014)
Detect cardiac disease	53	SNAP	100 pmol/l	(Harris et al. 2017a)

N, number of cats; HCM, hypertrophic cardiomyopathy; CS, Cardioscreen NT-proBNP (Guildhay Ltd); CP, Cardiopet proBNP (IDEXX Ltd); SNAP, SNAP Feline proBNP (IDEXX Ltd); CC, Feline CardioCare NT-proBNP assay (Veterinary Diagnostics Institute)

6.6 Associations with feline characteristics

In cats, no associations between the concentration of NT-proBNP and breed (Wess *et al.* 2011), sex (Fox *et al.* 2011; Wess *et al.* 2011), age (Wess *et al.* 2011; Humm *et al.* 2013), BW (Wess *et al.* 2011), or BCS (Humm *et al.* 2013) have been previously reported. However, in dogs, considerable breed

variation has been reported (Sjöstrand *et al.* 2014; Couto *et al.* 2015). In people, both age and sex have been reported to influence the concentration of NT-proBNP (Redfield *et al.* 2002; Loke *et al.* 2003).

6.7 Associations with other diseases

In cats (Lalor *et al.* 2009), and in people (Luchner *et al.* 2005; Takase & Dohi 2014) the concentrations of NT-proBNP have been reported to increase with impaired renal function. In people with normal kidney function, it has been suggested that approximately 17% of secreted BNP is metabolized and excreted by the kidney, whereas the remaining BNP then either binds to its receptors or becomes inactivated by endopeptidases. Furthermore, in people, NT-proBNP has been reported to be excreted into urine without being metabolized (Takase & Dohi 2014). Systemic hypertension (Lalor *et al.* 2009; Bijsmans *et al.* 2017) and hyperthyroidism (Peterson & Ward 2007; Menaut *et al.* 2012; Sangster *et al.* 2014) increase the concentration of NT-proBNP in cats.

7. Troponin: a marker of myocardial injury

Cardiac troponins are biomarkers for myocardial damage. Troponin was discovered by the Japanese physiologist Setsuro Ebashi and his co-worker Ayako Kodama in 1965 (Ebashi & Kodama 1965).

7.1 The troponin complex and cardiac troponin I

The troponin complex belongs to the thin actin filament within the sarcomere, which is the contractile part of the cardiomyocyte (Figure 12). The sarcomere contains two protein filaments, thin actin filaments and thick myosin filaments. These filaments slide past each other when the cardiomyocyte contracts (Craig & Woodhead 2006).

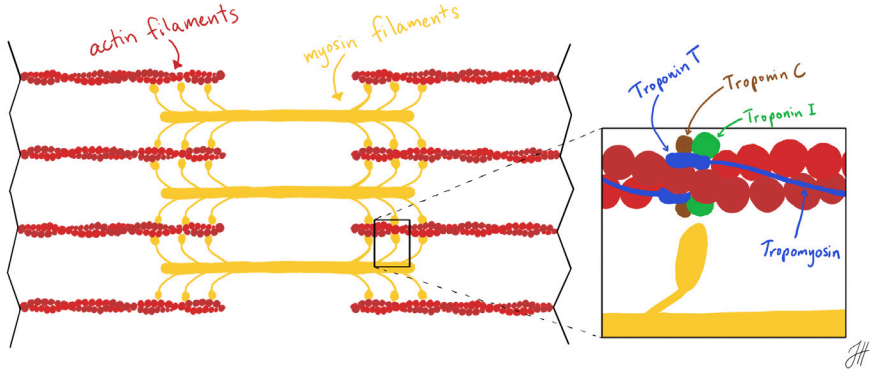


Figure 12. The troponin complex is comprised of troponin C, troponin I and troponin T. The complex is a small piece within the sarcomere, the contractile unit of the cardiomyocyte, with thick myosin filaments and thin actin filaments. Note that this is a simplified schematic figure, and the illustration does not correspond to molecular size or exact morphology. Illustration by Jenny Hanås.

The troponin complex, along with calcium ions, regulate the interaction between actin and myosin and thereby regulates the cardiomyocyte contraction. The complex consists of three isoforms: troponin C, which binds to calcium ions, troponin I (cTnI), which binds to actin to hold the actin-tropomyosin complex in place, and troponin T (Figure 12), which binds to tropomyosin forming a troponin-tropomyosin complex. Because cTnI and cardiac troponin T are specific to cardiomyocytes these are called *cardiac troponins* (cTn). Approximately 95% of troponins are bound to actin filaments in the sarcomere, with the remaining part floating free in myocyte cytoplasm enabling free troponin to easily be released into circulation (O'Brien *et al.* 2006; White 2011; Sternberg *et al.* 2019).

In people, cTnI appears in circulation 4−10 hours after myocardial injury with a peak at 12−48 hours (Jaffe *et al.* 1996). The half-life of circulating cTnI has been reported to be approximately two hours when only free cytosolic cTnI is released. The half-life has been reported to be considerably longer (4−10 days) if bound cTnI is released, due to a slow breakdown of the contractile apparatus, as is the case with irreversible myocardial injury with necrosis. The release of these contractile proteins from the myocardium has been reported to be proportional to the degree of myocardial injury (O'Brien *et al.* 2006; White 2011; Sternberg *et al.* 2019). Rapid increase and decrease of cTnI within a day may be due to release of the free cTnI and reversible myocardial damage (membrane injury with leakage of cTnI). In cats and dogs, the release kinetics of cTnI has been presumed to be similar to that in people (Langhorn & Willesen 2016). In dogs, the biological half-life has been reported to be approximately 70 minutes (Jaffe *et al.* 1996).

7.2 Immunoassays for cardiac troponin I

Immunoassays for cTnI measurement have been developed by several manufacturers. The assays apply antibodies targeting different amino acid sequences and, therefore, results between different assays are not comparable (Apple & Collinson 2012). Cardiac TnI has been reported to be well conserved among species (people, dogs, cats, and rats) (Rishniw *et al.* 2004), and human cTnI assays can be used for samples from cats. The cTnI gene has been cloned and sequenced in cats and dogs (Rishniw *et al.* 2004). Two human cTnI assays (one conventional cTnI assay and one high-sensitivity (hs) cTnI assay) have been validated for cats (Langhorn *et al.* 2013b;

Langhorn *et al.* 2019b). Importantly, significantly elevated cTnI concentrations—frequently much higher concentrations than found in primary cardiac diseases in cats and dogs—have also been found in non-cardiac conditions such as critical illness (Sharpe *et al.* 2020), traumatic myocardial injury (Schober *et al.* 1999; Biddick *et al.* 2020), heatstroke (Mellor *et al.* 2006), and snakebite (Pelander *et al.* 2010; Harjen *et al.* 2020). Therefore, to use cTnI assays to diagnose primary structural heart disease may be difficult, but cTnI concentration has been reported to provide prognostic information for both cardiac and non-cardiac conditions in cats and dogs (Fonfara *et al.* 2010; Hezzell *et al.* 2012; Langhorn *et al.* 2013a; Borgeat *et al.* 2014; Langhorn *et al.* 2014; Hamacher *et al.* 2015). In cats with HCM, cTnI has been reported to have prognostic value. (Borgeat *et al.* 2014; Langhorn *et al.* 2014)

7.2.1 Conventional troponin assays

In 1987, the first cTnI assays were described by Bernadette Cummins et al. (Cummins *et al.* 1987). In the 1990s, measurement of cTnI concentrations using commercial assays became possible. Conventional cTnI assays, in which cTnI concentrations in healthy individuals are negligible or unmeasurable, have been validated for use in cats and dogs (Langhorn *et al.* 2019b).

7.2.2 High sensitivity troponin I assays

Currently, a high sensitivity cTnI (hs-cTnI) assay is defined as enabling detection of cTnI concentrations in at least 50% of healthy individuals, with a CV ≤10% for the 99th percentile (Apple & Collinson 2012). In people, hs-cTnI assays have been recommended over conventional cTnI assays due to higher diagnostic accuracy for coronary artery disease (Roffi *et al.* 2016; Collet *et al.* 2021). In cats and dogs, one hs-cTnI assay (the ADVIA Centaur TnI-Ultra assay) has previously been validated (Langhorn *et al.* 2013b; Winter *et al.* 2014). In people, different cut-off values are used for the available hs-cTnI assays, because of varying capture and detection antibodies and a lack of standardization (Clerico *et al.* 2017; Apple *et al.* 2020).

7.3 Storage, stability and biological variation

Human serum cTnI concentrations, measured using an hs-cTnI assay, have been described to be stable for at least one year at –80°C (Egger *et al.* 2018). Previous research in people, using an hs-cTnI assay, indicated that cTnI does not have a circadian rhythm (Wildi *et al.* 2018). However, a recent small study has shown contrasting results with significantly higher cTnI concentrations in the morning and decreasing cTnI concentrations during the day, although this was not clinically relevant (Zaninotto *et al.* 2020). Biologic intra-individual variability in healthy patients using hs-cTnI assays has been reported to be low (Kozinski *et al.* 2017).

7.4 Troponin concentration in cats

In cats, studies have reported a positive association between cTnI concentrations and hypertrophy of the LV and with LAE (Connolly *et al.* 2003; Langhorn *et al.* 2014; Hori *et al.* 2018; Hertzsch *et al.* 2019). Conventional cTnI assays have shown that cats with HCM have higher cTnI concentrations than healthy cats (Herndon *et al.* 2002; Connolly *et al.* 2003) and cTnI concentrations are usually below the detection limit in healthy cats (Langhorn *et al.* 2019b). Studies have reported detection of low cTnI concentrations in healthy cats when using hs-cTnI assays (Langhorn *et al.* 2013b; Hori *et al.* 2018; Hertzsch *et al.* 2019). Currently, there are even more sensitive assays for cTnI available than the previously validated hs-cTnI assay (Langhorn *et al.* 2013b) in cats.

7.5 Associations with feline characteristics

In previous studies in cats, cTnI concentrations have not been associated with sex or breed (Langhorn *et al.* 2016; Hori *et al.* 2018; Hertzsch *et al.* 2019). However, breed differences have been reported in healthy dogs (Baumwart *et al.* 2007; LaVecchio *et al.* 2009). Some studies in cats report positive associations between cTnI concentrations and age (Serra *et al.* 2010), and BW (Hori *et al.* 2018), while other studies report no significant associations with either age (Hori *et al.* 2018; Hertzsch *et al.* 2019), BW, or BCS (Hertzsch *et al.* 2019). In people, male sex and age have been positively associated with cTnI (Venge *et al.* 2003; Love *et al.* 2016; Kimenai *et al.* 2018; Mueller *et al.* 2018; Clerico *et al.* 2019). In healthy dogs, and in dogs

with myxomatous mitral valve disease, cTnI have been reported to be positively associated with age (Oyama & Sisson 2004; Ljungvall *et al.* 2010).

7.6 Associations with other diseases

Circulating cTnI has been reported to be a sensitive and specific biomarker for myocardial injury because cTnI has not been found outside the myocardium (Thygesen *et al.* 2010). Although cTnI is specific to the myocardium, it is not specific for primary cardiac disease, and increased cTnI concentrations have been shown in cats with hyperthyroidism (Sangster *et al.* 2014), hypertension (Bijsmans *et al.* 2017), renal disease (Langhorn *et al.* 2019a) and critical illness (Sharpe *et al.* 2020).

8. Microribonucleic acid - microRNA

Microribonucleic acids (miRNAs) are small, approximately 22 nucleotides long, regulatory non-coding, single stranded, ribonucleic acids (RNAs) that function by regulating messenger RNA (mRNA) such as inhibiting translation or stability by controlling degradation. (Bartel 2009) The definition of noncoding RNA is that the RNA molecule functions without being translated into a protein. Thus, miRNAs have been reported to control processes such as cell growth, proliferation, differentiation, apoptosis, metabolism and homeostasis and interact in physiological and pathological processes ranging from embryonic development to neoplastic progression (Bartel 2004). In cardiac disease with myocardial remodelling, miRNAs have been reported to be involved in hypertrophy, apoptosis, fibrosis, aberrant conduction and angiogenesis (Small *et al.* 2010).

The first miRNAs were discovered in 1993 by Lee and colleagues in an invertebrate model organism, the roundworm *Caenorhabditis elegans* (C elegans) (Lee *et al.* 1993). Subsequent research reported that lin-4 and let-7 genes, which previously had been described to be required for *C. elegans* development, produced noncoding RNAs, including short RNAs approximately 22 nucleotides in length instead of mRNAs (Lee *et al.* 1993; Reinhart *et al.* 2000). Subsequently, research reported exact matches to let-7 RNA sequenced in people, as well as in other animal species. Thus, these let-7 miRNAs were reported to be highly conserved among animal species (Pasquinelli *et al.* 2000). Further studies have reported that these short noncoding RNAs were part of a larger class of small RNAs (Lagos-Quintana *et al.* 2001; Lau *et al.* 2001; Lee & Ambros 2001). When the first of these small noncoding RNAs were identified their functions were not known, and they were named ‘microRNAs’ (Lagos-Quintana *et al.* 2001; Lau *et al.* 2001; Lee & Ambros 2001).

Levels of miRNAs in serum have been reported to be stable and consistent among individuals of the same species (Chen *et al.* 2008b). Moreover, the number of miRNAs detected in an animal or organism have been reported to correlate well with organismal complexity, so that nematodes have less miRNA genes than mammals (Friedländer *et al.* 2014). It has been suggested that circulating miRNAs can be used as blood-based biomarkers to diagnose and monitor disease (Gilad *et al.* 2008; Mitchell *et al.* 2008; Cortez & Calin 2009).

8.1 Biogenesis of microRNA in animals

Biogenesis of miRNA in mammals is a two-step process with nuclear and cytoplasmic cleavage events, i.e. the canonical miRNA pathway (Figure 13). MicroRNA genes are transcribed in the nucleus by RNA polymerase II as long transcripts called primary transcripts (pri-) miRNA, and it is in this hairpin structure that miRNA sequences are embedded (Ha & Kim 2014). Afterwards, the microprocessor complex (Drosha a ribonuclease enzyme) cleaves pri-miRNA near the base of the primary stem loop resulting in an approximately 70 nucleotide hair-pin shaped shorter precursor molecule called the precursor- (pre-) miRNA. The pre-miRNA is then transported to the cytoplasm by the nucleocytoplasmic transport protein Exportin 5. In the cytoplasm, pre-miRNA is bound and cleaved by the endoribonuclease Dicer, in this process the loop region is recognized and both strands are cleaved from the loop. Short RNA fragments form the miRNA duplex, consisting of the mature 5p and 3p strands (Pong & Gullerova 2018). This duplex miRNA is associated with Argonaute (AGO) proteins forming the RNA-induced silencing complex (RISC). In the RISC complex one of the miRNA duplex strands is discarded and degraded (Kobayashi & Tomari 2016). The miRNA then guides the RISC to target mRNAs which then leads to gene silencing. When miRNAs bind to the mRNAs target this will lead to translation inhibition and/or mRNA degradation, resulting in no protein production (Vishnoi & Rani 2017; Pong & Gullerova 2018).

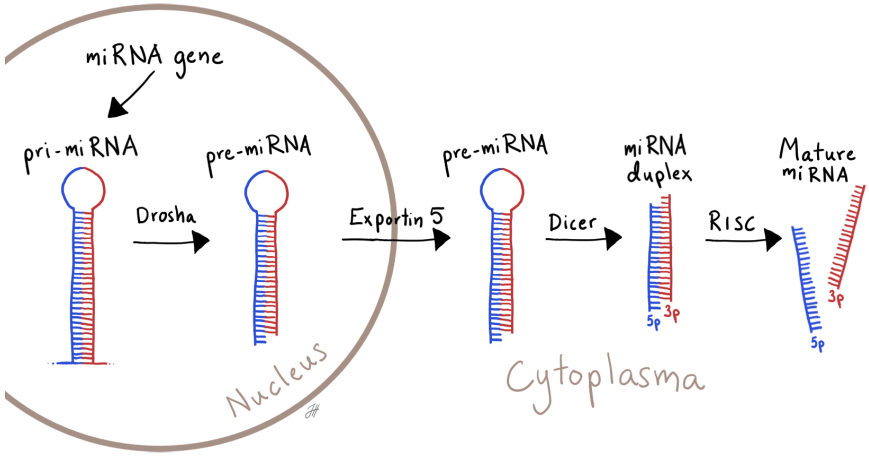


Figure 13. A schematic illustration of the canonical microRNA (miRNA) pathway, a two-step process with nuclear and cytoplasmic events for mammalian miRNA biogenesis. Note that this is a simplified schematic figure and that the illustration does not correspond to exact molecules. MiRNA, microRNA; pri-miRNA, primary transcripts microRNA; pre-miRNA; precursor microRNA; RISC, RNA-induced silencing complex; 5p, mature 5p strand; 3p, mature 3p strand. Illustration by Jenny Hanås.

8.2 Nomenclature

MicroRNAs that have been experimentally confirmed are assigned a number according to the order in which they were discovered, which is attached to the prefix “miR”. The first three letters of the miRNA signify the organism. For example, in the miRNA hsa-miR-21, hsa=*Homo sapiens*, confirmed miRNA number 21. Feline miRNA have the prefix fca=*Felis catus*. Mature miRNA is named as miR using a capitalized R, while mir using an un-capitalized r refers to both the miRNA gene and the precursor miRNA (i.e. pre-miRNA). When there are identical mature miRNA sequences, which originate from discrete precursor sequences and genomic loci, these are given identifiers that contain a numeric suffix like hsa-miR-219-1 and hsa-miR-219-2. Very closely related mature sequences which differ by only one or two nucleotides are named with a lettered suffix for example hsa-miR-130a and hsa-miR-130b (Bhaskaran & Mohan 2014). If determination of the predominantly expressed sequence is not possible, then identifiers as miR-502-5p (i.e. from the 5′ strand) and miR-502-3p (i.e. from the 3′ strand) may be assigned (Bhaskaran & Mohan 2014).

8.3 MicroRNA in biological fluids

Extracellular circulating miRNAs have been found in several biological fluids such as plasma, serum, saliva, colostrum, urine, tears, peritoneal fluid, bronchial lavage and ovarian follicular fluid. MicroRNAs have also been detected intracellularly in red blood cells (Rathjen *et al.* 2006) and erythrocyte-derived miRNAs have been reported to represent the majority of miRNAs expressed in whole blood (Chen et al. 2008a). Cells have been reported to release miRNA via small vesicles, called 'exosomes', thereby facilitating transport to other body tissues (Simons & Raposo 2009; Mathivanan *et al.* 2010).

8.4 Storage and stability

Extracellular miRNAs have been reported to be stable at room temperature for up to 4 days and over multiple freeze-thaw cycles (O'Brien *et al.* 2018). A report indicated that the stability of miRNAs in serum after long-time storage up to 10 years at –80° and –20°C was high (Grasedieck *et al.* 2012). RNA quality has been reported to decrease over time depending on sample collection, storage systems and temperature. Intracellular RNA may be degraded by endogenous nucleases (Esnault & Malter 1999). By using blood RNA tubes that contain specific reagents for stabilizing intracellular RNA at blood sampling, RNA molecules have been reported to be protected from degradation by RNases, which minimizes changes in RNA expression. Blood has been stored in these RNA tubes for at least 7–8 years at either −20°C or −70°C without significant change of purified RNA integrity using qRT-PCR analysis (PreAnalytiX 2018; Tang *et al.* 2019).

8.5 Techniques to study the transcriptome

The *transcriptome* of an organism is the sum of all RNA transcripts, comprising of mRNA, non-coding RNAs, and small RNAs in an individual or in a population of cells. An individual's information is recorded in the DNA of the individual's genome, which is expressed via transcription (Lowe *et al.* 2017). The first reported attempt to capture a partial human transcriptome from the brain was published in 1991 (Adams *et al.* 1991). Since then a rapid development of new technologies has increased the number of publications within the field of transcriptomics, with publications

of transcriptomes of, for example, different tissues, diseases or even single cells. Current techniques such as DNA-microarrays (Nelson 2001) and RNA-sequencing (Wang *et al.* 2009), which were developed in the mid-1990s and 2000s, are used to study the transcriptome (Lowe *et al.* 2017). RNA-sequencing has been influenced by the development of high-throughput sequencing technologies, which include next-generation sequencing (NGS) a massive parallel sequencing technique (Wang *et al.* 2009). The NGS perform rapid sequencing of millions of small fragments of DNA/RNA/miRNA in parallel, and with NGS it is possible to sequence, for example, the entire human genome within one day (Behjati & Tarpey 2013).

8.6 Bioinformatics

There are five steps in the bioinformatic analysis of miRNA sequencing data (Fu & Dong 2018): (1) data pre-processing, which includes quality filtering and 3′-adapter trimming; (2) mapping and annotation; (3) sequence analysis, which includes novel miRNA prediction and analysis of sequence variation of mature miRNAs isoforms; (4) differential expression analysis, which includes both known and novel miRNAs; and lastly, (5) functional analysis, which is based prediction of genes producing messenger RNA (mRNA) targeted by miRNA.

By using bioinformatic analysis, miRNAs are initially predicted in genome sequences that are based on structural features of miRNA. Different algorithms identify the hairpin structures in non-coding and non-repetitive regions of the genome, which are characteristic of miRNA precursor sequences. The previously-known miRNAs and precursors are important in these algorithms, because structures of known miRNAs are used to discriminate between true predictions and false positives (Bentwich 2005). There are many algorithms that can be used; mirDeep2 is one software program that is commonly used for the process (Friedländer *et al.* 2012).

miRBase is a database in which published miRNA sequences (known miRNA) are reported. More than 48885 mature miRNAs in 271 species have been identified and documented in miRBase (Kozomara & Griffiths-Jones 2013). The miRBase database can provide an integrated interface for miRNA sequence data, annotation and predicted mRNA gene targets (Griffiths-Jones *et al.* 2006).

8.7 Target genes producing mRNA

There have been approximately 2500 mature miRNAs and 22500 protein coding mRNA genes reported; which enables approximately 50 million potential pair-wise interactions between mature miRNAs and mRNA (Kehl *et al.* 2017). In mammals, miRNAs have been reported to regulate mRNA gene expression by either inhibiting translation (which is the process by which protein is synthesised after transcription) and/or by inducing degradation of target mRNAs. The target recognition has been reported to be mediated by the *seed region*, a 6–8 nucleotide sequence at the 5′ end of the mature miRNA, but the central and 3′ end of the miRNA may be involved (Bartel 2009). It has been reported that when miRNAs bind to mRNA, a perfect match along the whole mature miRNA sequence is not needed (Lewis *et al.* 2003). Therefore, a specific miRNA may target many different mRNAs (Selbach *et al.* 2008).

8.8 Feline microRNA transcriptome

A characterization of the feline total miRNA transcriptome (miRNAome) in several feline tissues has been published (Lagana *et al.* 2017). In that study, total miRNA was evaluated in 12 different feline tissues (CNS (brainstem, cerebellum, and cerebral cortex), testes, ovary, lip, tongue, lymph node, spleen, skin, lung, pancreas, liver and kidney), but whole blood was not evaluated. Furthermore, in that study, 271 candidate feline miRNA precursors, which encoded for 475 mature sequences were identified, as well as several novel feline-specific miRNAs. Some of the miRNAs identified were reported to be tissue/organ-enriched and tissue/organ-specific (Lagana *et al.* 2017).

In cats with HCM and compensated CHF, a previous study reported that miRNA profiles from serum samples showed a distinct miRNA expression pattern when cats with HCM were compared to healthy control cats. However, differences between cats with HCM and healthy controls were rather subtle, not exceeding a 4.1-fold difference for any of the identified miRNAs (Weber et al. 2015). In that study, different expressions of miR-381-3p, miR-486-3p, miR-4751, miR-476c-3p, miR-5700, miR-513a-3p, miR-320e, and miR-1246 were found in the HCM group, compared with healthy cats. Further analysis of differentially expressed miRNAs revealed 49 mRNA targets involved in cardiac hypertrophy (Weber *et al.* 2015).

Studies of HCM in people have reported that miRNAs are involved in the regulation of myosin gene expression, angiogenesis, vascular cell differentiation, and fibrosis (Kuster et al. 2013; Roncarati et al. 2014). The release of specific circulating miRNAs, miR-199a-5p,-27a and 29a, have been correlated to LV hypertrophy. Of these miRNAs, only miR-29a has been associated with both hypertrophy and fibrosis in people with HCM (Roncarati et al. 2014). The list of miRNAs associated with different stages of HCM in animals and people is increasing (Sabater-Molina et al. 2018; Scolari et al. 2021).

9. Aims

The major aims of this thesis were to assess potential associations between biomarkers (BP, NT-proBNP, cTnI and miRNA) and feline characteristics in healthy cats, and to compare concentrations of circulating cardiac biomarkers in healthy cats with concentrations in cats with HCM, with or without LAE.

The specific aims were to:

- Investigate if, and if so, how feline characteristics (breed, sex, age, BW and BCS) are associated with indirectly measured BP and PR, as well as with circulating plasma NT-proBNP and serum cTnI concentrations in healthy cats.
- Investigate how different clinical settings are associated with indirectly measured BP and PR in healthy cats.
- Compare NT-proBNP concentrations in healthy cats with concentrations in cats with HCM using ELISA and a POCT, and to assess if POCT results reflect ELISA results.
- Compare cTnI concentrations in healthy cats with concentrations in cats with HCM.
- Characterize the total transcriptome of miRNA (miRNAome) in whole blood in healthy NF and DSH cats, and compare the miRNAome in healthy cats and cats with preclinical HCM.

10. Comments on materials and methods

A brief description of materials and methods used is presented here. A more detailed description is provided in the respective papers. The present section also describes additional studies not included in these papers.

10.1 Study population (paper I–IV)

All studies included in this thesis were approved by Uppsala Animal Experiment Ethics Board, Sweden. Client-owned cats were prospectively recruited by information distributed to cat owners on webpages, at seminars for owners, or at the Evidensia Animal Clinic in Västerås. Informed written consent was obtained from the owner of each cat. For all four papers, parts of the sample of 100 healthy control cats and 39 cats with HCM were examined. The number of cats included in each paper is summarized in Table 6.

Table 6. Number of cats included in the different studies and shared by the four studies of the present thesis.

Paper	**All cats (N)**	**Healthy cats per breed (N)**			**Hypertrophic cardiomyopathy**	
	Total	Birman	NF	DSH	without LAE	with LAE
I	94	34	33	27	0	0
II	139	33	35	32	32	7
III	135	33	33	30	32	7
IV	12	0	3	3	5	1

N, number; NF, Norwegian Forest cat; DSH, Domestic Shorthair; LAE, left atrial enlargement.

For inclusion into the studies, cats had to either have echocardiographic evidence of HCM (paper II–IV) or be free from echocardiographic evidence of cardiac disease (paper I–IV). For healthy cats, only cats of non-pedigree DSH, pedigree Birman and NF cats were included (paper I–IV). Cats of any breed with preclinical or clinical HCM, stabilized as a sequel of CHF treatment, were allowed into the study (paper II–IV). Cats between 1 and 14 years of age were eligible for the study (paper I–IV). Individual cats selected for paper IV are presented in Table 7. One cat (code D1) was added in paper IV because a previously included cat had to be excluded due to failure in the RNA isolation process.

Table 7. Individual feline characteristics and basic echocardiographic variables in the 12 cats in paper IV

ID	A3	B3	C3	A1	B4	C2	A4	B2	C1	B1	C4	D1
Breed	DLH	DSH	DSH	NF	NF	NF	DSH	DSH	DSH	NF	NF	NF
Sex (NF/NM)	NM	NM	NM	NF	NF	NM	NM	NM	NM	NF	NM	NF
BCS (1-9)	7	7	5	7	6	7	5	7	5	6	6	5
Indoor/Outdoor	I	O	O	O	I	O	O	O	O	I	I	O
Age (years)	9.0	4.3	13.7	6.5	12	2.4	10.7	5.2	13.8	8.9	3.7	8.3
Weight (kg)	6.1	5.8	4.4	6	4.6	8.4	5.2	7.3	4.5	4.7	7.0	3.6
HCM/Healthy	HCM	HCM	HCM	HCM	HCM	HCM	Healthy	Healthy	Healthy	Healthy	Healthy	Healthy
Murmur (yes/no)	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No
LA/Ao	1.5	1.0	1.0	1.1	1.1	1.2	1.1	0.9	1.2	1.0	1.1	1.3
IVSd (mm)	8.9	6.4	6.3	6.5	5.8	9.1	3.9	4.7	4.0	3.3	4.3	4.0
IVSdinc (%)	118	59	65	60	51	109	-1	10	4	-16	3	9
LVPWd (mm)	6.9	7.3	7.4	6.4	4.8	9.4	3.9	4.7	4.0	3.3	4.2	3.4
LVPWdinc (%)	69	81	97	57	26	112	-1	9	4	-15	-2	-5
Sep2D (mm)	9.8	8.0	8.9	6.7	6.5	9.1	4.2	4.4	4.3	3.3	4.9	3.4

ID, identification of the individual cat; DLH, domestic longhair cat; DSH, Domestic Shorthair cat; NF, Norwegian Forest cat; NF, neutered female; NM, neutered male; BCS, body condition score; Indoor, indoor only (I); Outdoor, allowed outdoors (O); HCM, hypertrophic cardiomyopathy; LA/Ao, left atrial-to-aortic root diameter ratio ; IVSd, interventricular septum in diastole; $IVSd_{inc\%}$, percentage increase interventricular septum in diastole ; LVFWd, left ventricular free wall in diastole ; $LVFWd_{inc\%}$, percentage increase left ventricular free wall in diastole; Sep2D, two-dimensional echocardiographic measurement of septum.

10.2 Study design (paper I–IV)

All clinical examinations were performed according to a standardized protocol in a quiet examination room with the owner present. The procedures, in the order of performance, were: the cat was brought to the examination room together with the owner, a case history was obtained from the owner, BP was measured, a physical examination including cardiac auscultation and assessment of BCS using a 9-point scale (Laflamme 1997) and an echocardiographic examination were performed, and lastly blood samples were collected. A spontaneously voided urine sample was collected by the owners at home from a subset of cats.

10.2.1 Study design in Paper IV

Six healthy cats, three DSH and three NF cats were selected and matched with respect to breed, sex, age, and BW with a cat with preclinical HCM (Figure 14). One of the healthy DSH cats was matched with a Domestic Longhair (DLH) cat with preclinical HCM.

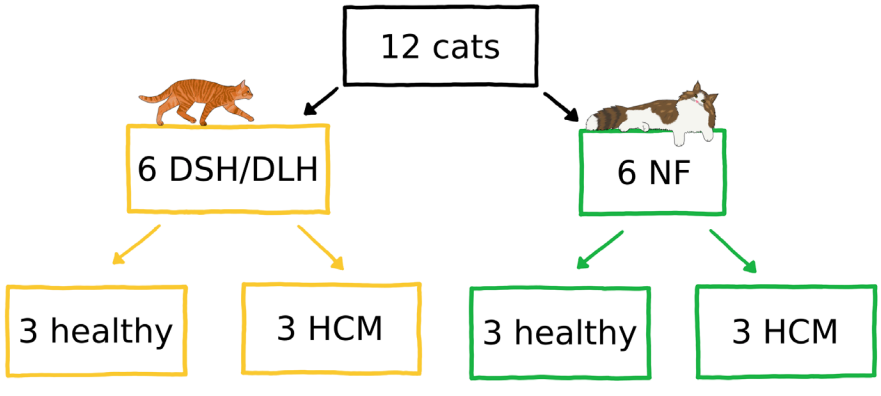


Figure 14. The study design in paper IV. DSH, Domestic Shorthair cat; DLH, domestic longhair cat; NF, Norwegian Forest cat; HCM, hypertrophic cardiomyopathy.

10.3 Indirect blood pressure measurement (paper I)

In this study, we investigated whether indirect BP results were affected by the clinical setting and if feline characteristics were associated with BP or PR variables. For acclimatization, the cats were allowed to adapt to the clinical environment for at least 10-15 minutes before BP was indirectly

measured using an automated high definition oscillometric (HDO) technique (Vet Memodiagnostic HDO monitor) with the cuff placed on the base of the tail (Brown *et al.* 2007; Acierno *et al.* 2018). Three clinical settings were used: (1) cat placed in its own carrier with the owner and veterinarian present (setting carrier-veterinarian-owner [Carrier-VO]); (2) cat placed in its own carrier and the owner measured BP when the veterinarian had left the room (setting carrier-owner [Carrier-O]); and (3) cat placed on the examination table, with both the owner and veterinarian present (setting table-veterinarian-owner [Table-VO]) (Figure 15) . The settings Carrier-VO and Carrier-O were always carried out sequentially, but in a randomized order, whereas the setting Table-VO was randomized to be performed either first or last.

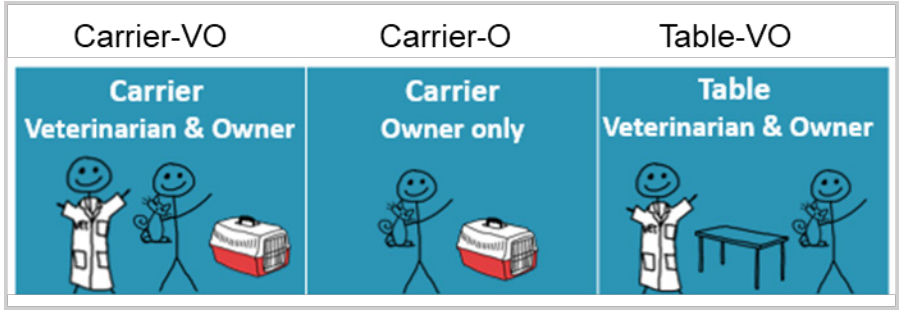


Figure 15. The clinical settings evaluated in the blood pressure study of healthy cats (paper I). Illustration by Jenny Hanås.

In setting Carrier-VO; the veterinarian performed 6 measurements, and in setting Carrier-O; the owner performed 6 measurements. In setting Table-VO; the veterinarian performed 4 measurements. For all three settings, SBP, MAP, DBP, and PR were recorded using the HDO device. No readings were excluded at the time of measurement. Failure to obtain a reading was noted by the veterinarian in setting Carrier-VO and Table-VO. In a subgroup of cats, evaluation of the arterial pulse pressure wave form (APPW) was performed after each measurement by the veterinarian. The APPW was assessed as 'adequate' if the pulse waves generated a bell curve pattern, and 'inadequate' if the APPW had substantial distortion (Taylor *et al.* 2017). For further evaluation on how feline characteristics are associated with indirectly-measured BP and PR only the two settings in the carrier were used.

10.4 Echocardiography (paper I–IV)

Echocardiographic examination was performed in all cats using an ultrasound system (IE33, Philips Ultrasound, Bothell, Washington), and continuous ECG (Thomas *et al.* 1993). The left atrial-to-aortic root diameter ratio (LA/Ao) was measured from the right 2-dimensional (2D) short-axis view (Hansson *et al.* 2002). End-diastolic and systolic LV dimensions (interventricular septum diastole [IVSd], LV internal diameter in diastole [LVIDd], LV free wall diastole [LVFWd], LV internal diameter in systole [LVIDs], and fractional shortening [FS]) were measured from M-mode and 2D images (Sahn *et al.* 1978; Thomas *et al.* 1993). Expected BW-dependent values for IVSd, LVIDd, and LVFWd were calculated (Haggstrom *et al.* 2016).

The diagnosis a HCM (paper II–IV) was made when a subjective impression of hypertrophy (diffuse or regional) with a non-dilated LV chamber was supported by increased M-mode and/or 2D diastolic LV wall dimensions of the IVSd, LVFWd or both (Luis Fuentes *et al.* 2020). Left atrial enlargement was defined as LA/Ao ≥1.5. Calculated deviations and LA/Ao were used to classify cats into three different groups: healthy cats, HCM without LAE, and HCM with LAE. Cats with subjectively normal LV morphology, no LAE, and <30% increase in BW-based predicted values for IVSd and LVFWd were classified as healthy cats (Haggstrom *et al.* 2016). Cats with subjective impression of LV hypertrophy and ≥30% increase in BW-based predicted values for IVSd or LVFWd and no LAE were classified as having HCM without LAE, and as having HCM with LAE if LA/Ao ≥1.5 (Abbott & MacLean 2006; Haggstrom *et al.* 2016).

10.5 Blood sampling and analyses (paper I–IV)

Blood was collected into tubes with EDTA, serum tubes, and into PAXgene tubes. Plasma was transferred to plastic cryotubes and aliquots were frozen at –80°C within 30 minutes of collection for storage. Serum was transferred to aliquots in plastic cryotubes and frozen at –80°C within 60 minutes of collection. Whole blood was collected in PAXgene tubes, frozen and stored at –20 °C.

Haematology and serum biochemistry profiles, including alanine aminotransferase activity, and concentrations of creatinine, glucose, and total

protein, were performed immediately after sampling. Total thyroxine and fructosamine concentrations were sent and analysed after each sampling.

10.6 Urine sampling and analyses (paper I)

Spontaneously voided urine samples were collected by the owner at home for a subgroup of cats. Urine samples were examined by urine dipstick analysis and urine specific gravity was measured using a digital refractometer.

10.7 Circulating cardiac biomarkers (paper II–IV)

10.7.1 Analysis of NT-proBNP using ELISA (paper II)

Plasma concentrations of NT-proBNP were batch analysed at Vet Med Labor GmbH, Division of IDEXX Laboratories, Ludwigsburg, Germany in duplicate using a previously validated second-generation ELISA for cats (Mainville *et al.* 2015). The reported assay interval for NT-proBNP concentration was 24–1500 pmol/l. Concentrations of NT-proBNP less than the lowest reported concentration were assigned a value of 24 pmol/l and concentrations above the highest reported concentration were assigned a value of 1500 pmol/l.

10.7.2 Analysis of NT-proBNP using POCT (paper II)

Plasma samples were also analysed using the POCT feline NT-proBNP (SNAP Feline proBNP, IDEXX Laboratories Inc). The samples were randomized, and the examiner was blinded to the sample's identity. Normal results were recorded when the density of the sample spot appeared lighter than the reference spot. Abnormal results were recorded when density of the sample spot appeared equal to or darker than reference spot, which according to the manufacturer occurs when concentrations are >100 pmol/l (IDEXX Reference Laboratories Inc) (IDEXX 2016). The POCT evaluation was performed on the same occasion by visual inspection and by using an automated evaluation. Visual inspection of POCT results was classified into 3 groups (lighter, equal, and darker) based on subjective evaluation of sample spot colour.

Inter observer variability (Thesis only)

We performed an inter-observer variability study in paper II. Results of the POCT evaluated by a trained examiner, an untrained examiner and an automated reading were compared. Twenty-one samples (seven with low concentration of NT-proBNP (all <24 pmol/l), seven with intermediate concentration (49–168 pmol/l) and seven with high concentrations (1007–1496 pmol/l)), were evaluated. The examiners were blinded to each other's POCT results, to the results obtained by the automated reader, and to the cat's identity.

10.7.3 Analytical performance of hs-cTnI assay (paper III)

The validation study was set up in concordance with the Clinical and Laboratory Standards Institute document EP15-A3 (Wayne 2014), and consisted of an imprecision study, limit of blank, linearity of dilution, and a stability study.

For the imprecision study, each sample was analysed three times every day at five different days to determine within run (CV_R), between run (CV_B), and within laboratory (CV_{WL}) repeatability. Linearity of dilution was performed in duplicates of pooled feline serum by dilution with physiological saline.

10.7.4 Analysis of Cardiac troponin I (paper III)

Serum concentrations of cTnI were batch analysed in duplicate at the Department of Clinical Chemistry and Pharmacology, Uppsala University Hospital, using a human two-step, double-monoclonal chemiluminescent microparticle immunoassay (CMIA) for detection of cTnI concentration (Abbott ARCHITECT ci16200 analyser). Reported assay interval for cTnI concentration was 2–50000 ng/l. Concentrations of cTnI less than the lowest reported concentration were assigned a value of 2 ng/l.

10.7.5 MicroRNAs (paper IV)

Isolation of total RNA and sequencing

Total RNA was extracted from PAXgene tubes. Molecules longer than 18 nucleotides consisted of mRNA and small RNAs such as miRNAs, which were purified. Libraries were prepared of total RNA and the libraries were quantified.

Next-generation sequencing (NGS)

Paired-end sequencing data was generated using a mid-output flow-cell, on Illumina NextSeq550 (Illumina Inc, San Diego, CA, USA). The paired-end sequencing enables sequencing of both ends of a fragment, thereby generating high-quality sequence data. By producing twice the number of reads in library preparation, sequences aligned and read as pairs enable a more accurate read alignment than single-read data.

Bioinformatics analysis and count generation

The sequenced data were converted and de-multiplexed, i.e., adaptors were removed, followed by trimming nucleotides added to the sequences from NGS. Reads shorter than 17 nucleotides were excluded from further processing. A quality control of the sequenced reads was performed. To verify that most reads mapped to miRNAs, reads were aligned to the feline genome using STAR (Dobin *et al.* 2012), and then assigned to a gene biotype using featureCounts (Liao *et al.* 2014). All reads could not be assigned a biotype, however of those that could be assigned a biotype the majority of reads were assigned to miRNAs. Further quality controls of sequencing reads were performed using fastQC and multiqc (Ewels *et al.* 2016). This fastQC sequence length distribution was consistent with miRNA sequencing.

Prediction of novel miRNAs and quantification of all miRNAs was performed using software package miRDeep2 (Friedländer *et al.* 2012). The feline genome, Felis catus 8.0, was used as reference genome. Mature and hairpin sequences were downloaded from miRBase (Kozomara & Griffiths-Jones 2014; Kozomara *et al.* 2019), with human (*Homo sapiens*) used as main reference, and mouse (*Mus musculus*) as distant relative reference and dog (*Canis familiaris*) as close relative reference. Main reference miRNAs identified in the dataset were classified as predicted known miRNAs, and miRNAs previously not described in the main reference were classified as novel miRNAs by miRDeep2. Only novel miRNAs with a miRDeep2 score of >5.0 was included in the count-file generated for subsequent differentially expressed miRNA analyses.

Differentially expressed miRNAs and statistical analyses (Paper IV)

MiRNAs with low expression were excluded. DESeq2 (Love *et al.* 2014) was used to calculate size factors and counts normalization was performed. A quality control of miRNA expression data were performed using a centred

principal component analysis and clustered heatmap (Kolde 2019). A differential expression analysis was performed using DESeq2 with a model including an interaction between breed and preclinical HCM (with design matrix: ~ breed + preclinical_HCM + breed:preclinical_HCM) for the following contrasts: effect of breed (NF vs DSH) in healthy cats, effect of preclinical HCM (HCM vs Healthy) irrespective of the breed (main effect), differential effect of preclinical HCM between breeds (interaction), effect of preclinical HCM (HCM vs Healthy) within the NF breed and the DSH/DLH cats. The *P*-values obtained by the Wald test are by default corrected for multiple testing using the Benjamini-Hochberg adjustment (BH-A) with a false discovery rate (FDR) ≤0.1.

10.8 Statistical analyses

Descriptive statistics of data for healthy cats for continuous variables in paper I and descriptive statistics of data for healthy cats and for cats with HCM for continuous variables in paper III are presented as mean ± SD. In paper I and III, one-way analysis of variance (ANOVA) for group comparisons of continuous variables were used followed by adjustment using Tukey's method for multiple comparisons. Descriptive statistics of data for healthy cats and cats with HCM are presented as medians and interquartile ranges (IQR) in paper II and IV. In paper II, the Kruskal-Wallis test was used followed by Bonferroni correction for multiple comparison between groups. Coefficient of variation (CV) is presented as percentage. A value of P <0.05 was considered significant for analyses in paper I-III. Statistical analyses used in paper IV are described above (see 10.7.5).

In paper I, analysing the effect of clinical setting on BP and PR results, each cat was subjected to multiple measurements and statistical analysis was performed using mixed linear models using the mixed procedure available in SAS (Littell 2006; *SAS/Stat User's Guide. Version 9.4.* 2017). The models included setting, sequence, interaction setting × sequence as fixed factors, and 'cat' as a random factor. For, further analysis of the effect of feline characteristics and life situation on BP and PR, the models included breed, age, sex, BW, BCS, indoor-only or allowed outdoors, number of cats in the household, and all two-way interactions between them. Models were built using stepwise procedures for general linear models (*SAS/Stat User's Guide.*

Version 9.4. 2017). Post hoc pairwise comparisons were adjusted using Tukey's method.

In paper II, statistical analyses were performed separately in 3 groups of cats: all cats, healthy cats, and HCM cats. Fisher's exact test was used for comparing proportions. The Kruskal–Wallis test was used to evaluate the effects of nominal and ordinal variables (sex, neutered or intact, BCS, presence or absence of SAM) on NT-proBNP concentrations, the overall association between the POCT results (intensity of colour in the POCT), and plasma NT-proBNP concentrations in all cats, and for evaluating the effect of breed in the healthy cat group. For the cats with HCM, linear regression was used to evaluate potential associations between NT-proBNP concentration and continuous variables (age, BW, SBP, echocardiographic measurements, HR obtained from clinical examination, and storage time of the plasma samples) after logarithmic transformation of plasma NT-proBNP concentration. Multiple regression analysis was performed. All variables were assessed as main effects; no interaction terms were considered in the model.

In paper III statistical analyses were performed separately in healthy cats and cats with HCM. Descriptive data for concentrations of cTnI were presented as median and interquartile ranges (IQR). For comparison of cTnI concentrations between healthy cats, cats with HCM without LAE and cats with HCM with LAE, the Kruskal–Wallis test was used followed by posthoc Wilcoxon tests. Multiple regression analysis was performed. All variables were assessed as main effects. Interactions breed*BW and breed*sex were evaluated but were not significant and thus not included in the model. ANOVA and multiple regression analyses were performed after logarithmic transformation of cTnI concentrations.

In paper II and paper III, the optimal cut-off, i.e. the combination of highest SE and SP, in NT-proBNP and cTnI concentration for identifying HCM was investigated using receiver operator characteristic (ROC) curves.

11. Results

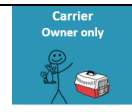
The main results from the thesis are summarized here. Further details are found in the respective papers.

11.1 BP measurement in different clinical settings (paper I)

11.1.1 Effect of clinical setting

In total there were 1116 BP measurements performed in 94 healthy cats in three clinical settings. The SBP did not differ between the settings ($P = 0.21$). However, the clinical setting had an impact on MAP, DBP, and PR (overall $P = 0.003$). In the setting Table-VO, MAP, DBP, and PR were higher than in the setting Carrier-O, ($P = 0.002$), and DBP and PR were also higher than in the setting Carrier-VO, ($P = 0.007$). Coefficient of variation for the obtained SBP, MAP, DBP, and PR results was significantly higher in the setting Table-VO than in the settings Carrier-VO and Carrier-O, (all P < 0 .0001, Table 8).

Table 8. Coefficient of variation of blood pressure variables for 94 healthy cats in the three clinical settings

Clinical settings	 Carrier-VO	 Carrier-O	 Table-VO
	Coefficient of variation (%)		
SBP	7.3[a]	9.1[a]	13.4[b]
MAP	8.4[a]	8.6[a]	14.3[b]
DBP	12.8[a]	12.2[a]	18.7[b]

Settings; Carrier-VO = cat placed in carrier with cuff on tail, both owner and veterinarian present, veterinarian performed the measurements; Carrier-O = cat placed in carrier with cuff on tail, owner alone performed the measurements; Table-VO = cat placed on examination table with cuff on tail, both owner and veterinarian present, veterinarian performed the measurements. Within each row, values with different superscripts were statistically different. SBP: systolic blood pressure; MAP: mean arterial blood pressure; DBP, diastolic blood pressure

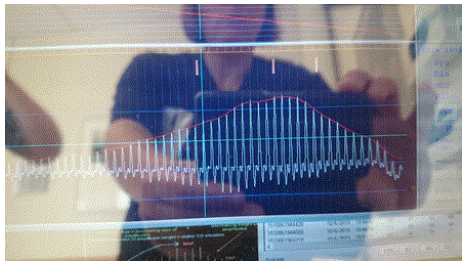


Figure 16. Adequate arterial pulse pressure wave form (APPW) from blood pressure measurement using high definition oscillometry (HDO). Photo: Sofia Hanås.

In 43/94 cats the APPW was available for evaluation (Figure 16). In the setting Carrier-VO, 90% of APPWs were subjectively evaluated as adequate, whereas 62% of the APPWs were evaluated as adequate for the setting Table-VO (Figure 17). The HDO device failed to obtain a BP reading and a new cuff inflation had to be performed in 55/94 cats. These errors occurred 65 times in setting Table-VO and 46 times in Carrier-VO.

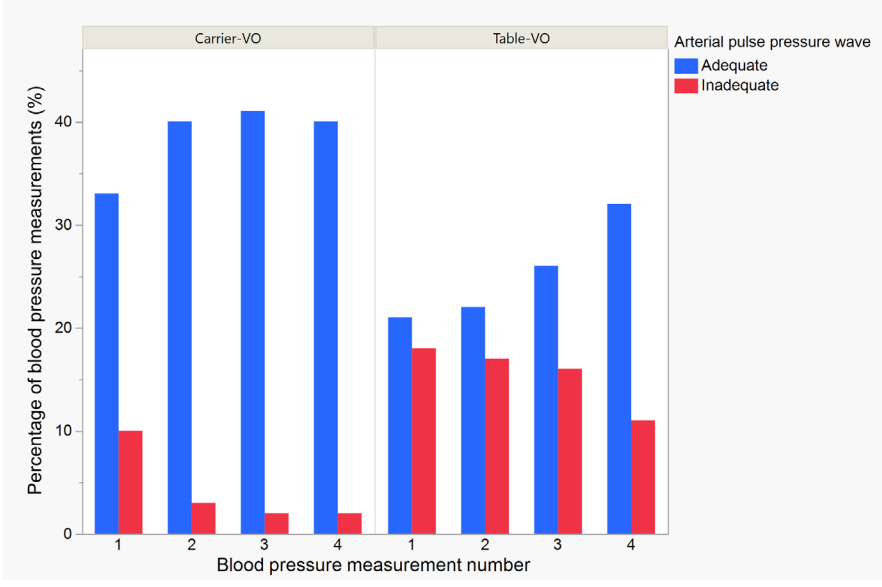


Figure 17 Percentage of adequate and inadequate arterial pulse pressure wave forms (APPW) in the settings Carrier-VO and Table-VO by order of sequential blood pressure measurements. Note the higher proportion of adequate APPW forms in setting Carrier-VO.

Carrier-VO = cat placed in carrier with cuff on tail, both owner and veterinarian present, veterinarian performed the measurements; Table-VO = cat placed on examination table with cuff on tail, both owner and veterinarian present, veterinarian performed the measurements.

Mean results for SBP in the different settings are presented in Figure 18.

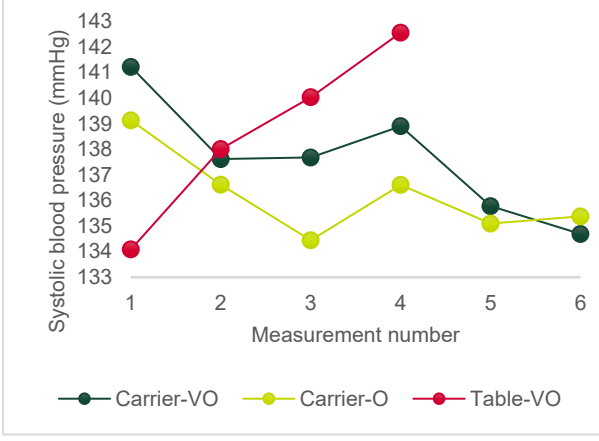


Figure 18. Mean arterial systolic blood pressure for the three clinical settings by order of sequential measurements, in 94 healthy cats. Note the increasing mean systolic blood pressure in the setting Table-VO per measurement number.

Carrier-VO = cat placed in carrier with cuff on tail, both owner and veterinarian present, veterinarian performed the measurements; Carrier-O = cat placed in carrier with cuff on tail, owner alone performed the measurements; Table-VO = cat placed on examination table with cuff on tail, both owner and veterinarian present, veterinarian performed the measurements.

11.1.2 Association with feline characteristics

The setting Table-VO had a higher CV for BP and PR, a larger proportion of inadequate APPW and more failures to obtain a BP reading than settings in the carrier. Therefore, for further evaluation as to how feline characteristics are associated with indirectly measured BP and PR, and of how feline characteristics were associated with BP and PR, only settings Carrier-VO and Carrier-O were used. In total, there were 1127 BP measurements performed in 94 healthy cats in the settings Carrier-VO and Carrier-O. After discarding the first measurement in each setting according to current guidelines, (Acierno *et al.* 2018), 939 measurements were left. Of these, 88 measurements from 39 cats were outliers and were excluded, leaving 4 to 10 measurements included per cat. Higher BP variables were found in NF and DSH cats than in Birman cats (Table 9. Blood pressure values per breed). Pulse rate was higher in Birman cats than in DSH cats. All

BP variables (Figure 19) and PR increased with age. Cats allowed outdoors had lower PR (137 ± 26) than cats living indoors only (157 ± 24, $P = 0.02$).

Table 9. Blood pressure values per breed (mean ± SD)

Blood pressure (mmHg)	All cats	Birman cats	Norwegian Forest cats	Domestic Shorthair cats
Number	94	34	33	27
SBP	136 ± 17	125 ± 12[a]	141 ± 17[b]	146 ± 14[b]
MAP	93 ± 11	85 ± 9[a]	96 ± 10[b]	98 ± 10[b]
DBP	69 ± 10	63 ± 8[a]	72 ± 9[b]	72 ± 9[b]

Within each row, values with different superscripts are statistically different. SBP, systolic blood pressure; MAP, mean arterial blood pressure; DBP, diastolic blood pressure. Number = number of cats.

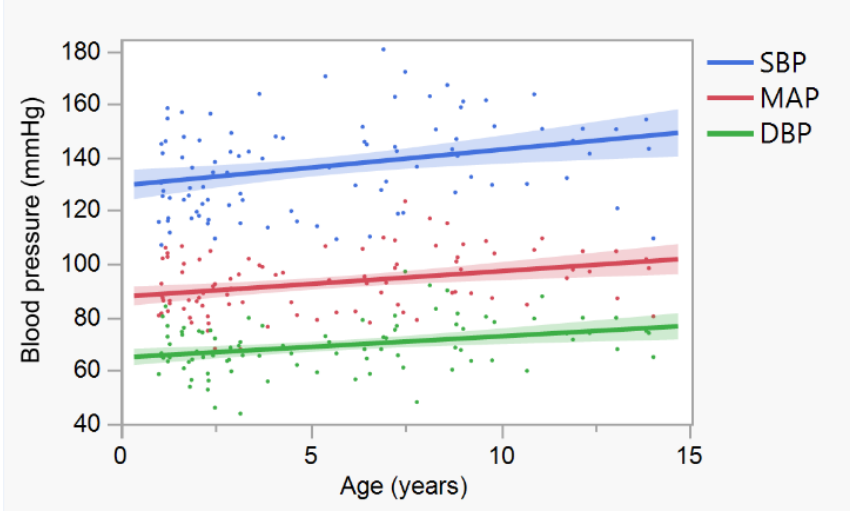


Figure 19. Systolic (SBP), diastolic (DBP) and mean arterial (MAP) blood pressure in 94 healthy cats by age. All pressures increased with age; adjusted $R^2 = 0.32, 0.32, 0.23$; $P = 0.01, 0.0003, 0.004$, respectively. Shaded areas indicate 95% confidence intervals of the fitted curves.

11.2 Analysis of circulating biomarkers (paper II-III)

11.2.1 NT-proBNP (paper II)

Healthy cats

The median NT-proBNP concentration in the group of healthy cats was <24 pmol/l with similar IQR for the Birman (<24–39 pmol/l), DSH (<24–38 pmol/l), and NF (<24–29 pmol/l) cats. The median NT-proBNP concentration was higher in male cats (25 pmol/l; IQR, <24–49) than in female cats (<24 pmol/l; IQR, <24–26; $P = 0.005$), Figure 20A-B. In 62/100 cats the NT-proBNP concentration was <24 ng/l.

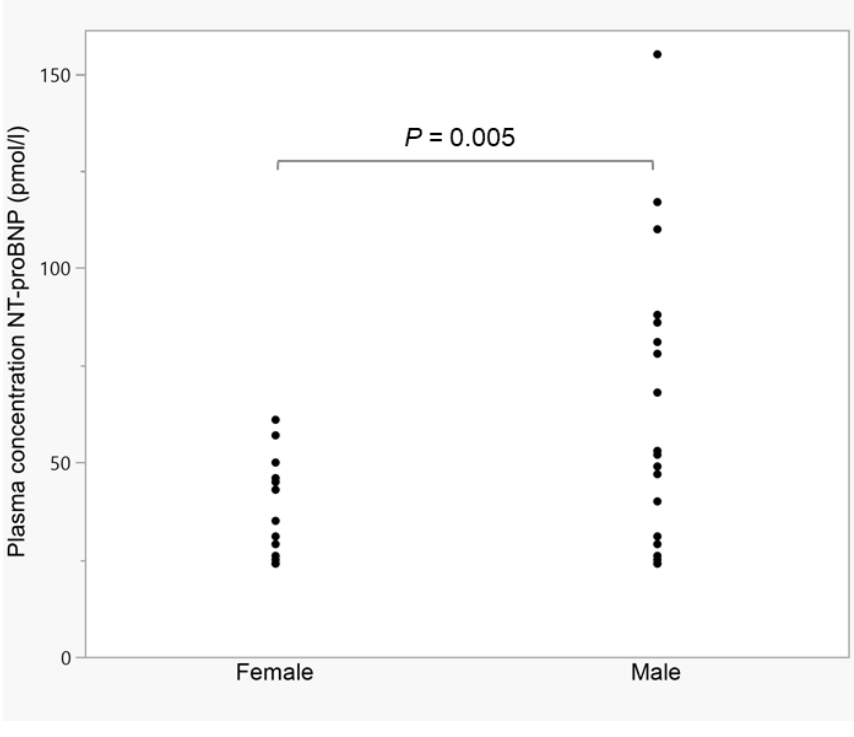


Figure 20A. Median NT-proBNP concentration in 100 cats by sex. Note that the NT-proBNP concentration was higher in male (N=43) cats compared to female (N=57).

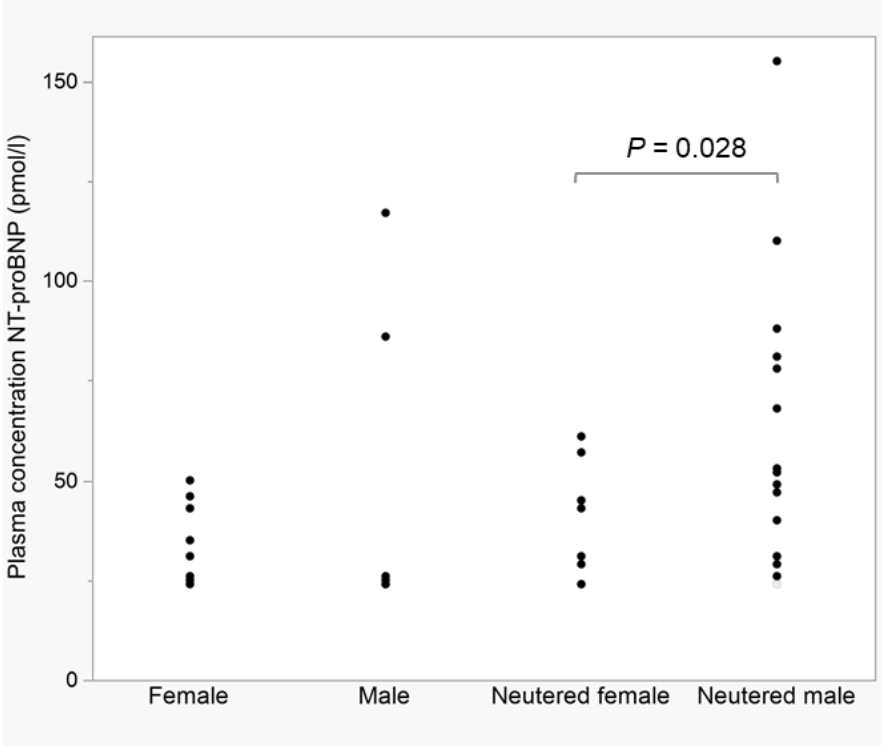


Figure 20B. Median NT-proBNP concentration in 100 intact and neutered female (N=22 and N=35, respectively) and intact and neutered male (N=10 and N=33, respectively) cats. Adjusted by Bonferroni correction.

Cats with HCM

Using multiple regression analysis, a positive association between plasma NT-proBNP concentration and increasing $LVFWd_{inc\%}$ ($P < 0.0001$), and higher NT-proBNP concentrations in male cats ($P = 0.002$) than in female cats were found. The final model included the variables $LVFWd_{inc\%}$, sex, and BW and the model had an adjusted $R^2 = 0.50$.

Comparison among groups

The median plasma concentration of NT-proBNP differed significantly among healthy cats (<24 (IQR <24–31) pmol/l), HCM without LAE (253 (IQR 52–456) pmol/l), and HCM with LAE (1496 (IQR 1007 to >1500) pmol/l (all $P < 0.0001$).

Evaluation of POCT results

In samples with sample spot colours lighter than the reference spot, the median plasma NT-proBNP concentration was (<24 (IQR <24–31) pmol/l), with equal colour of the sample spot the median plasma NT-proBNP concentration was (162 (IQR 100–217) pmol/l), and with darker colour of the sample spot the median plasma NT-proBNP concentration was (505 (IQR 336–1312) pmol/l). The median concentration of NT-proBNP differed significantly among all three groups (lighter, equal, darker) (Figure 21). The transition interval between normal and abnormal colour of the sample spot for the POCT was between 69 and 117 pmol/l.

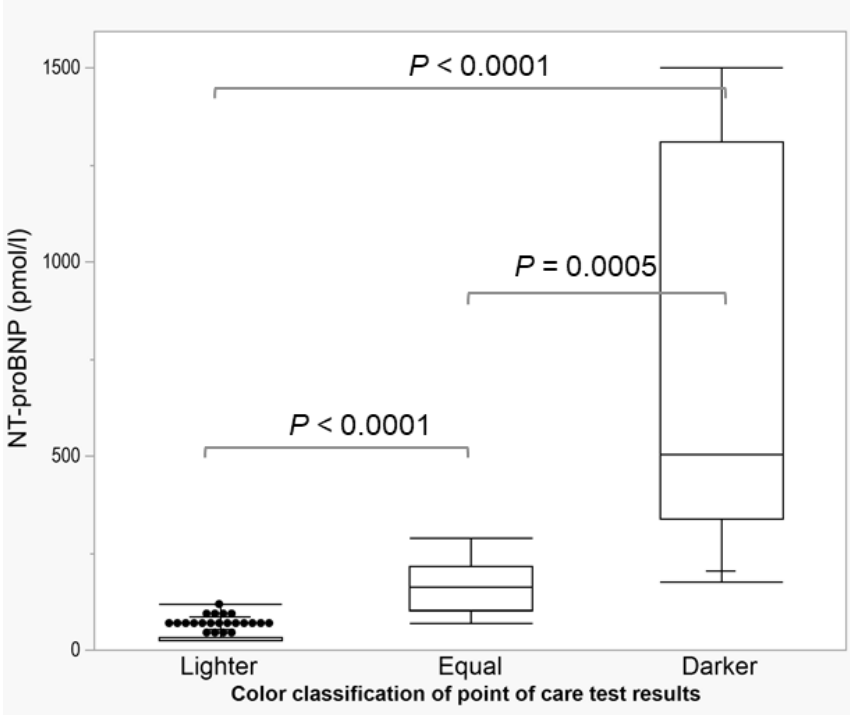


Figure 21. Median plasma concentrations of N-terminal-prohormone-B-type natriuretic peptide (NT-proBNP) by colour classification of point-of-care test results in the study population of 139 cats with (N=39) and without (N=100) hypertrophic cardiomyopathy (HCM). Number of POCT tests: lighter N=108, equal N=6 and darker N=25.

Healthy cats

Using visual inspection of the POCT, 98/100 (98%) healthy cats were classified as normal and two were classified as abnormal.

Cats with HCM

Using visual inspection of the POCT 29/39 (74%) cats with HCM were classified as abnormal using the POCT and 10/39 cats with HCM (26%) were classified as normal. All cats with HCM with LAE (N=7) were classified as 'darker' using the subjective visual evaluation of the sample spot colour on the POCT (Figure 21).

Sensitivity and specificity for detecting HCM

Using ≥100 pmol/l as a cut-off value for identifying abnormal samples, the ELISA and visual inspection of the POCT had SE of 72 and 74% and SP of 97 and 98%, respectively, for detecting HCM. The ELISA and the visual inspection of the POCT had a SE of 100% and SP of 97 and 98%, respectively, for detecting HCM cats with LAE, and a SE of 69%, and SP of 97 and 98%, respectively, for detecting HCM cats without LAE when compared to in healthy cats.

Inter-observer variability (Thesis only)

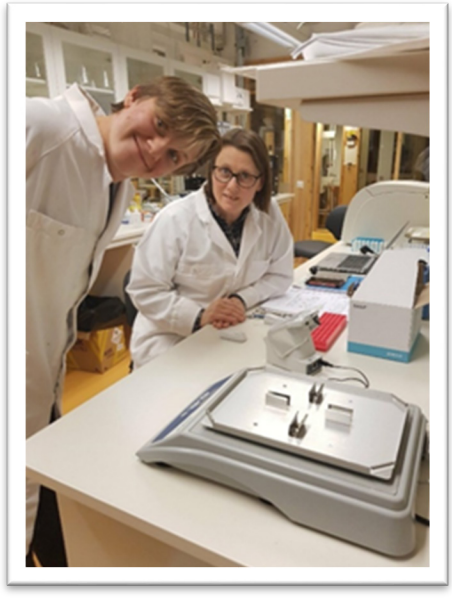


Figure 22. Double blinded inter-observer variability of point-of-care test showing the trained examiner and the untrained examiner. Photo Holger Hanås.

For all samples except one, the two examiners and the automated evaluation (Figure 22) reported the same result. One sample (ELISA concentration 168 pmol/l) was interpreted as normal by the trained examiner and abnormal by the untrained examiner and by the automated evaluation.

11.2.2 Cardiac troponin I (paper III)

Method validation (parts in Thesis only)

Within run, between run, and within lab assay CVs were <9% (Table 10). Assay results were adequately linear after dilution with recovery range 64–116% (Figure 23). Limit of blank (LOB) was 0.25 ng/l ± 0.12 (mean ± SD).

Table 10. Within run (CV_R), between run (CV_B), and within lab (CV_{VL}) coefficient of variation for a high sensitivity (hs) cardiac troponin I (cTnI) assay.

Analytical method: CMIA	**Coefficient of Variation %**	
Feline serum samples	cTnI 16.6 ng/l	cTnI 491.1 ng/l
Within run (CV_R)	5.1	4.0
Between run (CV_B)	6.8	5.4
Within lab (CV_{WL})	8.6	6.7
Internal human controls	cTnI 22.8 ng/l	cTnI 312.7 ng/l
Within run (CV_R)	5.1	5.7
Between run (CV_B)	5.6	6.2
Within lab (CV_{WL})	7.6	8.4

CMIA, chemiluminescent microparticle immunoassay; cTnI, cardiac troponin I.

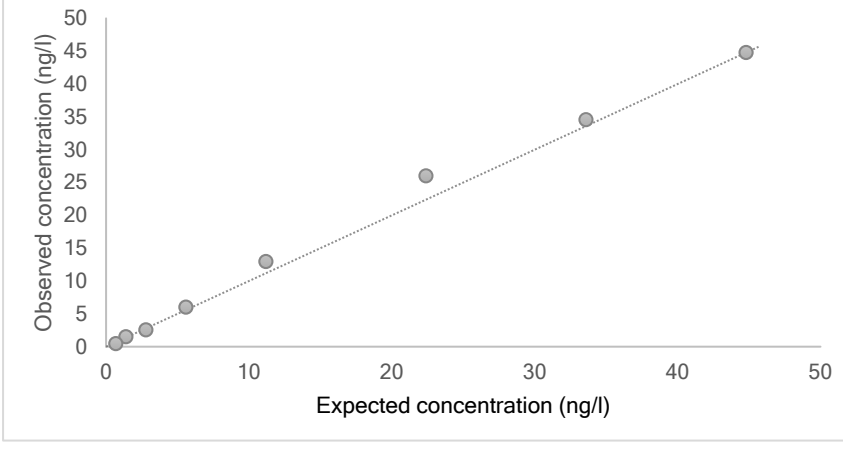


Figure 23. Concentration of cardiac troponin I after dilution of pooled feline serum. Line shows trend for y = x, meaning that the measured value corresponds to the expected value.

Healthy cats

Cardiac TnI was detectable in 89/96 (93%) of healthy cats, with higher concentrations in neutered males (7.5 (IQR 3.0–16.0) ng/l) than in intact females (3.7 (IQR 2.1–7.5) ng/l, $P = 0.032$). Intact male cats had a median cTnI concentration of 7.8 (IQR 5.5–10.5) ng/l. Using multiple regression analysis, cTnI concentration was associated with breed, sex, and HR. The final multiple regression model included breed, sex, HR, and $LVFWd_{inc\%}$, and had an adjusted $R^2 = 0.23$ and an overall $P < 0.0001$ (Table 11).

Table 11. Cardiac troponin I in healthy cats, by breed

Troponin I (ng/l)	All cats	Birman cats	Norwegian Forest cats	Domestic Shorthair cats
Number	96	33	33	30
Median	5.7	7.6[a]	4.0[b]	7.7[a]
IQR	(2.8-11.0)	(3.3-14.4)	(2.6-7.7)	(3.0-16.1)

On the row showing the median cardiac troponin I (cTnI) concentration, values with different superscripts are statistically different. Note that the cTnI concentration was higher in the Birman cats compared to the Norwegian Forest cats.

Cats with HCM

The median cTnI serum concentration was higher in cats with HCM and LAE (296 (92.0–642.0) ng/l) than in HCM without LAE (29.3 (13.3–46.5) ng/l) and in healthy cats (5.7 (2.8-11.0) ng/l), (all P =0.0003) (Figure 24). The seven cats with HCM with LAE had cTnI concentrations ranging from 56 to 1880 ng/l. Using multiple regression, cTnI concentration was positively associated with both $LVFWd_{inc\%}$ and LA/Ao (all $P = 0.010$). The final model included $LVFWd_{inc\%}$ and LA/Ao and had an adjusted $R^2 = 0.46$ and an overall $P < 0.0001$.

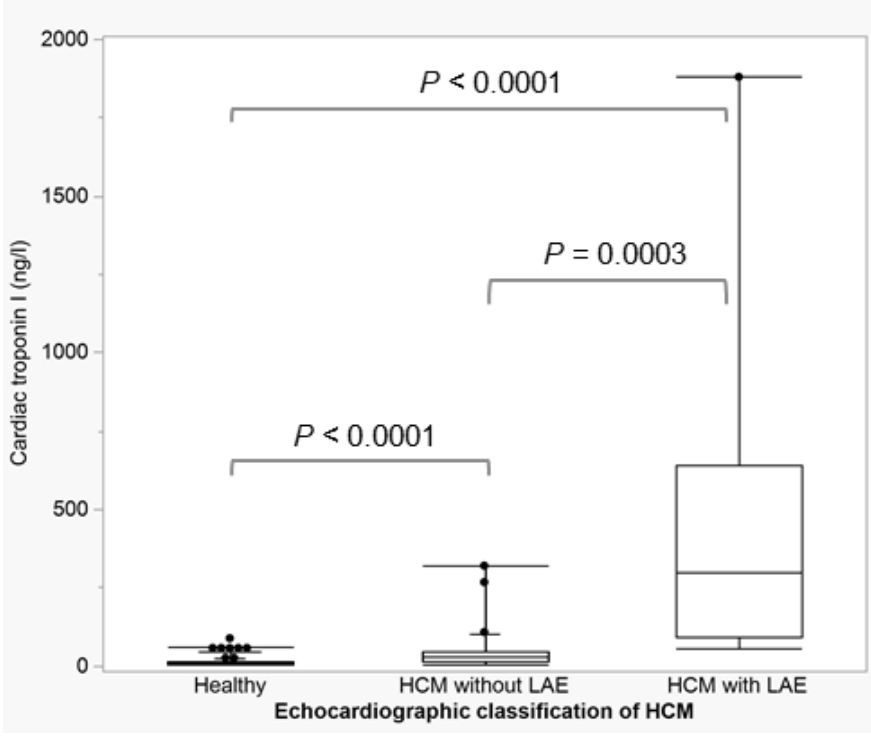


Figure 24. Median serum concentration of cardiac troponin I (cTnI) by echocardiographic classification. Healthy: N =96, HCM without LAE: N =32, and HCM with LAE: N =7.

11.3 MicroRNAs (paper IV)

11.3.1 Centred principal component analysis

The principal component analysis of the miRNA expression showed an effect of breed (Figure 25). In the principal component analysis, none of the 12 included cats were considered to be an outlier, therefore analyses were performed using 12 cats and 228 miRNAs.

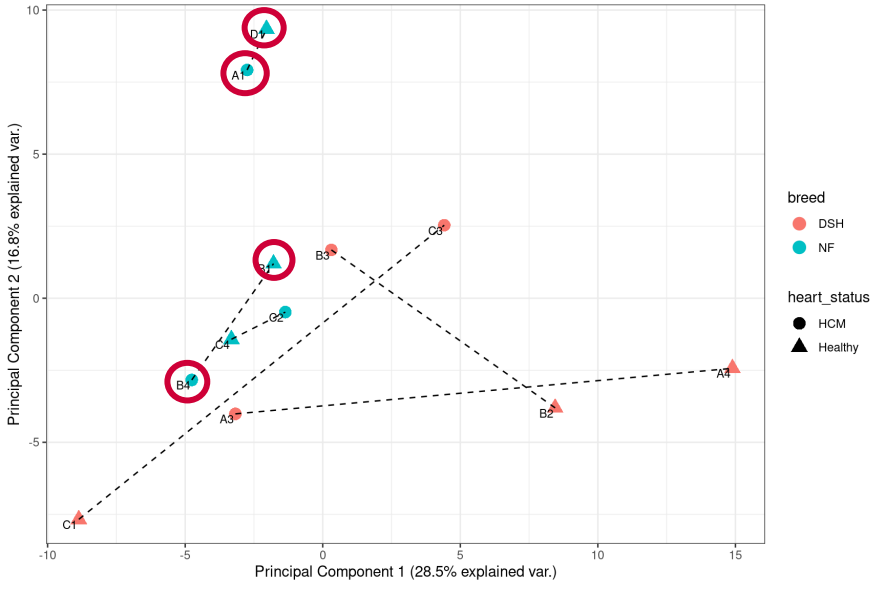


Figure 25. Principal component analysis of 228 mature microRNAs from the 12 cats, breed, sex, age and bodyweight matched. Note that all the NF cats and all DSH cats were separated. Matched cats are connected with dashed lines. Domestic shorthair (DSH) cats are marked with orange colour. There was one Domestic Longhair (DLH) cat with HCM, code A3, the other 5 cats were DSH. Norwegian Forest (NF) cats are marked with blue colour. Cats with hypertrophic cardiomyopathy (HCM) are marked as a filled dot. Healthy cats are marked as a filled triangle. Neutered females are circled by red rings. Illustration by Julie Lorent.

11.3.2 Prediction of novel miRNAs in feline whole blood

The prediction analysis identified 40 potentially novel miRNAs in cats in whole blood, which had previously not been identified in people, dogs, mice, or cats.

11.3.3 Identification of differentially expressed microRNAs

Effect of breed in healthy cats

Four miRNAs had significantly higher expression levels, and two miRNAs had significantly lower expression levels when the three healthy NF cats were compared to the three healthy DSH cats (Table 12).

Table 12. Significantly higher or lower expression levels of microRNAs when three healthy Norwegian Forest cats were compared to three healthy Domestic Shorthair cats

microRNA	baseMean	log2FoldChange	*P*	Adjusted *P*
fca-miR-151a-3p	936.64	-1.10	0.0001	0.018 *
fca-miR-3613-5p	10.84	3.33	0.0001	0.018 *
fca-let-7f-5p	6633.10	1.77	0.0003	0.023 *
fca-miR-98-5p	399.03	1.68	0.0004	0.024 *
fca-miR-330-5p	70.03	-1.42	0.0007	0.031 *
fca-miR-26b-5p	3968.11	1.04	0.0011	0.041 *

fca, Felis catus; miR, mature miRNA; let, lethal. Known microRNAs (miRNAs) are labelled with letters and numbers. *show significant Benjamini-Hochberg adjusted *P* using a threshold of 0.1.

No overall effect of preclinical hypertrophic cardiomyopathy

No miRNAs had significantly higher or lower expression levels when six healthy cats were compared to six cats with preclinical HCM.

Effect of preclinical HCM in Norwegian Forest cats

One miRNA; fca-miR-204-5p, had significantly lower expression levels, but no miRNA had significantly higher expression levels when the three NF cats with preclinical HCM were compared with the three healthy NF cats.

12. Discussion

12.1 Cat population

This thesis included a comparably large cohort of apparently healthy cats that were phenotypically and genetically different (Lipinski *et al.* 2008) by including Birman, NF, and the mixed-breed DSH cats. The reason was to evaluate, whether, and if so, how, feline characteristics variables were associated with the cardiac biomarkers BP, NT-proBNP, cTnI and miRNAs. We also included a cohort of cats with HCM to compare to healthy cats. This allowed the results to be compared with previous studies concerning biomarkers in cats with HCM. The main findings in the population of healthy cats were significant differences between *breed* and BP, cTnI, and miRNA, *sex* and NT-proBNP and cTnI, and *age* and BP and PR. In the population of cats with HCM, we also found that both NT-proBNP and cTnI may differentiate cats with HCM from healthy cats, and cats with HCM and LAE from cats with HCM without LAE.

To be able to obtain sample groups of appropriate size, enabling evaluation of associations between feline characteristics and cardiac biomarkers in healthy cats, we selected common breeds. Hypertrophic cardiomyopathy in cats has been reported to be highly prevalent in many breeds in addition to DSH cats (Luis Fuentes *et al.* 2020; Kittleson & Côté 2021). The chosen cat populations, with a wide range of ages, were thus relevant regarding the potential application of the results in clinical settings.

The original theme was to focus only on circulating cardiac biomarkers in healthy cats, and in cats with HCM. In cats with HCM, it was important to exclude hypertension as one of the differential aetiologies for hypertrophy. In cats with HCM, BP have been reported to be within normal reference

ranges (Oldach *et al.* 2021). Because of this BP was included (paper I) as a physiological biomarker.

12.2 Blood pressure in healthy cats (paper I)

We first investigated the effect of three clinical settings on BP and PR in healthy cats. In the first part of the study, the focus was on evaluating the performance of the oscillometric system used under clinical conditions in different clinical settings. The protocol thus deviated somewhat from current guidelines (Acierno et al. 2018). Previous studies in cats have reported that BP and HR may be affected by whether BP measurements are performed at home or at the veterinary clinic, with higher SBP and HR at the clinic than at home, possibly due to situational hypertension (Quimby et al. 2011; Nibblett et al. 2015; Acierno et al. 2018). It was found that when BP was measured in the setting Table-VO, the MAP, DBP, PR, and their CVs, but not the SBP, were higher than when the cat was placed in its own carrier for BP measurement. This may be explained by a potentially more stressful situation for the cat when it is placed on the table than when it is placed in the carrier. For SBP, which is the most commonly assessed BP variable in clinical practice, no difference was found among the three settings. Both MAP and DBP showed a decreasing pattern by measurement number for the settings in the carrier, a pattern that was not evident for the setting Table-VO, possibly explaining the difference among settings.

The higher CVs for BP variables and PR, when BP measurements were performed in the setting Table-VO, required further evaluation. Analysis of the APPW revealed that BP measurements performed in the setting Table-VO had a higher percentage of inadequate APPW curves than in the setting Carrier-VO. Inadequate APPW may be a result of movement or anxiety (Taylor *et al.* 2017). Information on the number of times that the oscillometric device failed to obtain readings/setting was also incorporated, because this might have impacted the cat by increasing stress levels due to more frequent cuff inflations, as the HDO failed to report adequate readings. In the setting Table-VO, the oscillometric device failed to obtain a BP result more often than in the setting Carrier-VO. More failures to obtain a BP reading, and a higher percentage of inadequate APPW in the setting Table-VO, compared to the setting Carrier-VO, may be explained by increased

mobility on the table and increased situational stress for the cat, eventually leading to higher CV.

12.3 Biomarkers and feline characteristics (paper I–IV)

Several associations between the cardiac biomarkers selected for this study and feline characteristics in healthy cats were identified. An association of *breed* was found for BP variables, cTnI serum concentrations, and differentially expressed miRNA in feline whole blood. Birman cats had higher cTnI concentrations than NF cats (paper III). Breed differences for the concentration of cTnI have previously been reported in dogs (Baumwart et al. 2007; LaVecchio et al. 2009), but have not yet been shown in cats (Langhorn et al. 2016; Hori et al. 2018; Hertzsch et al. 2019). Although cats from genetically distant breeds (Lipinski *et al.* 2008) were selected, plasma NT-proBNP concentration in healthy cats did not differ significantly between breeds (paper II). This finding is in accordance with a previous report in cats (Wess *et al.* 2011), but in contrast to studies in dogs (Sjöstrand *et al.* 2014; Couto *et al.* 2015). A possible cause for the lack of association between NT-proBNP concentrations and breed might be that NT-proBNP concentrations were only found in 38% of the healthy cat population. Further studies in other breeds may yield other results.

Norwegian Forest and DSH cats had significantly higher SBP, MAP and DBP than the Birman cats. In previous reports, no association between breed and BP variables has been reported in cats (Bodey & Sansom 1998; Lin *et al.* 2006; Payne *et al.* 2017). The results in paper I is, therefore, in accordance with previous studies in dogs where breed differences have been reported for both BP and PR (Bodey & Michell 1996; Hoglund *et al.* 2012). We decided to analyse this further by using a multivariable model, which included both breed and BW, as well as the two-way interactions between these variables. We found that breed was associated with all BP variables, whereas BW was not. The Birmans were the smallest cat breed, followed by the DSH cats, and NF cats were the largest. A smaller cat likely also has a thinner tail, and the smaller tail circumference in the Birmans, relative to the cuff size, might have contributed to the lower BP results found in this breed (Sparkes *et al.* 1999; Brown *et al.* 2007).

In paper IV, again, an association of *breed* was found for differences in the miRNA-expression patterns when healthy NF cats were compared to

healthy DSH cats. Breed differences in miRNA-expression patterns have previously been shown to influence miRNA-profiles in horse blood (Pacholewska et al. 2016), and in muscle tissue in cattle (Sadkowski et al. 2018). In dogs, no significant breed differences were found in healthy dogs when circulating miR-122 concentration (a sensitive and specific biomarker of liver injury) was evaluated (Oosthuyzen *et al.* 2018). Studies of feline miRNA are sparse and have not yet addressed potential breed differences (Ichii et al. 2014; Tamazian et al. 2014; Weber et al. 2015; Lagana et al. 2017). However, in a previous study evaluating miRNA on a human-based array, 11 miRNAs in serum were differentially expressed between healthy cats and cats with stable CHF caused by HCM (Weber *et al.* 2015). The breeds were not evenly distributed between healthy cats and HCM cats in the previous study (Weber *et al.* 2015); in that study, 7/12 healthy cats were NF, whereas there were no NF cats among the 11 cats with HCM. The differentially expressed miRNAs in the study by Weber et al. might, therefore, have been influenced by an effect of breed as well as by HCM.

The potential association with *breed* and differentially-expressed miRNAs identified in the miRNA study (paper IV) might have been influenced by *sex*, because all females belonged to the NF cat breed, thus the influence of sex on miRNAs could not be fully evaluated. In people, sex has been reported to influence miRNA-profiles. (Meder *et al.* 2014; Ameling *et al.* 2015; Rounge *et al.* 2018) Further studies are needed to evaluate the potential effect of sex on miRNAs to elucidate if the association with breed that we identified in paper IV might be an effect of sex or possibly a combination of both breed and sex.

Sex was found to be associated with plasma concentrations of NT-proBNP (paper II) and with serum concentrations cTnI (paper III). The plasma concentration of NT-proBNP in healthy cats was positively associated with male sex, and both sexes were comprised of both intact and neutered cats. Sex has previously not been reported to influence NT-proBNP concentration in cats (Fox et al. 2011; Wess et al. 2011). However, in dogs, the results have been ambiguous, varying from no effect of sex on NT-proBNP concentrations (Boswood et al. 2008) to higher concentrations in intact female dogs, compared to in intact male dogs (Wolf et al. 2013). The results from the latter study in dogs (Wolf et al. 2013) is in agreement with studies in people, where women have higher NT-proBNP concentrations than men (Redfield *et al.* 2002; Wang *et al.* 2002; Loke *et al.* 2003; Raymond

et al. 2003; Fragopoulou *et al.* 2010). Approximately 77% of healthy male cats, and 61% of healthy female cats in the present study were neutered, and the intact males seemed to have lower concentrations of NT-proBNP than neutered males. This may thus have affected the results with lower concentrations of NT-proBNP in female cats than in male cats. In rats, testosterone suppressed the release of NPs from atria (Deng & Kaufman 1993). An explanation for the discrepancy between our cat population and previous studies in people may therefore be the suggested suppressive effect of testosterone on the NT-proBNP concentration in men (Chang et al. 2007).

In paper III, *sex* was associated with cTnI serum concentrations, with higher cTnI concentrations in healthy neutered male cats than in intact female cats. In people, women have lower cTnI concentrations than men (Kubo *et al.* 2010; Kimenai *et al.* 2018; Mariathas *et al.* 2019; Giannitsis *et al.* 2020). By using a more sensitive cTnI assay in paper III than in previous studies in cats (Langhorn et al. 2016; Hori et al. 2018; Hertzsch et al. 2019), most of the healthy cats had detectable cTnI concentrations, and this may be an explanation for the breed and sex associations we identified. Although there were statistically significant differences in cTnI concentrations among both breed and sex in our study, these differences were small and likely not of relevance in a clinical situation. To investigate whether breed- and sex-specific reference intervals for cTnI concentrations would be beneficial for cats, further studies are warranted, possibly including more breeds.

Age had an impact on all BP variables. The SBP, MAP, and DBP increased with increasing age, which is in accordance with previous studies in cats (Bodey & Sansom 1998; Sansom *et al.* 2004; Bijsmans *et al.* 2015; Payne *et al.* 2017), dogs (Bodey & Michell 1996), and in people (Smulyan *et al.* 2001). In cats (McLeland *et al.* 2015; McLeeland 2019) and in people (Martin & Sheaff 2007), alterations in kidney vasculature have been reported to occur with age (fibrointimal hyperplasia and hyperplastic arteriolosclerosis) which leads to a progressive stiffening of arteries that may lead to increased SBP, as seen in the present study. In cats, there are also some studies that have shown no effect of age on indirect BP measurements (Kobayashi *et al.* 1990; Sparkes *et al.* 1999; Hori *et al.* 2019). Because BP is a highly variable physiologic biomarker that has been reported to be affected by situational hypertension, the variable BP results may be explained by the use of different BP devices, different handling techniques and different cat populations. For the circulating cardiac biomarkers cTnI and NT-proBNP,

no associations with age were found. For cTnI, this is in accordance with two previous reports in cats (Hori *et al.* 2018; Hertzsch *et al.* 2019), but in contrast to another report in cats, which showed positive associations with age in cats (Serra *et al.* 2010), and in healthy people (Venge *et al.* 2003; Clerico *et al.* 2019; Mariathas *et al.* 2019), healthy dogs (Oyama & Sisson 2004), and in dogs with mild myxomatous mitral valve disease (Ljungvall *et al.* 2010). For NT-proBNP, the lack of association with age in the study in paper II is in accordance with previous studies in cats (Wess *et al.* 2011; Humm *et al.* 2013). In people, age has been reported to be positively associated with NT-proBNP concentration (Redfield *et al.* 2002; Wang *et al.* 2002; Loke *et al.* 2003).

12.4 Cats with HCM (paper II–IV)

By comparing the concentrations of circulating cardiac biomarkers NT-proBNP and cTnI in healthy cats with concentrations in cats with HCM with or without LAE, we found that LV hypertrophy and LAE were associated with significantly increased NT-proBNP and cTnI concentrations. For miRNA we studied only a small proportion of the included cats from paper I-III, and there was only one miRNA that was differently expressed between the cats with preclinical HCM and the healthy cats within the NF breed.

In cats with HCM, serum concentration of cTnI and plasma concentration of NT-proBNP were positively associated with increased LV hypertrophy and LAE, in accordance with previous studies in cats (Fox et al. 2011; Tominaga et al. 2011; Wess et al. 2011; Hori et al. 2018; Hertzsch et al. 2019). The cTnI concentration and the NT-proBNP concentration also differed significantly between healthy cats, cats with HCM without LAE, and cats with HCM with LAE.

Evaluating the concentration of NT-proBNP using both an ELISA and a POCT, we found that the results from the POCT reflected those of the ELISA. The POCT could be used for a rough semi-quantitative evaluation of NT-proBNP concentration because NT-proBNP concentration differed significantly between the three groups of subjective classification of the colour of the sample spot (lighter=normal, equal=mildly abnormal, and darker=abnormal) at visual inspection of the POCT. This finding may increase the value of the POCT in the assessment of a suspected cardiac disease. In the present study, all cats with HCM with LAE had an abnormal

result (darker) on the POCT. In cats with HCM, LAE has been reported to be an indicator of worse prognosis (Rush et al. 2002; Payne et al. 2010; Schober et al. 2013; Payne et al. 2015a), and cats with preclinical HCM and LAE as well as increased NT-proBNP on the initial examination may have a higher risk for CHF, arterial thromboembolism or sudden cardiac death (Ironside et al. 2021). For cats with suspected heart disease, veterinarians may use the POCT to increase suspicion of cardiac disease, especially in the absence of access to sonographic examination of the heart. Additionally, in this thesis we reported that a trained and an untrained examiner reported similar results from POCT analysis, which implies that the feline POCT may be used without specific training.

In previous studies in cats (Borgeat *et al.* 2014; Langhorn *et al.* 2014), dogs (Fonfara *et al.* 2010; Ljungvall *et al.* 2010; Borgeat *et al.* 2014), and people (Antman *et al.* 1996; Kubo *et al.* 2011), cTnI concentrations as well as NT-proBNP concentrations have been reported to correlate with the severity of the heart disease, which is in accordance with the results in study II and III in the present thesis. We showed that the NT-proBNP and cTnI had significantly higher concentrations in cats with HCM with LAE than in cats with HCM without LAE.

Even though cTnI is heart-specific, increases of cTnI concentrations are not disease specific, and cTnI concentrations have been reported to increase in cats with other diseases such as hyperthyroidism (Sangster *et al.* 2014), hypertension (Bijsmans *et al.* 2017), and renal disease (Porciello *et al.* 2008; Langhorn *et al.* 2019a). In the present study, the exclusion criteria were strict regarding BP and blood analysis results to avoid including cats with non-cardiac–related diseases, but with the disadvantage of introducing the spectrum effect for NT-proBNP and cTnI studied in paper II and IIII. The studies were intended to be explorative, and further research including a mixed cat population is accordingly warranted (Usher-Smith *et al.* 2016). However, as per cTnI concentrations, it is important to note that other severe non-cardiac conditions have been reported to increase cTnI concentration in cats and dogs, e.g. critical illnesses (Mellor *et al.* 2006; Sharpe *et al.* 2020), trauma (Schober *et al.* 1999; Biddick *et al.* 2020), and snakebite intoxication (Pelander *et al.* 2010; Harjen *et al.* 2020), and cTnI concentrations are often much higher in these non-cardiac conditions than in cardiac diseases.

In the fourth paper, the miRNAome of feline whole blood was described in a small cohort of only 12 cats. MicroRNA are small non-coding RNAs

which have been suggested to be promising biomarkers for the diagnosis, prognosis, treatment and monitoring of disease, and for patient stratifications in people (Chen *et al.* 2008b; Mishra 2014). In feline whole blood, we identified 228 mature miRNAs, of which 40 were predicted to be potentially novel for cats. No miRNAs were differentially expressed when cats with preclinical HCM were compared to healthy cats. This is in contrast with a previous study in cats with HCM (Weber *et al.* 2015), and in studies in people with HCM (Roncarati *et al.* 2014; Fang *et al.* 2015; Ntelios *et al.* 2020), where distinct miRNA expression patterns in serum or plasma in individuals with HCM were found compared to those in healthy individuals. An explanation for the lack of differentially expressed miRNA in the DSH/DLH cats in the present study might be that the cats with HCM were all in the preclinical phase of the disease, and therefore not expressing enough miRNAs associated with a remodelled or damaged heart tissue into the blood. All cats with HCM included in the previous study had compensated clinical HCM at the time of serum sampling (Weber *et al.* 2015), whereas all cats in paper IV were in the preclinical phase of HCM and none of them had progressed to CHF. Expression patterns of miRNA have been reported to be enhanced as different diseases progress (Margue *et al.* 2015). Another explanation for the lack of differently expressed miRNA in DSH/DLH cats with HCM might be that too few cats were included.

In paper IV, each healthy cat was matched according to feline characteristics with a cat with preclinical HCM. The study design included a comparison for the potential effect of preclinical HCM irrespective of breed and within breed. The non-significant interaction between preclinical HCM and breed was kept in our final model because many miRNAs were compared, and they may be differently affected by the interaction. For comparison within the NF breed only one miRNA (fca-miR-204-5p) had significantly lower expression levels in cats with HCM compared to healthy cats. In previous reports in cats, pedigree cats such as the NF breed have presumably lower degrees of genetic heterozygosity than mixed breed cats within DSH/DLH cats (Menotti-Raymond et al. 2008; Matsumoto et al. 2021), which may increase the potential of identifying specific miRNAs as potential biomarkers for disease within the NF breed. In people, miR-204-5p has been reportedly involved in processes such as regulation of cardiac muscle myoblast proliferation (Xiao *et al.* 2012), cardiac muscle cell differentiation (Xiao *et al.* 2012), and regulation of blood vessel endothelial

cell migration (Zhang *et al.* 2017). In people, miR-204 has been reported to have higher expression levels in heart tissue from patients with HCM, compared to in healthy individuals (Kuster *et al.* 2013). An explanation for why miR-204 had lower expression levels in the present study may be that the samples were from whole blood and not from tissue, and miRNAs have been reported to be expressed in a tissue/organ-specific manner in cats (Lagana *et al.* 2017), mice (Lagos-Quintana *et al.* 2002; Roux *et al.* 2012), and people (Weber *et al.* 2010; Ludwig *et al.* 2016). The significance of the lower expression level of fca-miR-204-5p in NF cats with HCM in the present study merits further investigation.

13. Future perspectives

- Studies investigating BP measurement in healthy cats of other breeds than NF and Birman, preferably including these two breeds as well for comparison.
- Further investigation of the clinical performance of the NT-proBNP POCT test including a mixed cat population with heart disease and other comorbidies, such as hyperthyroidism and kidney disease, to evaluate the clinical performance of the POCT test.
- Explorative study in healthy cats of several breeds to further investigate the potential breed and sex effect on miRNA expression found in paper IV.
- Exploration of miRNA in cats with HCM and in healthy cats, using whole blood, serum and heart tissue if possible, including cats with different severities of HCM to evaluate differentially expressed miRNAs as the disease progresses.

14. Conclusions

We have shown that:

In healthy cats:

- Male cats had higher NT-proBNP concentrations than female cats when both sexes comprised of both intact and neutered cats.
- Neutered male cats had higher cTnI concentrations than intact female cats.
- Birman cats had higher cTnI concentrations than NF cats.
- An effect of breed was found with six miRNAs being differentially expressed between NF and DSH cats.
- Blood pressure increased with age and was higher in NF and DSH cats than in Birman cats.
- Blood pressure measured with the cat placed in its own carrier resulted in less variable BP and PR readings than when measuring BP with the cat placed on the table.

In cats with HCM:

- The POCT provided an estimate of NT-proBNP concentration similar to that found using the ELISA test.
- Plasma NT-proBNP and serum cTnI concentrations were higher than in healthy cats, and cats with LAE had higher concentrations than cats without LAE.
- One miRNA (fca-miR-204-5p) had lower expression level in NF cats with HCM compared to in healthy NF cats.

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Popular science summary

A biological marker (biomarker) is a biological measurement which can objectively measure and evaluate a physiological or pathological process in a cell or an individual. The use of biomarkers in research, as well as in clinical practice, improves the possibility of diagnosing diseases such as hypertrophic cardiomyopathy (HCM), thus enabling targeted monitoring and treatment for the disease and possibly resulting in more reliable prognosis. The research presented in this thesis primarily focuses on cardiac biomarkers and their association with feline characteristics in healthy cats, and contrasts concentrations of circulating cardiac biomarkers in healthy cats with concentrations in cats with HCM. Hypertrophic cardiomyopathy is the most common heart disease in cats, and the disease is characterised by thickening of the left ventricle of the heart.

Blood pressure (BP) is a physiologic cardiac biomarker, which can be used to evaluate the aetiology for left ventricular hypertrophy. Hypertension has been reported to increase LV hypertrophy due to increased systemic vascular resistance. Circulating cardiac biomarkers, N-terminal prohormone-B type natriuretic peptide (NT-proBNP) and cardiac troponin I (cTnI) are the most commonly used cardiac biomarkers in cats. N-terminal-prohormone-B-type natriuretic peptide is released into the bloodstream when cardiac walls are subjected to stretching. Cardiac TnI increases in serum if cardiac muscle cells are injured. MicroRNAs (miRNAs) are tissue-specific small molecules that regulate the protein content of the body's cells. In cats with HCM, several circulating miRNAs have been reported to have higher or lower expression levels in blood compared to healthy cats.

The objectives of this thesis were to investigate possible associations between cardiac biomarkers (BP, NT-proBNP, cTnI, and miRNA) and feline characteristics (breed, sex, age, body weight and body condition score) in

healthy domestic shorthair cats, Birman and Norwegian Forest cats. We also compared the concentrations of circulating cardiac biomarkers in healthy cats of these breeds with the concentrations in cats (regardless of breed) with HCM with and without left atrial enlargement (LAE). We found that in healthy cats, *breed* was associated with cTnI, miRNA and BP, *sex* was associated with the concentration of NT-proBNP and cTnI, and *age* was associated with BP. We also found that in cats with HCM, left ventricular hypertrophy and LAE were associated with higher concentrations of NT-proBNP and cTnI. One miRNA had lower expression levels in blood in NF cats with preclinical HCM than in healthy NF cats.

In summary, we found that there were associations between cardiac biomarkers and characteristics as breed, sex, and age in healthy cats. This needs to be taken into consideration when further evaluating these biomarkers. The fact that concentrations of the circulating cardiac biomarkers NT-proBNP and cTnI were significantly higher in cats with HCM and LAE than in cats with HCM without LAE opens up for future studies on their possible use for evaluating prognosis in cats with HCM. The biomarker NT-proBNP can be used to increase suspicion of cardiac disease in cats.

Populärvetenskaplig sammanfattning

En biologisk markör (biomarkör) är ett biologiskt mått som objektivt kan mäta och utvärdera en fysiologisk eller patologisk process i en cell eller en individ. Användningen av biomarkörer i forskning, såväl som i klinisk praxis, förbättrar möjligheten att diagnostisera sjukdomar som hypertrofisk kardiomyopati (HCM), vilket möjliggör en riktad behandling av sjukdomen och eventuellt en mer tillförlitlig prognos. Forskningen som presenteras i denna avhandling fokuserar främst på hjärtbiomarkörer och deras samband med kattegenskaper hos friska katter, och för att kontrastera jämförs koncentrationer av cirkulerande hjärtbiomarkörer hos friska katter med koncentrationer hos katter med HCM. Hypertrofisk kardiomyopati är den vanligaste hjärtsjukdomen hos katter. Sjukdomen kännetecknas av förtjockning av hjärtats vänstra kammare.

Blodtryck (BP) är en fysiologisk hjärtbiomarkör som används för att utvärdera orsaker till förtjockning av vänster kammare. Hos katt kan högt blodtryck medföra att vänster kammare förtjockas på grund av ett ökat vaskulärt tryck. Cirkulerande hjärtbiomarkörer såsom N-terminal prohormon-B-typ natriuretisk peptid (NT-proBNP) och kardiellt troponin I (cTnI) är de vanligaste hjärtbiomarkörerna hos katter. N-terminal-prohormon-B-typ natriuretisk peptid frisätts i blodet när hjärtväggarna utsätts för uttänjning. Kardiellt TnI ökar i serum om hjärtmuskelceller skadas. MikroRNA (miRNA) är vävnadsspecifika små molekyler som reglerar proteininnehållet i kroppens celler. Hos katter med HCM har flera cirkulerande miRNA rapporterats ha högre eller lägre uttrycks nivåer i blod jämfört med friska katter.

Målen med denna avhandling var att undersöka möjliga samband mellan hjärtbiomarkörer (NT-proBNP, cTnI, miRNA och blodtryck) och katters egenskaper (ras, kön, ålder, kroppsvikt och hull) hos friska huskatter, birmor

och norska skogkatter. Vi jämförde också koncentrationerna av cirkulerande hjärtbiomarkörer hos friska katter av dessa raser med koncentrationerna hos katter (oavsett ras) med HCM med och utan vänster förmaksförstoring (LAE). Vi fann att hos friska katter var ras associerad med cTnI, miRNA och BP, kön var associerad med NT-proBNP och cTnI, och ålder var associerad med BP. Vi fann också att hos katter med HCM var vänsterkammarhypertrofi och LAE associerade med högre koncentrationer av NT-proBNP och cTnI. Ett miRNA hade lägre uttrycksnivåer i blod hos NF-katter med preklinisk HCM än hos friska NF-katter.

Sammanfattningsvis fann vi att det fanns samband mellan hjärtbiomarkörerna och egenskaper såsom ras, kön och ålder hos friska katter. Detta bör beaktas vid fortsatt utvärdering av biomarkörer. Det faktum att koncentrationerna av de cirkulerande hjärtbiomarkörerna NT-proBNP och cTnI var signifikant högre hos katter med HCM och LAE än hos katter med HCM utan LAE öppnar upp för framtida studier av deras möjliga användning för att utvärdera prognos hos katter med HCM. Biomarkören NT-proBNP kan användas för att öka misstanken om hjärtsjukdom hos katt.

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STANDARD ARTICLE

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Influence of clinical setting and cat characteristics on indirectly measured blood pressure and pulse rate in healthy Birman, Norwegian Forest, and Domestic Shorthair cats

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Abstract

Background: Measured indirect blood pressure (BP) results in cats in a clinical environment might be affected by stress and characteristics of the cats.

Hypothesis: To investigate the influence of clinical setting, cat characteristics, and life situation on BP and pulse rate (PR) in healthy cats.

Animals: Ninety-four healthy Domestic Shorthair, Birman and Norwegian Forest cats.

Methods: Blood pressure measured by high-definition oscillometry in 3 settings: cat placed in its own carrier with veterinarian present; cat placed in carrier with owner alone present; and cat placed on table with veterinarian present. Statistical analyses were performed using mixed linear models.

Results: Systolic BP (SBP) did not differ among settings. Higher mean arterial pressure (MAP), diastolic BP (DBP), and PR were found when measurements were performed with cat placed on table, rather than in carrier. Coefficients of variation (CVs) higher for SBP, MAP, DBP, and PR when measured with cat placed on table than in carrier. Birman cats had lower BP than other breeds. Systolic BP, MAP, DBP, and PR increased with age. Cats allowed outdoors had lower PR than cats living strictly indoors.

Conclusion and Clinical Importance: No difference in SBP was found among settings, but measuring BP with the cat placed on the examination table gave higher MAP, DBP, PR, and CV than measuring BP with the cat in its carrier. Breed affected BP, with lower BP in Birman cats than other breeds. Blood pressure

Abbreviations: APPW, arterial pulse pressure wave; BCS, body condition score; BP, blood pressure; BW, body weight; Carrier-O, carrier-owner; Carrier-VO, carrier-veterinarian-owner; CI, confidence interval; CV, coefficient of variation; DBP, diastolic blood pressure; DSH, non-purebred Domestic Shorthair; FS, fractional shortening; HDO, high-definition oscillometry; HR, heart rate; IVSd, interventricular septum diastole; $IVSd_{inc\%}$, percentage increase interventricular septum diastole; LA, left atrium; LA/Ao, left atrial-to-aortic root diameter ratio; LV, left ventricular; LVFWd, left ventricular free wall diastole; $LVFWd_{inc\%}$, percentage increase left ventricular free wall diastole; LVIDd, left ventricular internal diameter diastole; $LVIDd_{inc\%}$, percentage increase left ventricular internal diameter diastole; LVIDs, left ventricular internal diameter systole; MAP, mean arterial blood pressure; NF, Norwegian Forest; PR, pulse rate; SBP, systolic blood pressure; Table-VO, table-veterinarian-owner; TOD, target organ damage; TT4, total thyroxine.

increased with age. Pulse rate was lower in cats allowed outdoors than cats living strictly indoors.

KEYWORDS
Birman, breed, oscillometry, pulse rate

1 | INTRODUCTION

Regulation of blood pressure (BP) is complex, involving the cardiovascular, nervous, renal, and endocrine systems. Excitement, stress, and anxiety in a clinical environment might increase BP by sympathetic activation (ie, situational hypertension[1]) in cats,[2] dogs,[3,4] and humans,[5] impairing the reliability of indirect BP measurements. The clinical setting has been shown to affect BP measurements in dogs, with higher systolic BP (SBP) and diastolic BP (DBP) results when performed by the veterinarian alone, compared to when the owner was present.[3] Few published studies have investigated how different clinical settings might affect measurements of BP and pulse rate (PR) in healthy cats.[6,7]

Breed differences have been identified for BP and PR in dogs,[3,8] whereas to the authors' knowledge, no studies have been specifically designed to investigate breed differences for these parameters in cats. Previous studies on other cat characteristics and their association with BP and PR measurements in healthy cats show variable results.[9-16]

In the clinical environment, BP measurements in cats are performed routinely by oscillometric or Doppler methods.[10,11,13,15,17] Indications for BP measurement in cats might be to identify and monitor systemic hypertension and hypotension in cats with primary disease processes that might affect BP, and potentially to screen for idiopathic hypertension in older cats.[1,18] Measurement of BP is indicated when evidence of target organ damage (TOD), such as hypertensive ocular TOD and left ventricular (LV) hypertrophy, is present, and hypertension thus might be suspected.[1,19]

Our primary aims were to investigate how different clinical settings (cat in a carrier with the owner and veterinarian present, cat in carrier with only the owner present, cat on the table with the owner and veterinarian present) and cat characteristics (breed, age, sex, body weight, and body condition) affect indirectly measured BP and PR in healthy cats. A secondary aim was to evaluate the potential influence of life situation (eg, single cat household, multi-cat household, being allowed outdoors, living strictly indoors) on BP and PR in healthy cats. We hypothesized that indirect BP and PR results would be affected by the clinical setting during measurement, cat characteristics, and life situation.

2 | MATERIALS AND METHODS

This prospective observational randomized study was approved by Uppsala Animal Experiment Ethics Board, Sweden (C137/13). Client-owned cats were recruited for the study by information distributed to cat owners on webpages, at seminars for owners, or at the recruiting clinic. Cats were examined at Evidensia Animal Clinic in Västerås in Sweden between September 2014 and June 2017. Informed written consent was obtained from the owner of each cat. The population has in part been described in a previous study.[20]

2.1 | Study design

Each cat was examined between 9:00 AM and 1:00 PM by a Swedish national veterinary specialist in internal medicine and cardiology (SH), according to a standardized protocol. Cats were brought directly to a quiet examination room upon arrival to the clinic, case history was obtained, and the cat was gently removed from the carrier and weighed on a digital scale. After an acclimatization period, BP measurements were performed in different clinical settings, followed by physical examination including assessment of body condition score (BCS; 1-9),[21] echocardiography, and blood sampling, in the same manner, and in the order described. All examinations were performed without sedation of the cats. Some cats had urinalysis performed.

2.2 | Inclusion criteria

Healthy Domestic Shorthair (DSH), Birman, and Norwegian Forest (NF) cats, aged 1-14 years, were included in the study. Cats were considered healthy if their physical examination did not identify any clinically relevant abnormalities, and if echocardiogram, hematology, and biochemistry results were within normal reference intervals. Where urine was available, urinalysis was performed and results were required to be within normal reference ranges for the cat to be included.

2.3 | Exclusion criteria

If findings indicating clinically relevant organ-related, or systemic diseases were detected, or if hematology or blood biochemistry results were outside reference intervals, the cat was excluded. Cats receiving medical treatment were excluded. If the BP protocol was not followed, the cat was excluded.

2.4 | Indirect blood pressure measurement

For acclimatization, all cats were allowed to adapt to the clinical environment for at least 10 to 15 minutes in the presence of their owners

(ie, allowed to roam freely) before BP measurement was performed.[1,18] Indirect BP was measured using high-definition oscillometry (HDO) device (Vet Memodiagnostic HDO monitor, S+B medVET, Babenhausen, Germany) with the C1 cuff applied to the base of the tail. Measurements were taken in 3 different clinical settings: 2 with the cat placed in its own carrier (a covered pet carrier) and 1 with the cat placed on the examination table (with rubber matting). The clinical settings were as follows: (a) cat placed in its carrier with the owner and veterinarian present in examination room. The veterinarian performed 6 measurements (setting carrier-veterinarian-owner [Carrier-VO]); (b) cat placed in its carrier and after instructing the owner on use of the device, the veterinarian left the room, and the owner performed 6 measurements (setting carrier-owner [Carrier-O]); and (c) cat placed on the examination table, with both the owner and veterinarian present. The veterinarian performed 4 measurements (table-veterinarian-owner [Table-VO]).

At least 10 seconds were allowed to elapse between all cuff inflations. The sequence of settings was randomized for all cats using Microsoft Excel. For practical reasons, the 2 settings with the cat placed in the carrier were always carried out sequentially, but in randomized order, whereas the setting with the cat placed on the table was randomized to be performed either first or last. In settings Carrier-VO and Carrier-O, the cuff was placed on the tail after the acclimatization period, and the cat was gently placed in the carrier, which then was closed. The cat had the cuff on the tail for 5 minutes before the measurement started. In setting Table-VO, the cat was gently placed on the table after the acclimatization period, the cuff was placed on the tail and the cat was gently held by the owner during measurements. The cat was allowed to settle and when it was still on the table, standing or resting in sternal recumbency, BP measurement started. For all settings, SBP, MAP, DBP, and PR were recorded using the HDO device. No readings were excluded at the time of measurement. If the HDO device failed to obtain a reading and a new cuff inflation had to be performed, it was noted as an error by the veterinarian in settings Carrier-VO and Table-VO. Errors could not be noted by the veterinarian in the setting Carrier-O, with the owner only present.

In some cats, subjective evaluation of the arterial pulse pressure wave (APPW) form was performed by the veterinarian after each measurement in settings Carrier-VO and Table-VO. The APPW form was assessed as "adequate" if the pulse waves generated a bell curve pattern, and "inadequate" if the waveform had substantial distortion (Supplement 1).[22]

2.5 | Echocardiography

Echocardiographic examination was performed using an ultrasound unit (IE33, Philips Ultrasound, Bothell, Washington) with a 4 to 12 MHz phased-array probe, and continuous ECG monitoring.[23] Left atrial-to-aortic root diameter ratio (LA/Ao) was measured from the right 2-dimensional (2D) short-axis view.[24] End-diastolic and systolic LV dimensions (interventricular septum diastole [IVSd], LV internal diameter in diastole [LVIDd], LV free wall diastole [LVFWd], LV internal diameter in systole [LVIDs], and fractional shortening [FS]) were measured from M-mode and 2D images.[23,25] Expected body weight (BW)-dependent values for IVSd, LVIDd, and LVFWd were calculated according to previously generated formulas for cats.[26] Mitral, tricuspid, aortic, and pulmonic valves were interrogated using spectral and color flow Doppler.[23,25]

2.6 | Blood and urine analyses for health assessment

Blood sampling was performed by venipuncture for analyses of selected hematology (hematocrit, hemoglobin, white blood cell count), and biochemistry tests (alanine aminotransferase [ALT] activity, and serum creatinine, glucose, total protein concentrations) at Evidensia Animal Clinic in Västerås using the ProCyte (IDEXX ProCyte Dx, IDEXX Laboratories, Inc., Westbrook, Maine) and Catalyst (Catalyst Dx Chemistry Analyzer, IDEXX Laboratories, Inc., Westbrook, Maine) systems. Serum samples for total thyroxine (TT4) and fructosamine concentrations were analyzed by chemiluminescence at the Clinical Pathology Laboratory at the University Animal Hospital of the Swedish University of Agricultural Sciences, using the Immulite (IMMULITE 2000, Siemens Healthcare GmbH, Erlangen, Germany) and Abbott Architect (Abbott Architect c4000, Abbott Park, Illinois) systems, respectively.

A voided urine sample from the cat was collected by the owner at home, if possible. Urine samples were examined by urine dipstick analysis (Siemens Multistix 10SG, Erlangen, Germany). Urine specific gravity was measured using a digital refractometer (Pocket refractometer, Atago, Tokyo, Japan).

2.7 | Statistical analyses

Statistical analyses were performed using commercially available software (SAS 2017, SAS Institute Inc., Cary, North Carolina),[27] (JMP Pro 12.2.0, SAS Institute Inc., Cary, North Carolina). Data on SBP, MAP, DBP, and PR are presented as mean ± SD. Coefficient of variation (CV) is presented as mean in percentage. Group comparisons for cat characteristics and echocardiographic variables were made using 1-way analysis of variance (ANOVA). Significance level was set at $P < .05$ and multiple comparisons were adjusted using Tukey's method.

When analyzing the effect of clinical setting on obtained indirect BP and PR results, readings for the first 4 measurements per setting initially were included to obtain a balanced data set (ie, the 2 last measurements for Carrier-VO and Carrier-O were excluded). In the next step, measurements with PR < 75 or > 300 pulses/min, regarded as outliers, were excluded.[28-30] Mean values, SD, and CV for SBP, MAP, DBP, and PR were determined for each cat in each setting. These summary statistics were subjected to analysis without transformation by mixed linear models using the mixed

TABLE 1 Cat characteristics, echocardiographic data, and laboratory variables in 94 healthy cats

Group	All cats	Birman	Domestic Shorthair	Norwegian Forest	*P*-value
Number	94	34	27	33	
Sex (F/M)	54/40	21/13	12/15	21/12	
Neuter status (F/NF/M/NM)	22/32/9/31	12/9/6/7	0/12/0/15	10/11/3/9	
Indoor/Outdoor	68/26	30/4	9/18	29/4	
Age (y)	5.4 ± 3.8	4.7 ± 3.9[a]	6.7 ± 4.3[a]	5.0 ± 3.0[a]	.11
BCS normal 4–5/ Overweight 6–7	48/46	23/11	11/16	14/19	
BW (kg)	4.6 ± 1.4	3.6 ± 0.7[a]	4.8 ± 1.0[b]	5.4 ± 1.6[b]	.0003
HR ausc (bpm)	158 ± 26	158 ± 20[a]	151 ± 28[a]	165 ± 27[a]	.11
LA/Ao	1.1 ± 0.1	1.1 ± 0.1[a]	1.1 ± 0.1[a]	1.1 ± 0.1[a]	.68
IVSd (mm)	3.8 ± 0.4	3.6 ± 0.4[a]	3.9 ± 0.4[b]	4.0 ± 0.4[b]	.0032
$IVSd_{inc}$ (%)	−0.5 ± 9.1	−1.1 ± 9.4[a]	0.4 ± 8.7[a]	−0.6 ± 9.4[a]	.82
LVIDd (mm)	16.2 ± 2.1	15.1 ± 1.6[a]	16.5 ± 1.8[b]	17.1 ± 2.3[b]	.011
$LVIDd_{inc}$ (%)	2.5 ± 10.3	1.1 ± 9.0[a]	3.4 ± 12.4[a]	3.3 ± 10.0[a]	.62
LVFWd (mm)	3.7 ± 0.5	3.5 ± 0.4[a]	3.8 ± 0.5[b]	3.9 ± 0.4[b]	.015
$LVFWd_{inc}$ (%)	−0.9 ± 9.6	−2.0 ± 9.5[a]	−0.2 ± 10.7[a]	−0.5 ± 8.8[a]	.74
FS (%)	50 ± 7	49 ± 7[a]	52 ± 8[a]	50 ± 7[a]	.15
Creatinine (mg/dL)	1.68 ± 0.3	1.80 ± 0.32[a]	1.58 ± 0.27[b]	1.64 ± 0.26[a,b]	.011
Hematocrit (%)	35 ± 5	35 ± 7[a]	35 ± 4[a]	37 ± 4[a]	.28
TT4 µg/dL (nmol/L)	2.4 ± 0.5 (30.6 ± 7.0)	2.3 ± 0.6[a] (28.9 ± 7.3)	2.7 ± 0.6[b] (34.3 ± 7.4)	2.3 ± 0.4[a] (29.4 ± 5.2)	.016
USG	1.053 ± 0.01 (N = 38)	1.057 ± 0.01[a] (N = 19)	1.053 ± 0.01[a] (N = 9)	1.047 ± 0.01[a] (N = 10)	.10

Note: The mean ± SD are shown for continuous variables. Within each row, values with different superscripts are statistically different between breeds. Tukey's adjustment for multiple comparisons was performed and a *P*-value of <.05 was considered significant. For urine-specific gravity (USG), results were available for a proportion of cats, and the number of cats is stated in the table.
Abbreviations: BCS, body condition score; BW, body weight; F, female; FS, fractional shortening; Indoor, indoor only; HR ausc, heart rate auscultation; IVSd, interventricular septum diastole; $IVSd_{inc}$, percentage increase interventricular septum diastole; LA/Ao, left atrial-to-aortic root diameter ratio; LVFWd, left ventricular free wall diastole; $LVFWd_{inc}$, percentage increase left ventricular free wall diastole; LVIDd, left ventricular internal diameter diastole; $LVIDd_{inc}$, percentage increase left ventricular internal diameter diastole; M, male; N, number of cats for which results were available; NF, neutered female; NM, neutered male; Outdoor, allowed outdoors; TT4, total thyroxine; USG, urine specific gravity.

TABLE 2 Blood pressure and pulse rate values in the different clinical settings

	SBP	MAP	DBP	PR
Setting	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Carrier-VO	138 ± 18[a]	94 ± 11[a,b]	70 ± 10[a]	152 ± 26[a]
Carrier-O	136 ± 18[a]	93 ± 12[a]	70 ± 11[a]	151 ± 25[a]
Table-VO	138 ± 19[a]	97 ± 14[b]	75 ± 13[b]	161 ± 32[b]
P-value	.21	.002	.011	.007
All	137 ± 18	95 ± 13	72 ± 12	155 ± 28

Note: Obtained SBP, MAP, and DBP and PR measurements in 94 healthy cats in the different clinical settings. Values are given as mean ± SD. Blood pressure is measured in mmHg, and PR in pulses per minute. Obtained BP and PR values were included for the first four measurements of each setting (in total 12 missing values), and thereafter measurements with PR <75 or >300 pulses/min, regarded as outliers, were excluded (in total 18 measurements). Within each column, values with different superscripts were statistically different between settings. Tukey's adjustment for multiple comparisons was performed and a *P*-value of <.05 was considered significant. The *P*-values presented are the highest of the pairwise comparisons within each column.
Abbreviations: BP, blood pressure; DBP, diastolic blood pressure; Carrier-O = cat placed in carrier with cuff on tail, owner alone performed the measurements; Carrier-VO = cat placed in carrier with cuff on tail, both owner and veterinarian present, veterinarian performed the measurements; MAP, mean arterial blood pressure; mmHg, millimeters of mercury; mmHg, millimeters of mercury; PR, pulse rate; SBP, systolic blood pressure; Table-VO = cat placed on examination table with cuff on tail, both owner and veterinarian present, veterinarian performed the measurements.

procedure in SAS.[27,31] Models included setting, sequence, and the interaction setting × sequence as fixed factors. Cat was set as random factor. If significant interactions were found, subsequent comparisons of least squares mean values were performed to investigate in which sequences the BP, PR, and CVs differed. Assumptions underlying analysis were checked by preparing

diagnostic plots. Post hoc comparisons were adjusted for multiplicity using Tukey's method. Although sample estimates of SD and CV are not expected to follow normal distributions, sample size was large enough to allow analyses using similar models as for mean values, and residual plots did not suggest extreme distributions.

Randomization of BP settings gave the following possible sequences: (Carrier-VO; Carrier-O; Table-VO); (Carrier-O; Carrier-VO; Table-VO); (Table-VO; Carrier-VO; Carrier-O); and (Table-VO; Carrier-O; Carrier-VO). Each of the 94 cats was subjected to each of the 3 clinical settings, resulting in 282 sessions.

Because of the high CVs for BP and PR measurements in setting Table-VO, only settings Carrier-VO and Carrier-O were included in evaluation of the effect of cat characteristics and life situation on measured BP and PR. Six measurements per setting were available. After discarding the first BP measurement in settings Carrier-VO and Carrier-O, measurements with >20% variation in SBP[1,18,22] and measurements with PR < 75 or > 300 pulses/min,[28-30] 1 mean value for SBP, MAP, DBP, and PR, respectively, was determined for each cat. Models then were built using stepwise procedures for general linear models, using the GLMselect procedure.[27] The models included the following variables and all 2-way interactions between them: breed, age, sex, BW, BCS, and life situation (ie, indoor only or allowed outdoor, number of cats in household). Age, BW, and number of cats in household were assessed as continuous variables. Body condition score was divided into 4 classes (4–7), sex into 4 classes (male, female, neutered male, neutered female), and breed was set as a classification variable. The first analyses were performed using the Lasso method,[32] because comparisons based on simulations indicated that the prediction power was slightly better for Lasso than for traditional methods, such as stepwise selection.[33] These analyses suggested that breed and age were associated with all BP

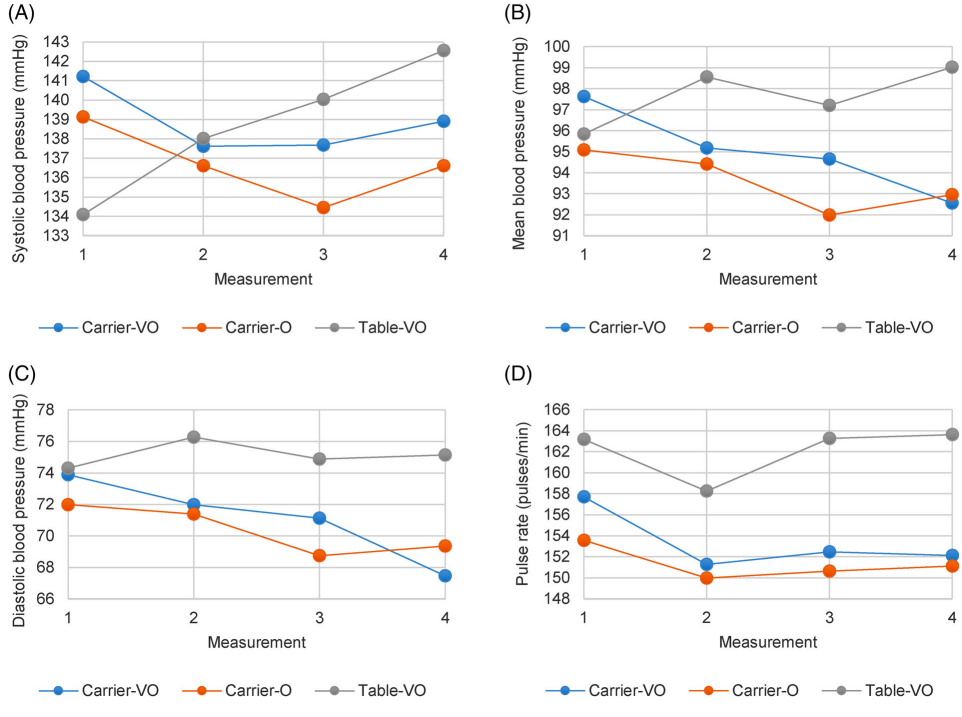

FIGURE 1 Arterial systolic (A), mean (B), and diastolic (C) indirect blood pressures, and pulse rate (D) in all cats (n = 94) in three different clinical settings by the number of measurement. Mean values are presented at each measurement. Obtained BP and PR values were included for the first four measurements of each setting. There were 12 missing values, one in the setting Carrier-O in the second measurement, and the others in the setting Table-VO in the second (N = 1), third (N = 3), and fourth (N = 7) measurement. Measurements with PR <75 or >300 pulses/min (N = 18) were regarded as outliers and excluded. Two of these were in the setting Carrier-VO in the fourth measurement: in the setting Table-Carrier-O they were in the first (N = 3) and fourth (N = 1) measurements, and in the setting Table-VO in the first (N = 4), second (N = 4), third (N = 1), and fourth (N = 3) measurements. Carrier-O = cat placed in carrier with cuff on tail, owner alone performed the measurements; Carrier-VO = cat placed in carrier with cuff on tail, both owner and veterinarian present, veterinarian performed the measurements; Table-VO = cat placed on examination table with cuff on tail, both owner and veterinarian present, veterinarian performed the measurements

variables and PR. Therefore, new stepwise analyses for SBP, MAP, DBP, and PR in which breed and age were forced into the model were performed to determine if any other variables contributed. The assumptions underlying the analyses were checked using diagnostic plots. Post hoc pairwise comparisons were adjusted for multiplicity using Tukey's method.

TABLE 3 Coefficients of variation for blood pressure variables and pulse rate

	Coefficient of variation (%)			
Settings	**SBP**	**MAP**	**DBP**	**PR**
Carrier-VO	7.3[a]	8.4[a]	12.8[a]	8.3[a]
Carrier-O	9.1[a]	8.6[a]	12.2[a]	7.4[a]
Table-VO	13.4[b]	14.3[b]	18.7[b]	12.7[b]

Note: Coefficient of variation (CV) for obtained SBP, MAP, and DBP variables and pulse rate (PR) in the three different clinical settings for the first four blood pressure measurements per setting in 94 healthy cats (in total 12 missing values, of these, one was in setting Carrier-O, and 11 in setting Table-VO). Measurements with PR <75 or >300 pulses/min, regarded as outliers, were excluded (in total 18 measurements, of these, two were in setting Carrier-VO, four in setting Carrier-O, and 12 in setting Table-VO). The CVs are given as mean values in percent. Within each column, values with different superscripts were statistically different between clinical settings. Tukey's adjustment for multiple comparisons was performed and a *P*-value of <.05 was considered significant.
Abbreviations: Carrier-O = cat placed in carrier with cuff on tail, owner alone performed the measurements; Carrier-VO = cat placed in carrier with cuff on tail, both owner and veterinarian present, veterinarian performed the measurements; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; PR, pulse rate; SBP, systolic blood pressure; Table-VO = cat placed on examination table with cuff on tail, both owner and veterinarian present, veterinarian performed the measurements.

3 | RESULTS

3.1 | Study population

Of 117 examined healthy cats, 23 were excluded. Five cats had kidney disease, 5 heart disease, 2 severe gingivitis, 2 congenital defects (diaphragmatic hernia and peritoneal-pericardial diaphragmatic hernia), 2 increased ALT activity, 1 hyperthyroidism, 2 treated with deslorelinacetate, and in 4 cats BP was not measured according to study protocol. Blood glucose concentration was mildly increased in 6 cats. However, serum fructosamine concentration, hematology, and other serum biochemistry variables were within normal limits, and thus these cats were included. Urine was available in 38 of 94 cats and results of urinalyses were within normal limits in all cats (Table 1).

Of the 94 included cats, 33 were NF, 34 Birman, and 27 non-purebred DSH. Cat characteristics, echocardiographic, and laboratory variables are presented in Table 1.

3.2 | Indirect blood pressure and pulse rate

In the data for analysis of effect of clinical setting on obtained BP and PR results, there were 12 missing BP values (11 in setting Table-VO; cats did not cooperate, and 1 in Carrier-O; owner missed 1 measurement). Because each of the 94 cats was subjected to each clinical setting, there were 1116 measurements. Of these, 18 measurements from 13 cats were identified as outliers and excluded (2 in setting Carrier-VO, 4 in Carrier-O, and 12 in Table-VO). The HDO device failed to obtain a reading and a new cuff inflation had to be performed in 55/94 cats. These errors occurred 46 times in setting Carrier-VO and 65 times in Table-VO.

TABLE 4 Blood pressure and pulse rate values divided per breed

Breed		**SBP**	**MAP**	**DBP**	**PR**
Domestic Shorthair	27	146 ± 14[a] (116-180)	98 ± 10[a] (77-123)	72 ± 9[a] (48-97)	141 ± 28[a] (90-215)
Birman	34	125 ± 12[b] (107-159)	85 ± 9[b] (68-106)	63 ± 8[b] (44-84)	159 ± 22[b] (104-193)
Norwegian Forest	33	141 ± 17[a] (109-170)	96 ± 10[a] (78-117)	72 ± 9[a] (58-92)	152 ± 25[a,b] (97-207)
P-value		<.0001	<.0001	.001	.02
All cats	94	136 ± 17 (107-180)	93 ± 11 (68-123)	69 ± 10 (44-97)	151 ± 26 (90-215)

Note: SBP, MAP, and DBP blood pressure and PR values in 94 healthy cats. Values are given as mean ± SD and (range), and are based on the clinical settings Carrier-VO and Carrier-O. Outliers have been discarded. Blood pressure was measured in mmHg and PR in pulses per minute. Within each column, values with different superscripts are statistically different between breeds. Tukey's adjustment for multiple comparisons was performed and a *P*-value of <.05 was considered significant. The *P*-values presented are the highest of the pairwise comparisons within each column.
Abbreviations: Carrier-O = cat placed in carrier with cuff on tail, owner alone performed the measurements; Carrier-VO = cat placed in carrier with cuff on tail, both owner and veterinarian present, veterinarian performed the measurements; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; PR, pulse rate; SBP, systolic blood pressure.

Systolic BP did not differ between settings (P = .21). Clinical setting had an impact on MAP, DBP, and PR (overall P = .003). Mean arterial BP, DBP, and PR were higher in setting Table-VO than Carrier-O (P = .002). Furthermore, DBP and PR were higher in setting Table-VO than Carrier-VO (P = .007). Sequence alone did not significantly affect BP variables or PR. The combined effect of setting and sequence was significant for SBP, MAP, and DBP (all P = .02). Subsequent comparisons among settings, within sequences, showed lower results for MAP in setting Carrier-O compared to Table-VO, when the owner measured BP last in the sequence (Table-VO; Carrier-VO; Carrier-O; P = .02).

Mean results for SBP, MAP, DBP, and PR in different settings for the first 4 measurements are presented in Table 2. Plots of SBP, MAP, DBP, and HR in different settings by measurement number are presented in Figure 1A-D.

3.3 | Variation in obtained indirect blood pressure and pulse rate measurements

The CVs for SBP, MAP, DBP, and PR in the different settings are presented in Table 3. Clinical setting had an impact with higher CVs for SBP, MAP, DBP, and PR results in setting Table-VO compared to both settings in the carrier (all P < .0001). Neither sequence nor the combined effect of setting and sequence affected the CV for BP values. The combined effect

TABLE 5 Association between indirect blood pressure and pulse rate, and cat characteristics and life situation in 94 healthy cats

Mean	Variable	Estimate	*P* value	95% confidence interval	
				Lower limit	Upper limit
SBP (mmHg)	Breed		<.0001		
	DSH	3.839		−3	11
	Birman	−16.029		−23	−9
	NF	Baseline breed			
	Age	1.006	.01	0.2	1.8
MAP (mmHg)	Breed		<.0001		
	DSH	−0.044		−5	5
	Birman	−11.358		−16	−7
	NF	Baseline breed			
	Age	0.769	.0031	0.3	1.3
DBP (mmHg)	Breed		.0001		
	DSH	−1.948		−6	2
	Birman	−8.592		−13	−5
	NF	Baseline breed			
	Age	0.687	.0039	0.2	1.1
PR (pulses/min)	Breed		.02		
	DSH	−4.596		−19	10
	Birman	7.488		−4	19
	NF	Baseline breed			
	Age	1.458	.036	0.1	2.8
	Life situation		.016		
	Indoor	16.462		4	29
	Outdoor	Baseline life situation			

Note: The effect of cat characteristics and life situation on indirect SBP, MAP, DBP, and PR using multivariable analysis in 94 healthy cats. A mean value from the measurements from settings Carrier-VO and Carrier-O was used for each variable. The final multiple regression model, including cat characteristics (breed, age, sex, BW, and BCS), and life situation (indoor only/allowed outdoor, and number of cats in household) had an adjusted R^2 of 0.32 for SBP, 0.32 for MAP, 0.23 for DBP, and 0.14 for PR. In this table, variables that remained significant in the final model are reported. A P-value of <.05 was considered significant.

Abbreviations: Carrier-O = cat placed in carrier with cuff on tail, owner alone performed the measurements; Carrier-VO = cat placed in carrier with cuff on tail, both owner and veterinarian present, veterinarian performed the measurements; DBP, diastolic blood pressure; DSH, Domestic Shorthair; Indoor, indoor only; MAP, mean arterial blood pressure; NF, Norwegian Forest; Outdoor, allowed outdoor; PR, pulse rate; SBP, systolic blood pressure.

of setting and sequence affected the CV for PR results (P = .04). Subsequent comparison among settings, within sequences, showed lower CV for PR in settings Carrier-O and Carrier-VO compared to setting Table-VO, when the owner measured BP first in sequence Carrier-O; Carrier-VO; Table-VO; P = .02). The APPW was available for evaluation in 43/94 cats (Supplement 1, Figure S1A-C). The percentage values of adequate and inadequate APPWs were 90% and 10%, respectively, in setting Carrier-VO, and 62% and 38%, respectively, in setting Table-VO.

3.4 | Effect of cat characteristics and life situation on indirect blood pressure and pulse rate measurements

Because each of the 94 cats was subjected to each carrier setting with 6 measurements per setting, and there was 1 missing result, there were 1127 measurements (Supplement Figure S2A-D). After discarding the first measurement in each setting, there were 939 measurements. Of these, 88 measurements from 39 cats were identified as outliers and excluded, leaving 4 to 10 included measurements per cat. Mean BP and PR measurements for each breed are shown in Table 4. Ten cats had mean SBP > 160 mmHg, of which 6 were NF cats and 4 were DSH cats. Of these 10 cats, 9 of 10 cats had a BP recording of <150 mmHg in 1 of the settings during the study.

Results of multivariable analyses of BP and PR vs cat characteristics (breed, age, sex, BW, BCS) and life situation (indoor only, allowed outdoor, number of cats in household) are presented in Table 5. All indirect BP variables were lower in Birmans than in NF and DSH cats (SBP, MAP, DBP; adjusted R^2 = 0.28, 0.26, 0.16, respectively). The PR was higher in Birman than DSH cats (adjusted R^2 = 0.06). With increasing age, all indirect BP variables (SBP, MAP, DBP; adjusted R^2 = 0.32, 0.32, 0.23; P = .01, .0003, .004, respectively), as well as PR (adjusted R^2 = 0.10; P = .04) increased. Cats allowed outdoors had lower PR (adjusted R^2 = 0.14; P = .02) as compared with cats living indoors only. Mean PR (pulses/min) ± SD was 157 ± 24 (= 68) for cats living strictly indoors, and 137 ± 26 (=26) for cats allowed outdoors.

4 | DISCUSSION

In this group of healthy cats, MAP, DBP, and PR were higher when indirect BP was measured with the cat placed on the examination table (Table-VO) compared to 1 or both settings in the cat's own carrier (Carrier-VO and Carrier-O). The CVs for SBP, MAP, and DBP as well as PR were higher in setting Table-VO compared to both settings with the cat placed in the carrier. Breed and age had an effect on BP and PR variables, with lower BP in the Birman cats than in NF and non-purebred DSH cats, and increasing BP and PR with increasing age.

The variation in recorded results was higher for all BP variables, as well as PR, when measurements were performed with the cat placed on the table compared to both settings with the cat in its own carrier (Table 3). In the first part of the study, our focus was to evaluate performance of the oscillometric system under clinical conditions in different clinical settings, and therefore the first measurement was not discarded. A higher percentage of inadequate APPWs in setting Table-VO than in setting Carrier-VO (Supplement 1, Figure S1A-C) might contribute to the higher variation in setting Table-VO. Moreover, the HDO device failed to obtain readings more often in the setting Table-VO than in the setting Carrier-VO and additional cuff inflations had to be performed, which could have had an impact on the stress of the cat. Inadequate APPWs with following high CVs could be attributed to movement or anxiety, which may have been exacerbated by failure to obtain readings and requirement for more cuff inflations during measurements on the table.[22,34,35] Overall, these factors indicate that results from setting Table-VO were less reliable in our study. A potentially more stressful situation also might explain the higher results of MAP and DBP in setting Table-VO compared to 1 or both settings in the carrier (Table 2). However, albeit statistically significant, differences in recorded values among settings were small and likely not of relevance to the individual cat in a clinical situation.

In contrast to MAP and DBP, no difference was found among settings for SBP. Although MAP and DBP showed a decreasing pattern by measurement number for both settings in the carrier, but not for setting Table-VO, this difference in pattern was not as evident for SBP, which might explain the lack of difference among settings (Figure 1A-C). Hence SBP, the most commonly assessed variable in clinical practice,[1] did not differ among settings in our study of healthy cats. Because the degree of situational hypertension has been reported to be larger in cats with hypertension than in healthy cats,[2] investigation of the effects of different clinical settings on BP recordings in cats with hypertension would be of interest.

For PR, recorded results were lower in both settings in the carrier compared to setting Table-VO and a decreasing pattern similar to observations for MAP and DBP was evident in the carrier settings, but not in setting Table-VO (Figures 1B-D). The first measurement had a higher percentage of inadequate APPW, which might be caused by cuff inflation surprising the cat, leading to movement (Figure S1B- S1C). Again, situational stress might initiate early pressor and tachycardic responses, thereby causing early increases in BP and PR, which is in accordance with previous studies in cats[2] and humans.[5] This observation emphasizes the importance of performing multiple measurements over a period of time and to exclude the first measurement as recommended.[1,2]

No significant effect of the sequence of measurements (created by randomization of settings) was found on BP measurements or PR. However, the interaction between setting and sequence was significant for SBP, MAP, and DBP, complicating interpretation of these findings. Subsequent evaluation showed lower MAP when the owner alone measured BP last with the cat in the carrier in sequence (Table-VO, Carrier-VO, Carrier-O) than with the cat placed on table first. An explanation might be that placing the cat on the table, after the acclimatization period, entails a change in the cat's situation, leading to increased BP.[2,7] The first set of measurements then was performed—a new event for the cat. The cat then was placed in the carrier,

potentially perceived as a secure place, before further measurements were performed. Cats often respond to confrontation by hiding, and the carrier might be considered a safe place to hide for many cats.[36,37] Finally, the veterinarian, who together with the owner had been present in both of the above settings, an unfamiliar person to the cat, left the room and final measurements were performed by the owner, a familiar person to the cat.[36,37] The order of these changes for the cat in the 3 settings in this specific sequence might explain the lower MAP in setting Carrier-O compared to Table-VO. In the clinical environment, being placed in the cat's own carrier, with its familiar smell, might thus cause less stress than being placed on the table.[36,37]

A significant interaction was found between setting and sequence for the CV of PR, with lower CV in both settings in the carrier than the setting on the table in sequence (Carrier-O, Carrier-VO, Table-VO). An explanation might be that the first measurements were performed by the owner alone, a familiar person, and that the cat had the possibility to hide in the carrier, leading to less perceived stress in this setting.[36,37] Thereafter, the veterinarian entered the room, and further measurements were performed by the veterinarian (setting Carrier-VO), but the cat remained in the carrier. The measurement procedure was already known to the cat, presumably inducing less anxiety for the cat in this setting.[2,36,37] The third setting, Table-VO, led to a change in the cat's situation (ie, being removed from carrier), which might lead to increased stress and the possibility for movement.[2,7]

The Birman breed had lower SBP, MAP, and DBP than NF and DSH cats (Table 4). In previous studies, no effect of breed was found for BP.[9,10,16] The Birman cats also had higher PR than DSH cats in our study. In dogs, breed differences have been reported for both BP and PR.[3,8] A previous study showed that women had comparatively lower BP and higher HR than men.[38] Studies indicate that lower BP in women might be caused by shorter length of the arterial tree and smaller LV diameter, giving a lower stroke volume in comparison with men.[38,39] In our study, standard cuff size for cats (cuff C1) was used. In the multivariable model, including breed, BW and the 2-way interaction between them, breed was associated with BP variables, whereas BW was not, indicating that the lower BP in the Birman cats was mainly a breed effect, and less dependent on smaller body size. However, breed and BW are highly covariate, and a smaller tail circumference in Birmans, relative to the cuff size, might have contributed to lower results in this breed.[15,18]

In all 3 breeds, mean SBP was within previously published reference ranges for cats obtained using oscillometric devices,[10,13,17] whereas mean DBP was under or at the lower end of previously published reference ranges.[10,17] The HDO performs a real-time analysis of the arterial oscillations to obtain pulse amplitudes and measures SBP, MAP, and DBP.[40] The lowest mean DBP was found in Birmans. This breed has a relatively low risk of being overweight[41] and also had the lowest proportion of overweight cats in our study. In people, it is important to measure DBP in relation to simultaneous magnitude of SBP. Diastolic hypertension occurs predominantly with obesity, insulin resistance, and hyperlipidemia and might evolve into systolic-diastolic hypertension, a potential increased risk for future diabetes.[42] Obesity is a risk factor for diabetes mellitus in cats and is an important health problem facing domestic cats.[43] Future studies on DBP in overweight and obese cats would be of interest, and for such studies, our results using healthy cats could serve as a foundation.

In our study, including cats of a wide range of ages, all BP variables increased with increasing age, in accordance with previous studies in cats,[9-12] dogs,[8] and humans.[38] However, this is not a consistent finding in the literature and some studies of cats have shown no effect of age on indirect BP measurements.[13-15] These variable results might be caused by different cat populations, use of different BP devices, and different handling techniques of cats during measurements. Kidney disease can be a cause for hypertension in both cats and humans. Alterations in renal vasculature, such as fibrointimal hyperplasia and hyperplastic arteriolosclerosis, are found both in healthy cats and in cats with chronic kidney disease.[44] In people and in cats,[45] aging leads to progressive stiffening of arteries caused by vascular remodeling with progressive intimal thickening, which can lead to an increase in SBP with age,[46] although these changes were found to be indistinguishable from similar lesions of aging in normotensive patients.[47] In 1 study, hypertensive cats were not more likely to be affected by fibrointimal hyperplasia than normotensive cats.[44] The process of vascular aging in cats and people is associated with vascular remodeling and inflammatory processes,[44,45,47] which might be an explanation for increasing BP with age. Pulse rate also increased with increasing age, which is in accordance with a previous study.[28] Heart rate also has been shown to increase with age in dogs.[8,48]

Pulse rate was lower in cats allowed outdoors than in cats living strictly indoors, possibly explained by cats allowed outdoors being more physically fit or more comfortable with different situations than cats living strictly indoors. However, this association was weak and should be interpreted with caution.

4.1 | Study limitations

Our study included healthy cats of 3 genetically distant breeds,[49] with relatively large group sizes. All cats were specifically recruited for the study. Cats visiting veterinary clinics for BP measurements comprise a great variety of breeds, including healthy cats as well as those with extra-cardiac diseases that might lead to hypertension. Therefore, our study population cannot be considered representative of the general population, and results cannot be extrapolated to a general cat population or to cats with hypertension.

Kidney disease is common in cats and might cause hypertension.[11,14,17] For practical reasons, urine samples were only available in 38/94 cats. Although serum creatinine concentration and hematocrit were within reference ranges in all cats, subclinical kidney disease might have been present in some cats, causing increased BP results.[11,18] In accordance with previous studies, Birman cats had serum creatinine concentrations in the upper end of normal reference ranges, which might have masked subclinical kidney disease.[50,51] Of the 94 cats, 10 had mean SBP > 160 mmHg, which might have been caused by situational stress or subclinical kidney disease. None of these cats were Birmans.

To limit the number of measurements for the individual cat and minimize the handling of each cat, 6 measurements were performed in each setting in the carrier (Carrier-VO, Carrier-O) and 4 measurements in the setting on the table (Table-VO). In the first part of the study, focusing on the performance of the oscillometric system under clinical conditions, measurements 5 and 6 in the carrier settings were excluded to obtain a balanced data set. The first measurement of each setting was included, again to evaluate the oscillometric device under clinical conditions. Thus, the protocol deviated from current *American College of Veterinary Internal Medicine* guidelines.[1] Because a large CV might affect findings by masking results, setting Table-VO was excluded in statistical analysis of effect of cat characteristics and life situation on SBP, MAP, DBP, and PR. However, exclusion of measurements might affect the data.

Inspection of the APPW form was not performed in all cats, which is a limitation, considering that if the APPW was deemed inadequate, the result from that measurement should have been excluded.[22] Thus artifacts, such as motion disturbances, that might have affected measurements could not be evaluated in these cats.[22,40]

Another potential limitation is the slight difference in time from cuff placement to measurement between carrier and table settings. In carrier settings, the cat had the cuff on the tail for 5 minutes in the carrier before measurements started, whereas on the table measurement started when the cat had settled and was still on the table. This procedure was chosen to avoid unnecessary handling of the cats on the table.

A few results in our study had weak associations, and these should be interpreted with caution.

5 | CONCLUSIONS

In our study of healthy cats, CVs were higher for indirect BP variables, as well as PR, when measurements were performed with the cat on the table compared to in the carrier. The SBP did not differ among the 3 clinical settings, whereas MAP, DBP, and PR were higher when measurements were performed on the table compared with the cat in its own carrier. The differences in recorded results for MAP, DBP, and PR among settings were small and likely not of clinical relevance for the individual cat. There was a higher percentage of inadequate APPW forms (evaluated by the veterinarian in some of the cats), when BP was measured on the table compared to in the carrier. Thus, measuring BP with the cat placed in its own carrier, thereby giving the cat the possibility to hide, might result in less variable BP. Blood pressure increased with increasing age and was lower in Birman cats than in both other breeds. Pulse rate was lower in cats allowed outdoors than in cats living strictly indoors.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the Uppsala ethical committee for animal research, Uppsala, Sweden C137/1.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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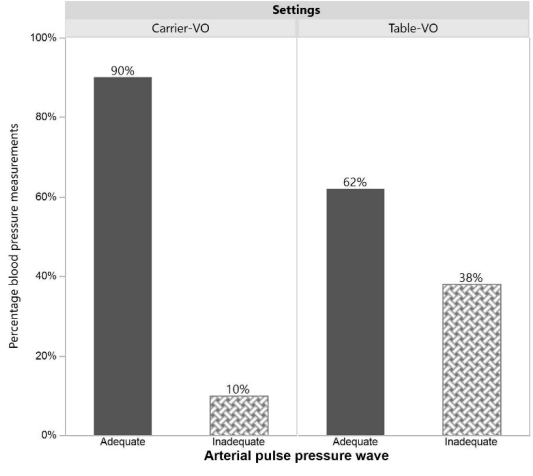
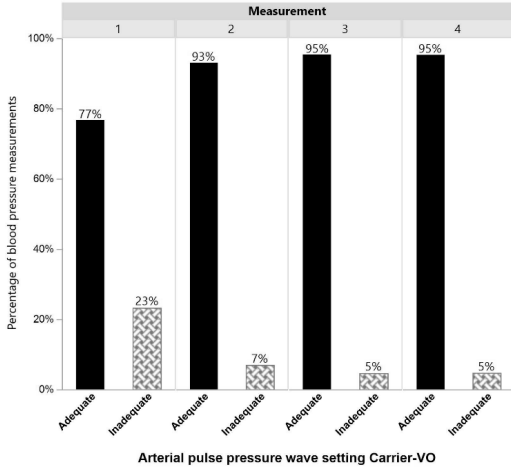
Supporting Information

Figure S1 Subjective evaluation of the arterial pulse pressure wave (APPW) form.

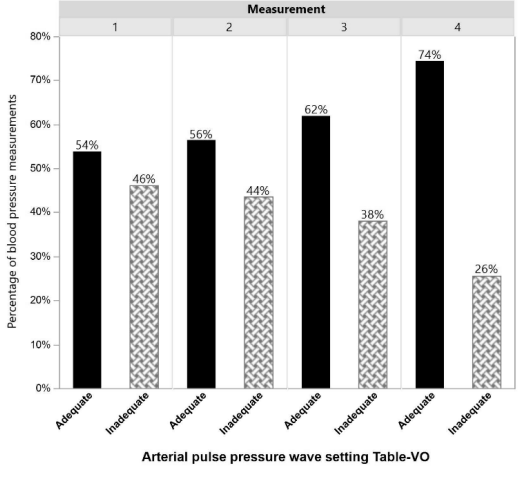
Subjective evaluation of the APPW, that is, a visual display of pulse waves detected during BP measurement, was performed in 43 of the 94 included cats. The APPW form was evaluated by the veterinarian in settings Carrier-VO and Table-VO after each measurement. An APPW form was evaluated as “adequate” if the pulse waves generated a pattern of a bell-curve, and “inadequate” if the waveform had significant distortion. When analyzing the APPW form in the clinical settings Carrier-VO and Table-VO, all obtained measurements for the first four measurements per setting were included, in order to obtain a balanced dataset, that is, the two last measurements for Carrier-VO were excluded. Furthermore, measurements with PR <75 or >300 pulses/min were regarded as outliers and were excluded. As each of the cats was subjected to two clinical settings, and there were 3 missing BP values, this resulted in a total of 341 measurements. Of these, 7 measurements were identified as outliers and were excluded. Results of the APPW form were evaluated as adequate in 255 BP measurements and as inadequate in 79 BP measurements.



(A) Percentage of BP measurements evaluated as adequate and inadequate, respectively, based on the APPW form in setting Carrier-VO is shown to the left, and in setting Table-VO to the right. The higher percentage of adequate APPW forms in setting Carrier-VO compared to setting Table-VO indicates more reliable measurements in the carrier.



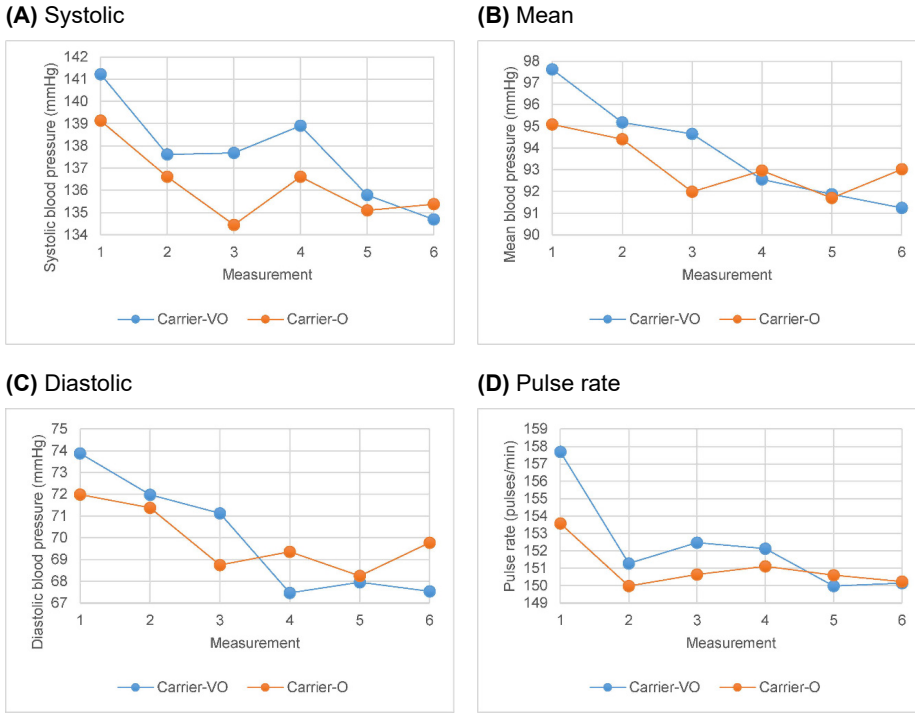
(B) Percentage of BP measurements evaluated as adequate and inadequate, respectively, in the setting Carrier-VO by number of measurement. In the setting Carrier-VO the percentage of BP measurements evaluated as adequate by number of measurement was >90% from the second measurement and onwards, indicating that the measurements in the carrier were reliable.



(C) Percentage of BP measurements evaluated as adequate and inadequate, respectively, in the setting Table-VO by number of measurement. In the setting Table-VO the percentage of BP measurement by number evaluated as inadequate was still 26% by the fourth measurement indicating less reliable measurements on the table in the present study.

Carrier-VO = cat placed in carrier with cuff on tail, both owner and veterinarian present, veterinarian performed the measurements; Table-VO = cat placed on examination table with cuff on tail, both owner and veterinarian present, veterinarian performed the measurements.

Figure S2 Obtained arterial systolic (A), mean (B), and diastolic (C) indirect blood pressures, and pulse rate (D) in all cats (n = 94) in the two clinical settings with the cat placed in its own carrier (Carrier-VO and Carrier-O) for six consecutive indirect blood pressure measurements. Mean values after exclusion of outliers (PR <75 or >300 pulses/min) are presented at each measurement.



Carrier-VO = cat placed in carrier with cuff on tail, both owner and veterinarian present, veterinarian performed the measurements; Carrier-O = cat placed in carrier with cuff on tail, owner alone performed the measurements.

II

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STANDARD ARTICLE

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Effect of feline characteristics on plasma N-terminal-prohormone B-type natriuretic peptide concentration and comparison of a point-of-care test and an ELISA test

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Abstract

Background: Increased plasma concentration of N-terminal-prohormone B-type natriuretic peptide (NT-proBNP) can be detected in cats with cardiac disease. Potential effects of feline characteristics on NT-proBNP concentration may influence clinical usefulness.

Objectives: To evaluate potential effects of feline characteristics on NT-proBNP plasma concentration and to compare NT-proBNP plasma concentrations in healthy cats with results in hypertrophic cardiomyopathy (HCM) cats with or without left atrial enlargement (LAE) using an ELISA and a point-of-care test (POCT), and assess if POCT results reflect ELISA results.

Animals: One hundred healthy cats of 3 breeds and 39 HCM cats were included.

Methods: Diseases other than HCM were excluded by physical examination, blood pressure measurement, echocardiography, hematology, and serum biochemistry.

Results: Higher NT-proBNP concentrations were found in males than in females in healthy (P = .005) and in HCM cats (P = .0021), but breed had no effect on NT-proBNP concentrations. Using ≥100 pmol/L as a cutoff for abnormal samples, ELISA and POCT had similar sensitivity (SE; 72 and 74%) and specificity (SP; 97 and 98%) for detecting cats with HCM, cats with HCM and LAE (SE, both 100%; SP, 97 versus 98%), and cats with HCM without LAE (SE, both 69%; SP, 97 versus 98%), respectively, when compared to healthy cats.

Conclusions and Clinical Importance: Breed had no effect on plasma NT-proBNP concentrations, but higher concentrations were found in male than in female cats.

Abbreviations: 2D, 2-dimension; ALT, alanine aminotransferase; Ao, aorta; BCS, body condition score; BNP, B-type natriuretic peptide; BW, body weight; CHF, congestive heart failure; CV, coefficient of variation; DSH, Domestic Shorthair; FS, fractional shortening; HCM, hypertrophic cardiomyopathy; HDO, high-definition oscillometric device; HR, heart rate auscultation; IQR, interquartile range; IVSd, interventricular septum diastole; $IVSd_{inc\%}$, percentage increase interventricular septal diameter in end diastole; LA, left atrium; LA/Ao, left atrial-to-aortic root diameter ratio; LV, left ventricle; LVFWd, left ventricular free wall diameter in end diastole; $LVFWd_{inc\%}$, percentage increase of left ventricular free wall diameter in end diastole; LVIDd, left ventricular internal diameter in end diastole; $LVIDd_{inc\%}$, percentage increase of left ventricular internal diameter in end diastole; LVIDs, left ventricular internal diameter in systole; NF, Norwegian Forest; NP, natriuretic peptide; NT-proBNP, N-terminal-prohormone-B-type natriuretic peptide; proBNP, prohormone-BNP; POCT, point-of-care test; ROC, receiver operator characteristic curve; SAM, systolic anterior motion; SBP, systolic blood pressure; SE, sensitivity; SP, specificity; T4, thyroxine.

The ELISA and POCT had similar SE and SP for detecting HCM. Both tests could identify all HCM cats with LAE but not all HCM cats without LAE.

KEYWORDS
biomarker, cardiomyopathy, feline, NT-proBNP

1 | INTRODUCTION

A high prevalence of cardiac disease has been reported in cats, and affected individuals may develop severe dyspnea because of congestive heart failure (CHF), as well as arterial thromboembolism and sudden death.[1,2] Hypertrophic cardiomyopathy (HCM) is the most common cardiac disease in cats,[3,4] and this form of cardiomyopathy is characterized by hypertrophy of the left ventricle (LV).[5] Cardiac disease remains preclinical in many affected cats, and suspicion of cardiac disease may occur when the clinician incidentally discovers a murmur, gallop sound, or arrhythmia.[3,4,6]

Cardiac biomarkers in blood samples may be measured as part of clinical assessment, especially when echocardiography is not available. B-type natriuretic peptide (BNP) is produced as prohormone-BNP (proBNP) by cardiomyocytes in response to wall stretch.[7] Inactive proBNP is cleaved intracellularly before release from the cardiomyocyte into biologically active BNP, which has a short half-life, and inactive more stable N-terminal-proBNP (NT-proBNP), which usually is measured in clinical settings.[8]

A quantitative ELISA that measures NT-proBNP concentration is available for cats, as is a semiquantitative point-of-care test (POCT).[9,10] The ELISA test is offered by a commercial laboratory (IDEXX Laboratories Inc, Westbrook, Maine), whereas the POCT offers a quick assessment of NT-proBNP concentration in the clinic. The POCT assay results are normal or abnormal based on a cutoff NT-proBNP concentration of approximately 100 pmol/L according to the manufacturer (SNAP Feline proBNP, IDEXX Laboratories Inc). The transition interval when the POCT becomes positive has been reported to range between 108 and 200 pmol/L.[9,11] Abnormal ELISA or POCT NT-proBNP results in cats have been shown to be associated with cardiac disease, implying that further diagnostic evaluation by echocardiography is indicated.[5,9-14] Previous studies have reported that both quantitative and semiquantitative NT-proBNP concentrations can discriminate cats with cardiac disease from healthy cats. Sensitivity (SE) has been described to vary between 65.4 and 93.9%, and specificity (SP) between 87.8 and 100%.[9-12,14,15] These studies included cats with various cardiac diseases.

In dogs, considerable interbreed variation exists in concentration of NT-proBNP.[16-19] Studies specifically designed to investigate breed variation in NT-proBNP concentration in cats have not been performed previously. In 1 study designed to investigate NT-proBNP concentration in cats with HCM of different severity, breed had no influence on NT-proBNP concentration.[14] In that study, several breeds were included, often comprising only a few cats per breed.[14] Furthermore, little information is available concerning other characteristics such as sex, age and body condition score (BCS) on plasma NT-proBNP concentration.[12,14,20]

We hypothesized that plasma concentrations of NT-proBNP (1) vary with characteristics such as breed, sex, age, body weight (BW) and BCS; (2) may differentiate cats with HCM with or without left atrial enlargement (LAE) from healthy cats; and (3) POCT results reflect ELISA results. Our aims were to assess potential effects of feline characteristics on NT-proBNP plasma concentration, to compare NT-proBNP concentrations in healthy cats with concentrations in HCM cats with or without LAE using ELISA and a POCT, and to assess if POCT results reflect ELISA results.

2 | MATERIALS AND METHODS

2.1 | Recruitment

The study was approved by the Uppsala Animal Experiment Ethics Board. Cats were recruited prospectively to participate in the study through information for cat owners provided on webpages, at seminars or at the recruiting clinic. Client-owned cats were examined at Evidensia Animal Clinic in Västerås between September 2014 and June 2017. Clinically healthy cats and cats with murmurs or previously diagnosed HCM were examined for possible inclusion in the study. Informed written consent was obtained from the owner of each cat.

2.2 | Inclusion criteria

Healthy cats of nonpurebred Domestic Shorthair (DSH), purebred Birman and Norwegian Forest (NF), and HCM cats of any breed were allowed into the study. Cats with preclinical or clinical HCM, stabilized as a consequence of CHF treatment, were allowed into the study. Cats aged between 1 and 14 years were eligible for the study. All healthy cats must have had normal echocardiogram. Diagnosis of HCM was based on characteristic findings on an echocardiogram as outlined below.[21-23]

2.3 | Exclusion criteria

Cats with increased mean systolic blood pressure (SBP) >160 mmHg, increased serum concentrations of thyroxine (TT4), creatinine, or fructosamine, increased alanine aminotransferase (ALT) activity, or some combination of these were excluded from the study. Cats with

decompensated CHF, thromboembolism, congenital cardiac disease, other acquired cardiovascular disorders, equivocal findings concerning the presence of hypertrophy of the LV, severe dental disease or clinically relevant organ-related or systemic diseases were not included in the study. All cats receiving medical treatment other than those in need of standard CHF treatment, standard antithrombotic treatment, medroxyprogesterone acetate, or deslorelin acetate were excluded from the study.

2.4 | Clinical examination and blood pressure measurement

All examinations were performed according to a standardized protocol in a quiet examination room by a single experienced veterinarian (S.H.). The healthy control cats were examined between 0900 and 1300 hours. The cats were allowed to adapt to the environment for 10-15 minutes together with their owners before SBP measurement.[24] An appropriate cuff was applied to the base of the tail and the cats then were allowed to rest in their carrier before SBP was indirectly measured using an automated high-definition oscillometric (HDO) device (VET Memodiagnostic HDO monitor, S+B medVET, Babenhausen, Germany).[25] Once consistent consecutive readings were obtained, 6 recordings were performed. Mean values for SBP were determined for each cat, after discarding recordings deviating more than 20%.[24]

After the SBP measurement, all cats underwent general physical examination. The BCS was recorded on a 9-point scale.[26]

2.5 | Echocardiography

Transthoracic 2-dimensional (2D), M-mode, color flow, and spectral Doppler echocardiographic evaluations were performed by an experienced echocardiographer, trained by a board-certified veterinary cardiologist, in unsedated cats placed first in right and then in left lateral recumbency using an ultrasound system (IE33, Philips Ultrasound, Bothell, Washington) equipped with a 4-12 MHz phased-array probe. Continuous electrocardiography monitoring was performed during echocardiographic examination. Images and loops from standardized imaging planes[21] were stored digitally. The left atrial-to-aortic root diameter ratio (LA/Ao) was measured in 2D from the right 2D short-axis view.[22] Diastolic and systolic LV dimensions (interventricular septum diastole [IVSd], LV internal diameter in end diastole [LVIDd], LV free wall diameter in end diastole [LVFWd], LV internal diameter in systole [LVIDs], and fractional shortening [FS]) were measured from M-mode and 2D using right parasternal short- and long-axis images.[21,23] Mitral, tricuspid, aortic, and pulmonic valves were interrogated using spectral and color-flow Doppler according to published recommendations.[21,23] Two-dimensional images of the LV outflow tract were used to identify the presence of systolic anterior motion (SAM) of the mitral valve.

The diagnosis HCM was made when a subjective impression of hypertrophy (regional or global) was supported by increased M-mode and 2D diastolic LV wall dimensions of the interventricular septum (IVSd), left ventricular free wall (LVFWd), or both defined as outlined below. Left atrial size was assessed using the LA/Ao as previously described.[22] Expected BW-dependent values for IVSd, LVIDd, and LVFWd were calculated according to previously generated formulas for cats.[27] Percentage deviation from these expected values was calculated according to the formula: $X_{inc}(\%) = ([\text{observed value-expected value}]/\text{expected value}) \times 100$, where X represents the variable in question. These calculated percentage deviations from expected BW-dependent values,[27] and the LA/Ao ratio were used to classify the cats into 3 different groups: healthy controls, HCM without LAE, and HCM with LAE. Cats with subjectively normal LV morphology, LA/Ao <1.5,[27,28] and <30% increase in BW-based predicted values for IVSd and LVFWd[27] were classified as healthy cats. Cats with subjective impression of LV hypertrophy and ≥30% increase in BW-based predicted values for IVSd or LVFWd were classified as having HCM without LAE if LA/Ao <1.5, and with LAE if LA/Ao ≥1.5. Cats that could not be classified according to the criteria specified above were excluded from the study.

2.6 | Blood collection and analysis

Blood was collected in ethylenediaminetetraacetic acid (EDTA) and serum tubes by venipuncture of the cephalic vein in unsedated minimally restrained cats. Hematology and serum biochemistry profiles, including ALT activity, creatinine, glucose, and total protein concentrations, were performed at Evidensia Animal Clinic in Västerås immediately after sampling using the ProCyte (IDEXX ProCyte Dx, IDEXX Laboratories, Inc) and Catalyst (Catalyst Dx Chemistry Analyzer, IDEXX Laboratories, Inc) systems. Total thyroxine and fructosamine concentrations were analyzed within a few days after each sampling at the Clinical Pathology Laboratory of the University Animal Hospital, Swedish University of Agricultural Sciences using the Immulite (IMMULITE 2000, Siemens Healthcare GmbH, Erlangen, Germany) and Abbott Architect (Abbott Architect c4000, Abbott Park, IL, USA) systems.

For later analysis of NT-proBNP, EDTA blood was centrifuged and plasma frozen at −80°C within 30 minutes of collection for storage. All samples were transported frozen (−80°C) to a commercial laboratory (Vet Med Labor GmbH, Division of IDEXX Laboratories, Ludwigsburg, Germany) for batch analysis in duplicate using a validated second-generation ELISA for cats (CardioPet NT-proBNP assay, IDEXX Laboratories, Inc).[29] The reported assay range for NT-proBNP concentration at the commercial laboratory was 24-1500 pmol/L. For statistical analyses, concentrations of NT-proBNP less than the lowest reported concentration were assigned a result of 24 pmol/L. Concentrations of NT-proBNP >1500 pmol/L were assigned a result of 1500 pmol/L.

All samples also were analyzed using the POCT feline NT-proBNP (SNAP Feline proBNP, IDEXX Laboratories Inc) according to the instructions of the manufacturer. The samples were randomized and the examiner (S.H.) performing the evaluation of the POCT was

blinded to the identity of the cats. Plasma was thawed and kept at room temperature for <30 minutes before analysis. Normal results were recorded when the density of the sample spot appeared lighter than the reference spot, according to the manufacturer's instructions (IDEXX Reference Laboratories Inc). Abnormal results were recorded when the density of the sample spot appeared equal to or darker than the reference spot, which, according to the manufacturer, occurs when concentrations are >100 pmol/L (IDEXX Reference Laboratories Inc). Evaluation of the POCT was performed on the same occasion by visual inspection and using an automated evaluation (SNAP Pro Analyzer, IDEXX Laboratories Inc). The examiner performed the visual inspection before checking and documenting the results from the automated evaluation. Visual inspection of the POCT results was classified into 3 groups (lighter, equal, and darker), based on subjective evaluation of the sample spot color. After all samples had been analyzed, concentrations of NT-proBNP measured by ELISA were revealed.

2.7 | Statistical analysis

Statistical analyses were performed using a commercially available software program (JMP, version 12.2.0, SAS Institute Inc, Cary, North Carolina). Group data are presented as medians and interquartile range (IQR). A value of $P < .05$ was considered significant, unless otherwise indicated.

Univariable analyses were performed separately in 3 groups of cats: all cats, healthy cats and HCM cats. Fisher's exact test was used for comparing proportions. The nonparametric Kruskal-Wallis test was used to evaluate the effects of nominal and ordinal variables (sex, neutered or intact, BCS, presence or absence of SAM) on NT-proBNP concentrations. Furthermore, the Kruskal-Wallis test was used to evaluate the overall association between the POCT results (intensity of color in the POCT) and the plasma NT-proBNP concentrations in all cats, and for evaluating the effect of breed in the healthy cat group. The Kruskal-Wallis test was used followed by Bonferroni correction for post hoc comparison between groups. These statistical analyses were repeated in the HCM cats after excluding 6 cats treated with cardiac medications (furosemide, enalapril, clopidogrel).

Spearman's ρ was used to evaluate potential associations between plasma NT-proBNP concentration and continuous variables (age, SBP, heart rate auscultation [HR] obtained from clinical examination, echocardiographic measurements, and storage time of the plasma samples) for any of the cat groups with a high rate of non-detectable NT-proBNP plasma concentrations.

TABLE 1 Feline characteristics, echocardiographic and laboratory variables in 139 cats with and without hypertrophic cardiomyopathy

	Healthy	HCM without LAE	HCM with LAE
Number	100	32	7
Sex (F/M)	57/43	12/20	1/6
Sex NF/F/NM/M	35/22/33/10	9/3/16/4	0/1/6/0
Age (years)	4.6 (2.1-8.7)[a]	5.3 (3.6-9.1)[a]	6.6 (3.0-9.0)[a]
Weight (kg)	4.3 (3.6-5.5)[a]	5.0 (4.1-5.9)[a]	5.1 (3.8-5.9)[a]
BCS normal 4-5/overweight 6-7)	49/50[1]	6/22[4]	5/2
HR (bpm)	160 (140-180)[a]	160 (143-200)[a]	180 (150-200)[a]
Murmur (yes/no)	4/96	30/2	7/0
SBP (mmHg)	135 (123-143)[a]	140 (131-148)[a]	130 (128-134)[a]
LA/Ao	1.1 (1.0-1.2)[a]	1.1 (1.0-1.2)[a]	1.6 (1.6-1.7)[b]
IVSd (mm)	3.8 (3.5-4.1)[a]	6.1 (5.3-6.4)[b]	6.8 (6.5-8.5)[b]
$IVSd_{inc}$ (%)	−0.6 (−6.3 to 5.8)[a]	52.7 (44.4-68.7)[b]	83.0 (63.5-114.6)[c]
LVIDd (mm)	15.9 (14.9-17.4)[a]	15.0 (13.2-16.5)[b]	14.7 (14.0-17.6)[ab]
$LVIDd_{inc}$ (%)	1.1 (−4.8 to 8.3)[a]	−5.6 (−18.4 to −2.1)[b]	−10.2 (−14.5 to 15.7)[ab]
LVFWd (mm)	3.7 (3.5-4.0)[a]	5.3 (4.4-6.2)[b]	7.7 (7.1-8.1)[c]
$LVFWd_{inc}$ (%)	−0.9 (−6.3 to 4.6)[a]	31.7 (14.8-58.1)[b]	105.3 (89.6-114.0)[c]
FS (%)	50 (46-56)[a]	60 (52-64)[b]	47 (41-59)[ab]
SAM (yes/no)	0/100	24/8	5/2

Note: Within each row, values with different letter superscripts are statistically different ($P < .017$) after Bonferroni correction, and values with the same superscript letter did not differ significantly. For description of the grading of hypertrophic cardiomyopathy, see the main text. Superscript numbers for specific comment for number of missing values: 1 = one missing value, 3 = three missing values, 4 = four missing values. The median and interquartile ranges are shown for continuous variables.

Abbreviations: BCS, body condition score; F, female; FS, fractional shortening; HCM, hypertrophic cardiomyopathy; IVSd, interventricular septum diastole; $IVSd_{inc\%}$, percentage increase in end-diastolic interventricular septal dimension; LA/Ao, left atrial-to-aortic root diameter ratio; LAE, left atrial enlargement; LVFWd, left ventricular free wall diameter in end diastole; $LVFWd_{inc\%}$, percentage increase in end-diastolic left ventricular free wall dimension; LVIDd, left ventricular internal diameter in end diastole; $LVIDd_{inc\%}$, percentage increase in end-diastolic left ventricular internal dimension; M, male; N, neutered; HR, heart rate auscultation; SAM, systolic anterior motion of the mitral valve; SBP, systolic blood pressure, basic echocardiographic data.

After logarithmic transformation of the concentration of NT-proBNP, linear regression was used to evaluate potential associations between plasma NT-proBNP concentration and continuous variables (age, BW, SBP, echocardiographic measurements, HR obtained from clinical examination, and storage time of the plasma samples) in any of the cat groups with a high rate of detectable NT-proBNP plasma concentrations. Variables with $P < .2$ in the univariable regression analysis were included in a multiple regression analysis as were BCS and sex. Multivariable analyses were performed in a backward stepwise manner, starting with all variables included in the model and then removing the variable with the highest P value until all of the remaining variables had a P value <.05. All variables were assessed as main effects; no interaction terms were considered in the model. The distribution of the residuals was assessed for normality by inspection of normal quantile plots. The adjusted R^2 is defined as the percentage of the total sum of squares that can be explained by the regression and it also considers the degrees of freedom for variables added.

The optimal cutoff (combination of highest SE and SP) in NT-proBNP concentration for identifying an abnormal POCT was investigated using receiver operator characteristic (ROC) curves.

3 | RESULTS

3.1 | Study population

Of 118 examined apparently healthy cats, 18 cats were excluded. Five of these cats had kidney disease, 5 had mild heart disease (4 cats had cardiac disease other than HCM and 1 cat had equivocal findings concerning presence of hypertrophy of the LV), 2 had severe dental disease, 2 had congenital defects (diaphragmatic hernia and peritoneal-pericardial diaphragmatic hernia), 2 had ALT activity above the reference range, and 1 had hyperthyroidism. Of 48 examined cats with murmurs or previously diagnosed HCM, 9 were excluded: 2 of these cats were normal, but did not meet the breed criteria for the healthy controls, 2 had congenital defects (ventricular septal defect and bicuspid Ao), 1 had equivocal findings concerning presence of hypertrophy of the LV, 1 was in decompensated CHF, 1 had kidney disease, 1 had hyperthyroidism, and 1 cat was treated with 4 times the normal dose of clopidogrel.

A total of 139 cats (69 males and 70 females) were included in the study: 100 healthy controls, 32 cats with HCM without LAE and 7 HCM cats with LAE. Three of the cats with HCM had experienced CHF and were stabilized by medical treatment. The healthy cats consisted of 35 NF, 33 Birman, and 32 DSH cats. The HCM cats comprised 13 breeds: 18 nonpurebred DSH, 5 Persian, 4 NF, 2 Bengal, 2 Maine Coon, 2 Ragdoll, and 1 each of the following breeds: British Shorthair, Cornish Rex, Exotic, Siberian, and Sphynx. Two healthy male cats were treated with deslorelin, and 1 of the female cats with HCM without LAE was treated with medroxyprogesterone acetate. Three of the cats with HCM without LAE were treated with enalapril, and 3 of the stabilized CHF cats were treated with furosemide and enalapril, of which 1 cat also received clopidogrel. Feline characteristics, concentration of NT-proBNP, and echocardiographic measurements of the 3 cat groups (healthy, HCM without LAE, and HCM with LAE) are presented in Table 1 and Figure 1. Feline characteristics, echocardiographic measurements, concentration of NT-proBNP, and POCT results of the healthy control cats of the 3 different breeds are presented in Table 2.

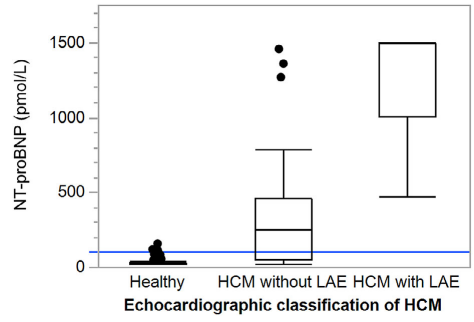


FIGURE 1 ELISA plasma concentration of NT-proBNP versus echocardiographic classification in the entire study population of 139 cats with and without hypertrophic cardiomyopathy. The NT-proBNP plasma concentration measured by ELISA compared to the echocardiographic classification are shown for normal healthy controls (n = 100), for cats with HCM without LAE (n = 32), and cats with HCM with LAE (n = 7). Blue line indicates 100 pmol/L, which was the cutoff value used for evaluation of normal and abnormal samples. Median NT-proBNP concentration for healthy was <24 (IQR <24-31) pmol/L, HCM without LAE 253 (IQR 52-456) pmol/L, and HCM with LAE 1496 (1007 to >1500) pmol/L differed significantly, $P < .0001$. HCM, hypertrophic cardiomyopathy; IQR, interquartile range; LAE, left atrial enlargement; NT-proBNP, N-terminal-prohormone B-type natriuretic peptide

Blood glucose concentration was mildly increased in 11/139 cats. However, serum fructosamine concentrations were measured and were within normal limits in all cats, as were hematology and other serum biochemistry variables.

3.2 | Evaluation of NT-proBNP plasma concentration measured by ELISA

The intra-assay coefficient of variation (CV) for the feline NT-proBNP ELISA was 5.2%-8.1%, and the interassay CVs were 7.2, 3.1, and 4.2%, respectively, for samples with mean concentrations of 57, 229, and 639 pmol/L, respectively.

3.3 | All cats

3.3.1 | Univariable analysis

The median NT-proBNP plasma concentration was higher in male (49 pmol/L; IQR, <24-300) than in female cats (<24 pmol/L; IQR, <24-40; $P < .0001$), and higher in cats with SAM ($P < .0001$) than in those that did not have SAM. The NT-proBNP concentration increased

TABLE 2 Feline characteristics and echocardiographic and laboratory variables in 100 healthy cats

Group	Birman	Domestic Shorthair	Norwegian Forest
Number	33	32	35
Sex (F/M)	21/12	14/18	22/13
Sex NF/F/NM/M	9/12/7/5	14/0/18/0	12/10/8/5
Age (years)	3.2 (1.5-7.4)[a]	7.1 (3.0-10.8)[a]	4.1 (2.1-7.2)[a]
Weight (kg)	3.4 (2.9-4.0)[a]	4.5 (4.0-5.5)[b]	5.4 (4.1-6.4)[b]
BCS normal 4-5/overweight 6-7)	21/11[1]	14/18	14/21
HR (bpm)	160 (140-164)[a]	140 (132-179)[a]	160 (140-180)[a]
Murmur (yes/no)	2/31	1/31	1/34
SBP (mmHg)	124 (116-131)[a]	140 (136-147)[b]	138 (124-145)[b]
LA/Ao	1.1 (1.0-1.2)[a]	1.1 (1.0-1.2)[a]	1.1 (1.0-1.2)[a]
IVSd (mm)	3.6 (3.4-3.8)[a]	3.9 (3.6-4.1)[b]	4.0 (3.8-4.3)[b]
$IVSd_{inc}$ (%)	−3.1 (−5.8 to 2.9)[a]	0.8 (−7.9 to 7.7)[a]	−0.3 (−6.4 to 5.9)[a]
LVIDd (mm)	15.3 (13.9-15.8)[a]	16.3 (15.0-18.3)[b]	16.4 (15.7-18.0)[b]
$LVIDd_{inc}$ (%)	0.1 (−4.6 to 7.7)[a]	2.5 (−5.6 to 10.5)[a]	1.7 (−4.0 to 7.2)[a]
LVFWd (mm)	3.5 (3.3-3.7)[a]	3.8 (3.5–4.0)[b]	4.0 (3.6-4.2)[b]
$LVFWd_{inc}$ (%)	−0.9 (−11.0 to 4.5)[a]	−1.4 (−5.7 to 6.8)[a]	−0.3 (−5.4 to 3.7)[a]
FS (%)	48 (45-56)[a]	51 (48-57)[a]	50 (46-56)[a]
SAM (yes/no)	0/33	0/32	0/35
NT-proBNP pmol/L (ELISA)	<24 (<24-39)[a]	<24 (<24-38)[a]	<24 (<24-29)[a]
POC test Normal/abnormal visual evaluation	33/0	31/1	34/1
POC test Normal/abnormal automated evaluation	30/3	30/2	35/0

Note: Within each row, values with different superscripts are statistically different ($P < .017$) after Bonferroni correction, and values with the same superscript letter did not differ significantly. Superscript numbers for specific comment for number of missing values; 1 = one missing value. The median and interquartile ranges are shown for continuous variables.

Abbreviations: BCS, body condition score; F, female; FS, fractional shortening; HR, heart rate auscultation; IVSd, interventricular septum diastole; $IVSd_{inc\%}$, percentage increase in end-diastolic interventricular septal dimension; LA/Ao, left atrial-to-aortic root diameter ratio; LVFWd, left ventricular free wall diameter in end diastole; $LVFWd_{inc\%}$, percentage increase in end-diastolic left ventricular free wall dimension; LVIDd, left ventricular internal diameter in end diastole; $LVIDd_{inc\%}$, percentage increase in end-diastolic left ventricular internal dimension; M, male; N, neutered; NT-proBNP, N-terminal-prohormone B-type natriuretic peptide; SAM, systolic anterior motion of the mitral valve; SBP, systolic blood pressure, basic echocardiographic data.

with increasing $IVSd_{inc\%}$ (R^2 = .22; $P < .0001$), $LVPWd_{inc\%}$ (R^2 = .21; $P < .0001$), and FS (R^2 = .07; P = .002).

3.4 | Healthy cats

The median NT-proBNP concentration in the healthy cats was <24 pmol/L in all 3 breeds with similar IQR across the breeds (Birman, <24-39; DSH, <24-38; NF, <24-29 pmol/L). Three of the healthy controls had concentrations >100 pmol/L (110, 117, and 155 pmol/L, respectively).

3.4.1 | Univariable analysis

Median NT-proBNP concentration was higher (P = .005) in male (25 pmol/L; IQR, <24-49) than in female cats (<24 pmol/L; IQR, <24-26).

3.5 | Cats with HCM

Of the 39 cats with HCM, 11 cats had an NT-proBNP concentration <100 pmol/L (range, <24-69 pmol/L).

3.5.1 | Univariable regression analysis

N-terminal-proBNP concentrations increased with increasing $IVSd_{inc\%}$ (R^2 = .17; P = .006), percentage increase of LVFWd ($LVFWd_{inc\%}$; R^2 = .36; $P < .0001$), and LA/Ao (R^2 = .17; P = .007), and with decreasing SBP (R^2 = .11; P = .02).

3.5.2 | Multiple regression analysis

The effect of increasing $LVFWd_{inc\%}$ ($P < .0001$) on NT-proBNP concentration was maintained in the multiple regression analysis.

Furthermore, the final model also included sex (males had higher concentrations, P = .002). The final model included the variables $LVFWd_{inc\%}$, sex, and BW had an adjusted R^2 of .50.

Only marginal changes of the results presented above were found after excluding the 6 cats treated with any cardiac medication (furosemide, enalapril, clopidogrel) from the data set, except that $IVSd_{inc\%}$ was no longer significantly associated with plasma concentration of NT-proBNP.

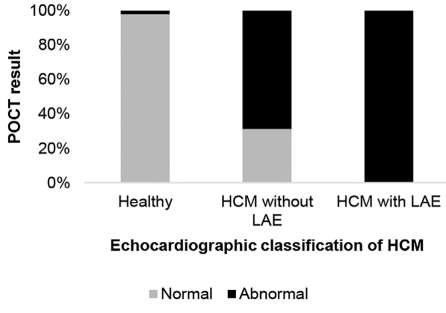


FIGURE 2 Visual evaluation of point-of-care test versus echocardiographic examination in all cats. The result of the visual evaluation of the POCT compared to the echocardiographic examination for normal healthy controls (n = 100), cats with HCM without LAE (n = 32), and cats with HCM with LAE (n = 7). The number of normal/abnormal test results for healthy controls (98/2), HCM cats without LAE (10/22), and HCM cats with LAE (0/7). Gray = normal test and black = abnormal test. HCM, hypertrophic cardiomyopathy; LAE, left atrial enlargement; POCT, point-of-care test

3.6 | Evaluation of POCT NT-proBNP test results

3.6.1 | All cats

The ELISA concentration of NT-proBNP was higher in cats when the subjective evaluation of the sample spot was darker than when the sample spot color was equal to the control spot (P < .0001), and higher in cats when the subjective evaluation of the sample spot was equal to the control spot compared to when it was lighter (P < .0001) (Figure 3). The transition interval between normal and abnormal for the POCT was between 69 and 117 pmol/L. A ROC curve showed that the optimal cutoff value (combination of highest SE and SP) for ELISA NT-proBNP concentration to identify an abnormal POCT was 110 pmol/L.

3.6.2 | Healthy cats

By visual inspection of the POCT, 98/100 healthy cats were classified as normal and 2/100 as abnormal (ELISA NT-proBNP concentrations in these cats were 110 and 155 pmol/L, respectively; Figures 2 and 3).

By automated evaluation of the POCT, 95/100 healthy cats were evaluated as normal and 5/100 healthy cats as abnormal (corresponding ELISA NT-proBNP concentrations in these cats were 49, 61, 78, 88, and 155 pmol/L, respectively).

3.6.3 | Cats with HCM

By visual inspection of the POCT, 29/39 (74%) HCM cats were classified as abnormal using the POCT (corresponding plasma NT-proBNP

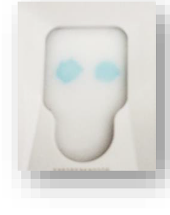
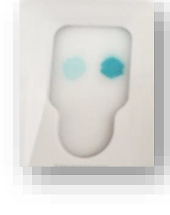
NT-proBNP	Lighter	Equal	Darker
			
Evaluation	Normal	Abnormal	Abnormal
NT-proBNP concentration (pmol/L)	24 (24-31)[a]	162 (100-217)[b]	505 (336-1312)[c]
No of POCT	108	6	25

FIGURE 3 Classification of point of care test results based on subjective evaluation of the color. Results of the subjective evaluation of the POCT test in 139 cats; the sample spot is located to the right and the reference spot to the left in each example. The outcome "lighter" is indicative of a normal NT-proBNP concentration. The outcomes "equal" or "darker" are interpreted as abnormal NT-proBNP concentrations. For the column NT-proBNP concentrations per group, the median and interquartile ranges are shown. Values with different superscripts are statistically different (P < .0001). NT-proBNP, N-terminal-prohormone B-type natriuretic peptide; POCT, point-of-care test

concentrations using the ELISA were 69 pmol/L in 1 cat and >100 pmol/L in the other 28 cats), and 10 HCM (26%) cats were classified as normal (all had ELISA concentrations <100 pmol/L; Figures 2 and 3).

By the automated evaluation, 29/39 (74%) HCM cats were classified as abnormal using the POCT (corresponding ELISA NT-proBNP concentrations were >100 pmol/L in 27 cats, and 61 and 69 pmol/L, respectively, in 2 cats), and 10/39 (26%) HCM cats were classified as normal (corresponding ELISA NT-proBNP concentrations were <100 pmol/L in 9 cats and 168 pmol/L in 1 cat).

3.6.4 | SE and SP for detecting HCM

Using ≥100 pmol/L as a cutoff value for identifying abnormal samples, the ELISA and visual inspection and automated evaluation of the POCT had SE of 72 and 74% (both visual inspection and automated evaluation) and SP of 97, 98, and 95%, respectively, for detecting HCM when healthy cats were compared to all cats with HCM. The ELISA and the visual inspection and automated evaluation of the POCT had a SE of 100% and SP of 97, 98, and 95%, respectively, for detecting HCM cats with LAE, and a SE of 69%, and SP of 97, 98, and 95% respectively, for detecting HCM cats without LAE (Figure 2) when the specific groups were compared to healthy cats.

4 | DISCUSSION

Male cats had higher plasma NT-proBNP concentrations than did female cats in our population of cats. The NT-proBNP concentration in healthy cats was not affected by age, BW, BCS, or breed. In all cats, and in the cats with HCM, the NT-proBNP concentration increased with increasing IVSd and LVFWd. Measuring NT-proBNP using the POCT and the ELISA tests showed similar SE and SP for detecting HCM. The ELISA and the POCT results were abnormal in all HCM cats with LAE, but not in all HCM cats without LAE.

Median concentration of NT-proBNP was higher in healthy male cats than in female cats in our study. This finding contrasts with studies in people in which the NT-proBNP concentration has been reported to be higher in women than in men,[30-32] and with studies in dogs, which have shown ambiguous results such as no effect of sex on the NT-proBNP concentration,[33] or a higher concentration in female dogs.[34,35] In previous studies in cats, sex has not been reported to significantly influence the NT-proBNP concentration.[12,14] Testosterone also may be a factor. A suppressive effect of testosterone has been suggested to contribute to the difference in BNP concentration between men and women,[36] and in rats, testosterone has been shown to suppress the release of NPs.[37] In our study, most of the male cats (77%) were neutered, which may be a reason for the different results compared to those in humans. A significant difference was found between sexes in the healthy cat group, and the reason for this difference hopefully will be further explored in future studies.

Although the healthy cats were from 3 genetically distant breeds,[38] with >30 cats/breed (see Table 2), no significant difference in NT-proBNP concentration was found among the breeds, which is in accordance with a previous study that included several different breeds with variable and often small breed group sizes.[14] This finding is in contrast to studies in dogs, in which significant interbreed differences have been found in NT-proBNP concentrations.[19,39]

In the cats with HCM, lower SBP was associated with higher concentrations of plasma NT-proBNP. A possible explanation for this finding may be that cats with more prominent HCM might have impaired cardiac output leading to lower, but still within normal variation, SBP.[40] Furthermore, NPs regulate several physiological processes, such as arteriolar relaxation and promotion of urinary sodium excretion (natriuresis), which potentially might lower the SBP further.[41]

Left ventricular hypertrophy, LAE or both were associated with significantly increased NT-proBNP concentrations in cats with HCM in our study. Similar findings have been described previously in cats,[12,14,15] dogs,[34] and people.[42-44] The POCT had similar SE and SP as did the ELISA for diagnosing HCM in cats, which is in agreement with previous studies in which SE of the tests ranged between 71%-92% and SP between 94%-100% for detecting cardiac disease.[12,14,15] The test results obtained by visual inspection of the POCT were similar to the automated evaluation. The POCT in our study had a SE of 74% with visual inspection or automated evaluation for detecting HCM, and the SP was slightly lower when analyzed by automated evaluation (95%) compared to visual inspection (98%) of the NT-proBNP concentration. However, our study design, with a study population consisting of either cats with HCM or healthy cats, and excluding cats with equivocal findings concerning presence of hypertrophy of the LV, other diseases than HCM, and cats with abnormal blood test results, might have overestimated the accuracy of the test and thereby resulting in higher SE and SP for the ELISA and the POCT test than if the test had been used in the general feline population. Differences in SE (65%-84%) and SP (83%-100%) in POCT results in previous studies of cats[9,11] may be explained by different study populations including cats with different cardiac diseases and different disease severities. In our study, the observed echocardiographic measurements were related to BW-dependent expected values both in the healthy cats and in the cats with HCM.[27] Evaluations of the accuracy of the POCT for assessing HCM in cats categorized using echocardiographic measurements adjusted for BW have not been published previously. In our study, none of the HCM cats with LAE had a normal POCT result, whereas 31% of the HCM cats without LAE had a normal POCT result (see Figure 2). The POCT showed a higher false-negative rate than true positive rate, which implies that a cat with a normal POCT may need further evaluation by echocardiography to exclude mild HCM. Similar results have been reported previously for both the POCT and the quantitative ELISA in cats with cardiac disease.[9,11,14]

Evaluation of the color of the sample spot at visual inspection of the POCT (see Figure 3) showed that NT-proBNP plasma concentration differed significantly among the 3 POCT groups: lighter (normal), equal (mildly abnormal) and darker (abnormal). This finding may increase the value of the POCT for the assessment of whether cardiac disease is likely or not, because the results are readily available and give an estimate of the NT-proBNP concentration.

In our study, cats with severe dental disease obvious on clinical examination were excluded because periodontitis is an inflammatory disease that causes pain, gingival bleeding, and may impact overall health.[45] In people, patients with periodontitis have been shown to have higher serum NT-proBNP concentrations than individuals without periodontitis and the greater the extent of periodontal destruction, the higher the concentration of NT-proBNP in serum.[46]

4.1 | Study Limitations

Blood was collected only once from each cat. Therefore, biological variation in NT-proBNP concentration within an individual cat could not be evaluated. Studies have indicated a high biological variation for NT-proBNP concentrations in cats, dogs, and humans, and this variability may affect the SE and SP.[47-49] The healthy control cats were examined in the morning to decrease the effect of diurnal variation among the cats included in the study. Cats with HCM were not examined at a standardized time of day because of the clinical condition of the cat and for practical reasons.

Plasma samples were stored at −80°C up to 4 years before batched analyses were performed, but storage time was not shown to be associated with the NT-proBNP plasma concentration. Furthermore, previous studies have shown that NT-proBNP concentrations are stable at the freezing temperatures used in our study.[50-52] It thus is unlikely that storage had any major effect on the results.

The POCT becomes abnormal within a transition interval rather than a set cutoff value, which is a limitation. In our study, the transition interval was found to between 69 and 117 pmol/L, which is slightly lower than a previous study in which the transition occurred between 108 and 122 pmol/L[9] but different from another study in which the transition interval was between 150 and 200 pmol/L.[11] However, the SE and the SP for the ELISA and the POCT were similar, therefore the POCT results seem reliable.

In our study, the cats with HCM without LAE generally were mildly affected and few cats had HCM with LAE. Furthermore, a few of the cats included were treated with deslorelin, medroxyprogesterone acetate, or cardiac medications. Three of the HCM cats with LAE were on medical treatment for previous CHF, and plasma concentrations of NT-proBNP in cats treated for CHF may decrease after stabilization of CHF.[53]

The design of our study, using selected inclusion and exclusion criteria, may have influenced the accuracy of the test and thereby overestimated SE and SP. Our study was intended as an explorative study, and further research in a mixed cat population is warranted.

Although no differences in NT-proBNP concentration were found among the 3 genetically distant breeds in the study, differences among other breeds cannot be excluded.

5 | CONCLUSIONS

Male cats had higher plasma NT-proBNP concentrations than did female cats, but other characteristics, such as breed, age, BW, and BCS, were not associated with plasma NT-proBNP concentration.

The POCT gave a quick semiquantitative estimate of NT-proBNP concentration, with similar SE and SP to those found using the quantitative ELISA test for detecting HCM in cats. Both tests could identify all HCM cats with LAE but not all HCM cats without LAE.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Uppsala Ethical Committee for Animal Research, Uppsala, Sweden C137/13.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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Cardiac biomarkers may be used to support diagnosis of heart diseases such as hypertrophic cardiomyopathy, a common heart disease in cats. Results presented herein elaborate on associations between cardiac biomarkers and feline characteristics in healthy cats, and between healthy cats and cats with hypertrophic cardiomyopathy. The biomarkers N-terminal-prohormone-B-type natriuretic peptide, cardiac troponin I, microRNA, and blood pressure were studied. Cardiac biomarkers were associated with breed, sex, and age and their concentrations differ between healthy cats and cats with hypertrophic cardiomyopathy.

Sofia Hanås underwent her postgraduate education at the Department of Clinical Sciences, SLU, Uppsala. Her undergraduate degree was obtained at the Faculty of Veterinary Medicine, SLU, Uppsala.

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