UNIVERSIDADE DE LISBOA
FACULDADE DE MEDICINA VETERINÁRIA

PLASMA AND URINARY METANEPHRINE AND NORMETANEPHRINE IN HEALTHY CATS – A PILOT STUDY

MARIA TERESA CARNEIRO PREGO MARQUES ALEXANDRE

ORIENTADOR:
Doutor Rodolfo Assis Oliveira Leal

2022

UNIVERSIDADE DE LISBOA
FACULDADE DE MEDICINA VETERINÁRIA

PLASMA AND URINARY METANEPHRINE AND NORMETANEPHRINE IN HEALTHY CATS – A PILOT STUDY

MARIA TERESA CARNEIRO PREGO MARQUES ALEXANDRE

DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

JÚRI
PRESIDENTE:
Doutor António José de Freitas Duarte

ORIENTADOR:
Doutor Rodolfo Assis Oliveira Leal

VOGAIS:
Doutora Maria Teresa da Costa Mendes Vitor Villa de Brito
Doutor Rodolfo Assis Oliveira Leal

2022

DECLARAÇÃO RELATIVA ÀS CONDIÇÕES DE REPRODUÇÃO DA DISSERTAÇÃO

Nome: Maria Teresa Carneiro Prego Marques Alexandre

Título da Tese ou Dissertação: PLASMA AND URINARY METANEPHRINE AND NORMETANEPHRINE IN HEALTHY CATS – A PILOT STUDY

Designação do curso de Mestrado ou de Doutoramento: Mestrado Integrado em Medicina Veterinária

Área científica em que melhor se enquadra (assinale uma):

[X] Clínica
[ ] Produção animal e Segurança Alimentar
[ ] Morfologia e Função
[ ] Sanidade Animal

Declaro sobre compromisso de honra que a tese ou dissertação agora entregue corresponde à que foi aprovada pelo júri constituído pela Faculdade de Medicina Veterinária da ULISBOA.

Declaro que concedo à Faculdade de Medicina Veterinária e aos seus agentes uma licença não-exclusiva para arquivar e tornar acessível, nomeadamente através do seu repositório institucional, nas condições abaixo indicadas, a minha tese ou dissertação, no todo ou em parte, em suporte digital.

Declaro que autorizo a Faculdade de Medicina Veterinária a arquivar mais de uma cópia da tese ou dissertação e a, sem alterar o seu conteúdo, converter o documento entregue, para qualquer formato de ficheiro, meio ou suporte, para efeitos de preservação e acesso.

Retenho todos os direitos de autor relativos à tese ou dissertação, e o direito de a usar em trabalhos futuros (como artigos ou livros).

Concordo que a minha tese ou dissertação seja colocada no repositório da Faculdade de Medicina Veterinária com o seguinte estatuto (assinale um):

1. [X] Disponibilização imediata do conjunto do trabalho para acesso mundial;
2. [ ] Disponibilização do conjunto do trabalho para acesso exclusivo na Faculdade de Medicina Veterinária durante o período de [ ] 6 meses, [ ] 12 meses, sendo que após o tempo assinalado autorizo o acesso mundial*;

* Indique o motivo do embargo (OBRIGATÓRIO)

Nos exemplares das dissertações de mestrado ou teses de doutoramento entregues para a prestação de provas na Universidade e dos quais é obrigatoriamente enviado um exemplar para depósito na Biblioteca da Faculdade de Medicina Veterinária da Universidade de Lisboa deve constar uma das seguintes declarações (incluir apenas uma das três):

1. É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTA TESE/TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.

Faculdade de Medicina Veterinária da Universidade de Lisboa, 27 de Julho de 2022.

Assinatura: 

Agradecimentos

Hoje, tenho de começar por agradecer aos meus pais, que sempre me garantiram a melhor educação e me apoiaram ao longo das várias etapas da minha vida. Tenho também muito a agradecer aos meus restantes familiares, avós, tios, primos, irmão e namorado, sem os quais não seria a pessoa que sou hoje, tanto a nível pessoal como profissional.

Gostaria, igualmente, de agradecer a todos os meus amigos que de alguma forma desempenharam um papel importante na minha vida, especialmente àqueles que viveram comigo os últimos seis anos da minha vida e que partilham a minha paixão pela medicina veterinária.

Quero também deixar um agradecimento especial a todos os que me ajudaram a concretizar este trabalho, começando pelas pessoas que partilharam o meu pedido de adesão ao estudo e relevando as que decidiram participar. Gostaria de estender este agradecimento especial aos dez gatinhos saudáveis que tornaram possível a realização deste projeto.

Ao meu professor e orientador Rodolfo Oliveira Leal, à Joana Dias e ao Nuno Santos, um muito obrigada. Obrigada pelo tempo despendido a ajudar-me, pela aprendizagem que me proporcionaram, pela boa disposição e excelente ambiente de trabalho. Foi esta equipa que reforçou para mim o sentido da expressão popular portuguesa “correr por gosto não cansa”.

Expresso ainda a minha gratidão ao Dr. Rui Lemos, que desempenhou um papel essencial na triagem dos pacientes para o estudo, e à minha colega Joana Bento, que sempre se mostrou disponível para me ajudar nesta fase do projeto. Desejo, juntamente, agradecer à enfermeira Élia Cosme, que dispôs do seu tempo para me auxiliar na obtenção das amostras biológicas.

Quero aqui deixar o meu apreço a todos os membros do Hospital Escolar Veterinário, uma vez que eu, assim como todos os restantes estagiários, fui desde logo muito bem recebida e integrada por todos os médicos, enfermeiros, auxiliares e rececionistas. Estendo este apreço aos membros do Laboratório de Análises Clínicas Prof. Dr. Braço Forte, com um especial agradecimento à Dr. Salomé Gonçalves.

É com enorme gratidão que refiro a Professora Sara Galac, um elemento essencial para a realização deste estudo, que sempre se prontificou a colaborar comigo.

Finalmente, agradeço ao Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA) da Faculdade de Medicina Veterinária, Universidade de Lisboa, o financiamento do presente trabalho (código da bolsa: UIDB/00276/2020).

METANEFRINAS E NORMETANEFRINAS PLASMÁTICAS E URINÁRIAS EM GATOS SAUDÁVEIS – UM ESTUDO-PILOTO

Resumo

O feocromocitoma (PCC) felino é considerado raro e a literatura é limitada a poucos relatos de casos clínicos. Em humanos e cães, o diagnóstico bioquímico de PCC é baseado na medição de metanefrinas (MN) e normetanefrinas (NMN) plasmáticas (PL) e/ou urinárias (U), mas pouco se sabe sobre estes biomarcadores em gatos.

Este estudo piloto tem como objetivo avaliar a viabilidade da medição das PL-MN/NMN e U-MN/NMN em gatos, utilizando cromatografia líquida acoplada à espectrometria de massa em tandem (LC-MS-MS). Além disso, pretende aferir a estabilidade das U-MN/NMN sob refrigeração (+4ºC) por 24h.

Foi realizado um estudo piloto transversal, utilizando um grupo de 10 gatos saudáveis, com idades entre os 4 e 10 anos. Amostras de EDTA-plasma e urina foram imediatamente armazenadas a -80ºC após colheita, até à medição das PL-MN/NMN e U-MN/NMN. Para cada gato, uma amostra de urina adicional foi refrigerada (UR) por 24h antes do armazenamento a -80ºC. Amostras de plasma e urina coletadas de um gato com diagnóstico definitivo prévio de PCC (PheoCat) também foram submetidas para análise. A creatinina urinária (Creat) foi medida nas mesmas amostras de urina para calcular os rácios U-MN/Creat e U-NMN/Creat.

Relativamente à população de gatos adultos saudáveis, a mediana das PL-MN e PL-NMN foi 2,73nmol/L (IQR=2,37) e 7,02nmol/L (IQR=5,2), respetivamente. Os rácios U-MN/Creat e U-NMN/Creat tiveram medianas de 70µg/g (IQR=70) e 139µg/g (IQR=77), respetivamente. O PheoCat teve valores de PL-MN de 3,68nmol/L, PL-NMN de 66,27nmol/L, rácio U-MN/Creat de 179 µg/g e rácio U-NMN/Creat de 1262 µg/g. Nenhum destes valores se sobrepôs às medianas obtidas nos gatos saudáveis. As U-MN/NMN revelaram ser estáveis sob refrigeração por 24h, uma vez que não houve diferença estatística entre U-MN vs UR-MN e U-NMN vs UR-NMN (p= 0,329 e p= 0,813, respetivamente).

Este é o primeiro estudo que afere as PL-MN/NMN e U-MN/NMN por LC-MS-MS em gatos adultos saudáveis e contribuirá para o diagnóstico bioquímico de PCC felino no futuro. O PheoCat teve um aumento substancial de todos os parâmetros medidos quando comparado com os gatos saudáveis, principalmente da PL-NMN e rácio U-NMN/Creat, destacando a aplicabilidade clínica destes biomarcadores no diagnóstico de PCC em gatos.

PALAVRAS-CHAVE: Feocromocitoma; Metanefrinas plasmáticas e urinárias; Cromatografia líquida com espectrometria de massa em tandem; Gatos saudáveis.

PLASMA AND URINARY METANEPHRINE AND NORMETANEPHRINE IN HEALTHY CATS – A PILOT STUDY

Abstract

Feline pheochromocytoma (PCC) is considered to be rare and literature is limited to a few case-reports. In humans and dogs, biochemical diagnosis of a PCC is based on the measurements of plasma (PL) and/or urinary (U) metanephrine (MN) and normetanephrine (NMN) but little is known about these biomarkers in cats.

This pilot study aims to evaluate the feasibility of PL-MN/NMN and U-MN/NMN measurement in cats, using liquid chromatography with tandem mass spectrometry (LC-MS-MS). Furthermore, it intends to assess the U-MN/NMN stability under refrigeration (+4ºC) for 24h.

A cross-sectional pilot study was conducted, using a group of 10 healthy cats, with ages ranging from 4 to 10 years. After sampling, EDTA-plasma and urine samples were stored at -80ºC until measurement of PL-MN/NMN and U-MN/NMN. For each cat, an additional urine sample was refrigerated (UR) for 24h before storage at -80ºC. Leftover plasma and urine samples collected from a cat with a confirmed diagnosis of PCC (PheoCat) were also submitted for analysis. Urinary creatinine (Creat) was measured in the same spot urine samples in order to calculate U-MN/Creat and U-NMN/Creat ratios.

With regard to the population of healthy adult cats, the PL-MN and PL-NMN median values were 2.73nmol/L (IQR=2.37) and 7.02nmol/L (IQR=5.2), respectively. U-MN/Creat and U-NMN/Creat ratios had medians of 70µg/g (IQR=70) and 139µg/g (IQR=77), respectively. Results obtained from the PheoCat revealed a PL-MN of 3.68nmol/L, PL-NMN of 66.27nmol/L, U-MN/Creat ratio of 179 µg/g and U-NMN/Creat ratio of 1262 µg/g. None of these values overlapped with the medians obtained from the healthy cats. The U-MN/NMN proved to be stable under refrigeration for 24h, as there was no statistical difference between U-MN vs UR-MN and U-NMN vs UR-NMN ($p= 0.329$ and $p= 0.813$, respectively).

This is the first study reporting both PL-MN/NMN and U-MN/NMN measurements by LC-MS-MS in adult healthy cats and will contribute to the biochemical diagnosis of feline PCC in the future. The PheoCat had a substantial increase in all the measured parameters, particularly PL-NMN and U-NMN/Creat ratio, when compared to the healthy cats, highlighting the clinical applicability of these biomarkers in the diagnosis of PCC in cats.

KEYWORDS: Pheochromocytoma; Plasma and urinary metanephrines; Liquid chromatography with tandem mass spectrometry; Healthy cats.

Table of contents

List of figures

List of tables

List of graphs

List of abbreviations and symbols

AADC - Aromatic-l-amino acid decarboxylase

ACTH - Adrenocorticotropic Hormone

ANS – Autonomic Nervous System

BID – *Bis in die*

CKD – Chronic Kidney Disease

CNS – Central Nervous System

COMT - Catecholamine-0-methyltransferase

Creat – Creatinine

CRF - Corticotropin Releasing Factor

CT – Computed Tomography

DHB - Dopamine-β-hydroxylase

DOPA - 3,4-dihydroxyphenylalanine

Epi – Epinephrine

GC – Glucocorticoids

HPLC - High Performance Liquid Chromatography

IQR – Interquartile range

MAO - Monoamine oxidase

MN – Metanephrine

NE - Norepinephrine

NMN – Normetanephrine

LC-MS-MS - Liquid Chromatography with tandem mass spectrometry

LDDST – Low Dose Dexamethasone Suppression Test

PBZ – Phenoxybenzamine

PCC – Pheochromocytoma

PL - Plasma

PMNT - Phenylethanolamine-N-methyltransferase

PO – *Per os*

PRA – Plasmatic Renin Activity

SBP – Sistolic Blood Pressure

TH - Tyrosine Hydroxylase

U – Urinary

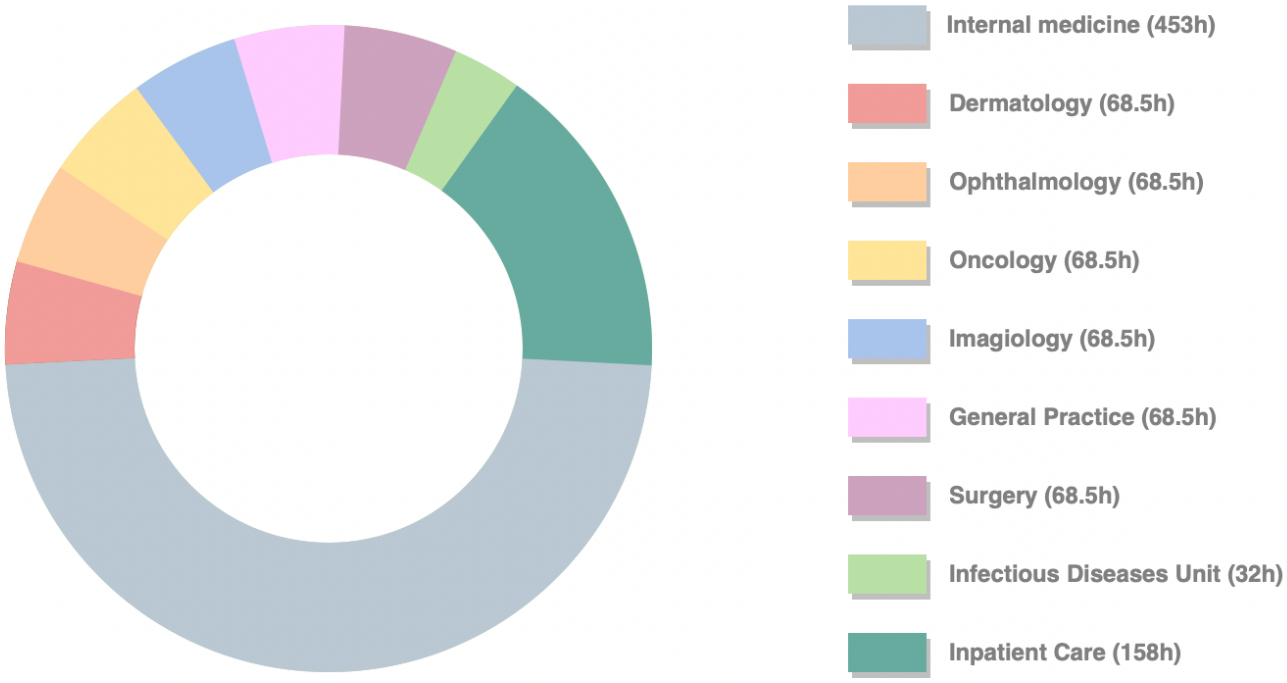
UR – Refrigerated Urine

USG – Urine Specific Gravity

1. TRAINEESHIP REPORT

My curricular internship had a duration of six months, with a total of 1054 hours (h). It took place at the Veterinary Hospital of the Faculty of Veterinary Medicine (HEV) - University of Lisbon, from September 2021 to March 2022. My internship was divided into two different periods. In the first three months, I was assigned to the department of Internal Medicine, in which I joined the Referral Internal Medicine Service (SMIR) team. In the second trimester, I was scheduled on a rotation basis across the different hospital departments along with the other trainees, which allowed me to acquire skills in different fields of the small animal practice (graph 1).

***Graph 1*- Distribution of time spent in each department.**



Legend: h – hours.

This trainee period was essential for the accomplishment of the current work, since it was during the time spent in the hospital that I was able to recruit the animals enrolled in the study and to perform the medical evaluation required and sample collection.

1.1. Internal Medicine

Internal Medicine has always been the area that most fascinated me in the Clinic of Companion Animals. For that reason, I decided to do my internship under the mentorship of Prof. Dr. Rodolfo Oliveira Leal, Dipl.ECVIM-CA, his resident Dr. Joana Dias and his intern Dr.

Nuno Santos. During the three months I spent on the Internal Medicine Service, I gained an understanding of how to diagnose and treat the most common diseases of the endocrine, digestive, urinary, hematologic and respiratory systems. Furthermore, I closely contacted with difficult and challenging cases.

Throughout these months, we started the day-work with a medical round, focusing on the inpatient care animals. In these rounds, each clinical case was presented and discussed in a multidisciplinary approach. In the course of the morning, I accompanied Prof. Rodolfo both in his first-time consultations and follow-ups. Under his supervision, I was allowed to participate in the anamnesis collection of the patients, which I would then discuss with the professor right before the consultation. Once a week, I also assisted in endoscopies, which allowed me to gain experience in recognizing the main endoscopic alterations in upper endoscopies, colonoscopies, rhinoscopies and tracheobronchoscopies. I had the opportunity to actively participate in the elaboration of the consultations and endoscopy reports. On top of this, we performed other procedures such as bronchoalveolar lavages, joint and bone marrow punctures, tracheal stent placement, among others. During the afternoon, we talked through the clinical cases we had seen in the morning and discussed the most likely differential diagnoses, the adequate complementary exams to perform, the most appropriate treatment plan and prognosis.

In this period, I had the chance to take part in a weekly journal club and book club. These activities consisted in a group presentation of an article and chapter of the Textbook of Veterinary Internal Medicine, assigned to each one of us.

Furthermore, I was assigned the elaboration of a poster with the title “Corpos estranhos traqueobrônquicos – Um diferencial importante na prática clínica: A propósito de quatro casos clínicos”, which was submitted to communication at *Congresso Internacional Hospital Veterinário Montenegro*, in October 2021 (Annexe 1).

1.2. Dermatology

I spent two weeks in the dermatology department, during which I worked with Prof. Dr. Ana Mafalda Lourenço and Dr. Hugo Pereira. Under their guidance, I was responsible for taking the patient’s anamnesis, doing a complete physical and dermatological examination and performing some skin diagnostic tests, such as superficial and deep skin scrapings, trichograms, fine-needle aspiration cytology and analysis of skin and ear canal cytology. I also learned how to collect and send skin, hair and ear samples to bacteriological and mycological culture. Additionally, I witnessed a few other procedures including Wood’s lamp examination, taking of skin biopsies and video otoscopy.

In between consultations, we addressed the main skin diseases of dogs and cats, their diagnostic approach and treatment. We also talked through the most common products for dermatological use, which will be very useful for my future work as a veterinarian.

1.3. Ophthalmology

The two weeks spent in the ophthalmology department provided me with a better knowledge of the major diseases affecting the eye of dogs and cats. I attended consultations with Prof. Dr. Esmeralda Delgado and Dr. Ana Marta Amorim, where I got to do several diagnostic tests performed in the eye, such as tonometry, ocular examination, Schirmer tear test and fluorescein stain test. During these weeks, I acquired knowledge of the most common products used in ophthalmological treatment. Furthermore, I took part in a small number of ophthalmic surgeries, such as eye enucleation and phacoemulsification.

1.4. Oncology

During the two weeks I spent in the Oncology department, under Dr. Gonçalo Vicente's and Dr. Ana Coelho's guidance, I was able to learn more about the diagnosis and treatment modalities of the most common malignancies in small animals and to participate in the preparation and execution of chemotherapy plans. I was also allowed to discuss more challenging cases and innovative approaches to cancer in small animals. I took part in first-time referral consultations and follow-ups, in which diagnostic imaging was often used as a diagnostic and/or monitoring tool. Additionally, I assisted in the performance of several procedures such as thoracocentesis, abdominocentesis and fine needle aspiration.

1.5. Imagiology

In this rotation, I spent two weeks attending ultrasonography exams with Dr. Rui Lemos Ferreira, Dr. Ana Filipe and Dr. Rui Máximo. I was responsible for positioning the patients and doing the trichotomy. During this time, I gained experience in identifying the various abdominal organs on ultrasound and in recognizing the most prevalent abnormal findings. I assisted in the performance of ultrasound-guided percutaneous needle biopsies, ultrasound-guided fine needle aspirations and subcutaneous ureteral bypass lavages. I was also introduced to some notions of echocardiography.

1.6. Surgery

I joined the surgery and anesthesiology team for two weeks, during which I assisted in the performance of several soft tissue, orthopedic, ophthalmic, neurologic and oral surgeries. Throughout these weeks, I was responsible for the patients' admission, intravenous catheter placement, pre-surgical medication, trichotomy and skin antisepsis, and post-surgical patients' monitoring. I was given the opportunity to perform some other procedures such as anesthetic induction, maintenance and animal intubation. Furthermore, I took the role of surgeon assistant for one or two surgeries a day, where I got to learn more about different surgical techniques from Dr. Miguel Ramos, Dr. António Martinho, Dr. António Ferreira, Dr. Miguel Carreira, Dr. Leonor Iglesias, Dr. Rita Rosa, Dr. Esmeralda Delgado and Dr. Lisa Mestrinho. I became more aware of the most appropriate anesthesia and analgesia protocols used in small animals during these two weeks' stay and also from a lecture given by Dr. Ana Teresa Reisinho.

1.7. General Practice Service

During my stay in the general practice service, I got to see the most common complaints that lead owners to consultation. I also gained an understanding of how to cope with different types of owners, from those who have little interest and/or knowledge to those who are very committed and/or informed. I acquired skills in several procedures such as blood sampling, cystocentesis, intravenous catheter placement, fluid system preparation and placement, glycemia measurement, blood pressure assessment, urethral catheterization, thoracocentesis, abdominocentesis, electrocardiography, among others. I was allowed to collect the patients' anamnesis, do the list of differential diagnoses and discuss the most appropriate diagnostic and treatment plans with the veterinarian responsible. I also participated in routine consultations, where I got to practice my clinical examination competencies and implement the appropriate vaccination and deworming plans. I was allowed to take part in the conversations with the owners that addressed the euthanasia issue, assisting in its execution.

1.8. Infectious Diseases Unit

During one-week stay in the Infectious Diseases Unit, I had contact with a national referral infectious diseases unit. I dealt with inpatient care cases of canine parvoviruses, canine leptospirosis, feline panleukopenia, feline retroviruses, feline caliciviruses and multiresistant bacterial infections. Other than this, noninfectious diseases in patients with unknown

vaccination status were also addressed in this unit. I progressively became more accustomed to the individual protection equipment and protocols required in this unit.

1.9. Inpatient Care

I was assigned two nights per month and two 12-hour day shifts in Inpatient Care. Each shift started with a medical round in which the clinical cases were discussed in group. Along with the nurse, I was responsible for feeding the patients and doing their clinical examination and drugs administration. In this service, I was able to perform several procedures including intravenous catheter placement, fluid system preparation and placement, glycemia measurement, blood pressure assessment, urethral catheterization, nasogastric tube feeding placement, among others. I witnessed life-threatening situations which required prompt measures such as fluid supplementation, blood transfusion, oxygen supplementation and cardiopulmonary resuscitation. I also attended emergency consultations that allowed me to become more acclimated to urgent situations like seizures, trauma, sepsis, dyspnea, along with others.

Scientific communications

The paper “Plasma and Urinary Metanephrine and Normetanephrine in Healthy Cats – a Pilot Study” has been submitted to the *Journal of Veterinary Internal Medicine* (Annexe 2).

The paper “Pheochromocytoma confirmed by Immunohistochemistry in a Cat – a Case-Report” has been submitted to publication (Annexe 3).

The abstract “Plasma and Urinary Metanephrine and Normetanephrine in Healthy Cats – a Pilot Study” has been accepted for oral presentation at the *European College of Veterinary Internal Medicine – Companion Animals (ECVIM-CA) Congress*, taking place in September 2022 (Annexe 4).

2. LITERATURE REVIEW

2.1. The Adrenal Glands

The adrenal glands are two symmetric, flattened, bilobed endocrine organs located in the retroperitoneal space just cranial to the kidneys (Dyce et al. 2010). In cats, adrenals are generally more oval or cylindrical and more uniform in both size and shape than in dogs (Nyland et al. 2015). The topography of the adrenal glands is the same for both species. The left gland is dorsolateral to the aorta and its vein drains into the left renal vein. The right gland is dorsolateral to the caudal vena cava, into which the right adrenal vein drains directly. Both glands are diffusely supplied by branches from adjacent arteries. The nerve fibers that go into the adrenal glands are preganglionic and provided by the splanchnic nerves that enter the abdominal cavity (Dyce et al. 2010).

On abdominal ultrasound, the adrenal glands usually appear as uniformly hypoechoic structures craniomedial to the kidneys. On occasion, a distinct layering referred to as the corticomedullary junction may be seen in dogs and less commonly in cats (Nyland et al. 2015). The adrenal glands are not typically seen in normal abdominal radiographs of dogs and cats but mineralization of the adrenal glands, sometimes visible radiographically, is an incidental finding in old cats (Thrall 2018).

Each adrenal gland is divided into two separate entities with different embryonic origins – *medulla* and *cortex* – each of which produces different types of hormones. The adrenal medulla arises from the neuroectoderm and produces catecholamines such as norepinephrine (NE) and epinephrine (Epi). The adrenal cortex arises from the mesodermal coelomic epithelium and is divided into three different zones – *zona glomerulosa*, *zona fasciculata* and *zona reticularis*. The cortex is responsible for the production of two major types of steroid hormones - the mineralocorticoids, produced by the zona glomerulosa, and the glucocorticoids (GC), produced mostly by the zona fasciculata but also by the zona reticularis (Petroff and Greco 2020).

The hormone receptors for catecholamines are found on the target cell surface, while steroid receptors are intracellular, due to their hydrophilic and lipophilic natures, respectively. The main mechanisms by which catecholamines exert their intracellular effects culminate in enzyme activity alterations and, consequently, short-term changes in cell function. On the other hand, steroids act by repressing or inducing specific gene transcription (Engelking 2012). On account of this, the hormones of the adrenal medulla are involved in the very short-term “fight or flight” response, while the cortical hormones have a longer-term homeostatic role, which enables the body to cope with stress (Hinson et al. 2010).

In mammals, the conventional view holds that the two different endocrine tissues are clearly separated into an outer steroid-producing cortex and a central medulla. However, it is well accepted now that this is an oversimplification, and the two cell types are instead in closer contact than previously thought (Bornstein and Bornstein 2008). There is a close vascular relation between the adrenal medulla and cortex, since the chromaffin cells are located close to the venous sinuses that drain the adrenal cortex, therefore being exposed to venous effluent that contains high concentrations of cortisol (Galac 2017).

Regarding to the adrenal cortex, aldosterone exerts 90% of the mineralocorticoid activity of adrenal secretions and is a key regulator of sodium, potassium, and body fluid balance. The hormone angiotensin II from the renin-angiotensin-aldosterone system (RAAS) and the increase of extracellular K+ concentrations are the strongest secretagogues for aldosterone (Ames 2019). Synthesis and release of GC are regulated by the hypothalamic-pituitary-adrenal axis. The corticotropin releasing factor (CRF) produced by the hypothalamus stimulates the release of adrenocorticotropic hormone (ACTH) from the pituitary gland into the bloodstream. ACTH then increases the production of GC in the adrenal cortex. An increase in GC concentration inhibits the secretion of CRF by the hypothalamus via a negative-feedback control system. The major GC is cortisol (Petroff and Greco 2020). In the adrenal cortical cells, a number of biosynthetic pathways allow some synthesis of androgens and estrogens. Although the amount of sex steroids produced by the adrenal cortex under normal conditions is low, significant amounts can be synthesized under pathological conditions (Petroff and Greco 2020).

2.2. The Adrenal Medulla

The adrenal medulla is a modified neural tissue that occupies the central portion of the adrenal gland. The adrenal medulla is the equivalent of a modified sympathetic ganglion and its activity is regulated by a direct neural input, functioning as an interface between the neuronal and endocrine systems (Hinson et al. 2010).

The cells of the adrenal medulla that synthesize catecholamines are classified as pheochromocytes or chromaffin cells, based on the formation of colored pigments within the cells when exposed to potassium dichromate. There are two major types of catecholamines produced in the chromaffin cells – *epinephrine (Epi)* and *norepinephrine (NE)*. The cells that produce Epi are distinguishable from those that synthesize NE, as the type of chromaffin granules present is different for each cell type (Petroff and Greco 2020).

2.2.1. Catecholamine Syntheses

Catecholamines are molecules that contain a catechol structure (ortho-dihydroxybenzene) and a side chain with an amino group (Reusch 2015). These biogenic amines include Epi (CNS, adrenal medulla), NE (CNS, ANS, adrenal medulla) and dopamine (CNS) (Engelking 2012).

The synthesis of catecholamines begins with the hydroxylation and decarboxylation of either one of the aminoacids phenylalanine or tyrosine, although most synthesis begins with tyrosine, which is derived from food or formed from phenylalanine in the liver. The biosynthetic pathway of catecholamine synthesis starts with the conversion of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) via tyrosine hydroxylase (TH) (Petroff and Greco 2020). This first step is the committed rate-limiting step and is feedback-inhibited by NE (Galac 2017). DOPA is then converted to dopamine through the enzymatic activity of aromatic-l-amino acid decarboxylase (AADC). Both these transformations occur within the cytosol. The conversion of dopamine to NE occurs within the chromaffin granule via dopamine-β-hydroxylase (DHB) (Petroff and Greco 2020). Either TH or DHB can be activated by ACTH (Engelking 2012). In most sympathetic postganglionic neurons, NE is the final product and remains in the NE granule until secretion (Galac 2017). However, the adrenal medulla expresses an additional enzyme responsible for the conversion of NE to Epi back in the cytoplasm - phenylethanolamine-N-methyltransferase (PMNT). Epi then moves into an epinephrine granule for storage before its release. Cortisol that comes from the centripetal blood flow from the adrenal cortex is responsible for the induction of PMNT gene expression (Galac 2017). Cells of the adrenal medulla that synthesize Epi are exposed to higher concentrations of GC than cells that produce NE. The latter receive their blood supply by arteries that bypass the adrenal cortex (Fitzgerald, 2018). Although the enzyme PNMT is also found in other tissues than the adrenal medulla, the amount of Epi from extra-adrenal sources is small. This means that under physiological conditions, nearly all of the Epi in the circulation comes from the adrenal medulla, whereas circulating NE is mostly derived from postganglionic sympathetic neurons (Reusch 2015). In Figure 1 is a schematic representation of the catecholamine synthesis process.

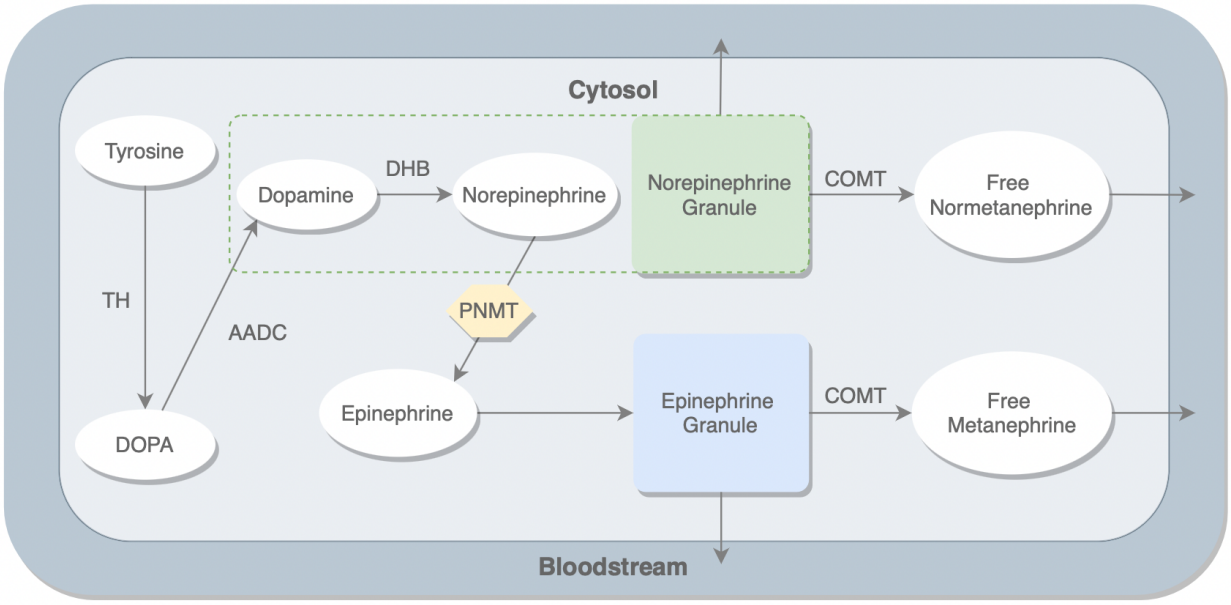


***Figure 1*- Schematic *r*epresentation of catecholamine synthesis and metabolism in the adrenal medulla** (source: *Cunningham's Textbook of Veterinary Physiology*). (AADC) - aromatic-l-amino acid decarboxylase; (COMT) - catecholamine-0-methyltransferase; (DHB) - dopamine-β-hydroxylase; (DOPA) - tyrosine to 3,4-dihydroxyphenylalanine; (PNMT) - phenylethanolamine-N-methyltransferase; (TH) - tyrosine hydroxylase.

Fetal and neonatal adrenals secrete predominantly NE, followed by a gradual increase in the proportion of Epi secreted, which varies from species to species (Engelking 2012). In dogs, the proportion of Epi/NE is 70/30 and in cats is 60/40 (Galac 2017). A variety of other compounds are packaged together with the catecholamines inside the granules, including chromogranins, enkephalins, adrenomedullin, neuropeptide Y, vasoactive intestinal peptide (VIP), pituitary adenylate cyclase activating polypeptide and ACTH, but the physiologic roles for substances other than the catecholamines have not been well established yet (Reusch 2015). For instance, adrenomedullin appears to reduce adrenal aldosterone secretion and to increase vascular nitric oxide production but its role in cardiovascular control is still being investigated (Engelking 2012). Chromogranin A, on the other hand, is widely used as a diagnostic marker for tumors of neuroendocrine origin and seems to have an important role in the storage and release of catecholamines (Elias et al. 2010).

2.2.2. Catecholamine Release

The adrenal medulla displays features of both neuronal and endocrine systems, as catecholamines are released by exocytosis into the bloodstream upon stimulation of the chromaffin cells by acetylcholine from the preganglionic nerve fibers of the sympathetic nervous system. Therefore, Epi and NE are released into the circulation as a hormone instead of being released into the synaptic cleft as a neurotransmitter (Hinson et al. 2010).

The neurotransmitter responsible for the stimulation of the adrenal medulla is acetylcholine, which acts on nicotinic receptors. Acetylcholine acts to increase the rate of catecholamine synthesis and also stimulates the release of catecholamine-containing storage granules (Hinson et al. 2010).

The response to acute stress can be particularly marked, because each preganglionic sympathetic neuron that supplies the adrenal medulla affects a number of chromaffin cells, which means the signal is greatly amplified (Petroff and Greco 2020). The release signal is triggered by physiologic stress, which has been subclassified as either emotional, biochemical or physical. Anxiety and fear bring about emotional stress. Acute hypoglycemia, hypoxemia, hemorrhage or other fluid losses and changes in blood pH are responsible for the biochemical stress. Physical stress occurs in situations of pain, trauma, exposure to extreme temperatures and exercise (Engelking 2012; Galac 2017). The main biochemical factor that influences catecholamine secretion is hypoglycemia (Petroff and Greco 2020).

The adrenal medulla is highly vascularized and directly connected to the abdominal aortic system, allowing catecholamines to rapidly spread and reach their target tissues (Galac 2017).

2.2.3. Catecholamine Metabolism

Catecholamines circulate in plasma in loose association with albumin and exhibit a short plasma half-life of about 1-2 minutes. They are inactivated predominantly in the liver, via the enzymes catecholamine-0-methyltransferase (COMT) and monoamine oxidase (MAO) (Engelking 2012). They are also metabolized in the kidney and, on a smaller scale, in the gastrointestinal tract.

In addition to the extra-adrenal metabolism of circulating catecholamines, there is also a continuous metabolism of Epi and NE that physiologically leak from their storage granules within the chromaffin cells. This adrenal metabolic process leads to the production of O-methylated metabolites metanephrine (MN) and normetanephrine (NM), via the enzyme COMT (Galac 2017) (Fig.1). In contrast to the catecholamines, that are released only intermittently, these metabolites are constantly leaking into the circulation (Reusch 2015). The primary metabolite of dopamine is 3-methoxytyramine (3MT), which has been the subject of some studies on the diagnosis of PCC in human medicine (Smy et al. 2021). Vanillylmandelic acid (VMA) is the end product of catecholamine metabolism. It is produced almost exclusively in the liver, from catecholamines and metanephrines, and excreted in urine (Li et al. 2022).

2.2.4. Catecholamine Biological Effects

The primary actions of catecholamines on metabolism allow animals to face situations of acute stress. Catecholamines act by binding to adrenergic receptors located in the cell membrane of most cells of the body. From there, signal transduction to intracellular sites takes place via G-proteins (Reusch 2015). Their action depends on the receptor type as well as the number of receptors in the tissue (Petroff and Greco 2020). There are two major types of adrenergic receptors – *α* and *β* - which are then divided into two major groups each – *α1, α2, β1* and *β2*. Although all adrenergic receptors are responsive to both Epi and NE, the responses to the two catecholamines are different: α1 and α2 receptors respond more to NE; β2 receptors respond more to Epi; and β1 receptors respond to NE and Epi about equally (Engelking 2012). Because the metabolic effects of catecholamines are mediated mainly by β2 receptors, Epi plays a much more important role in the control of intermediary metabolism than NE (Petroff and Greco 2020). Table 1 discriminates the major biological responses of target tissues to catecholamines.

In a general sense, the effects of catecholamines on glucose metabolism are similar to those of glucagon and opposite to those of insulin, therefore increasing the blood glucose concentrations. Hepatic glycogenolysis and gluconeogenesis are the most preponderant factors involved in this increase. Epi also stimulates glycogenolysis in skeletal muscle, but lactate is produced instead of glucose. The liver then takes up lactate and converts it to glucose. Additional effects on glucose metabolism include the inhibition of insulin secretion and stimulation of glucagon secretion by the pancreas (Petroff and Greco 2020).

The effect of Epi on blood pressure is variable and depends on its plasma concentration. At low concentrations, Epi mainly stimulates β2 receptors, leading to vasodilation. At higher concentrations, the effect on α receptors dominates, causing vasoconstriction instead. The catecholamine effects are also modulated by reflex mechanisms. For example, an increase in heart rate is limited by simultaneous vagal stimulation (Reusch 2015).

Epinephrine promotes lipolysis through the activation of a lipase enzyme, increasing the blood concentration of free fatty acids. Glucocorticoids potentiate the effect of Epi on lipolysis during the stress response (Petroff and Greco 2020).

***Table 2*- Major biological effects of catecholamines in target tissues.**

Major Responses to Catecholamines in different Target Tissues		
Target Tissue	**Response**	**Receptor Type**
Adipose Tissue	Lipolysis	β2
Liver	Glycogenolysis Gluconeogenesis Lipolysis	β2
Pancreas	Insuline and glucagon* secretion increase	β2
	Insuline* and glucagon secretion decrease	α2
Skeletal Muscle	Glycogenolysis	β2
Cardiovascular system	Positive chronotropism and inotropism	β1
	Vasoconstriction	α2
	Vasodilation in coronary arteries, skeletal muscle arterioles, liver and veins	β2
Kidney	Renin and Erythropoietin secretion increase	β1
Bronchioles	Bronchodilation	β2
Eye	Mydriasis	α1
	Relaxation of ciliary muscle for far vision	β2
Central Nervous System	Stimulation Alertness increase	α2
Skin	Piloerection and sweating increase	α2
Gastrointestinal tract	Motility decrease	β2
	Sphincter contraction	α1
Urinary Bladder	Detrusor relaxation	β2
	Sphincter contraction	α2
Spleen	Capsule contraction	α1
	Capsule relaxation	β2
Other tissues	Increased calorigenesis and K+ uptake	β2

Source: *Cunningham's Textbook of Veterinary Physiology.*
Legend: *Primary effect in pancreatic islet tissue.

2.3. Pheochromocytoma

2.3.1. History and Background

Pheochromocytoma (PCC) is an often-malignant catecholamine-producing neuroendocrine tumor arising from chromaffin cells of the adrenal medulla (Galac 2017). Catecholamine-secreting tumors can also originate from extra-adrenal sites such as sympathetic and parasympathetic paraganglia, where they are referred to as paragangliomas (Lunn and Boston 2020). The rarity of PCCs is notable across species, with the exception of the rat, in which the prevalence of PCC may exceed 30% in some lab strains (Tischler et al. 2004). In humans, PCCs and paragangliomas are considered to be rare tumors, as their combined prevalence varies between 0.2 and 0.6%, in autopsy series (Lenders et al. 2014). In dogs, PCCs account for approximately 0.01-0.1% of all canine tumors, although this is probably an underestimation (Galac and Korpershoek 2017). Recently, genetic screening revealed that 20% to 30% of human patients with PCC have germline mutations (Reusch 2015). However, the potential presence of germline mutations in dogs remains unknown (Galac and Korpershoek 2017). Unlike canine PCCs, the majority of human PCCs are considered to be benign, as the traditional position of the World Health Organization (WHO) is to base the diagnosis of malignancy on the presence of metastasis rather than on the presence of local invasion (Tischler et al. 2006). In dogs, the growth rate of PCCs seems to be much higher than in humans and either local invasion or distant metastasis usually qualifies for the definition of malignancy (Reusch 2015). Metastasis is reported in up to 40% of affected dogs and vascular invasion in as many as 82% of cases (Lunn and Boston 2020). A high frequency of concurrent neoplasia has been identified in these patients in two case series, being the percentages of coexisting neoplasia 50% and 54%, respectively (Gilson et al. 1994; Barthez et al. 1997). Most PCCs in dogs are unilateral (>90%) and their size is extremely variable, ranging from a few millimeters to more than 15 centimeters (Reusch 2015). PCC usually is diagnosed in older dogs, and no clear breed predilection has been found (Lunn and Boston 2020).

2.3.2. Pathophysiology

The clinical signs in patients with a PCC can be explained by the known actions of catecholamines or, less frequently, by the tumor size and its invasiveness (Reusch 2015). Catecholamine secretion from PCCs is highly variable with regard to relative amounts and types of catecholamines and their time of release. In humans, PCCs are categorized as with either noradrenergic or adrenergic phenotypes. In the adrenergic phenotype, PNMT is

expressed and the tumor secretes NE and Epi in various proportions. In the noradrenergic phenotype, PNMT is lacking and tumors produce predominantly NE (Eisenhofer et al. 2010). The majority of PCCs in humans produce more NE than Epi (Reusch 2015).

Information on the pathophysiological aspects of canine PCC is scarce. However, many of the findings in dogs appear to be similar to those in humans (Reusch 2015). The concentrations of NE are increased in most dogs with PCC, whereas a minority of dogs reveal an increase in Epi (Kook et al. 2007; Salesov et al. 2015). Therefore, it is likely that in canine PCC, NE is also the predominant catecholamine.

In a general sense, tumor size in humans is positively correlated with the amount of catecholamines and metanephrines released into the circulation (Eisenhofer et al. 2005). As in humans, there seems to be a correlation between severity and presence of clinical signs and tumor size in dogs, as very small PCCs are more often an incidental finding and very serious clinical signs are more often associated with large tumors (Reusch 2015).

In addition to catecholamines and metanephrines, PCCs may secrete a multitude of other peptides which may add abnormalities to the clinical picture and/or counterbalance catecholamine effects (Reusch 2015). For example, hypercalcemia may occur in case of parathyroid hormone–related peptide (PTHrp) secretion.

As in humans, clinical signs occur paroxysmal in most dogs with PCC, pointing to sporadic and unpredictable catecholamine secretion. Some dogs reveal more constant clinical signs, which are most likely associated with continuous catecholamine release at a lower rate (Reusch 2015). True nonsecretory PCCs are very rare, although some may present as subclinical. In humans, this can happen as a result of small tumor size, extensive functional tissue loss due to necrosis and hemorrhage, paroxysmal symptoms with long asymptomatic periods, low medical awareness, and counteraction of the effects of catecholamines by co-secreted peptides (Mannelli et al. 2012).

2.3.3. Clinical Manifestations

Dogs with PCC most often have intermittent episodes of collapse, weakness, and/or panting (Gilson et al. 1994; Barthez et al. 1997). The episodes are paroxysmal and may occur from several times per day to only at intervals of weeks or months. This, and the fact that the clinical signs can mimic or be obscured by other concurrent disorders, dictate that the PCC diagnosis requires a high degree of clinical alertness (Maher and McNiel 1997). The episodes can vary from mild to life-threatening and may progress with time (Galac 2017). Clinical signs related to catecholamine excess are categorized in Table 2.

Clinical manifestations of a space-occupying PCC may be related to invasion of the vena cava, which can lead to ascites, hind limb edema and distension of the caudal epigastric

veins, or may be due to invasion of the aorta, leading to painful and weak hind limbs paraparesis, absence of the femoral pulse and cold distal extremities (Galac 2017; Gilson et al. 1994). As PCCs have a heterogeneous structure, they are predisposed to episodes of bleeding, necrosis and spontaneous tumor rupture. This can lead to retroperitoneal hemorrhage associated with lethargy, tachypnea, tachycardia, weakness, pale mucous membranes and abdominal pain (Whittemore et al. 2001). Metastatic disease can cause organ-specific clinical signs as PCCs may metastasize to the liver, kidneys, spleen, regional lymph nodes, lungs, heart, bone, and the CNS (Galac 2017).

Physical findings greatly depend on the presence or absence of secretory activity by the tumor at the time of clinical examination but are most often unremarkable. When signs are present, panting and tachypnea are the most common, followed by weakness, tachycardia, and cardiac arrhythmias (Barthez et al. 1997).

***Table 2* – Categories of clinical signs in canine pheochromocytoma.**

	Clinical Signs
Cardiorespiratory System and/or Systemic Hypertension	Tachypnea; Panting; Tachycardia; Cardiac arrhythmias; Collapse; Pale Mucous Membranes; Nasal, Gingival or Ocular Hemorrhage; Acute Blindness
Neuromuscular System	Weakness; Anxiety; Pacing; Muscle Tremors; Seizures
Nonspecific	Anorexia; Weight Loss; Lethargy
Miscellaneous	Polyuria/polydipsia; Vomiting; Diarrhea; Abdominal Pain

Source: *Canine and Feline Endocrinology.*

2.3.4. Diagnostic Evaluation

PCC has been called the great mimic, as similar clinical signs are produced by many other, more common clinical conditions (Galac and Korpershoek 2017). The ante-mortem diagnosis of PCC is usually challenging since clinical signs are often non-specific, episodic and unnoticed by the owners. Therefore, clinical awareness represents a crucial initial step in the diagnosis of PCC.

An adrenal tumor may be classified as cortical or medullary, functional or nonfunctional and benign (adenoma) or malignant (carcinoma), based upon its origin, endocrine activity and

malignancy, respectively (Lunn and Boston 2020). Therefore, the diagnosis of a PCC should be a step-by-step approach.

In humans, hypertension is one of the key features of PCC, affecting 80% to 90% of the patients (Prejbisz et al. 2011). In dogs, blood pressure measurements are available in fewer than 50 patients with PCC, in which a little more than 50% had hypertension (Reusch 2015). The American College of Veterinary Internal Medicine (ACVIM) provides a consensus statement for dogs and cats in which blood pressure is classified based upon the risk of target-organ damage into four categories (Acierno et al. 2018). According to this guideline, and similar to the situation in humans, the increase in blood pressure in dogs with PCC may range from mild to severe (Reusch 2015).

Abdominal and thoracic imaging are required to identify and characterize the primary mass, define the degree of local invasion, and look for distant sites metastases for the purpose of staging the tumor (Gregori et al. 2015). Computed Tomography (CT) is the imaging study of choice for the detection of primary adrenal masses and intra-abdominal metastasis (Gilson et al. 1993). Moreover, CT is an accurate method for the detection of vascular invasion by malignant adrenal neoplasms in dogs, with 92% sensitivity and 100% specificity in one study (Schultz et al. 2009).

Endocrine tests should be performed to determine if the adrenal mass is functional and to predict its most likely origin. These include a Low Dose Dexamethasone Stimulation Test (LDDST) for the diagnosis of hypercortisolism (Boland and Barrs 2017); measurement of Plasmatic Renin Activity (PRA) and aldosterone for the diagnosis of aldosteronoma (Schulman 2010); and measurement of fractionated metanephrines (MN and NMN) in urine (U) and/or plasma (PL) for the diagnosis of PCC (Pacak et al. 2007). The origin of an adrenal tumor can also be determined by percutaneous ultrasound-guided fine-needle aspiration, which is still a controversial and not routinely performed procedure in veterinary medicine (Pey et al. 2020).

Initial screening for PCC requires biochemical evidence of inappropriate catecholamine production (Pacak et al. 2007). In humans, measurement of plasma-free metanephrines and urinary fractioned-metanephrines are the most sensitive tests for diagnosis, and the most suitable for reliable exclusion of PCC (Lenders et al. 2005; Galac and Korpershoek 2017). The question of whether urine or plasma is best is still somewhat controversial, but plasma metanephrines are recommended more often as a test of choice in humans (Hickman et al. 2009). In dogs, there is also no consensus about the use of plasma or urine samples, but it has been suggested that urine is superior to plasma (Salesov et al. 2015). Regardless of the sample, there is a strong preference for NMN determination over MN for canine PCC diagnosis (Galac and Korpershoek 2017). The measurement of urinary metanephrines in dogs has been established using spot urine samples and their concentrations are correlated with urinary creatinine concentration (Sasaki et al. 2021).

However, neither endocrine tests nor cytology are reliable in distinguishing benign from malignant neoplasia (Bertazzolo et al. 2014). Only macro- or microscopic invasiveness and metastatic tendency detectable by diagnostic imaging and histopathology combined with immunohistochemistry can be considered reliable indicators of malignancy (Labelle et al. 2004). Moreover, the definitive diagnosis of a PCC can only be established by histopathology and immunohistochemistry (Lunn and Boston 2020).

Macroscopically, PCCs usually present as partially encapsulated red-brown nodules. Malignant PCCs tend to be larger than benign tumors, and are often associated with extensive hemorrhage and necrosis. Furthermore, hyperplastic nodules in the adrenal medulla are often multiple, whereas PCCs are usually solitary (Miller 2017).

Microscopically, PCCs are generally similar across species, consisting of packets of polyhedral neoplastic cells separated by fine fibrovascular stroma that tend to be larger than nonneoplastic chromaffin cells (Tischler et al. 2004). These cells usually have a pale amphophilic cytoplasm and a variable mitotic index. Immunohistochemically, the neoplastic cells express generic neuroendocrine markers such as chromogranin. This immunoreactivity can be useful in distinguishing poorly differentiated PCCs from adrenocortical carcinomas (Miller 2017). As the histological features of benign and malignant PCCs may overlap, invasion through the adrenal capsule or the presence of distant metastases are necessary criteria to define malignancy (Miller 2017).

2.3.5. Treatment and Prognosis

Adrenalectomy is the treatment of choice for PCC both in dogs and cats and should be performed as soon as possible after diagnosis. Exceptions to this include very old and debilitated patients, patients with tumors considered to be unresectable due to massive local invasion and patients with metastasis (Reusch 2015). This is a demanding and high-risk procedure and hence should only be performed by an experienced surgeon-anesthesiologist team (Reusch 2015). There is an overall 60% post-operative complication rate in dogs with PCC undergoing adrenalectomy. These complications can include significant blood pressure variations, tachyarrhythmias, intraoperative hemorrhage and death (Kyles et al. 2003). Moreover, the drainage of the right adrenal vein directly into the caudal vena cava can make it difficult for a surgeon to perform a safe right adrenalectomy (Hinson et al. 2010).

Phenoxybenzamine (PBZ) is an α-adrenergic antagonist that irreversibly binds to both α-1 and α-2 adrenergic receptors and blocks the response to circulating catecholamines (Plumb 2011). In humans and dogs, PBZ is typically administered before adrenalectomy to minimize the deleterious effects of excessive catecholamine secretion that occurs during the perioperative period (Herrera et al. 2008; Jacques et al. 2014). In humans, the traditional

preoperative approach is to block the effects of catecholamines for at least 10 to 14 days prior to surgery (Chen et al. 2010). In dogs, the current agreement consists of a 2-week treatment, with a starting dose of 0.25 mg/kg BID that is increased every 2 to 3 days until a final dose of 1 mg/kg BID is achieved. Treatment should be implemented in dogs with hypertension as well as in dogs that are normotensive at the time of examination (Reusch 2015). Peri-operative mortality has been shown to be significantly lower in dogs pretreated with PBZ compared to untreated dogs (18% versus 48%) (Herrera et al. 2008).

Effective treatment options for inoperable, metastatic, or recurrent canine PCCs are lacking (Musser et al. 2018). Medical treatment with PBZ alone can be considered in these patients (Galac 2017). The results of a recent study suggest that toceranib may have biological activity in dogs with primary and metastatic PCCs (Musser et al. 2018). Treatment with metaiodobenzylguanidine labeled with radioactive iodine ([131I] MIBG) has been reported to result in partial remissions of PCC in humans (Safford et al. 2003). In dogs, this treatment has only been applied once and has led to a clinically stable disease for 4 months (Bommarito et al. 2011). In human medicine, some patients with metastasized PCC make a full or partial response to chemotherapy, but in general few patients benefit from this treatment (Nomura et al. 2009). To the author's knowledge, treatment of malignant PCC with chemotherapy has not yet been reported in dogs.

The prognosis for PCC depends on the tumor size, endocrine activity, presence of metastases, local invasion, age and presence of concurrent diseases (Reusch 2015). A median survival time of 53 weeks has been reported in dogs following adrenalectomy, with some living for 2-3 years (Schwartz et al. 2008; Gilson et al. 1994). Dogs treated exclusively with PBZ can only live about a year after diagnosis (Reusch 2015).

2.3.6. Pheochromocytoma in Cats – What we know so far

Adrenal neoplasia is an uncommon finding in cats, accounting for 0.2% of all feline neoplasms (Lunn and Boston 2020). Primary hyperaldosteronism is the most frequently described clinical syndrome associated with adrenal tumors in cats (Daniel et al. 2015). On the other hand, PCCs are extremely rare and knowledge is limited to a few case-reports (Henry et al. 1993; Chun et al. 1997; Calsyn et al. 2010; Daniel et al. 2015; Wimpole et al. 2010; Cervone 2017). To the author's knowledge, only six cats with histologically confirmed PCC and two cats with suspected PCC without histopathology confirmation have been reported. Polydipsia/polyuria in 3/8 cats and hypertension in 2/8 cats were the most common abnormalities described (Melián and Pérez-López 2019). Other reported clinical signs include aggression, agitation, hyphema, inappetence, lethargy, vomiting, tachypnea and seizures

(Gunn-Moore and Simpson 2013). Therefore, PCC should be considered in cats exhibiting polyuria/polydipsia, weakness and/or hypertension, especially if an adrenal mass is identified.

The diagnosis of feline PCC is challenging as clinical manifestations are often unnoticed by the owners. While it has been estimated that approximately 50% of PCCs are discovered incidentally in dogs, the percentage of feline PCCs diagnosed as an incidental finding is unknown (Melián and Pérez-López 2019). In the majority of cats, the physical examination has been reported to be relatively normal. Documented abnormalities include the presence of a heart murmur, palpable abdominal mass and poorly kept hair coat (Gunn-Moore and Simpson 2013). Routine laboratory testing for feline PCC is usually unremarkable, being the most common finding low Urine Specific Gravity (USG), although increased ALT activity and/or hypertriglyceridemia can also occur (Melián and Pérez-López 2019). Assessment of the blood pressure is important since it has been elevated in at least two cats with PCC. However, hypertension may also be present in cases of hypercortisolism and aldosteronoma, which are two major differential diagnoses of adrenal masses, and in other highly prevalent diseases in cats (Table 3) (Gunn-Moore and Simpson 2013).

***Table 3*- Main differential diagnoses of adrenal masses and hypertension in cats.**

	Differential Diagnoses
Adrenal Mass	Hypercortisolism Aldosteronoma Pheochromocytoma Sex hormone-secreting tumor Nonsecretory tumor
Hypertension	Chronic Kidney Disease Hyperthyroidism Aldosteronoma Hypercortisolism Acromegaly Pheochromocytoma

Source: *Clinical Endocrinology of Companion Animals.*

Initial testing for feline PCC should include the measurement of fractionated metanephrines in urine and/or plasma, as in dogs and humans. However, little is known about these biomarkers in cats, as there are only two published studies concerning this. One study focused on the measurement of plasma metanephrines by HPLC, establishing a first-line guide reference range for PL-MN/NMN in healthy cats and providing rationale for further studies regarding its applicability in feline PCC diagnosis (Wimpole et al. 2010). And one other validated an ELISA for the measurement of U-NMN in cats (Srithunyarat et al. 2018). An

increased in the concentration of PL-NMN was documented in one cat with a suspected PCC (Wimpole et al. 2010).

Adrenalectomy is the treatment of choice for feline PCC (Reusch 2015). As in dogs, PBZ should be administered for days to weeks before adrenalectomy to minimize the deleterious effects of excessive catecholamine secretion. In cats, PBZ at a dose of 2.5 mg per cat PO BID has been used to treat patients with urethral obstruction and may also be helpful in those with PCC (Melián and Pérez-López 2019).

The prognosis for cats with PCC appears highly variable (Gunn-Moore and Simpson 2013). As in dogs, concurrent neoplasia is common in cats, having occurred in approximately 50% of the case-reports (Melián and Pérez-López 2019). Therefore, care should be taken to assess any cat with a PCC for concurrent neoplasia, as this exerts a great influence on the prognosis. In a retrospective study with 3 confirmed cases of feline PCC (ante-mortem or on necropsy), the median survival time was 20 weeks (Daniel et al.1015). In one case, the cat was alive 36 months postsurgery (Calsyn et al.2010). In other case, the cat developed fatal thromboembolic disease following surgery and was euthanized (Chun et al. 1997).

2.4. Metanephrine and Normetanephrine – Laboratory Methodologies

Nowadays, methodologies used to measure metanephrines allow the separation of MN and NMN into fractionated components that can be measured individually, both in plasma and urine (Eisenhofer 2003). Metanephrines in urine are mainly in the sulfur-conjugated form. Thus, most centers measure fractionated total (free and sulfur-conjugated form) metanephrines in urine, unlike plasma metanephrines, in which only the free form is usually measured (Ahn et al. 2021). Ideally, urinary metanephrines should be measured in a 24-hour urine collection, performed while the patient is at rest. As this is not practical in veterinary medicine, the measurement of urinary metanephrines in dogs has been established using spot urine samples and their concentrations are correlated with urinary creatinine concentration (Sasaki et al. 2021).

Liquid chromatography with electrochemical detection (LC–ECD) was initially the method of choice for the measurement of metanephrines in humans. LC–ECD has since been largely superseded by liquid chromatography with tandem mass spectrometry (LC–MS-MS) or immunoassay methods (Weismann et al. 2015). However, first reports from inter-laboratory proficiency programs suggest that immunoassays may be less precise and accurate than LC-MS-MS (Pillai et al. 2009; Pillai and Callen 2010). LC-MS-MS has seen enormous growth in clinical laboratories during the last years as it offers analytical specificity superior to that of immunoassays or conventional high performance liquid chromatography (HPLC) for low

molecular weight analytes and has higher throughput than gas chromatography-mass spectrometry (GC-MS) (Grebe and Ravinder 2011). Moreover, the clean-up and extraction steps in the HPLC assay are relatively laborious and slow and it is known that some drugs interfere with this methodology (Sasaki et al. 2021). Therefore, LC-MS-MS has become progressively more popular in biochemical analysis due to its high detection sensitivity, specificity and simplicity. Assays such as mineralo- and glucocorticoids, sex steroids, metanephrines and 25-hydroxyvitamin D highlight the advantages of LC-MS-MS (Grebe and Ravinder 2011).

Catecholamines are unstable at high temperatures and pH, hence urine samples must be collected into chilled containers with hydrochloric acid (Pinto et al. 2020). However, studies that assessed the stability of urinary metanephrines in humans and dogs under different conditions have proved that, unlike catecholamines, metanephrines are relatively stable and special preservation of the samples is not necessary if they are assayed or frozen within a week (Sasaki et al. 2021; Willemsen et al. 2007). This is of great importance because these assays are infrequently requested and reliable diagnostic methods are not widely available in veterinary medicine, making sample transport to a more specialized diagnostic facility almost always required.

3. FELINE PHEOCHROMOCYTOMA – A CASE-REPORT

An 8-year-old neutered male, European domestic shorthair cat was referred for consultation at the Referral Internal Medicine Service of the Veterinary Teaching Hospital – Faculty of Veterinary Medicine - University of Lisbon (PheoCat - Fig 2). Being a stray cat in the past, he was adopted 4 years before consultation and had been living in a multi-house cats' apartment since, with no access to the outside. As part of the previous medical history, the patient had undergone full-mouth extractions one month after adoption, due to gingivostomatitis associated to a calicivirus infection. Vaccination and deworming were not current and retroviral status was negative. The patient was referred from the Hospital Veterinário de Massamá with a 4-month history of progressive polyphagia, weight loss, polyuria/polydipsia, weakness and severe hypertension.

***Figure 2*- Photograph of the PheoCat** (original).

Geriatric patients experiencing weakness are more likely to suffer from degenerative diseases of the cardiovascular, musculoskeletal, neurological and endocrine systems, in addition to neoplastic conditions. The chief differential diagnoses of polyuria/polydipsia considered in this patient were liver disease, chronic kidney disease (CKD), pyelonephritis, diabetes mellitus, hyperthyroidism, hyperaldosteronism, hypercortisolism, pheochromocytoma, hypercalcemia and acromegaly (Galac 2017). Of these, diabetes mellitus and hyperthyroidism were the most suitable differentials as these conditions are most often associated with polyphagia and weight loss in cats.

A preliminary diagnostic investigation had already been performed by the assistant veterinarian. An abdominal ultrasound detected a heterogeneous nodular structure located caudal to the celiac artery and cranial mesenteric artery, suspected of having its origin in the left adrenal gland (Fig 3).

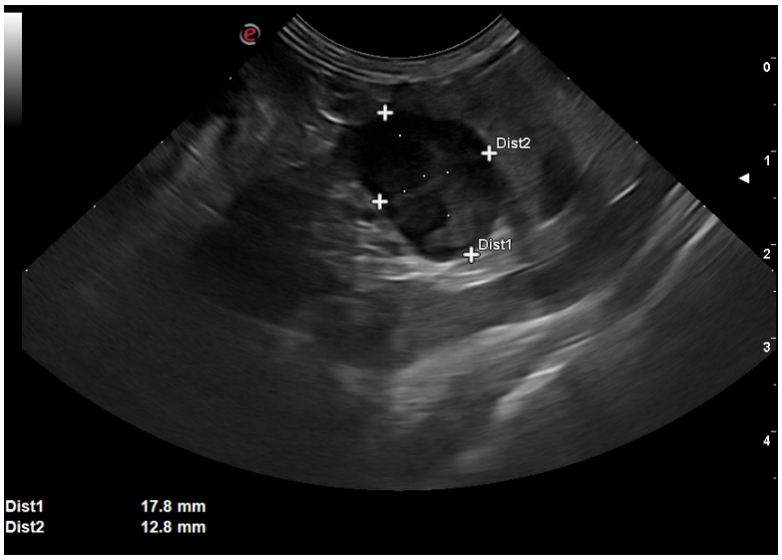


Figure 3 – **Abdominal ultrasound showing a heterogeneous nodular structure suspected of having its origin in the left adrenal gland of the PheoCat** (original).

An abdominal CT confirmed the presence of a mass arising from the left adrenal, with 17 mm of diameter and with no signs of vascular invasion, associated with cachexia and retroperitoneal edema/mild effusion (Fig 4). A thoracic CT detected a pulmonary lesion suspected to have an inflammatory/infectious and, less probably, a neoplastic origin.

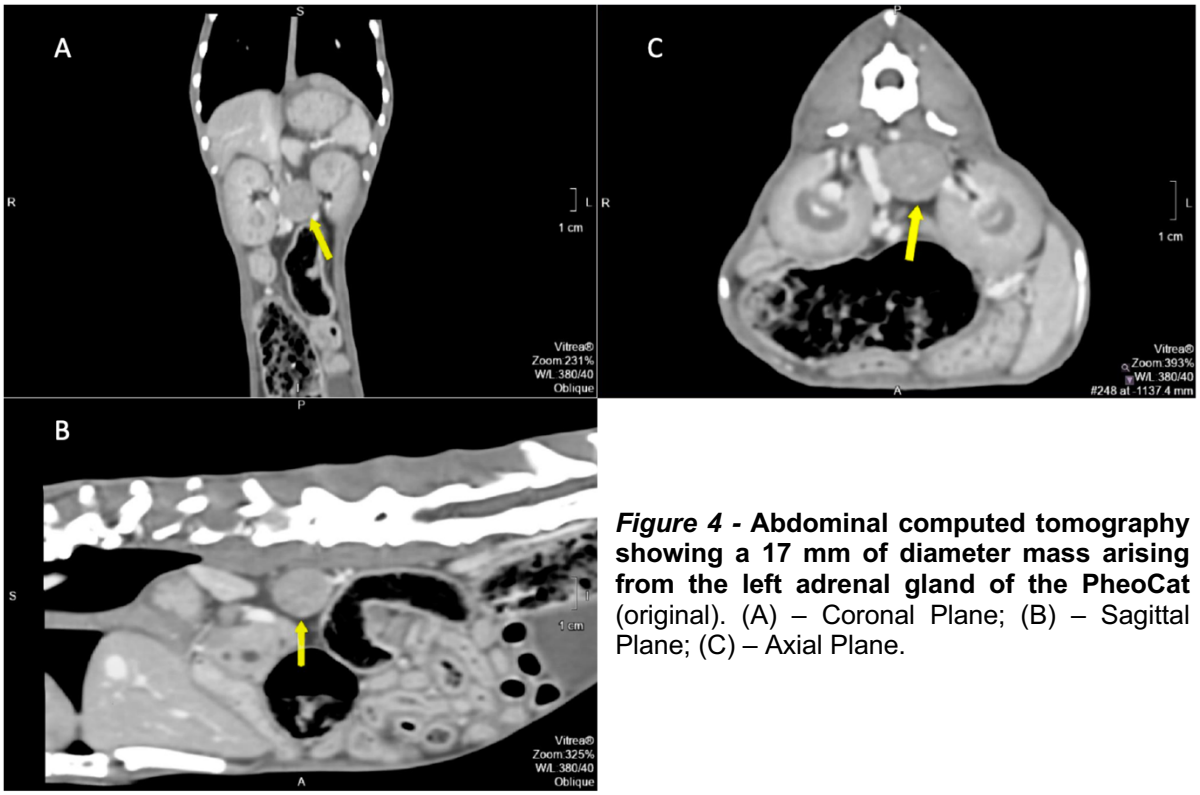


Figure 4 **- Abdominal computed tomography showing a 17 mm of diameter mass arising from the left adrenal gland of the PheoCat** (original). (A) – Coronal Plane; (B) – Sagittal Plane; (C) – Axial Plane.

The main differential diagnoses of an adrenal mass associated with hypertension in this patient were cortical functional tumors (aldosteronoma, hypercortisolism or sex hormone-producing tumor), pheochromocytoma and a nonsecretory adrenal tumor associated to a concurrent disease such as acromegaly and CKD (Gunn-Moore and Simpson 2013).

The first clinical examination revealed a weigh of 3.3 Kg, lethargy, normal mucous membranes with refill time <2 seconds, cardiopulmonary auscultation with a grade III/IV heart murmur on a scale of VI, soft and non-painful abdominal palpation and a SBP of 180-200mmHg.

The hematological investigation was unremarkable and the biochemistry panel revealed a mild azotemia (creatinine = 2.5 mg/dL), hyperkalemia (6 mmL/L) and hypernatremia (172 mmL/L), with no other significant abnormalities (including total T4).

The urine analysis revealed slight pyuria (2-3 WBC/field 400X), hematuria (8-10 RBC/field 400X), rare granular casts, bacteriuria and minimally concentrated urine (USG = 1021). Multidrug- resistant *Pseudomonas aeruginosa* was isolated from urine collected by cystocentesis (>1x10^3 ufc/mL). An antibiotic sensitivity test was performed and the patient started appropriate antibiotic treatment for the urinary tract infection (marbofloxacin, 10mg PO SID). Additionally, treatment with amlodipine was started for hypertension control (0.625mg PO SID).

Taking into account the patient's medical history and the results of the previous diagnostic tests performed, an endocrine investigation was conducted to assess whether the adrenal tumor identified was functional or not and to predict its most likely origin (Table 4).

A Low Dose Dexamethasone Stimulation Test (LDDST) was performed since it is considered the test of choice for the diagnosis of hypercortisolism in cats due to its high sensitivity and moderate specificity (Boland and Barrs 2017; Chiaramonte and Greco 2007). As there was an appropriate negative feedback response to exogenous glucocorticoid administration (T8< 1 µg/dL), hypercortisolism was found unlikely. Moreover, cortisol-secreting adrenal tumors are particularly unlikely in this species.

Aldosterone and plasmatic renin activity (PRA) were also measured. Aldosterone measurement is the diagnostic hallmark of primary hyperaldosteronism both in cats and humans, and should be interpreted in combination with potassium concentration and PRA. In primary hyperaldosteronism, an increased aldosterone/renin ratio and decreased potassium concentration are expected (Schulman R. 2010). In this patient, the measurements of aldosterone (151.62 pg/mL) and PRA (0.86 ng/mL/h), combined with the serum potassium concentration of 6 mmL/L, indicated that aldosteronoma was unlikely. Although the measured aldosterone value was higher than the upper limit of the reference range, it would be expected a minimal PRA and a concurrent hypokalemia in case of autonomous secretion of aldosterone by the adrenal gland (Schulman 2010).

Considering these results, it was decided to measure the plasmatic metanephrines using the HPLC methodology. PL-MN (4,60 nmol/L) and PL-NMN (54,87 nmol/L) measurements were compatible with the diagnosis of feline PCC. Even though a reference range for plasma metanephrines in cats is yet to establish, these values are 4,16 and 15,63 times higher than the PL-MN and PL-NMN mean values previously reported in healthy cats (Wimpole et al. 2010). The patient started PBZ (2.5 mg PO BID) and had its weight, SBP and creatinine and potassium blood levels monitored every two weeks.

Table 4 **- Diagnostic tests performed during the endocrinological investigation of the adrenal mass identified in the PheoCat.**

TEST	METHOD	RESULT	CONCLUSION
LDDST	Chemiluminescence	**Cortisol (µg/dL):** **T0** = 3.2 (RR: 2-5) **T4** = 1 (RR: <1.4) **T8** = <1 (RR: <1.4)	Hypercortisolism unlikely
Renin and Aldosterone	RIA	**Aldosterone** = 151.62 pg/mL (RR= 15-102) **PRA** = 0.86 ng/mL/h (RR= 0.4-1.9) **Aldosterone/Renin Ratio** = 2.44	Aldosteronoma unlikely
Plasma Metanephrines	HPLC	**PL-MN**: 4,60 nmol/L **PL-NMN**: 54,87 nmol/L	Compatible with Pheochromocytoma

Legend: (LDDST) - Low Dose Dexamethasone Suppression Test; (T0) – immediately before dexamethasone injection; (T4, T8) – 4 and 8 hours after dexamethasone injection, respectively; (RIA) – radioimmunoassay; (PRA) – Plasmatic Renin Activity; (HPLC) – High Performance Liquid Chromatography; (PL–MN) – Plasmatic metanephrine; (PL-NMN) – Plasmatic Normetanephrine; (RR) – Reference Range.

Since surgery is the only definitive treatment for PCC and the definitive diagnosis can only be established by histopathology and immunohistochemistry, left adrenalectomy was performed after the patient's cardiovascular stabilization.

A pre-surgical echocardiography was performed. This exam confirmed the existence of a hypertrophic cardiomyopathy phenotype, with diffuse and moderate hypertrophy throughout the left ventricle and the presence of tachycardia with regular and concordant rhythm. Normal systolic and diastolic functions were preserved, as there were no signs of dilatation or left overload. Therefore, no remarkable contraindication for the surgical procedure was found, but special attention to blood pressure and cardiac rhythm during the surgical procedure was vital.

Repeating a pre-surgical abdominal and thoracic CT was ideal to assess the stabilization state of the lesions previously observed on the diagnostic imaging tests. However, due to financial constraints and since only 4 months had passed since the last CT, a pre-surgical abdominal ultrasound and thoracic X-rays were conducted instead. No significant differences from the previous tests performed were found on these exams.

Eight weeks later (and while on PBZ), as the SBP had been stable for a month at around 150 mmHg and creatinine blood levels had lowered and stabilized at around 1,7 mg/dL (IRIS Stage 2 CKD) as well as potassium blood concentrations (5,6 mmL/L), it was performed the unilateral left adrenalectomy. Standard laparotomy allowed access to the adrenal mass which was severely attached to the caudal vena cava wall (Fig 5). Therefore, adrenalectomy with a partial cavectomy was performed. The surgical procedure went without noteworthy incidents. Only a transient hypotension occurred at the end of the procedure, when the vena cava was partially occluded. During the recovery after surgery, the patient experienced hypothermia (34ºC) and hypotension (120/80mmHg). However, the temperature reached 37.4ºC and the blood pressure rose up to 140/90 mmHg over the afternoon. PBZ was given for the last time the evening before surgery and no supplementation was needed post-op. Once the patient was progressively more active, hospital discharge was given 2 days after surgery.

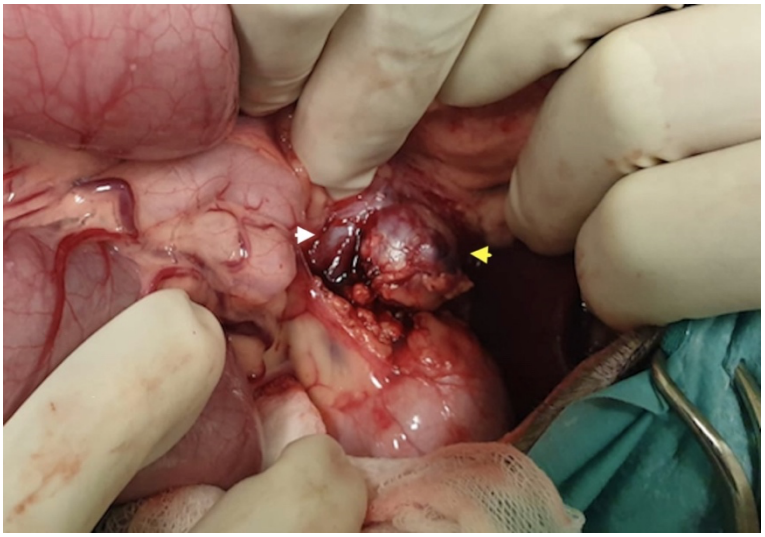


Figure 5 **– Intraoperative image of the adrenal mass (yellow arrow) and the caudal vena cava (white arrow) partially compressed by the surgeon's hands** (original).

The patient recovered well at home although hyporexia and polyuria/polydipsia remained present. The patient had its blood pressure reassessed weekly after surgery and the measurement results were 185/135, 162/106, 159/113, 136/102 and 125/88 mmHg for each week, representing a sequential decrease of the SBP. Since the patient had persistent normotensive values, revaluation was extended to every 2-3 months.

The left adrenal gland was submitted for histopathology analysis and immunohistochemistry for chromogranin A and synaptophysin, at the Veterinair Pathologisch Diagnostisch Centrum – Utrecht University. The biological material sent for analysis consisted of a solid, light brown fragment of elastic tissue with approximately 1.5 x 1.7 x 1.4 cm in size.

The fragment was preserved in formol and had a longitudinal section with a light brown to brown cut surface (Fig 6).

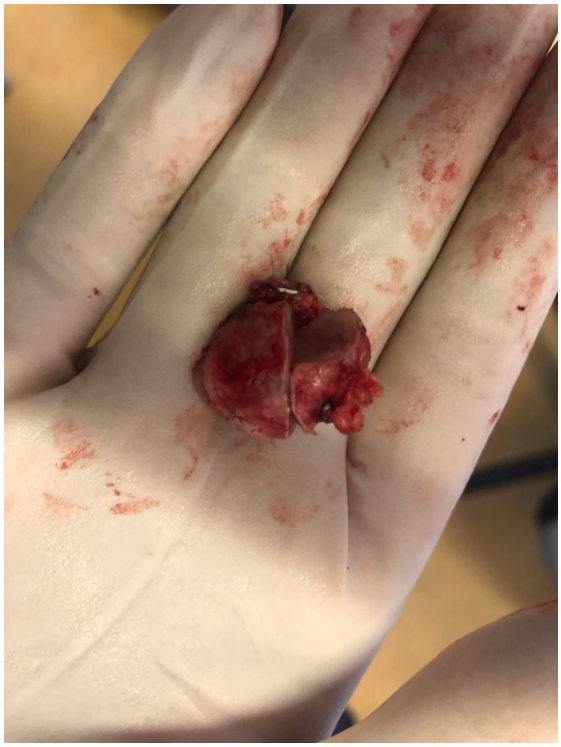


***Figure 6* - Photograph of the left adrenal gland submitted for histopathology analysis** (original).

The histopathology investigations revealed the presence of a narrow band of cortical tissue with extracapsular foci of cortical tissue hyperplasia. The adrenal gland consisted mainly of trabeculae of neoplastic cells although cells organized in solid nests could also be observed. Fibrovascular septa were occasionally observed, within which palisade neoplastic cells could be noticed. The neoplastic cells were characterized by the presence of several, large, elongated oval nuclei located in a limited amount of amphophilic cytoplasm. These nuclei were mainly homogeneously light although, at times, marked nucleoli could be observed (Fig 7). The neoplastic cells seemed to have a low mitotic index.

Immunohistochemical stains were positive for a large number of neoplastic cells for both synaptophysin and chromogranin-A, therefore confirming the neuroendocrine origin of the tumor (Fig 8).

The conclusion drawn by the histopathologist was that the material sent for analysis consisted of an adrenal gland with a pheochromocytoma.

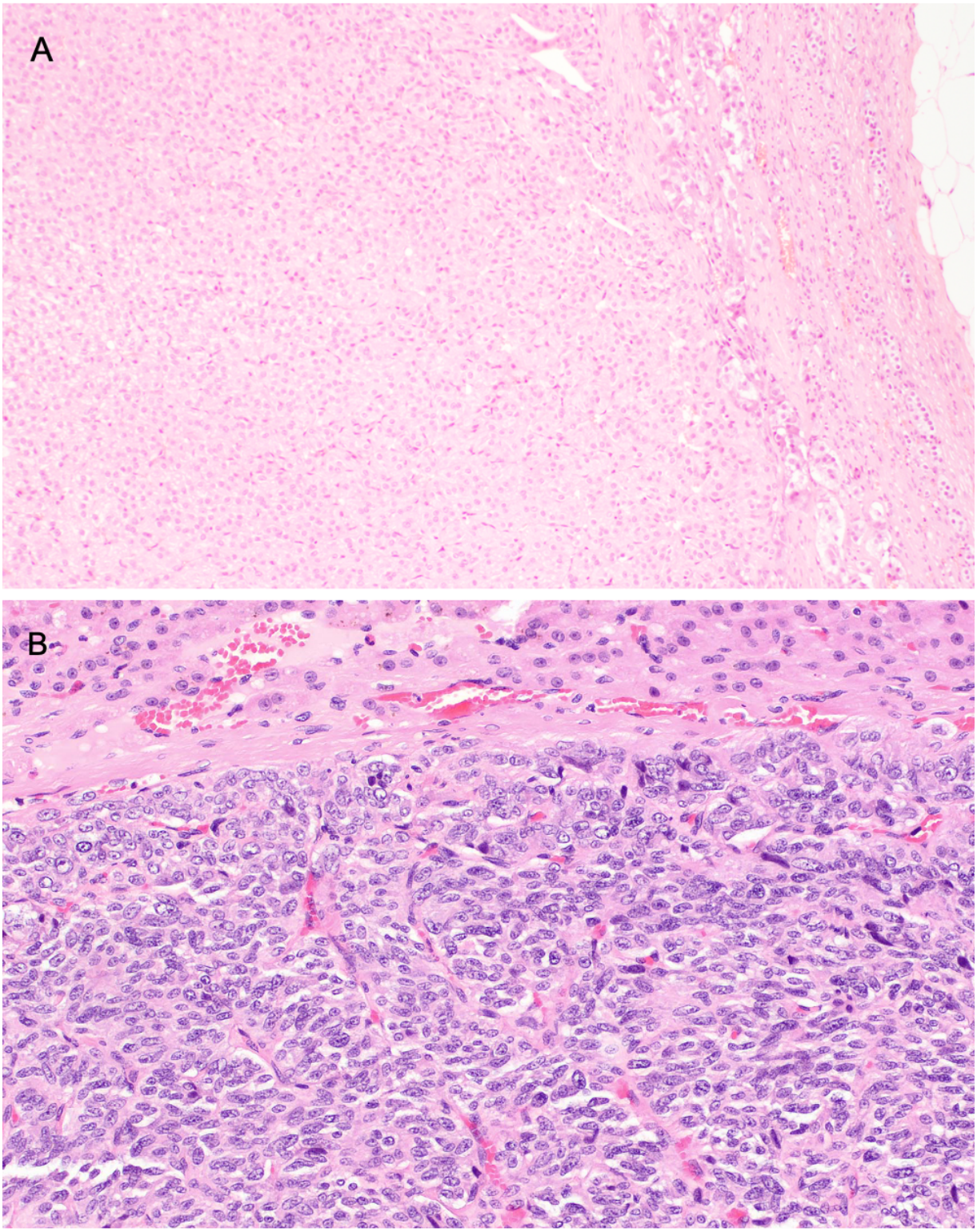


Figure 7 **- Photomicrographs of the left adrenal gland of the PheoCat (Hematoxylin - Eosin Stain)** (original). (A) – presence of a narrow band of normal cortical tissue compressed by the neoplastic tissue with pheochromocytoma histopathological features (10x); (B) – presence of trabeculae of neoplastic cells with several, large, oval nuclei located in a limited amount of amphophilic cytoplasm (20x).

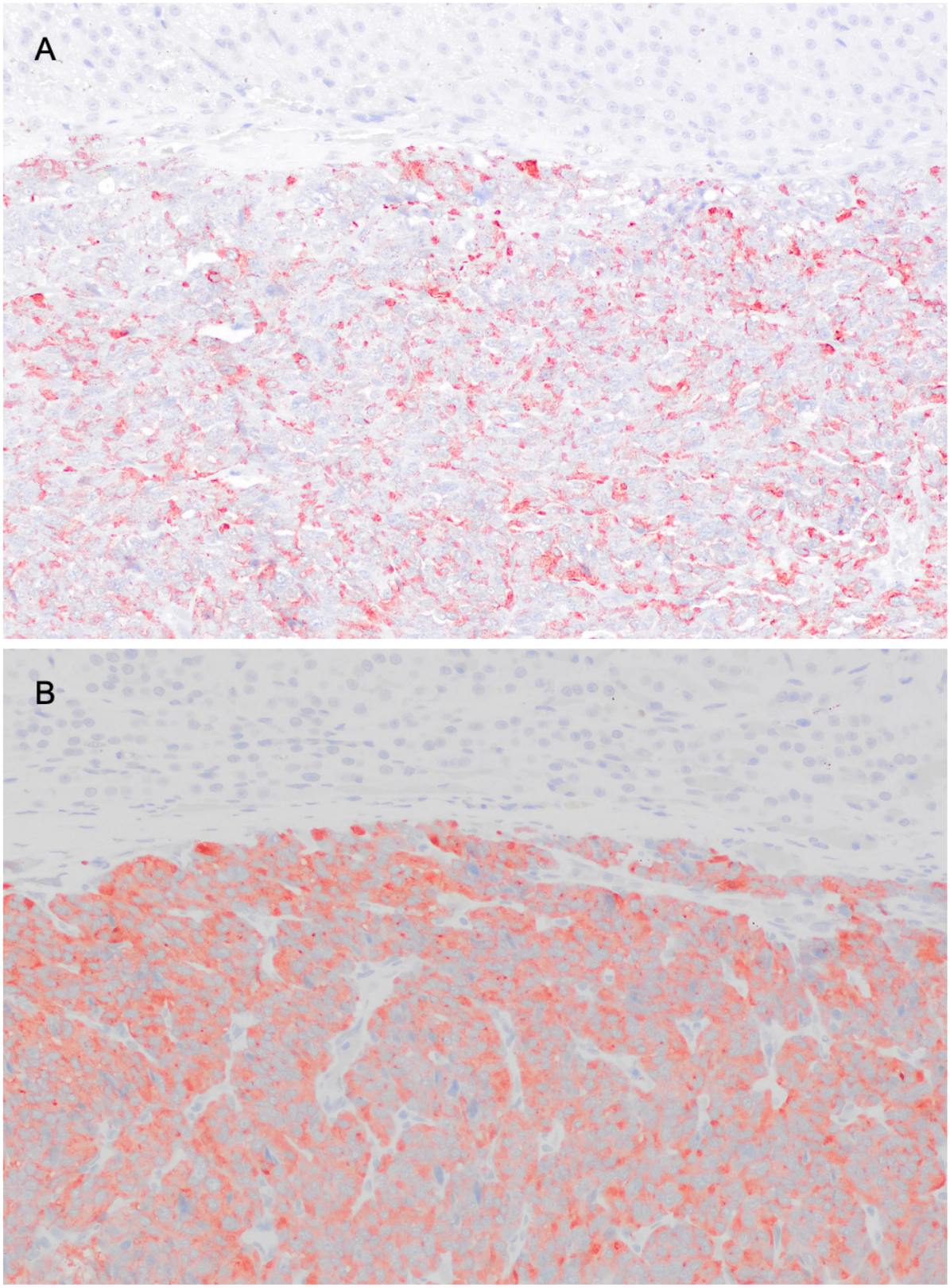


***Figure 8* – Immunohistochemistry of the left adrenal gland of the PheoCat** (10X) (original). (A) – Chromogranin A; (B) – Synaptophysin. (A, B) – positivity of a large number of cells for both immunohistochemical markers.

Two months after the surgery, the patient was consulted at the Referral Internal Medicine Service for hyporexia associated with an important weight loss (2.6Kg) and prostration. Oral ulceration was identified on clinical examination, which raised the suspicion of stomatitis recurrence. However, aggravation of a systemic condition such as the CKD could not be excluded. It was also detected a borderline hypertension (SBP≈180 mmHg), that could be justified by the oral discomfort. In the blood analysis performed, there was a massive increase of the urea (171. 8 mg/dL), which could be from the stomatitis or the CKD. At this point, creatinine values were stable at around 2.3 mg/dL (IRIS Stage 2 CKD) and proteinuria and hyperphosphatemia were absent. In the urine analysis, bacteriuria was again present. However, as the cat was apparently asymptomatic with a stable azotemia, and since treatment of subclinical bacteriuria with antimicrobials is presently discouraged, no antibiotic treatment was established (Weese et al. 2019). At this stage, it was solicited an abdominal ultrasound that revealed the presence of intestinal thickening and lymphadenopathy, that weren´t accompanied by digestive signs such as vomiting or diarrhea.

Seven months after the surgery, the patient came to consultation for prostration, noisy breathing, left exophthalmos and sneezing. On clinical examination, it was observed an 8% dehydration, oral ulcers, cachexia and absence of the left pupillary reflex. The main differential diagnosis considered were inflammatory orbital disease, zygomatic salivary gland disease, neoplasia of the orbit or other surrounding tissues and PCC metastasis in the CNS. A CT scan of the head and chest was recommended but the owner elected for euthanasia.

The survival time after the diagnosis of PCC and after adrenalectomy was 9 and 7 months, respectively. In addition to the PCC, the patient had a stabilized CKD (IRIS stage II) without hyperphosphatemia nor proteinuria, for which a renal diet was being fed; a multidrug-resistant *Pseudomonas aeruginosa* bacterial cystitis, apparently asymptomatic and causing no aggravation of the azotemia; a chronic gingivostomatitis associated with a positive calicivirus status; and intestinal thickening and lymphadenomegaly on abdominal ultrasound with no digestive signs associated. Because no further diagnostic tests were performed, it was not possible to reach a definitive diagnosis of the condition that led to euthanasia. The presence of inflammatory or neoplastic orbital disease was considered likely, but worsening of a previously known condition could not be excluded.

Discussion

This case-report describes the presence of feline PCC, confirmed on histopathology and immunohistochemistry, increasing the number of reported cases in the literature. Feline PCC is considered to be rare and literature is limited to a few case-reports (Henry et al. 1993; Chun et al. 1997; Calsyn et al. 2010; Daniel et al. 2015; Wimpole et al. 2010; Cervone 2017).

To the author's knowledge, only six cats with histologically confirmed PCC and two cats with suspected PCC without histopathology confirmation have been previously reported in literature. Of these, only one case was confirmed by immunohistochemistry (Calsyn et al. 2010) meaning this case-report accounts for the second-one described in the literature. Polydipsia/polyuria and systemic hypertension were the most common abnormalities described, having been reported in 3/8 cats and in 2/8 cats, respectively (Melián and Pérez-López 2019). In this case report, the patient's clinical signs of PCC included polyuria/polydipsia and severe hypertension, but also polyphagia, weight loss and weakness.

A step-by-step approach was used in the diagnostic evaluation process of this case. An abdominal and thoracic CT were performed to identify and characterize the primary mass, define the degree of local invasion, and look for distant sites metastases for the purpose of staging the tumor. A mass arising from de left adrenal gland was identified on the abdominal CT, with no signs of local invasion. The thoracic CT detected a pulmonary lesion suspected to have an inflammatory/infectious and, less probably, a neoplastic origin. Endocrine tests were then performed to determine if the adrenal mass was functional and to predict its most likely origin. The LDDST revealed a pattern that allowed us to exclude hypercortisolism. The measurements of PRA and aldosterone showed that aldosteronoma was also unlikely since we were expecting an hyperaldosteronemia with concurrent low PRA which was not observed. Adrenocortical tumors have the potential for synthesizing and secreting a variety of steroid products other than cortisol and aldosterone, such as androgen, estrogen, and progestogen. (Reusch 2015). Some cats have had excesses in progestagens with conventional signs of feline hyperadrenocorticism. A few cats have had increased androgen concentrations with typical male territorial urine spraying behavior, unusual strong urine odor, and aggressiveness (Rossmeisl et al. 2000; Boag et al. 2004; Millard et al. 2009). As the clinical signs observed in this cat were not consistent with those reported in cats with excessive sex hormones secretion, and a relatively limited number of cats with increased secretion of sex hormones from adrenal gland tumors has been described in the literature, endocrine testing for sex hormones was judged unnecessary.

Initial screening for PCC should include measurement of fractionated metanephrines in urine and/or plasma. In cats, there are only two published studies concerning these biomarkers, one of which established a first-line guide reference range for PL-MN/NMN in healthy cats (Wimpole et al. 2010). According to this study, PL-MN (4,60 nmol/L) and PL-NMN (54,87 nmol/L) measurements in this patient were compatible with the diagnosis of feline PCC.

The patient underwent left adrenalectomy and adrenal gland was submitted to standard histopathology and immunohistochemistry. Immunohistochemical assessment of chromogranin A and synaptophysin aimed to evaluate if the tumor had a neuroendocrine origin. Chromogranin is involved in the production and processing of catecholamines whereas

synaptophysin is a membrane component of synaptic vesicles in neurons and neuroendocrine cells (Berthez et al. 1997). As immunohistochemical stains were positive for a large number of neoplastic cells for both synaptophysin and chromogranin-A, it was confirmed the neuroendocrine origin of the tumor.

Histopathology and immunohistochemistry revealed that the material sent for analysis was consisted with an adrenal gland with a pheochromocytoma.

Conclusion

This is a case-report of a feline PCC confirmed by standard histopathology and immunohistochemistry for chromogranin A and synaptophysin.

More than increasing the number of cats reported as having PCC, this case-report highlights that pheochromocytoma should be included in the list of differential diagnosis of an adrenal mass in the cat.

4. PLASMA AND URINARY METANEPHRINE AND NORMETANEPHRINE IN HEALTHY CATS

4.1. Introduction and Objectives

In humans, the measurement of plasma-free metanephrines and urinary fractioned-metanephrines are the most sensitive tests for diagnosis, and the most suitable for reliable exclusion of PCC (Lenders et al. 2005; Galac and Korpershoek 2017). The question of whether urine or plasma is best is still somewhat controversial, but plasma metanephrines are recommended more often as a test of choice in humans (Hickman et al. 2009).

In dogs, there is also no consensus about the use of plasma or urine samples, but it has been suggested that urine is superior to plasma (Salesov et al. 2015). Regardless of the sample, there is a strong preference for NMN determination over MN for canine PCC diagnosis (Galac and Korpershoek 2017). The measurement of urinary metanephrines in dogs has been established using spot urine samples and their concentrations are correlated with urinary creatinine concentration (Sasaki et al. 2021).

In cats, there are only two published studies concerning the measurement of metanephrines. One addresses the plasma-free metanephrines levels using HPLC and establishes a first-line guide reference range for PL-MN/NMN in healthy cats, providing rationale for further studies in the clinical applicability of these biomarkers in the diagnosis of feline PCC (Wimpole et al.2010). The other evaluates an ELISA for metanephrines in feline urine (Srithunyarat et al. 2018).

LC-MS-MS has seen enormous growth in clinical laboratories during the last years as it offers analytical specificity superior to that of immunoassays or conventional HPLC methodology for low molecular weight analytes (Grebe and Ravinder 2011). Moreover, the clean-up and extraction steps in the HPLC assay are relatively laborious and slow and it is known that some drugs interfere with this methodology. Therefore, LC-MS-MS has become more popular in biochemical analysis due to its high detection sensitivity and specificity, in addition to its simplicity (Sasaki et al. 2021). LC-MS-MS has been formerly approved for PL-MN/NMN and U-MN/NMN measurement in dogs and humans (Sasaki et al. 2021; Gostelow et al. 2013; Eisenhofer et al. 2019). To the best of the authors' knowledge, this methodology has not yet been performed in cats for the measurement of metanephrines.

This pilot study aims to evaluate the feasibility of PL-MN/NMN and U-MN/NMN measurement using LC-MS-MS in adult healthy cats. Furthermore, this study compares the values obtained from healthy cats with those from a cat with a confirmed diagnosis of PCC (PheoCat). As a means to evaluate the stability of feline urinary metanephrines, this study also

intends to assess whether different storage conditions affect results, by comparing measurements obtained from urine samples immediately stored at -80ºC with samples refrigerated for 24h at +4ºC before storage at -80ºC.

4.2. Material and methods

4.2.1. Animals

A cross-sectional pilot study was conducted, including a total of 10 clinically healthy cats with ages ranging from 4 to 10 years, recruited among students and staff from the Veterinary Teaching Hospital – Faculty of Veterinary Medicine – University of Lisbon. The study was approved by the local ethical committee (Annexe 5). With the owner's consent, all cats were submitted to a thorough physical examination, complete bloodwork (including SDMA, total T4 and ionogram), urine analysis with protein-to-creatinine ratio evaluation, abdominal ultrasound, and systolic blood pressure (SBP) assessment (Annexe 6). Cats were excluded in case of hypertension (SBP mean >160 mmHg measured in an oscillometric device), azotemia, hyperthyroidism, electrolytic imbalances, proteinuria and abnormal findings in the abdominal ultrasound. Ultrasonographic evaluation of adrenal gland size in two bodyweight categories, performed by an experienced sonographer, was comprised in the inclusion criteria, with a tolerated maximum of 3,9 mm thickness for cats weighing ≤ 4kg and 4,8 mm for those weighing > 4-8 Kg (Pérez-López et al. 2021).

Plasma-free and Urinary Metanephrines

For each cat, two blood samples (2x1mL) and two urine samples (2x1mL) were collected for the measurement of fractionated plasma-free metanephrines and urinary metanephrines. After sampling, both EDTA-blood samples were centrifugated and promptly stored at -80ºC (PL - 2 aliquots), along with one of the urine samples (U) (Fig 9).

Urinary stability assessment

Apart from the urine sample immediately frozen (-80ºC), an additional urine sample was refrigerated (UR) for 24h before storage at -80ºC (Fig 9).

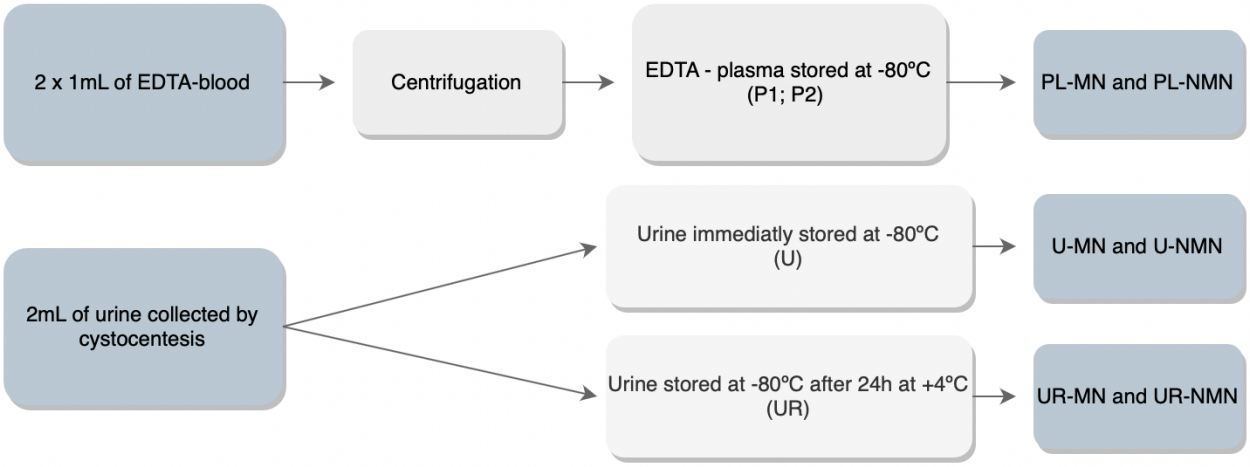


Figure 9 - Sampling method used in the population of healthy cats. (PL-MN) - Plasma metanephrine; (PL-NMN) - Plasma normetanephrine; (U-MN and U-NMN) - Urinary metanephrine and normetanephrine in urine samples immediately stored at -80oC; (UR-MN and UR-NMN) - Urinary metanephrine and normetanephrine in urine samples refrigerated (+4ºC;24h).

4.2.2. PheoCat

Also with the owner's consent, stored (-80ºC) leftover plasma and urine samples collected from an 8-year-old neutered male, European domestic shorthair cat previously diagnosed with PCC (PheoCat) at the Internal Medicine Service – Faculty of Veterinary Medicine – University of Lisbon, were submitted for analysis. The diagnosis was based on the increase in PL-MN/NMN measurements (performed by HPLC) and was confirmed by histopathology and immunohistochemistry (chromogranin A and synaptophysin). The plasma and urine samples were collected prior to any treatment.

All samples were preserved at -80ºC until measurement of PL-MN/NMN and U-MN/NMN by LC-MS-MS at the Algemeen Medisch Laboratorium, Belgium. Samples were shipped on dry ice. Urine creatinine (Creat) was measured in the same spot urine samples in order to calculate the U-MN/Creat and U-NMN/Creat ratios.

4.2.3. Statistical Analysis

All the collected data were recorded in Microsoft Office Excel version 16.59. Descriptive statistics and statistical tests were performed using the commercial statistical software IBM SPSS Statistics for Windows, version 28.0.1.0. P-values <0.05 were considered significant for all tests. Normality tests were not performed since it was not appropriate to accept normality given such a small number of observations (N=10). Assuming the data were not normally

distributed, median and interquartile ranges (IQR) were used to obtain a summary of the distribution of scores. Statistical difference between refrigerated and immediately frozen urine samples was determined using the Wilcoxon signed-rank test for paired samples.

4.3. Results

4.3.1. Sample Population

A total of 10 healthy cats were recruited, with a median age of 6 years (IQR=4.5). Of these, 3 cats were males and 7 were females. All cats were neutered. Physical examination, blood and urine analysis performed were unremarkable in all cats. All cats presented normal-sized adrenal glands and unremarkable findings on the abdominal ultrasound.

4.3.2. Plasma-free and Urinary Metanephrine and Normetanephrine

The PL-MN and PL-NMN median values of the population of healthy cats were 2.73nmol/L (IQR=2.37) and 7.02nmol/L (IQR=5.2), respectively. The U-MN/Creat ratio median value was 70µg/g (IQR=70) while the U-NMN/Creat ratio median value was 139µg/g (IQR=77) (Table 5).

The results obtained from the PheoCat revealed a PL-MN of 3.68nmol/L and a PL-NMN of 66.27nmol/L, which correspond to 1,3- and 9,4-times the median value obtained from the healthy cats' population. The PheoCat U-MN/Creat ratio value was 179µg/g and the U-NMN/Creat ratio value was 1262µg/g, corresponding to 2.55- and 9,07-times the median value from the population of healthy cats (Table 5).

None of the results obtained from the PheoCat overlapped with the median values determined for the healthy cats. Nevertheless, overlap between the results of the PheoCat and the results of the 10 healthy cats occurred in 4/10 cats for PL-MN and in 1/10 cat for U-MN/Creat. The PL-NMN and U-NMN/Creat measurements of all healthy cats were significant lower when compared to the ones of the PheoCat, hence no overlap occurred with these parameters (Graph 2).

***Table 5* - Plasma and urinary metanephrines by LC-MS-MS in a population of 10 healthy cats and in the PheoCat.**

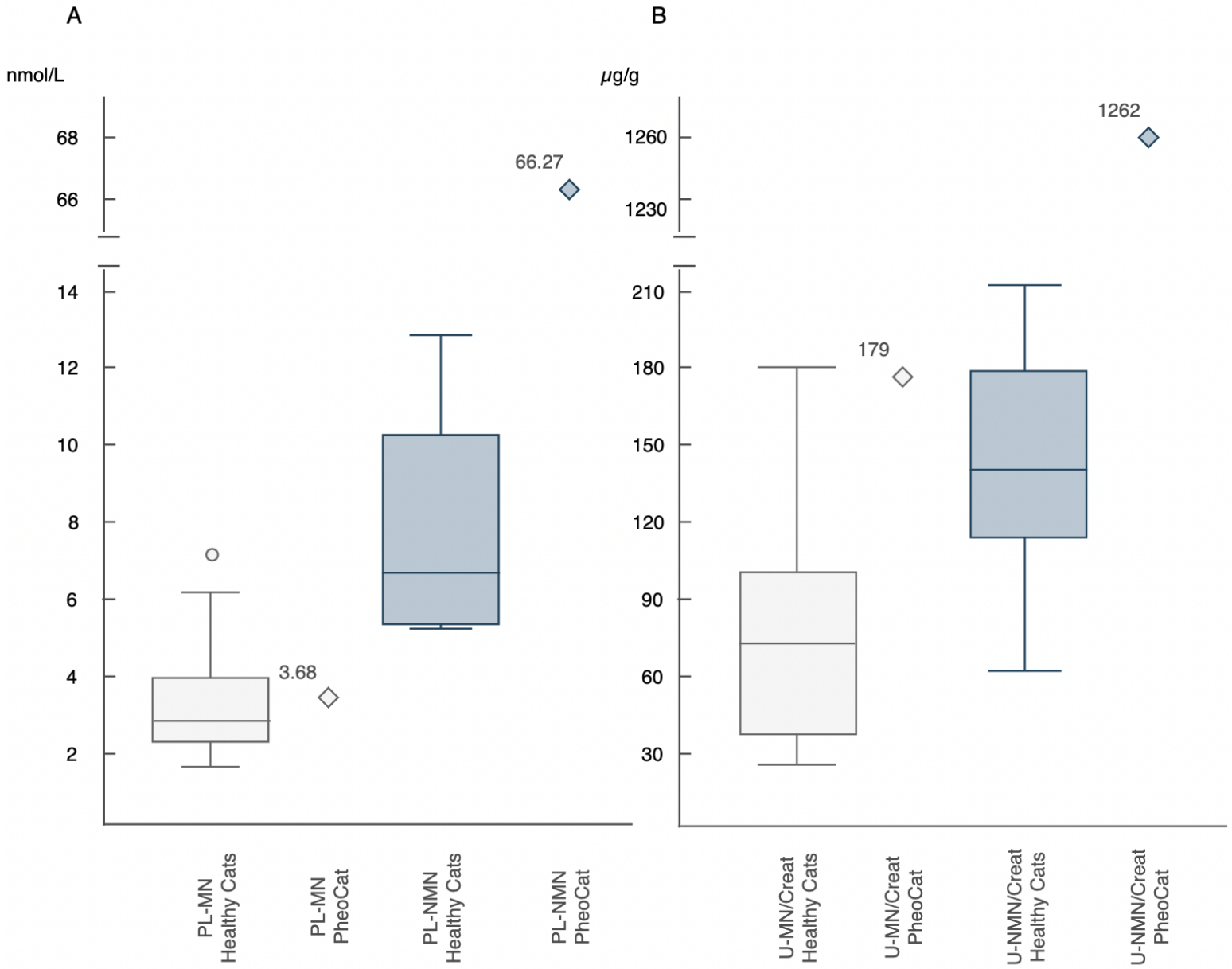
	PL-MN (nmol/L)	PL-NMN (nmol/L)	U-MN/Creat ratio (μg/g)	U-NMN/Creat ratio (μg/g)
1	1.71	**10.36**	36	**147**
2	4.02	**5.83**	64	**80**
3	6.16	**7.87**	182	**210**
4	3.73	**8.96**	100	**191**
5	7.24	**11.02**	73	**181**
6	2.58	**6.18**	24	**59**
7	2.42	**5.19**	84	**131**
8	1.77	**5.24**	67	**130**
9	2.32	**5.35**	119	**168**
10	2.88	**13.02**	31	**115**
Median (IQR)	2.73 (2,37)	**7.02 (5,2)**	70 (70)	**139 (77,25)**
PheoCat	3.68	**66.27**	179	**1262**

Legend: (IQR) - Interquartile range; (PL-MN) - Plasma metanephrine; (PL-NMN) - Plasma normetanephrine; (U-MN/Creat ratio) - Urinary metanephrine-to-creatine ratio; U-NMN/Creat ratio) - Urinary normetanephrine-to-creatinine ratio (U-NMN/Creat ratio).

4.3.3. Urinary Metanephrines Stability

A Wilcoxon signed-rank test for paired samples was used to evaluate the feline U-MN/NMN stability under 24h of refrigeration. These parameters proved to be stable at least for 24h at +4ºC, as there was no statistical difference between U-MN vs UR-MN and U-NMN vs UR-NMN, being the p-values 0.329 and 0.813, respectively (Table 6).

***Graph 2*- Boxplot representation of the distribution of plasma (A) and urinary (B) metanephrines in a population of 10 healthy cats and in the PheoCat.**



Legend: A) - (PL-MN) - plasma metanephrine; (PL-NMN) - plasma normetanephrine. (B) - (U-MN/Creat) - urinary metanephrine-to-creatinine ratio; (U-NMN/Creat) - normetanephrine-to-creatinine ratio.

***Table 6* - Urinary metanephrines in urine samples immediately preserved at -80ºC in comparison to those refrigerated for 24h before storage at -80ºC.**

	1	2	3	4	5	6	7	8	9	10	**Median (IQR)**
U-MN (ng/mL)	105	325	711	400	138	69	467	193	305	85	**249 (316,75)**
UR-MN (ng/mL)	104	329	722	392	142	68	473	192	314	84	**253 (313,25)**
U-NMN (ng/mL)	435	408	820	763	345	169	731	376	432	316	**420 (401,25)**
UR-NMN (ng/mL)	449	4015	847	747	339	173	731	369	443	301	**429 (405,5)**

Legend: (U-MN and U-NMN) - Urinary metanephrine and normetanephrine in urine samples immediately preserved at -80ºC; (UR-MN and UR-NMN) - Urinary metanephrine and normetanephrine in urine samples refrigerated for 24h before storage at -80ºC.

4.4. Discussion

This pilot study was the first to report the measurement of plasma and urinary metanephrines by LC-MS-MS in healthy cats and in a cat with a confirmed diagnosis of PCC, highlighting the clinical applicability of these biomarkers in the diagnosis of feline PCC.

It is presently an agreement among experts in both veterinary and human medicine, that initial testing for PCC should include the measurement of fractionated metanephrines in urine and/or plasma (Pacak et al. 2007; Galac and Korpershoek 2017). The LC-MS-MS methodology has previously been approved in dogs and humans for the measurement of plasma and urinary metanephrines, and reference ranges are reported in these species (Sasaki et al. 2021; Gostelow et al. 2013; Eisenhofer et al. 2019). To the authors' knowledge, LC-MS-MS had never been performed in cats before for the measurement of neither plasma nor urinary metanephrines.

This pilot study intended to assess the normal values of feline plasma and urinary metanephrines using the new simple LC-MS-MS methodology and to compare these values with those from a cat with a definitive diagnosis of PCC. The study's results go in line with the research that has been made in the scope of the biochemical diagnosis of human and canine PCC, supporting the feasibility of PL-MN/NMN and U-MN/NMN measurement in cats by LC-MS-MS.

The plasma and urinary metanephrines median values in the population of 10 healthy cats were different from those of healthy dogs, reinforcing the relevance of determining species-specific reference ranges (Sasaki et al. 2021; Gostelow et al. 2013). The results

obtained from the PheoCat were significantly higher when compared to the healthy cats', particularly with concern to PL-NMN and U-NMN/Creat ratio. This, and the fact that none of the values measured in the PheoCat overlapped with the medians obtained from the healthy cats' group, highlight the clinical applicability of these findings. Overlap with the PheoCat occurred in 4/10 cats for PL-MN, in 1/10 cat for U-MN/Creat, and in none of the cats for both PL-NMN and U-NMN/Creat, supporting that NMN is a better potential biomarker than MN for the diagnosis of feline PCC. These results are in accordance with the consensus that there is a strong preference for NMN determination over MN for the diagnosis of canine PCC, whether the sample is plasma or urine (Galac and Korpershoek 2017). No clear superiority of plasma over urine and vice versa was observed in this study, but a larger population of cats would be needed for a conclusion to be drawn on this subject.

In order to evaluate the stability of feline urinary metanephrines under different storage conditions, this study compared measurements obtained from urine samples immediately stored at -80ºC with measurements obtained from samples refrigerated (+4ºC) for 24h before freezing. The results revealed that urinary metanephrines were not significantly different in urine samples that underwent a 24h refrigeration process in comparison to those which did not. Thus, this study suggests that feline urinary metanephrines are stable for, at least, 24h under refrigeration temperatures. This finding is in agreement with previous studies that reported that urinary metanephrines are relatively stable in humans and dogs (Sasaki et al. 2021; Willemsen et al. 2007). Because these assays are infrequently requested and reliable diagnostic methods are not widely available in veterinary medicine, this is of great importance since sample transport to a more specialized diagnostic facility is almost always required.

This pilot study supports the feasibility of PL-MN/NMN and U-MN/NMN measurement in cats by LC-MS-MS and suggests that urinary metanephrines are stable for a period of 24h under refrigeration. The PheoCat had a substantial increase in all the measured parameters, particularly PL-NMN and U-NMN/Creat ratio, when compared to the healthy cats' population, highlighting the clinical applicability of these biomarkers in the diagnosis of feline PCC. Further investigations into plasma and urinary metanephrines levels in a larger population of cats is required to establish a reference range for this species.

4.5. Conclusion

This is the first study reporting both PL-MN/NMN and U-MN/NMN measurements by LC-MS-MS in adult healthy cats and will contribute to the biochemical diagnosis of feline PCC in the future.

This work project arose from the need to do an appropriate diagnosis of a PCC in a cat that was referred to the Referral Internal Medicine Service of the Veterinary Teaching Hospital – Faculty of Veterinary Medicine - University of Lisbon. This patient was presented with a 4-month history of progressive polyphagia, weight loss, polyuria/polydipsia, weakness and severe hypertension and had an abdominal mass confirmed to have its origin in the left adrenal gland. After investigation of whether this mass was functional or not and exclusion of hypercortisolism and aldosteronoma, the next step in the step-by-step diagnostic approach to an adrenal mass should include the measurement of fractionated metanephrines in urine and/or plasma, as it is in dogs and humans. However, as PCC is an extremely rare condition in cats, literature about these biomarkers in this species is scarce. Based on one previous study that reported PL-MN/NMN by HPLC in healthy cats, the PL-MN and PL-NMN measurements in this patient were compatible with the diagnosis of feline PCC (Wimpole et al. 2010). After adrenalectomy, the left adrenal gland was submitted to histopathology and immunohistochemistry for chromogranin A and synaptophysin, which confirmed that the mass excised consisted of a pheochromocytoma.

Nowadays, the most accurate methodology to measure plasma and urinary metanephrines in humans and dogs is LC-MS-MS (Grebe and Ravinder 2011). Furthermore, a study reporting both plasma and urinary metanephrines in a population of healthy cats and in a cat with a confirmed diagnosis of PCC was lacking. Taking this into account, we seized the opportunity of having plasma and urine samples stored at -80ºC from a cat with a confirmed diagnosis of PCC and developed a pilot-study with concern to plasma and urinary metanephrine and normetanephrine in healthy cats. Even though this study relied on a small population of 10 healthy cats, results supported the feasibility of PL-MN/NMN and U-MN/NMN measurement in cats by LC-MS-MS. Further investigations into plasma and urinary metanephrines levels in a larger population of cats are required to establish a reference range for this species. The cat with a PCC had a substantial increase in all the measured parameters, particularly PL-NMN and U-NMN/Creat ratio, highlighting the clinical applicability of metanephrines, specially normetanephrine, in the diagnosis of feline PCC.

5. REFERENCES

Acierno M, Brown S, Coleman A, Jepson R, Papich M, Stepien R, Syme H. 2018. ACVIM consensus statement: Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. J Vet Intern Med. p 1-20. DOI:10.1111/jvim.15331.

Ahn J, Park JY, Kim G, Jin SM, Hur KY, Lee SY, Kim J. 2021. Urinary Free Metanephrines for Diagnosis of Pheochromocytoma and Paraganglioma. Endocrinol Metab; 36. p 697-701. DOI: https://doi.org/10.3803/EnM.2020.925.

Ames MK, Atkins CE, Pitt B. 2019. The renin-angiotensin-aldosterone system and its suppression. J Vet Intern Med; 33(2). p 363 – 382. DOI: https://doi.org/10.1111/jvim.15454.

Barthez P, Marks S, Woo J, Feldman E, Matteucci. 1997. Pheochromocytoma in dogs: 61 cases (1984-1995). J Vet Intern Med; 11(5). p 272-278. DOI: 10.1111/j.1939-1676. 1997.tb00464. x.

Bertazzolo W, Didier M, Gelain ME, Rossi S, Crippa L, Avallone G, Roccabianca P, Bonfanti U, Giori L, Fracassi F. 2014. Accuracy of cytology in distinguishing adrenocortical tumors from pheochromocytoma in companion animals. Vet Clin Pathol; 43(3). p 453-9. DOI:10.1111/vcp.12161.

Boag AK, Neiger R, Church DB. 2004. Trilostane treatment of bilateral adrenal enlargement and excessive sex steroid hormone production in a cat. J Small Anim Pract; 45. p 263–266. DOI:10.1111/j.1748-5827.2004.tb00234.x.

Boland L, Barrs V. 2017. Peculiarities of feline hyperadrenocorticism: Update on diagnosis and treatment. JFMS; 19. p 933–947. DOI:10.1177/1098612X17723245.

Bommarito DA, Lattimer JC, Selting KA, Henry CJ, Cohen M, Johnson GC. 2011. Treatment of a malignant pheochromocytoma in a dog using 131I metaiodobenzylguanidine. J Am Anim Hosp Assoc; 47. p 188 – 194. DOI: 10.5326/JAAHA-MS-5605.

Bornstein ME, Bornstein SR. 2008. Cross-talk between adrenal medulla and adrenal cortex in stress. Ann N Y Acad Sci; 1148. p 112-7. DOI: 10.1196/annals.1410.053.

Calsyn JDR, Green RA, Davis GJ, Reilly CM. 2010. Adrenal pheochromocytoma with contralateral adrenocortical adenoma in a cat. JAAHA; 46. p 36-42. DOI: 10.5326/0460036.

Cervone M. 2017. Concomitant multiple myeloma and probable phaeochromocytoma in a cat. JFMS Open Reports; 3. p 1-6. DOI:10.1177/2055116917719209.

Chen H, Sippel R, O'Dorisio MS, Vinik A, Lloyd R, Pacak K. 2010. The NANETS Consensus Guideline for the diagnosis and management of neuroendocrine tumors: pheochromocytoma, paraganglioma & medullary thyroid cancer. Pancreas 39(6). p 775 -783. DOI:10.1097/MPA.0b013e3181ebb4f0.

Chun R, Jakovljevic S, Morrison WB, DeNicola DB, Cornell KK. 1997. Apocrine gland adenocarcinoma and pheochromocytoma in a cat. J Am Anim Hosp Assoc; 33 (1). p 33 - 6. DOI: 10.5326/15473317-33-1-33.

Daniel G, Mahony O, Markovich J, Appleman E, Monaghan K, Lawrence Y, Fiocchi E, Weaver K, Johnston A, Barton B. 2015. Clinical findings, diagnostics and outcome in 33 cats with adrenal neoplasia (2002-2013). JFMS. DOI: 10.1177/1098612X15572035.

Dyce KM, Sack WO, Wensing JG. 2010. Textbook of Veterinary Anatomy. Fourth Edition. Missouri (MO): Elsevier. p 448 – 449.

Eisenhofer G. 2003. Editorial: Biochemical Diagnosis of Pheochromocytoma—Is it Time to Switch to Plasma-Free Metanephrines? J Clin Endocrinol Metab 88(2). p 550–552. DOI: 10.1210/jc.2002-021913.

Eisenhofer G, Lenders J, Goldstein D, Mannelli M, Csako G, Walther M, Brouwers F, Pacak K. 2005. Pheochromocytoma catecholamine phenotypes and prediction of tumor size and location by use of plasma free metanephrines. Clin Chem;51(4). p 735-44. DOI: 10.1373/clinchem.2004.045484.

Eisenhofer G, Pacak K, Huynh TT, Qin N, Bratslavsky G, Linehan W, Mannelli M, Friberg P, Grebe S, Timmers H, Bornstein S, Lenders J. 2010. Catecholamine metabolomic and secretory phenotypes in phaeochromocytoma. Endocr Relat Cancer; 21;18(1). p 97-111.DOI: 10.1677/ERC-10-0211.

Elias S, Delestre C, Courel M, Anouar Y, Montero-Hadjadje M. 2010. Chromogranin A as a crucial factor in the sorting of peptide hormones to secretory granules. Review Cell Mol Neurobiol; 30(8). p 1189-95. DOI: 10.1007/s10571-010-9595-8.

Engelking LR. 2012. Metabolic and Endocrine Physiology. Third Edition. Wyoming (WY): Teton NewMedia.

Fitzgerald P. 2018. Adrenal Medulla and Paraganglia. In: Greenspan's Basic & Clinical Endocrinology. Tenth Edition. New York (NY): McGraw-Hill Education.

Galac S. 2017. Pheochromocytoma. In: Textbook of Veterinary Internal Medicine: Diseases of the Dog and the Cat. Eight Edition. Missouri (MO): Elsevier. p 4447-4457.

Galac S, Korpershoek E. 2017. Pheochromocytomas and paranganglioma in humans and dogs. Veterinary and Comparative Oncology. DOI: 10.1111/vco.1229113.

Gilson SD, Withrow S, Wheeler S, Twedt D. 1993. Pheochromocytoma in 50 dogs. J Vet Intern Med; 8 (3). p 228-232. DOI: 10.1111/j.1939-1676. 1994.tb03222. x.

Gilson SD, Withrow S, Orton EC. 1994. Surgical treatment of pheochromocytoma: technique, complications, and results in six dogs. Vet Surg;23(3). p 195-200. DOI:10.1111/j.1532-950x.1994.tb00472.x.

Grebe S, Ravinder S. 2011. LC-MS/MS in the Clinical Laboratory – Where to From Here? Clin Biochem Rev; 32(1): 5–31. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3052391/pdf/cbr_32_1_005.pdf.

Gregori T, Mantis P, Benigni L, Priestnall SL, Lamb CR. 2015. Comparison of computed tomographic and pathologic findings in 17 dogs with primary adrenal neoplasia. Vet. Radiol. Ultrasound; 56(2). p 153–159. doi:10.1111/vru.12209.

Gunn-Moore D, Simpson K. 2013. Pheochromocytoma in cat. In: Clinical Endocrinology of Companion Animals. First Edition. New Jersey (NJ): John Wiley & Sons.

Henry CJ, Brewer Jr WG, Montgomery RD, Groth Jr AH, Cartee RE, Griffin KS. 1993. Clinical vignette. Adrenal pheochromocytoma. J Vet Intern Med; 7: 199 - 201. DOI: 10.1111/j.1939-1676. 1993.tb03188. x.

Herrera MA, Mehl ML, Kass PH, Pascoe PJ, Feldman EC, Nelson RW. 2008. Predictive Factors and the Effect of Phenoxybenzamine on Outcome in Dogs Undergoing Adrenalectomy for Pheochromocytoma. J Vet Intern Med;22. p 1333–1339. DOI:10.1111/j.1939-1676.2008. 0182.xJ.

Hickman P, Leong M, Chang J, Wilson S, McWhinney B. 2009. Plasma free metanephrines are superior to urine and plasma catecholamines and urine catecholamine metabolites for the investigation of phaeochromocytoma. Pathology; 41(2):173-7. DOI: 10.1080/00313020802579284.

Hinson J, Raven P, Chew S. 2010. The Adrenal Glands Part I: The Adrenal Medulla. In: The Endocrine System. Second Edition. Missouri (MO): Elsevier.

Jacques WM, Lenders QD, Eisenhofer G, Gimenez-Roqueplo AP, Stefan KGB, Murad MH, Naruse M, Pacak K, Young WF. 2014. Pheochromocytoma and Paraganglioma: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab; 99 (6). p 1915–1942. DOI: https://doi.org/10.1210/jc.2014-1498.

Kook PH, Boretti F, Hersberger M, Glaus T, Reusch C. 2007. Urinary catecholamine and metanephrine to creatinine ratios in healthy dogs at home and in a hospital environment and in 2 dogs with pheochromocytoma. J Vet Intern Med 21(388). DOI: 10.1892/0891-6640(2007)21[388: ucamtc]2.0.co;2.

Kyles A, Feldman E, De Cock HEV, Kass PH, Mathews K, Hardie E, Nelson R, Ilkiw J, Gregory C. 2003. Surgical management of adrenal gland tumors with and without associated tumor thrombi in dogs: 40 cases (1994-2001). J Am Vet Med Assoc;223(5). p 654-62. DOI:10.2460/javma.2003.223.654.

Labelle P, Kyles AE, Farver TB, de Cock HEV. 2004. Indicators of Malignancy of Canine Adrenocortical Tumors: Histopathology and Proliferation Index. Veterinary Pathology; 41(5). p 490–497. DOI:10.1354/vp.41-5-490.

Lenders J, Eisenhofer G, Mannelli M, Pacak K. 2005. Phaeochromocytoma. Lancet; Vol 366. p 665-675. DOI: https://doi.org/10.1016/S0140-6736(05)67139-5.

Lenders J, Duh QY, Eisenhofer G, Gimenez-Roqueplo AP, Grebe S, Murad M, Naruse M, Pacak K, Young W. 2014. Pheochromocytoma and Paraganglioma: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab; 99(6). p 1915–1942. DOI: 10.1210/jc.2014-1498.

Li H, Zhang G, Wang W, Jiao LL, Chen CB, Huo JR, Wu W. 2022. Detection of catecholamine metabolites in urine based on ultra-high-performance liquid chromatography–tandem mass spectrometry. Biochemical Chromatography; 36. DOI: https://doi.org/10.1002/bmc.5280.

Lunn KF, Boston SE. 2020. Tumors of the Endocrine System. In: Withrow & MacEwen's Small Animal Clinical Oncology. Sixth Edition. Missouri (MO): Elsevier. p 565-575.

Maher ER, McNiel EA. 1997. Pheochromocytoma in dogs and cats. Vet Clin N Am; 27:2; p 359-380. DOI: https://doi.org/10.1016/S0195-5616(97)50037-4.

Mannelli M, Lenders J, Pacak K, Parenti G, Eisenhofer G. 2012. Subclinical phaeochromocytoma. Best Pract Res Clin Endocrinol Metab;26(4). p 507-15. DOI: 10.1016/j.beem.2011.10.008.

Melián C, Pérez-López L. 2019. Other adrenal cortical tumors and pheochromocytoma. In: Feline Endocrinology. First Edition. Milano (MI): Editorial Edra.

Millard RP, Pickens EH, Wells KL. 2009. Excessive production of sex hormones in a cat with an adrenocortical tumor. JAVMA;234. p 505–508. DOI:10.2460/javma.234.4.505.

Miller M. 2017. Endocrine System. In: Pathologic Basics of Veterinary Disease. Sixth Edition. Missouri (MO): Elsevier.

Musser L, Taikowski K, Johannes C, Bergman P. 2018. Retrospective evaluation of toceranib phosphate (Palladia®) use in the treatment of inoperable, metastatic, or recurrent canine pheochromocytomas: 5 dogs (2014–2017). BMC Veterinary Research; 14(1). DOI:10.1186/s12917-018-1597-7.

Nomura K, Kimura H, Shimizu S, Kodama H, Okamoto T, Obara T, Takano K. 2009. Survival of Patients with Metastatic Malignant Pheochromocytoma and Efficacy of Combined Cyclophosphamide, Vincristine, and Dacarbazine Chemotherapy. J Clin Endocrinol Metab; 94 (8). p 2850–2856. DOI: https://doi.org/10.1210/jc.2008-2697.

Nyland TG, Neelis DA, Mattoon JS. 2015. Adrenal Glands. In: Small Animal Diagnostic Ultrasound. Third Edition. Missouri (MO): Elsevier. p 541-553.

Pacak K, Eisenhofer G, Ahlman H, Bornstein S, Gimenez-Roqueplo AP, Grossman AB, Kimura N, Mannelli M, McNicol AM, Tischler A. 2007. Pheochromocytoma: recommendations for clinical practice from the First International Symposium. Nat Clin Pract Endocrinol Metab; 3(2):92-102. DOI: 10.1038/ncpendmet0396.

Pascaline P, Alessia D, Rossi F, Mortier J, Kafka U, Veraa S, Groth A, MacLellan M, Marin C, Fracassi F. 2020. Safety of percutaneous ultrasound-guided fine-needle aspiration of adrenal lesions in dogs: Perception of the procedure by radiologists and presentation of 50 cases. J Vet Intern Med. p 1-10. DOI:10.1111/jvim.15743.

Pérez-López L, Wagner AM, Saavedra P, Jaber J, Melián C. 2021. Ultrasonographic evaluation of adrenal gland size in two body weight categories of healthy adult cats. JFMS; 23(8). P 804-808. DOI: https://doi.org/10.1177/1098612X20974962.

Petroff B, Greco D. 2020. Endocrine Glands and Their Function. In: Cunningham's Textbook of Veterinary Physiology. Sixth Edition. Missouri (MO): Elsevier.

Pillai D, Ross HA, Kratzsch J, Pedrosa W, Kema I, Hoad K, Rouaix N, Fathi M, Nader H, Mathian B, Grouzmann E. 2009. Proficiency test of plasma free and total metanephrines: report from a study group. CCLM; 47(6). DOI:10.1515/CCLM.2009.162

Pillai D, Callen S. 2010. Pilot quality assurance programme for plasma metanephrines. Ann Clin Biochem; 47. p 137–142. DOI:10.1258/acb.2009.009153.

Pinto L, Kramer C, Camargo J, Canani L, Gross J, Leitão C. 2020. J Endocrinol Investig; 43. p 53-56. https://doi.org/10.1007/s40618-019-01076-9.

Plumb DC. 2011. Plumb's Veterinary Drug Handbook. Seventh Edition. Wisconsin (WC): PharmaVet Inc.

Prejbisz A, Lenders J, Eisenhofer G, Januszewicz A. 2011. Cardiovascular manifestations of phaeochromocytoma. Journal of Hypertension; 29(11). p 2049–2060. DOI: 10.1097/hjh.0b013e32834a4ce9.

Reusch C. 2015. Pheochromocytoma and Multiple Endocrine Neoplasia. In: Canine and Feline Endocrinology. Fourth Edition. Missouri (MO): Elsevier.

Rossmeisl JH, Scott-Moncrieff JC, Siems J, Snyder PW, Wells A, Anothayanontha L, Oliver JW. 2000. Hyperadrenocorticism and hyperprogesteronemia in a cat with an adrenocortical adenocarcinoma. JAAHA; 36. p 512–517. DOI:10.5326/15473317-36-6-512.

Safford SD, Coleman RE, Gockerman JP, Moore J, Feldman JM, Leight GS Jr, Tyler D, Olson J Jr. 2003. Iodine −131 metaiodobenzylguanidine is an effective treatment for malignant pheochromocytoma and paraganglioma. Surgery; 134(6). p 956 – 962. DOI: 10.1016/s0039-6060(03)00426-4.

Salesov E, Boretti FS, Sieber-Ruckstuhl NS, Rentsch KM, Riond B, Hofmann-Lehmann R, Kircher PR, Grouzmann E, Reusch CE. 2015. Urinary and Plasma Catecholamines and Metanephrines in Dogs with Pheochromocytoma, hypercortisolism, nonadrenal disease and in healthy dogs. J Vet Intern Med; 29:597-602. DOI: 10.1111/jvim.12569.

Sasaki N, Ikenaka Y, Inoue Y, Ichise T, Nagata N, Ishizuka M, Nakayama S, Nakamura K, Takiguchi M. 2021. Urinary free metanephrines measurement in dogs with adrenal gland

diseases using a new simple liquid chromatography tandem mass spectrometry method. J Vet Med Sci; 83(4). P 648-655. DOI: https://dx.doi.org/10.1292%2Fjvms.20-0508.

Schulman R. 2010. Feline Primary Hyperaldosteronism. Vet Clin Small Anim; 40. p 353–359. DOI: 10.1016/j.cvsm.2009.10.006.

Schultz RM, Wisner ER, Johnson EG, MacLeod JS. 2009. Contrast-enhanced computed tomography as a preoperative indicator of vascular invasion from adrenal masses in dogs. Vet Radiol Ultrasound;50. p 625–629. DOI: 10.1111/j.1740-8261.2009.01593.x.

Schwartz P, Kovak J, Koprowski A, Ludwig L, Monette S, Bergman P. 2008. Evaluation of prognostic factors in the surgical treatment of adrenal gland tumors in dogs: 41 cases (1999–2005). AVMA; 232(1). DOI: https://doi.org/10.2460/javma.232.1.77.

Smy L, Kushnir M, Frank E. 2021. A high sensitivity LC-MS/MS method for measurement of 3-methoxytyramine in plasma and associations between 3-methoxytyramine, metanephrines, and dopamine. JMSACL;21. p 19-26. DOI: 10.1016/j.jmsacl.2021.08.001.

Srithunyarat T, Svensson A, Hanås S, Höglund OV, Hagman R, Lilliehöök I, Olsson U, Ljungvall I, Häggström J, Holst BS, Pettersson A, Höglund K. 2018. Evaluation of an ELISA for metanephrines in feline urine. J Vet Diagn Invest; Vol. 30(6). p 887–893. DOI: 10.1177/1040638718793168.

Tischler A, Powers J, Alroy J. 2004. Animal models of pheochromocytoma. Histol Histopathol; 19. p 883-895. URL: https://digitum.um.es/digitum/bitstream/10201/21602/1/Animal %20models %20of%20pheochromocytoma.pdf.

Tischler A, Kimura N, Mariemcnicol A. 2006. Pathology of Pheochromocytoma and Extra-adrenal Paraganglioma. N.Y. Acad. Sci; 1073. p 557–570. DOI: 10.1196/annals.1353.059.

Thrall D. 2018. Textbook of Veterinary Diagnostic Radiology. Seventh Edition. Missouri (MO): Elsevier.

Weese JS, Blondeau J, Boothe D, Guardabassi LG, Gumley N, Papich M, Jessen LR, Lappin M, Rankin S, Westropp JL, Sykes J. 2019. International Society for Companion Animal Infectious Diseases (ISCAID) guidelines for the diagnosis and management of bacterial urinary tract infections in dogs and cats. Vet J ;247. p 8–25. DOI:10.1016/j.tvjl.2019.02.008.

Weismann D, Peitzsch M, Raida A, Prejbisz A, Gosk M, Riester A, Willenberg HS, Klemm R, Manz G, Deutschbein T, Kroiss M, Darr R, Bidlingmaier M, Januszewicz A, Eisenhofer G, Fassnacht M. 2015. Measurements of plasma metanephrines by immunoassay vs liquid chromatography with tandem mass spectrometry for diagnosis of pheochromocytoma. Eur J Endocrinol; 172(3). p 251–260. DOI:10.1530/EJE-14-0730.

Whittemore JC, Preston C, Kyles A, Hardie E, Feldman E. 2001. Nontraumatic rupture of an adrenal gland tumor causing intra-abdominal or retroperitoneal hemorrhage in four dogs. J Am Vet Med Assoc; 219(3). p 329–333. DOI:10.2460/javma.2001.219.329.

Willemsen J, Alec Ross H, Lenders J, Sweep F. 2007. Stability of Urinary Fractionated Metanephrines and Catecholamines during Collection, Shipment, and Storage of Samples. Clinical Chemistry; 53:2. p 268 –272. DOI: https://doi.org/10.1373/clinchem.2006.075218.

Wimpole JA, Adagra C, Billson M, Pillai D, Foster D. 2010. Plasma free metanephrines in healthy cats, cats with non-adrenal disease and a cat with suspected phaeochromocytoma. JFMS; 12, 435-440. DOI: 10.1016/j.jfms.2009.10.010.

6. ANNEXES

Annexe 1 – Poster submitted to communication at *Congresso Internacional Hospital Veterinário Montenegro*.

CORPOS ESTRANHOS TRAQUEOBRÔNQUICOS EM CÃES – UM DIFERENCIAL IMPORTANTE NA PRÁTICA CLÍNICA: A PROPÓSITO DE QUATRO CASOS CLÍNICOS

M. T. Prego[1], M. J. Dias[1,2], P. L. Marques[1], N. Santos[1], D. Bota[3], R.O. Leal[1, 2]

[1]HEV –Hospital Escolar Veterinário, Faculdade de Medicina Veterinária, Universidade de Lisboa, Avenida da Universidade Técnica, 1300-477, Lisboa, Portugal
[2]Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, ULisboa, Lisboa, Portugal
[3]Hospital Veterinário do Restelo, Rua Gregório Lopes 1513, 1449-010 Lisboa, Portugal

INTRODUÇÃO

Os **corpos estranhos traqueobrônquicos** são pouco frequentes em cães e gatos. Usualmente apresentam dimensões pequenas e alojam-se nos brônquios, causando uma broncopneumonia responsiva aos antibióticos que recidiva aquando da sua interrupção [1]. A traqueobroncoscopia surge muitas vezes como o exame complementar de diagnóstico para a identificação e remoção de corpos estranhos traqueobrônquicos [2].

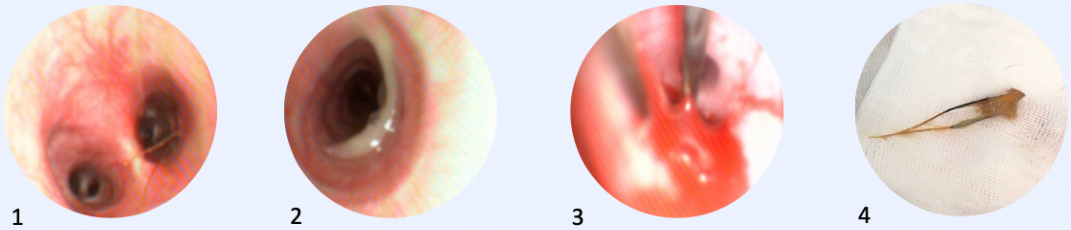
OBJETIVO

Sensibilizar os Médicos Veterinários para a possibilidade da ocorrência de corpos estranhos traqueobrônquicos como causa de tosse em cães.

CASOS CLÍNICOS

Foram reunidos os casos referenciados para traqueobroncoscopia com diagnóstico final de corpo estranho traqueobrônquico em dois centros de referência da área metropolitana de Lisboa, entre Setembro de 2018 e Agosto de 2021.
No total reuniram-se os dados referentes a **quatro cães**: um Setter Inglês com 11 meses, um Braco Alemão com 2 anos e um Labrador Retriever e Cane Corso, ambos com 6 anos. O Setter Inglês apresentava crises de **engasgos e tosse emetizante com hemoptise de aparecimento agudo** após passeio no campo. O Labrador Retriever, o Cane Corso e Braco Alemão apresentavam **tosse crónica refratária** (> 2 semanas) ao tratamento com antibiótico.
O Setter Inglês, o Labrador Retriever e o Braco Alemão realizaram **radiografias torácicas**. O Setter não revelava alterações significativas enquanto que o Labrador Retriever e o Braco Alemão apresentavam um padrão bronco-intersticial difuso inespecífico.
O Cane Corso realizou uma **tomografia computorizada torácica** que revelou uma suspeita de corpo estranho brônquico.

TRAQUEOBRONCOSCOPIA



1 2 3 4

Todos os cães apresentavam a mucosa brônquica eritematosa (1) e com friabilidade aumentada. O Labrador Retriever apresentava ainda espessamento focal da mucosa do brônquio principal esquerdo e o Cane Corso secreções brônquicas purulentas (2). No Setter Inglês identificaram-se três praganas, uma no brônquio principal direito (RPB), uma no brônquio referente ao lobo médio (RB2) e outra no brônquio correspondente ao lobo caudal direito (RB4). O Cane Corso apresentava duas praganas no brônquio correspondente ao lobo caudal direito (RB4). No Labrador foi identificada uma pragana no brônquio principal esquerdo (LPB) e no Braco Alemão, uma no lobo caudal esquerdo (LB2).
Em todos os casos as **praganas** foram removidas sem incidentes e com recurso a uma pinça de corpo estranho (3 e 4).

CONCLUSÃO

- ✓ Nesta série de quatro casos, todos os cães eram de **idade jovem ou adultos** e apenas um deles tinha história pregressa compatível com corpo estranho;
- ✓ Contrariamente às radiografias torácicas, apenas a tomografia computorizada permitiu a identificação de um corpo estranho;
- ✓ **A presença de corpos estranhos traqueobrônquicos deve fazer parte da lista de diagnósticos diferenciais de tosse;**
- ✓ **A realização de uma traqueobroncoscopia é recomendada em casos de tosse refratária.**

[1] Ettinger et al. 2017. Textbook of Veterinary Internal Medicine. 8th Edition. Missouri (MO): Elsevier
[2] Tenwolde AC, Johnson LR, Hunt GB. 2010. The role of bronchoscopy in foreign body removal in dogs and cats: 37 cases (2000–2008). J Vet Intern Med; 24: 1063–1068
"Este trabalho teve o apoio da FCT - Fundação para a Ciência e Tecnologia IP - bolsa UIDB / 00276/2020. O autor declara não haver conflito de interesses "

Annexe 2 – Abstract of the paper submitted to the *Journal of Veterinary Internal Medicine* - "Plasma and Urinary Metanephrine and Normetanephrine in Healthy Cats – a Pilot Study".

Plasma and Urinary Metanephrine and Normetanephrine in Healthy Cats – a Pilot Study

Maria Teresa Prego[1], Maria Joana Dias[1,2], Rui Lemos Ferreira[1], Salomé Gonçalves[2], Tiago Domingues[3], Greet Junius[4], Evi Van den Steen[4], Sara Galac[5], Rodolfo Oliveira Leal[1,2].

[1] Veterinary Teaching Hospital; Faculty of Veterinary Medicine – University of Lisbon; Av. Universidade Técnica, 1300-171 Lisboa.

[2] CIISA – Centro de Investigação Interdisciplinar em Sanidade Animal – Faculty of Veterinary Medicine – University of Lisbon; Av. Universidade Técnica, 1300-171 Lisboa.

[3] Department of Statistics and Operations Research; Faculty of Sciences - University of Lisbon; Campo Grande 016, 1749-016 Lisboa.

[4] AML – Algemeen Medisch Laboratorium - Emiel Vloorsstraat 9, 2020 Antwerpen, Belgium.

[5] Department of Clinical Sciences – Utrecht University - Yalelaan 108, 3508 TD Utrecht.

ABSTRACT

Feline pheochromocytoma (PCC) is rare and literature is limited to a few case-reports. In dogs, biochemical diagnosis of PCC is based on plasma (PL) and/or urinary (U) metanephrine (MN) and normetanephrine (NMN) but little is known about these biomarkers in cats.

This pilot study evaluates the feasibility of PL-MN/NMN and U-MN/NMN measurement in cats by Liquid Chromatography with tandem mass spectrometry (LC-MS-MS) and the U-MN/NMN stability under refrigeration (+4ºC).

A cross-sectional study was conducted, using a group of 10 healthy adult cats. Each cat had a urine and a plasma sample immediately stored at -80ºC and a urine sample refrigerated (UR) for 24h before freezing. Plasma and urine samples from a cat with a confirmed diagnosis of PCC (PheoCat) were also submitted for analysis. Urinary creatinine (Creat) was measured to calculate the urinary ratios.

The PL-MN and PL-NMN median values of the population of cats were 2.73nmol/L (IQR=2.37) and 7.02nmol/L (IQR=5.2), respectively. U-MN/Creat and U-NMN/Creat had medians of 70µg/g (IQR=70) and 139µg/g (IQR=77), respectively. The PheoCat had a PL-MN of 3.68nmol/L, PL-NMN of 66.27nmol/L, U-MN/Creat of 179µg/g and U-NMN/Creat of 1262µg/g. There was no statistical difference between U-MN vs UR-MN and U-NMN vs UR-NMN (p=0.329 and p=0.813, respectively).

The PheoCat had a substantial increase in all the measured parameters, particularly PL-NMN and U-NMN/Creat, highlighting the clinical applicability of these findings. This is the first study reporting both PL-MN/NMN and U-MN/NMN measurements by LC-MS-MS in healthy cats and will contribute to the biochemical diagnosis of feline PCC in the future.

KEYWORDS: Feline Pheochromocytoma; Plasma and Urinary Metanephrines; Liquid Chromatography with tandem mass spectrometry; Healthy cats.

ABBREVIATIONS: LC-MS-MS, Liquid Chromatography with tandem mass spectrometry; MN, Metanephrine; NMN, Normetanephrine; PCC, Pheochromocytoma; PheoCat, cat with a pheochromocytoma; PL, Plasma; U, Urinary; UR, Refrigerated Urine.

Annexe 3 – Abstract of the paper submitted to *the journal Topics in Companion Animal Medicine* - "Pheochromocytoma confirmed by Immunohistochemistry in a Cat – a Case-Report".

Pheochromocytoma confirmed by Immunohistochemistry in a Cat – a Case-Report

Maria Teresa Prego[1], Maria Joana Dias[1,2], Lisa Mestrinho[1,2], Guy Grinwis[3], Sara Galac[4], Rodolfo Oliveira Leal[1,2].

[1] Veterinary School Hospital; Faculty of Veterinary Medicine – University of Lisbon; Av. Universidade Técnica, 1300-171 Lisboa.

[2] CIISA – Centro de Investigação Interdisciplinar em Sanidade Animal – Faculty of Veterinary Medicine – University of Lisbon; Av. Universidade Técnica, 1300-171 Lisboa.

[3] Veterinair Pathologisch Diagnostisch Centrum - Department of Biomolecular Health Sciences - Utrecht University; Yalelaan 1, 3584 CL Utrecht.

[4] Department of Clinical Sciences – Utrecht University; Yalelaan 108, 3508 TD Utrecht.

ABSTRACT

Feline pheochromocytoma (PCC) is considered to be rare and literature is limited to a few case-reports. An 8-year-old neutered male, European domestic shorthair cat was referred for consultation with a 4-month history of progressive polyphagia, weight loss, polyuria/polydipsia, weakness and severe hypertension. An abdominal ultrasound detected a heterogeneous nodular structure suspected of having its origin in the left adrenal gland. An abdominal computed tomography (CT) confirmed the presence of a mass arising from the left adrenal gland, with 17 mm of diameter and with no signs of vascular invasion. A Low Dose Dexamethasone Stimulation Test (LDDST) was performed, excluding hypercortisolism. Measurements of aldosterone and Plasma Renin Activity (PRA), combined with the serum potassium concentration, indicated that aldosteronoma was also unlikely. An increase in plasma metanephrine (PL-MN) and normetanephrine (PL-NMN) values (4,60nmol/L and 54,87nmol/L) in association with diagnostic imaging findings supported the diagnosis of pheochromocytoma. The patient underwent unilateral left adrenalectomy and the left adrenal gland was submitted for histopathology standard analysis and immunohistochemistry for chromogranin A and synaptophysin. Results were consistent with PCC. To the author's knowledge, this case-report emerges as the second published case of feline PCC confirmed by immunohistochemistry.

KEYWORDS: Pheochromocytoma; Cat; Case-Report; Immunohistochemistry.

ABBREVIATIONS: LDDST, Low Dose Dexamethasone Stimulation Test; PBZ, Phenoxybenzamine; PCC, Pheochromocytoma; PRA, Plasma Renin Activity; SBP, Systolic Blood Pressure

Annexe 4 – Abstract accepted for oral presentation at the *European College of Veterinary Internal Medicine – Companion Animals (ECVIM-CA) Congress.*

Plasma and Urinary Metanephrine and Normetanephrine in Healthy Cats – a Pilot Study

MT. Prego[1], MJ. Dias[1,2], RL. Ferreira[1], S. Gonçalves[2], T. Domingues[3], G. Junius[4], E. Van den Steen[4], S. Galac[5], RO. Leal[1,2].

[1] Veterinary School Hospital; Faculty of Veterinary Medicine – University of Lisbon; Av. Universidade Técnica, 1300-171 Lisboa

[2] CIISA – Centro de Investigação Interdisciplinar em Sanidade Animal – Faculty of Veterinary Medicine – University of Lisbon; Av. Universidade Técnica, 1300-171 Lisboa

[3] Department of Statistics and Operations Research - Faculty of Sciences of the University of Lisbon - Campo Grande 016, 1749-016 Lisboa

[4] AML – Algemeen Medisch Laboratorium - Emiel Vloorsstraat 9, 2020 Antwerpen, Belgium

[5] Department of Clinical Sciences – Utrecht University - Yalelaan 108, 3508 TD Utrecht

Feline pheochromocytoma is considered to be rare and literature is limited to few case-reports. In humans and dogs, biochemical diagnosis of a pheochromocytoma is based on measurements of plasma (PL) and/or urinary (U) metanephrine (MN) and normetanephrine (NMN) but little is known about these biomarkers in cats.

This pilot study aims to evaluate the feasibility of PL-MN/NMN and U-MN/NMN measurement in cats, using Liquid Chromatography with tandem mass spectrometry (LC-MS-MS). Furthermore, it intends to assess the U-MN/NMN stability under refrigeration (+4ºC) for 24h.

A cross-sectional pilot study was conducted, using a group of healthy adult cats recruited among students and staff from a Veterinary Teaching Hospital. The study was approved by the local ethical committee. With owner's consent, all cats were submitted to a physical examination, complete bloodwork, urine analysis, abdominal ultrasound, and systolic blood pressure assessment. Cats were excluded in case of abnormal results were identified. After sampling, EDTA-plasma and urine were stored at -80ºC until measurement of PL-MN/NMN and U-MN/NMN. U-MN/Creatinine and U-NMN/Creatinine ratios were then calculated. For each cat, an additional urine sample was refrigerated (UR) for 24h before storage at -80ºC. Leftover plasma and urine samples collected from a cat with a confirmed diagnosis of pheochromocytoma (PheoCat) were also submitted for analysis. Non-parametric tests were used for data analysis.

A total of 10 healthy cats were recruited, with a median age of 6 years (IQR=4.5). The PL-MN and PL-NMN median values were 2.73nmol/L (IQR=2.37) and 7.02nmol/L (IQR=5.2), respectively. U-MN/Creatinine and U-NMN/Creatinine ratios had medians of 70µg/g (IQR=70) and 139µg/g (IQR=77), respectively. Results obtained from the PheoCat revealed a PL-MN of 3.68nmol/L, PL-NMN of 66.27nmol/L, U-MN/Creatinine ratio of 179 µg/g and U-NMN/Creatinine ratio of 1262 µg/g. None of these values overlapped with the medians obtained from healthy cats.

The U-MN/NMN proved to be stable under refrigeration for 24h, as there was no statistical difference between U-MN vs UR-MN and U-NMN vs UR-NMN (p= 0.329 and p= 0.813, respectively).

This pilot study supports the feasibility of PL-MN/NMN and U-MN/NMN measurement in cats and urinary stability after 24h stored under refrigeration. The PheoCat had a substantial increase of all the measured parameters (particularly PL- and U-NMN) when compared to the healthy cats, highlighting the clinical applicability of these findings. This is the first study reporting both PL-MN/NMN and U-MN/NMN measurements by LC-MS-MS in adult healthy cats and will contribute to the biochemical diagnosis of feline pheochromocytoma in the future.

Annexe 5 – Document of the study's approval by the CEIE (Comissão de Ética para a Investigação e Ensino).

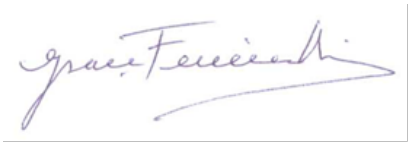
Universidade de Lisboa
Faculdade de Medicina Veterinária

TO WHOM IT MAY CONCERN

Subject: Evaluation of the Research project – N/Ref 002/2022

We hereby inform you that the CEIE (Comissão de Ética para a Investigação e Ensino/Ethical Committee for Research and Teaching), after evaluation of the activities that involve animal manipulation and welfare, within the scope of the research project entitled: "Plasmatic and urinary metanephrines in healthy cats - a pilot study", considered that the ethical and animal welfare subjects are safeguarded, according to the current legislation and the code of good practices. Therefore, the CEIE approved the implementation of the experimental protocol that will be held at the Faculty of Veterinary Medicine facilities.

Sincerely,



Graça Ferreira-Dias

President of the CEIE
(Full Professor)

April 7th, 2022

Av. Universidade Técnica – Polo Universitário Alto da Ajuda - 1300-477 LISBOA - **PORTUGAL**
☎ (351-21) 3652800 • ③ (351-21) 3652810 • http://www.fmv.utl.pt

Annexe 6 – Template of the Informed Consent Statement.

DECLARAÇÃO DE CONSENTIMENTO INFORMADO

Tendo sido solicitada a minha autorização para a participação do meu animal no estudo **avaliação das metanefrinas e normetanefrinas urinárias e plasmáticas em gatos saudáveis**, entendi que:

- a participação do meu animal no estudo é totalmente voluntária;

- a participação do meu animal no estudo pode ser revogada em qualquer momento, sem dar qualquer explicação;

- as colheitas de sangue e urina efectuadas ao meu gato servirão para averiguar gratuitamente o seu estado de saúde (check-up de gato adulto/geriátrico), autorizando que o remanescente seja utilizado para fins de investigação;

- a informação final obtida no estudo poderá ser consultada após pedido ao investigador responsável, se assim o desejar.

- a privacidade dos meus dados e dos dados do meu animal será respeitada no caso dos resultados serem utilizados em comunicações escritas ou orais, ou em publicações, que possam resultar do estudo.

Dou ainda fé de:

- ter lido a informação que me foi entregue

- ter-me sido transmitida oralmente a informação incluída nesta declaração

- ter podido fazer as perguntas que entendi por necessárias sobre o estudo

- ter recebido informação suficiente sobre o estudo

- ter compreendido que posso retirar o meu animal do estudo quando quiser e sem dar explicações.

Assim autorizo a divulgação da informação recolhida no estudo para o propósito do mesmo, com a salvaguarda do respeito pela privacidade dos meus dados e do meu animal, concedendo livremente a minha autorização para participação do meu animal.

Nome Proprietário _____

CC/BI nº _____

Local e Data _____

Assinatura do proprietário: _____