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Diagnostic imaging features of unusual systemic metastasis of canine and feline mammary gland tumours

Características imagiológicas de metástases incomuns de neoplasias mamárias em cães e gatos

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Mestrado Integrado em Medicina Veterinária
Instituto de Ciências Biomédicas de Abel Salazar
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Área Científica: Medicina e/ou cirurgia de animais de companhia

Orientador: Professora Doutora Cláudia Sofia Narciso Fernandes Baptista
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ABSTRACT

This document was prepared in the context of the 6th year of the Integrated Master's Degree in Veterinary Medicine at the Institute of Biomedical Sciences Abel Salazar, University of Porto. Uncommon sites of distant metastasis of mammary gland neoplasms in dogs and cats is a poorly documented topic in the scientific literature, which prompted this work out of curiosity to learn more about this subject. Thus, the main objective of this literature review was to compile the imaging characteristics of mammary tumour metastases in uncommon sites, specifically the heart, liver, eye, and bone, with the aim of optimizing their diagnosis in veterinary medicine. For this purpose, it was considered that a broad knowledge of the neoplastic process would be fundamental for understanding the topic, and thus the epidemiology, risk factors, diagnosis, and treatment of mammary neoplasms were also addressed, as well as highlighting the importance of animals as study models for human cancer. To integrate theory with practice, a clinical case, observed during the externship, of a malignant epithelial neoplasia of the mammary gland of a bitch with distant metastases (lymph nodes and spleen) was also included. With this work, it is intended to contribute to the implementation of clinical staging protocols for mammary tumours in dogs and cats that may enable earlier and more effective diagnosis of this type of neoplasia.

KEYWORDS: tumours; mammary gland; uncommon sites metastasis; imaging; dogs; cats.

RESUMO

Este documento foi elaborado no âmbito do 6º ano do Mestrado Integrado em Medicina Veterinária do Instituto de Ciências Biomédicas Abel Salazar da Universidade do Porto. Os locais incomuns de metastização de neoplasias da glândula mamária, no cão e no gato, são um assunto pouco documentado na literatura científica, pelo que este trabalho surgiu da curiosidade em saber mais sobre este tema. Assim, o principal objetivo desta revisão bibliográfica foi compilar as características imagiológicas das metástases de tumores mamários em locais incomuns, nomeadamente no coração, fígado, olho e osso, com o intuito de potenciar o seu diagnóstico em medicina veterinária. Para o efeito, considerou-se que o conhecimento alargado sobre o processo neoplásico seria fundamental para a compreensão do tema, pelo que também se abordou a epidemiologia, fatores de risco, diagnóstico e tratamento das neoplasias mamárias, assim como se salientou a importância dos animais enquanto modelos de estudo para o cancro humano. De forma a integrar a teoria com a prática, incluiu-se também um caso clínico, observado durante o estágio, de uma neoplasia maligna epitelial da mama de uma cadela com metástases no baço e gânglios linfáticos. Com este trabalho pretende-se, assim, contribuir para a implementação de protocolos clínicos de estadiamento de tumores mamários no cão e no gato que possibilitem um diagnóstico mais precoce e eficaz deste tipo de neoplasia.

PALAVRAS-CHAVE: tumores; glândula mamária; metástases em locais incomuns; imagiologia; cães; gatos.

CASUISTRY AND ACTIVITIES DEVELOPED

Over the course of 16 weeks, I completed a curricular internship at Anicura Valencia Sur Veterinary Hospital, where I had the opportunity to meet an extremely professional and competent team of specialists in all areas of the hospital. I rotated through the different services available, from the Intensive Care Unit, Internal Medicine, Cardiology, Ophthalmology, Neurology, Oncology, Dermatology, Imaging and Surgery, with the opportunity to shadow each service for an average of 2 weeks.

Table 1: Specification of medical clinical cases, their number, and their percentage.

Medicine			
Service/Speciality		**nº cases**	**%**
Cardiology	Pericardial effusion	4	12%
	Cardiac tamponade	1	3%
	Hypertrophic cardiomyopathy	4	12%
	Hereditary Ventricular Arrhythmia of the German Shepherd	1	3%
	Degenerative Mitral Valve Disease	10	29%
	Atrial fibrillation	2	6%
	Pulmonary hypertension	3	9%
	Ectopic ventricular complexes	1	3%
	Persistent ductus arteriosus	1	3%
	Aortic stenosis	2	6%
	Pulmonary stenosis	2	6%
	Pleural effusion	2	6%
Total		**34**	100%

Service/Speciality		**nº cases**	**%**
Neurology	Horner's syndrome	1	8%
	Myasthenia Gravis	1	8%
	Recurrent meningoencephalitis	1	8%
	Syringohydromyelia	1	8%
	Entrance Exam Crisis	1	8%
	Bilateral otitis	1	8%
	Meningoencephalitis of unknown origin	1	8%
	Disc herniation	5	42%
Total		**12**	100%

Service/Speciality		**nº cases**	**%**
Digestive	DGV Gastric dilation and volvulus	4	27%
	Hematochezia	1	7%
	Hemorrhagic gastroenteritis	1	7%
	Congenital megaesophagus	1	7%
	Diaphragmatic hernia	1	7%
	Intestinal foreign body	2	13%
	IBD	2	13%
	Gastric ulcer	1	7%
	Palatal fistula	1	7%
	Pyloric Perforation	1	7%
Total		**15**	100%

Service/Speciality		**nº cases**	**%**
Respiratory	Aspiration pneumonia	2	17%
	Laryngeal Paralysis	4	33%
	Tracheal collapse	2	17%
	Pneumonia	1	8%
	Nasopharyngeal polyp	1	8%
	Pulmonary hemorrhage	1	8%
	Pyothorax	1	8%
Total		**12**	100%

Service/Speciality		**nº cases**	**%**
Endocrine	Intrahepatic Portosystemic Shunt	1	7%
	Pancreatitis	6	40%
	Cholangiohepatitis	1	7%
	Cushing	1	7%
	Diabetes Mellitus	4	27%
	Hyperthyroidism	1	7%
	Chronic liver disease	1	7%
Total		**15**	100%

Service/Speciality		**nº cases**	**%**
Urinary	Multidrug-resistant UTI	1	11%
	Cystitis	1	11%
	Urethral obstruction	1	11%
	Chronic kidney failure	3	33%
	Acute kidney failure	2	22%
	Ectopic ureter	1	11%
Total		**9**	100%

Service/Speciality		nº cases	%
Oncology	Epitheliotropic cutaneous lymphoma	1	7%
	Lung adenocarcinoma	1	7%
	Bladder carcinoma	1	7%
	Heart hemangiosarcoma	1	7%
	Ceruminous cell carcinoma	1	7%
	Lung carcinoma	1	7%
	Mast cell tumor	1	7%
	Vagina leiomyoma	1	7%
	Nasal cavity chondrosarcoma	1	7%
	Intestinal mast cell tumor	1	7%
	Mammary gland carcinoma	1	7%
	Multiple myeloma	1	7%
	Foreskin mass	1	7%
	Plasmacytoma	1	7%
Total		**14**	100%

Service/Speciality		nº cases	%
Infectious	Leishmaniasis	5	45%
	PIF	1	9%
	Parvovirus	3	27%
	Leptospirosis	1	9%
	Giardia	1	9%
Total		**11**	100%

Service/Speciality		nº cases	%
Ophtalmology	Descemetocoelium	3	13%
	Indolent ulcer	8	35%
	Corneal perforation	3	13%
	Cataracts	2	9%
	Corneal sequestration	1	4%
	Glaucoma	3	13%
	Nasolacrimal obstruction	2	9%
	Ciliary mucocele	1	4%
Total		**23**	100%

Service/Speciality		nº cases	%
Theriogeneology	Pyometra	3	60%
	Prostatitis	1	20%
	Eclampsia	1	20%
Total		**5**	100%

Service/Speciality		nº cases	%
Hematology	Immune-mediated hemolytic anemia	3	75%
	Immune-mediated thrombocytopenia	1	25%
Total		**4**	100%

Service/Speciality		nº cases	%
Toxicology	Ingestion of rodenticides	5	56%
	Gabopentin poisoning	1	11%
	Cocaine intoxication	1	11%
	Marijuana intoxication	1	11%
	Intoxication with antipsychotics	1	11%
Total		**9**	100%

Service/Speciality		nº cases	%
Skeletal muscle	Bilateral ulnar-radius fracture caused by being run over	1	25%
	Kneecap dislocation	2	50%
	Femoral dislocation with acetabulum fracture	1	25%
Total		**4**	100%

Table 2: Specification of surgery clinical cases, number, and percentage of them.

Surgery			
Type of Surgery		**nº cases**	**%**
Soft Tissue Surgery	Cystectomy	1	6%
	Lung lobulectomy	1	6%
	Laterization of the arytenoids	6	35%
	Splenectomy	1	6%
	Typhlectomy	1	6%
	Ventral slot	1	6%
	Dorsal hemilaminectomy	5	29%
	Radical mastectomy	1	6%
Total		17	100%

Type of Surgery		**nº casos**	**%**
Minimally invasive	Laparoscopy ovariectomy	1	100%
Total		1	100%

Type of Surgery		**nºcases**	**%**
Ophthalmology	Phacoemulsification Faceectomy	13	62%
	Blepharoplasty	5	24%
	Amniotic membrane implant placement	3	14%
Total		21	100%

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“Não tenhamos pressa, mas não percamos tempo” José Saramago

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ABREVIATURAS

%	Percentage
BC	Body condition
CBC	Complete blood count
CEUS	Contrast-enhanced ultrasound
Cm	Centimetres
COX	Cyclo-oxygenase
CRT	Capillary refill time
CT	Computed Tomography
DDAVP	Desmopressin
DOX	Doxorubicin
ECM	Extracelular matrix
ER	Oestrogen Receptor
FNA	Fine needle aspiration
FNABs	Fine Needle aspiration biopsy
HER-2	Human epidermal receptor
IMC	Inflammatory mammary carcinoma
IV	Intravenous
K/µL	Thousands per. cubic millilitre
Kg	Kilogram
LN	Lymph nodes
mg/Kg	Milligrams per kilogram
MGT	Mammary gland tumours
mL/h	Millilitre per hour
MMPS	metalloproteases
MRI	Magnetic Resonance imaging
MST	Median survival Time
NIR	Near infrared fluorescence
NSAIDs	Nonsteroidal anti-inflammatory drugs
OPN	Secreted phosphoprotein 1
OS	Overall survival
OST	Overall survival time
PET	Positron Emission Tomography
PFS	Progression-free survival

PPV	Positive predictive value
PR	Progesterone receptor
q4h	Every 4 hours
QID	four times a day
RIA	Radioimmunological methods
RON	Macrophage – stimulating protein receptor.
RPM	Respirations per minute
SID	once daily
SLN	Sentinel Lymph node
TopBP1	Topoisomerase IIβ binding protein 1
US	Ultrasound
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
WoAUC	Wash-out area under the curve

1. INTRODUCTION

1.1. Epidemiology

Mammary neoplasia is highly prevalent in intact adult female dogs and older sterilised female dogs and is the second most common tumour in dogs. In Portugal, mammary tumours are the most common cancer in female dogs, according to the latest Animal Cancer Registry (ACR) from 2021. This registry, published by Vet-OncoNet, compiles data from an extensive network of veterinary laboratories across the country, covering 75% of all animal cancer cases in the country. (Carvalho et al., 2023) Otherwise in cats, mammary neoplasia is less common but more than 85% are malignant. In dogs, approximately 50% are benign and the other 50% are malignant and 50% of these will metastasize. Male dogs can also present mammary tumours but are far less common when compared with female dogs. (Murphy, 2008). Female dogs have a susceptibility to developing mammary tumours that is approximately 62 times higher than that of male dogs. (Ferreira et al., 2023)

The overall incidence of mammary gland tumours (MGTs) in cats is lower than in dogs. In female cats, MGTs are the third most common type (after skin tumours and lymphomas). The annual incidence rate for these tumours is 25.4 per 100,000 cats, accounting for 12% of all tumours in cats regardless of sex. In dogs, the estimated annual incidence rate of malignant MGT in intact female dogs is 257.7 per 100,000. (Vail et al., 2020)

Mammary neoplasms are one of the most common cancers in humans, dogs and cats, but are rare in other species. Mammary tissue is highly susceptible to carcinogenesis due to the various changes it undergoes throughout a female's life, including puberty, pregnancy, lactation and menopause (in women). These changes are influenced by various growth factors and hormones.(Zhelavskyi & Dmytriv, 2023)

In dogs, the incidence of the different histological types is as follows: i) benign tumours (51%) of which 45.5% are fibroadenomas, 5% simple adenomas and 0.5% benign mesenchymal tumours; ii) carcinomas (45.5%) of which 16.9% are solid carcinomas, 15.4% are adenocarcinomas, 8.6% are papillary adenocarcinomas and 4% are anaplastic carcinomas; iii) sarcomas and mixed malignant tumours (3.5%). (*schematised in Table A1*) (Baba & Câtoi, 2007).

Table A1: Incidence of the different histological forms of MGTs in dogs.

Type of Tumour	Percentage	Subtypes
Benign Tumours	51%	- Fibroadenomas: 45.5%
		- Simple Adenoma: 5%
		- Benign Mesenchymal Tumours: 0.5%
Carcinomas	45.5%	- Solid Carcinomas: 16.9%
		- Adenocarcinomas: 15.4%
		- Papillary Adenocarcinomas: 8.6%
		- Anaplastic carcinomas: 4%
Sarcomas	3.5%	- Malignant Mixed Tumours

In cats, adenocarcinomas are more common, then carcinomas and sarcomas. (Baba & Câtoi, 2007)

1.2. Risk factors

In dogs and cats, three main factors have been identified that play an important role in the risk of MGT: age, hormone exposure and breed. (Vail et al., 2020)

The risk of MGT in dogs increases with age and becomes clinically significant at 7 or 8 years of age. This risk continues to increase until the age of 11-13 years. Dogs diagnosed with malignant tumours are usually between the ages of 9 and 11 years, while those with benign tumours are usually between the ages of 7 and 9 years. (Vail et al., 2020) Dogs with a median age of 10 years were more likely to have larger (≥ 3 cm) and malignant mammary tumours. (Dolka et al., 2024) The peak incidence of tumours also varies according to the lifespan of different breeds. Larger breeds have a shorter natural lifespan, which means they tend to be diagnosed at a younger age than smaller breeds. These differences may be even more pronounced in high-risk breeds such as English Springer Spaniels. In cats, as in dogs, mammary neoplasia is predominantly seen in middle-aged to older cats, with the average age of diagnosis being between 10 and 12 years. (Vail et al., 2020)

In both normal and neoplastic mammary glands, tissue growth is significantly influenced by hormones such as oestrogen, progesterone, prolactin and growth factors. Progesterone actively suppresses myometrial activity, promotes the growth of endometrial glands and supports the development of mammary alveolar tissue. Oestrogen contributes significantly to oncogenesis by stimulating the mitotic activity of the mammary epithelium. In combination with oestrogen, progesterone plays a crucial role in the development of MGT by acting as a proliferative factor. When associated with prolactin, it increases the number of oestrogen receptors, facilitating the mitotic action of this hormone. There is no doubt that hormones are a critical risk factor for MGTs. (Rueda et al., 2024).

In addition, the presence of oestrogen and progesterone receptors in neoplastic mammary tissue has been demonstrated in dogs, and their expression is more common in benign than in malignant mammary tumours. (Benavente, Bianchi, et al., 2016)

Consequently, dogs that undergo spaying before their first oestrus cycle exhibit a markedly reduced risk of developing MGTs over the course of their lifetime, with an estimated incidence of only 0.5%. Nevertheless, the protective efficacy of ovariohysterectomy (OHE) appears to diminish over the initial few oestrus cycles, with some studies suggesting that the benefits may be negligible beyond four years of age. (Vail et al., 2020)

Similarly, ovarian hormones play a pivotal role in the development of mammary tumours in cats. Cats that remain sexually intact are at a sevenfold higher risk of developing mammary tumours

compared to those that have been spayed. The protective effect of OHE declines rapidly during the first few years. Specifically, risk reductions of 91%, 86%, and 11% are observed in cats that undergo OHE before 6 months, between 7 and 12 months, and between 13 and 24 months, respectively. No further benefit is observed after the 24-month mark. In addition to the endogenous ovarian hormonal influence, exposure to exogenous progestins also contributes to an increased risk of mammary tumours in cats. (Vail et al., 2020)

Considering the aforementioned information, it can be reasonably concluded that sex hormones represent a significant risk factor for tumour development in cats and dogs. Furthermore, the occurrence of pseudocyesis and the use of progestins as contraception may contribute to the formation of these tumours. The administration of exogenous progesterone to dogs or cats stimulates the synthesis of growth hormones in the mammary gland, resulting in lobuloalveolar proliferation and the subsequent hyperplasia of the myoepithelial and secretory elements. This results in the formation of benign nodules in young animals. A prolonged exposure to progesterone can result in malignant metaplasia of the mammary gland tissues. In pseudocyesis, the non-pregnant female exhibits elevated serum prolactin levels and/or sensitivity, accompanied by a precipitous decline in progesterone. In bitches with recurrent pseudocyesis, high prolactin concentrations can result in milk retention and the potential development of mammary neoplasms. (Rueda et al., 2024)

In dogs, MGTs are more prevalent in smaller breeds. Purebred dogs, including Poodles, Chihuahuas, Dachshunds, Yorkshire terriers, Maltese, and Cocker spaniels, are frequently considered to be at an elevated risk of developing MGTs within the small category. Nevertheless, some larger breeds, including the English Springer spaniel, English setter, Brittany spaniel, German shepherd, pointer, Doberman pincher, and Boxer, are also at an increased risk. In cats, Siamese cats have an elevated risk for various tumour types, including malignant glandular tumours (MGTs). (Vail et al., 2020)

In addition to genetic predisposition, other risk factors contribute to the development of MGT, including body weight and obesity. During puberty (approximately 9-12 months of age), body weight exerts a significant influence on the risk of developing mammary tumours in later life. A low body weight during this period confers a significant degree of protection against tumour development. (Vail et al., 2020) In dogs, obesity is also recognised as a risk factor, as it may be linked to a decrease in the serum concentration of sex hormones bound to globulin, which in turn increases the serum concentration of free oestrogen. Furthermore, adipose tissue may contribute to increased oestrogen production through the aromatase-mediated conversion of androgens. (Rueda et al., 2024)

1.3. Diagnosis

The early diagnosis of mammary gland neoplasms is of paramount importance. This involves a comprehensive physical examination, laboratory tests, imaging diagnostics, and a microscopic evaluation of the nodule. These steps are essential for establishing an appropriate treatment plan to prevent recurrence or metastasis. It is of great importance to gather information regarding the onset and progression of the lesion, previous treatments, and the patient's reproductive history. Following a comprehensive physical examination to ascertain the patient's overall condition, a detailed examination of the mammary gland should be conducted. This involves palpating the mammary chains and regional lymph nodes to identify nodules or changes in size, shape, or consistency, thereby defining the lesions macroscopically. Subsequently, a fine needle cytopathological examination (FNA) of the nodules and lymph nodes should be conducted as an additional diagnostic measure. There is no evidence to suggest that any mammary chain is more susceptible to neoplastic processes. Nevertheless, some studies have indicated that approximately 60% of neoplasms in dogs occur in the last two pairs of glands—the caudal and inguinal abdominal glands—due to their larger volume of glandular tissue. (Rueda et al., 2024)

It is of the utmost importance to re-evaluate the mammary chain under general anaesthesia to ensure comprehensive detection and inclusion in surgical plans. This necessitates a meticulous assessment of both mammary chains. Dogs with affected glands may be misdiagnosed with mastitis or severe dermatitis due to the absence of discrete, well-circumscribed tumours. Instead, the entire mammary chain may appear oedematous, swollen, warm, and painful. Given the potential for metastatic spread associated with MGTs, particularly in the absence of histological confirmation of benign disease, it is strongly recommended that staging be conducted before initiating treatment. A standard diagnostic evaluation and staging procedure involves a complete blood count (CBC), serum biochemistry, three-view thoracic radiographs, and fine-needle aspiration (FNA) of regional lymph nodes (LNs). To enhance the precision of the procedure, it is also possible to consider the use of sentinel lymph node mapping and biopsy. (Vail et al., 2020).

The regional lymph node is defined by its anatomical location, whereas the sentinel lymph node (SLN) is the initial lymph node to receive lymphatic flow from a solid neoplasm. The regional draining lymph node, which is determined by anatomical location and the presence or absence of metastasis affecting normal lymphatic flow, may differ from the actual SLN. The identification of the SLN typically necessitates the use of specialised techniques, such as direct or indirect lymphography. Palpation alone is an inadequate method for the detection of lymph node metastases. In a study of dogs with palpable solid tumours, up to 85% of regional metastatic lymph nodes were undetectable by palpation alone. Although the clinical relevance of this phenomenon remains unclear, early metastases may be present as isolated cells (submicrometastases up to 0.2 mm) or micrometastases

(0.2–2.0 mm) without palpably enlarged lymph nodes. (Souza et al., 2023) Micrometastasis is defined as the systemic dissemination of a small number of tumour cells that are insufficient in number to be identified by screening tests. It is postulated that this represents a significant factor in the development of metastatic disease in patients initially diagnosed as LN-negative.(Collivignarelli et al., 2021). In the past, the practice of sampling the regional anatomic lymph node to assess metastatic disease has been a standard procedure. However, mounting evidence suggests that the regional anatomic lymph node is frequently not the sentinel lymph node. Consequently, due to the potential for aberrant lymphatic drainage from the tumour, the regional anatomic lymph node may not serve as the primary draining lymph node or sentinel lymph node. Consequently, the identification of the SLN using indirect lymphography for canine mammary tumours provides valuable insights into the precise drainage pattern and SLN location. This knowledge is clinically significant for both surgeons and oncologists. For surgeons, it facilitates the selection of appropriate surgical excision techniques, while also aiding in the establishment of an accurate post-surgical prognosis. (Collivignarelli et al., 2021).

To date, SLN mapping in companion animal oncology has not reached a definitive standard, and there is a lack of established gold standard procedures. A single published study has explored the application of contrast-enhanced ultrasound (CEUS) for sentinel lymph node (SLN) mapping following local contrast injection in dogs with head and neck neoplasms. Furthermore, several research groups have reported successful SLN mapping using contrast-enhanced computed tomography (CT) lymphography following local contrast injection in dogs with neoplasms affecting the head and neck, perianal region, and mammary glands. (Favril et al., 2019). The primary techniques of SLN mapping are schematized in Tables A2 and A3.

Table A2: The primary techniques for preoperative sentinel lymph node mapping based on the information from (Beer et al., 2023).

Technique	**Description**
Lymphoscintigraphy	Utilizes radioactive tracers for sentinel lymph node (SLN) mapping, recognized as the standard procedure. Involves preoperative planar imaging and/or intraoperative use of a handheld gamma probe. Technetium-99m (Tc-99m) labelled colloids are commonly used as radiotracers. These tracers exhibit prolonged retention at the injection site due to their particulate nature. Accumulation of radioactivity in SLNs is observed during preoperative scanning. Limitations include the inability to confirm metastasis in "hot" SLNs and limited access to radioactive tracers in veterinary clinics due to high cost and equipment constraints. Lack of precise resolution hinders the identification of individual nodes, particularly in regions like the head and neck.
Indirect Radiographic Lymphography	Involves administration of a radio-opaque contrast agent via peritumoral injection for identifying sentinel lymph nodes (SLNs) in veterinary cancer patients. Following injection, the contrast agent is transported toward SLNs through the lymphatic system and can be observed through serial radiographs. SLNs remain radio-opaque for a period ranging from several days to months until the contrast agent is eliminated from the lymphatic system.

Computed tomography lymphography	Aims to accurately depict the anatomical location of SLNs and provide detailed visualization of surrounding tissue. Similar to radiographic lymphography, uptake in SLNs occurs within minutes post-injection.
CEUS	Widely used in human medicine and increasingly adopted in veterinary practice. Utilized for assessing blood flow and tissue perfusion in abdominal organs. Involves injecting small gas-filled microbubbles enclosed in a lipid shell into peritumoral tissue for lymphatic visualization. These lipid-based contrast agents rapidly enter lymphatics and reach SLNs within minutes. Microbubbles tend to remain confined to the first lymph node of the basin, with minimal spill-over to second-tier lymph nodes. Upon excitation by grayscale or Doppler ultrasound, microbubbles oscillate, resulting in a signal intensity several times higher than surrounding body tissue.

Table A3: The primary techniques for intraoperative SLN mapping based on the information from (Beer et al., 2023)

Technique	**Description**
Colorimetric SLN Mapping Using Blue Dye	Initial method for intraoperative SLN mapping in dogs involving the injection of methylene blue dye. Relying on direct visualization of SLNs without specialized detection equipment. Commonly used dyes include methylene blue dye, isosulfan blue, and patent blue dye. Ascending lymph vessels and corresponding SLNs become stained blue within 5–10 minutes post-peritumoral injection, enabling visualization if exposed or directly beneath the skin. Not recommended as a primary technique due to limited sensitivity and specificity. Often used as a supplementary method alongside CT lymphography and/or lymphoscintigraphy. Studies in human and canine oncology support combined techniques for improved accuracy in SLN mapping, suggesting a dual application of blue dye with TC-99m or radiographic lymphography.
Near-infrared (NIR) Imaging	Rapidly evolving field offering real-time visualization of lymphatic structures and SLNs in human and veterinary medicine. NIR's ability to penetrate tissue up to a few centimetres with minimal absorption by body tissues makes it suitable for transcutaneous SLN identification. Indocyanine green (ICG) is the preferred fluorescent dye. NIR-based SLN mapping is technically simpler, more reliable, and associated with higher SLN detection rates compared to conventional techniques. The smaller particle size of fluorescent agents facilitates faster spillage into secondary lymph nodes, contributing to enhanced accuracy.

MGTs are staged according to the T (tumour), N (lymph node), and M (metastasis) systems. At present, most oncologists employ a modified version of Owen's original staging system. The disease progresses from stage I to III as the primary tumour size increases from less than 3 cm to between 3 and 5 cm to larger than 5 cm. These size categories are associated with significant changes in prognosis and outcome. The presence of lymph node metastasis indicates stage IV disease, regardless of the size of the primary tumour. In contrast, the presence of distant metastasis signifies stage V disease. This staging approach applies to dogs with epithelial tumours (non-inflammatory), with the exclusion of sarcomas. (*table A4 and table A5*) (Vail et al., 2020)

Table A4: Staging System of MGT in dogs adapted from Withrow and MacEwen's Small Animal Clinical Oncology, 6th Edition.

Stage	Tumour Size	LN status	Metastasis
Stage I	T1 < 3 cm	N0	M0
Stage II	T2 3-5cm	N0	M0
Stage III	T3 >5cm	N0	M0
Stage IV	Any	N1 (positive)	M0
Stage V	Any	Any	M1 (metastasis)

Table A5: Staging system of MGT in cats (adapted from Withrow and MacEwen´s Small Animal Clinical Oncology, 6th Edition (Vail et al., 2020)

Stage	Tumour Size	LN Status	Metastasis
Stage I	T1 < 2 cm	N0	M0
Stage II	T2 2-3cm	N0	M0
Stage III	T1 or T2 T3 >3cm	N1 (positive) N0 or N1	M0 M0
Stage IV	Any	Any	M1

Currently, histopathological diagnosis is considered the gold standard for accurately diagnosing MGTs. (Zhelavskyi & Dmytriv, 2023). (*see histologic classification in annexe A*). It is recommended that a histopathological evaluation be conducted postoperatively in all cases. In addition to evaluating the primary tumour, a comprehensive examination of all mammary glands and regional lymph nodes is of paramount importance. In cases involving multiple nodules, a histopathological diagnosis of each nodule is beneficial for prognosis determination and identification of potential additional treatment strategies. (Rueda et al., 2024)

The diagnosis of malignant mammary tumours involves the evaluation of several criteria, including tumour type, nuclear and cellular pleomorphism, mitotic index, the presence of necrotic areas, lymphatic and peritumoral invasion, and regional metastatic lymph nodes. The histological grading system for canine mammary carcinoma quantifies anaplasia, tubule formation, mitotic activity, and nuclear pleomorphism (Table A6). The total of all individual values determines the histological grade of the malignancy. The histological grade is a prognostic factor, with higher grades indicating a poorer outcome and shorter survival rate. (Vazquez et al., 2023)

In veterinary medicine, immunohistochemistry is used to identify markers that can reveal the biological behaviour of tumours. This helps in determining prognostic and predictive factors for neoplasms. For canine and feline mammary carcinomas, the immunohistochemical panel should include the expressions of oestrogen receptor (ER), progesterone receptor (PR), Ki-67, and COX-2. (Rueda et al., 2024)

Table A6: Criteria used for the histologic grading of malignancy in canine mammary carcinomas. Based on (Vazquez et al., 2023)

Tubule Formation	Nuclear Pleomorphism	Mitosis
Tubule formation >75%: 1 point	Uniform or regular small nucleus and occasional nucleoli: 1 point	0-9: 1 point
Moderate formation of tubular arrangement (10-75%) admixed with areas of solid tumour: 2 points	Moderate degree of variation in nuclear size and shape, hyperchromatic nucleus (some of which can be prominent): 2 points	10-19: 2 points
Minimal or no tubule formation (<10%): 3 points	Marked variation in nuclear size and hyperchromatic nucleus, often with one or more prominent nucleoli: 3 points	>20: 3 points
Total Score	**Grade of malignancy**	
3-5	I (low) well differentiated	
6-7	II (intermediate) moderately differentiated	
8-9	III (high) poorly differentiated	

1.4. Prognosis

The prognostic factors most consistently linked with prognosis are tumour size and LN involvement. (Vail et al., 2020)

Dogs with tumours larger than 40cc (approximately 3.4 cm in diameter) have significantly poorer outcomes in both remission and survival compared to those with smaller tumours. The prognosis worsens gradually as tumour size increases. In cats, small tumours (less than 2 cm) can be effectively treated with surgery alone, specifically radical mastectomy, which has been shown to result in an MST of over 3 years. In contrast, cats with tumours larger than 3 cm have an MST of only 6 months, while those with tumours between 2 and 3 cm in diameter have an average survival time of 2 years. (Vail et al., 2020)

The condition of the local LN is a significant prognostic factor. The presence of micrometastases, defined as cell clusters between 0.2 and 2.0 mm in diameter, did not significantly influence the outcome in comparison to dogs without lymph node metastasis. The status of the local lymph nodes is of paramount importance when determining the necessity of adjuvant or systemic therapy for dogs with MGTs. (Vail et al., 2020)

Canine patients classified as stage IV or V, as well as those with nodules exceeding 5 cm (T3), demonstrate inferior prognoses and shorter survival durations. It is noteworthy that dogs in clinical stage IV exhibited a median survival time of 331 days, whereas those in stage I had a median survival time of 1,149 days. (Rueda et al., 2024)

The expression of specific genes, receptors, or proteins may undergo alterations during the malignant progression of feline mammary tumours. The molecular markers, which can be detected by immunohistochemistry, may prove to be a valuable prognostic indicator in certain cases. Please refer to Table A7 for further details. (Giménez et al., 2010)

Table A7: Molecular Markers in Feline Mammary Tumours based on the information from (Giménez et al., 2010)

Molecular Marker	Function/Role	Findings in Feline Mammary Tumours	Prognostic Value
Cyclin A	Regulates cell cycle	Abnormalities found in 7/8 carcinomas (small study); involvement detected in 48.6% of cases (large study)	Associated with tumorigenesis
Protein p53	Tumour suppressor maintains genome stability	Mutations and nuclear accumulation in 18.9% of cases; lower reactivity in adenomas compared to adenocarcinomas; overexpression in 32.5-33% of carcinomas	Indicates malignancy; overexpression correlates with worse prognosis
RON	Stimulates carcinoma cell invasiveness	High expression in 20% of carcinomas	Like human breast cancer, indicating invasiveness
(HER-2)	Involved in cell growth and differentiation	Low expression in normal glands, increased in benign tumours, elevated in carcinomas; associated with shorter survival times	Prognostic value, correlates with aggressive features and poor prognosis
VEGF	Promotes tumour growth, invasion, and metastasis	Higher expression in poorly differentiated carcinomas	Indicates more aggressive tumours, useful as a prognostic marker
COX-2	Involved in prostaglandin synthesis	Expression correlates with a poorer prognosis	Indicates poor prognosis, associated with many cancers
TopBP1	Similar to the human BRCA2 gene	Increased expression in higher-grade tumours	Potential role in tumour progression indicates higher malignancy

The combined use of these molecular markers provides valuable prognostic information, which may be used to inform treatment decisions in feline mammary tumours.

The histological classification of the tumour significantly influences the prognosis following surgery. The median survival time (MST) for anaplastic carcinoma and carcinosarcoma is 3 months, while that for solid carcinoma is 8 months, comedocarcinoma 14 months, and adenosquamous

carcinoma 18 months. The prognosis for dogs with benign tumours or carcinomas in benign mixed tumours is excellent. Dogs with complex carcinoma and simple tubular carcinoma exhibited prolonged survival, with MST not reached during a two-year monitoring period. Conversely, dogs with simple tubulopapillary carcinoma, intraductal papillary carcinoma, mixed tumour of carcinoma, and malignant myoepithelioma face over 10 times the risk of tumour-related death. The most aggressive form of mammary carcinoma is inflammatory mammary carcinoma (IMC), with an MST of less than six months and an 86% incidence of distant metastases. (Shelly Waltman, 2022)

1.5. Management

Following surgical removal, it is recommended that thoracic radiographs (3 views) and abdominal ultrasonography be performed every three months during the first year, with subsequent monitoring at four- to six-month intervals. In the case of animals undergoing chemotherapy, the monitoring frequency is contingent upon the specific chemotherapeutic protocol employed. During each treatment, a physical examination, complete blood count (CBC), and biochemistry profile (including at least renal parameter assessment) should be conducted. (Vail et al., 2020)

1.6. Metastasis

Metastasis is defined as the spread of cancerous cells from a primary tumour to secondary sites, where they form visible masses. This dissemination occurs through various routes, including the haematogenous, lymphatic, and peritoneal pathways. For metastasis to occur, cancer cells must first leave the primary tumour, breach the basement membrane, and enter the circulation. They must then resist cell death and immune responses. Upon reaching distant organs, the cancer cells adapt to the new environment, potentially influenced by signals from the primary tumour. The secondary sites may serve as the destination or temporary shelters for cancer cells, which often remain dormant before resuming growth. Upon reactivation, metastatic cells receive signals that promote proliferation and angiogenesis, allowing them to develop into measurable lesions. (Vail et al., 2020)

Following the successful growth of the primary tumour, the next critical step in the metastatic process is intravasation, whereby a cancer cell infiltrates the vascular or lymphatic circulation. This necessitates the cell's motility and capacity to interact with the extracellular matrix (ECM), which can be achieved through enzymatic degradation (mesenchymal invasion) or slipping between ECM fibres (amoeboid invasion). Collective invasion, observed in specific cancers, entails the localized expansion of a tumour into adjacent tissues, although it typically leads to limited distant metastasis. Metastatic tumours may utilise a variety of invasion mechanisms at different stages of progression. Matrix proteases and metalloproteases (MMPs) play a pivotal role in mesenchymal invasion, with their expression correlating with tumour grade and metastatic potential. Emerging evidence suggests that

matrix-degrading enzymes and growth factors may be supplied by inflammatory cells recruited by the tumour rather than being produced by tumour cells themselves. The process of intravasation is completed when a cancer cell successfully enters the vascular or lymphatic circulation.(Vail et al., 2020)

Throughout metastatic progression, tumour cells must evade immune detection and destruction. In hosts with intact immune systems, immunosurveillance eliminates many cancer cells from the primary tumour, circulation, and distant metastatic sites. Tumour cells employ diverse mechanisms to evade immune responses.(Vail et al., 2020)

The capture of circulating tumour cells at distant sites is believed to occur through two distinct yet overlapping mechanisms. These entrapment mechanisms involve the capture of tumour cells within small vessels based on their size and interactions mediated by receptors between the tumour cell and the host vasculature. Evidence supporting the entrapment theory is derived from studies utilising single-cell imaging, particularly in the context of metastasis to the liver and brain, where large tumour cells were observed to be entrapped within small blood vessels. This indicates that the initial vascular bed encountered may influence the site of metastasis. Alternatively, certain adhesion molecules may be essential for the initial arrest of tumour cells in the microenvironment of secondary metastatic sites. Both mechanisms contribute to the initial colonization of distant organs, with the dominant mechanism being determined by the tumour type or target organ. (Vail et al., 2020)

Upon reaching distant sites, cancer cells may either promptly exit the circulation into the target organ or remain within it. Regardless of their location, these cells must adapt to survive in a new microenvironment. Studies have indicated that cancer cells can initially arrest at multiple organs but experience a significant decrease in survival rates within hours, with only a small fraction remaining viable within days. The capacity of cancer cells to flourish following arrest determines the selectivity of organ involvement in metastasis. The "seed and soil" hypothesis, initially proposed by Paget and subsequently developed by Fidler and others, posits that the success of metastasis hinges on the interactions between the cancer cell, or "seed," and the tumour microenvironment, or "soil."(Vail et al., 2020)

The success of secondary metastasis is contingent upon the influence of the primary tumour and the subsequent arrival of metastatic cells. The concept of the premetastatic niche posits that before the arrival of most metastatic cells at a given site, the primary tumour modifies the local microenvironment. This modulation involves the mobilisation and recruitment of specific bone marrow-derived cells, primarily myeloid cells expressing VEGFR, to the secondary site. It is noteworthy that instances of metastatic tumour arrest frequently coincide with areas where these recruited myeloid-derived cells were initially mobilised. The targeting of VEGFR-positive cells has demonstrated efficacy in preventing metastatic development in animal models, suggesting potential therapeutic avenues for anti-metastatic treatment.(Vail et al., 2020)

Once tumour cells have established themselves in a distant site, they must proliferate and adapt to their new surroundings. While cancer cells typically exit the circulation and proliferate in the new organ, they can also multiply within blood vessels, a process termed intravascular metastases. Adaptation to the environment is a crucial factor in both tumour growth and metastatic lesion progression in both scenarios. Tumour-induced alterations in the stroma play a central role in this adaptation, leading to the production of growth factors and signals essential for further tumour growth. These stromal-derived factors are of vital importance for the proliferation, progression and angiogenesis of tumour cells. The recognition of the significance of tumour-stromal interaction suggests that the targeting of the "induced" tumour-stroma may hold promise for the development of novel cancer treatments.(Vail et al., 2020)

The most common cause of death associated with MGT is the dissemination of the primary mammary tumour to distant organs. Therefore, it is of great importance to detect this condition at an early stage. The metastatic process is facilitated by the distribution of blood and lymphatic flow within the mammary parenchyma. The lymphatic route is of particular significance, with lymph nodes and fine lymphatic vessels in the axillary and inguinal regions. These vessels facilitate the dissemination of neoplasms to the lungs and other parenchymatous organs, including the liver, via the thoracic and abdominal cavities. (Baba & Câtoi, 2007)

1.6. Treatment

The primary treatment for dogs' MGTs primarily relies on surgical intervention. Successful outcomes are achievable in cases without lymphatic or distant metastasis involvement, or those with less aggressive histological types. Excision performed before identifying distant metastasis does not extend survival but may improve quality of life, particularly in cases with ulcerated or painful lesions. Various surgical techniques are utilized, including lumpectomy, mammectomy (simple mastectomy), regional mastectomy, unilateral or bilateral mastectomy, with or without concurrent lymph node removal, and ovariohysterectomy. The concept of a "wide excision" varies but generally involves a 2-cm lateral margin, adjusted according to the patient and tumour size. Depth of excision may extend to include abdominal muscular fascia and/or portions of the abdominal wall, depending on tumour size and fixation. Care must be taken during abdominal surgery to avoid penetrating the tumour capsule to prevent the direct spread of tumour cells. Complete excision is crucial for malignant MGTs, with additional surgery considered if incomplete excision occurs. In dogs with clinical stage II to V mammary tumours, unilateral or bilateral surgery may be recommended, while stage I cases may benefit from regional mastectomy. Considering the lymphatic drainage of the mammary chain often renders lumpectomy and mammectomy unsuitable. Decision-making regarding which mammary gland to remove during regional mastectomy is typically guided by lesion localization and lymphatic drainage

patterns. Total unilateral mastectomy involves removing all glands from a mammary chain with ipsilateral superficial regional lymph nodes, indicated for multiple tumours, M3 lesions, and tumours with adverse prognostic factors. For dogs with multiple MGTs, extensive resections like regional mastectomy, unilateral chain mastectomy, or bilateral mastectomy may be pursued. These are usually performed through two surgical procedures, 4 to 6 weeks apart. Regional mastectomy entails the simultaneous removal of mammary glands sharing a common lymphatic and venous drainage network, indicated for single lesions with favourable clinical prognostic factors. These include a maximum size of 3 cm (T1), absence of adherence to adjacent tissues, no ulceration or signs of inflammation, and slow growth. (Cassali et al., 2020; Vail et al., 2020)

While ovariohysterectomy does not significantly affect survival or recurrence rates in most cases of canine mammary tumours, it can be beneficial in specific scenarios, such as ER-positive tumours or when addressing elevated oestrogen levels. This procedure can be performed in conjunction with tumour removal or as a subsequent surgical procedure. Dogs with distant metastases or inflammatory carcinoma are not surgical candidates. Palliative surgery may be a potential option for dogs in stage V of the disease, presenting with ulcerated tumours or pain, to improve their comfort and quality of life. (Cassali et al., 2020)

Systemic treatments, including chemotherapy, are available for MGTs in dogs. However, clinical studies in this area are limited. Despite the paucity of extensive research, chemotherapy is frequently recommended for dogs at higher risk of distant metastasis. The risk is assessed based on factors such as histological type, grade, clinical stage, and molecular prognostic markers identified through immunohistochemistry. Specifically, dogs with aggressive histologic types, including micropapillary carcinoma, solid carcinoma, carcinosarcoma, and pleomorphic lobular carcinoma, typically require adjuvant chemotherapy regardless of other considerations. Furthermore, chemotherapy is recommended for all grade III carcinomas and for dogs with clinical stages IV and V that exhibit metastasis in lymph nodes or lungs. Some studies have reported improved outcomes in dogs treated with chemotherapy alone or in combination with non-steroidal anti-inflammatory drugs (NSAIDs). (*table A8*) (Cassali et al., 2020; Vail et al., 2020)

Table A8: Summary of Published Studies Reporting Benefits from Systemic Therapy in Dogs with Malignant from (Vail et al., 2020)

Tumour stage	Grade/Histology	Treatment	Comments and Effect
Stage III-IV	Any grade Various carcinomas	Cyclophosphamide, 5-fluorouracil vs none	Evidence level: 2 DFS: p<0.01
Stage I-IV	Grade 3 Various carcinomas	NSAID: firocoxib vs none	Evidence level 2 DFS: p= 0.015
Stage IV-V	Grade: NA	Carboplatin +/- NSAIDs vs none	Evidence level 2 OS: p=0,07

Advanced	Inflammatory carcinomas	NSAIDS +/- chemotherapy vs None	Evidence level 3 Palliative intent OS: p=0.01
Advanced	Inflammatory carcinomas	NSAID (piroxicam) vs doxorubicin	Evidence level 3 Palliative intent PFS: <0.01
Stage I	Grades 1,2 Complex and mixed carcinomas, PR+	Hormonal Tx Antiprogestin (anglepristone) vs none	Evidence level 1 p=0.002-0.02
Stage I-IV	Grade 2	Hormonal Tx OHE vs intact	Evidence level 1 DFS: p= 0.001
Stage III-IV	Grade 2,3 Various carcinomas	DDAVP (desmopressin) vs none	Evidence level 1 DFS: P= 0.001
NA	Extraskeletal osteosarcoma, including mammary	Doxorubicin or cisplatin vs none	Evidence level 3 Mixed primary sites OS: p=0.02

Note: Evidence level 1: Prospective randomized trial; level 2: Prospective, nonrandomized trial; level 3: Retrospective; level 4: Case report(s); level 5: Extrapolation from human breast cancer studies. Significance set at p = 0.1

The most used drugs in this context include carboplatin, doxorubicin (DOX), and gemcitabine, which are sometimes combined with cyclophosphamide and COX-2 inhibitors. The overexpression of COX-2 in canine mammary tumours is associated with more aggressive tumours and a poorer prognosis. Studies have indicated that COX-2 inhibitors may be beneficial in improving outcomes in advanced neoplastic mammary gland disease, with positive results observed in clinical trials involving piroxicam for inflammatory carcinomas. Finally, a prospective, randomized trial documented a significant improvement in survival in dogs with histologic grade II or III carcinoma treated with perioperative desmopressin. (Cassali et al., 2020; Vail et al., 2020)

The criteria for selecting and implementing chemotherapy are not standardized. Establishing these criteria requires a consensus process and more randomized controlled trials. Additionally, there is a need to develop and evaluate new antineoplastic compounds that could help canine mammary cancer patients with fewer adverse effects. (Vazquez et al., 2023)

Systemic hormonal treatments like tamoxifen, a selective estrogenic receptor modulator, are widely used for treating breast cancer in women. However, research on the use of tamoxifen in canines with malignant mammary tumours and healthy female dogs has revealed substantial adverse effects. In one study, dogs administered 1 mg/kg of tamoxifen orally experienced a 56% incidence rate of complications, including pyometra, vulvar inflammation, and symptoms of pseudopregnancy. Another study, which administered doses between 0.5 mg/kg and 0.8 mg/kg per day to healthy female dogs, reported similar adverse outcomes such as vulvar inflammation, vaginal discharge, retinitis, and

pyometra. Due to these significant side effects, tamoxifen is not recommended for treating mammary cancer in dogs. (Vazquez et al., 2023)

In the context of feline MGTs, surgical intervention is more clearly defined than in canine cases. The recommended approach is a chain mastectomy, either unilateral for cats with a single tumour or a staged bilateral chain mastectomy for those with bilateral tumours. Several studies have demonstrated that bilateral radical mastectomy significantly improves overall survival (OS) compared to unilateral mastectomy. Cats undergoing bilateral mastectomy exhibited a lower recurrence rate (20%) compared to those with unilateral mastectomy (46.7%). Furthermore, the rates of lymph node and distant metastasis were higher in the unilateral group (55%) than in the bilateral group (35.6%). The median disease-free progression and specific disease survival times were also longer for the bilateral group (542 and 1140 days, respectively) compared to the unilateral group (289 and 473 days, respectively). This further supports the conclusion that a more extensive surgical approach confers survival benefits. (Cassali et al., 2020; Vail et al., 2020)

Despite these benefits, complication rates are higher for bilateral mastectomy, whether performed in one stage (40.6%) or two stages (35.7%), compared to unilateral mastectomy (21.3%). Nevertheless, bilateral mastectomy remains the recommended treatment for cats with mammary cancer, with the option of a two-stage procedure to reduce complications. Each case should be individually assessed to determine the optimal surgical strategy. (Cassali et al., 2020; Vail et al., 2020)

Given the high malignancy rate of mammary carcinoma and the poor prognosis associated with lymph node (LN) metastasis, a thorough LN assessment is crucial. This may involve techniques such as ultrasound-guided fine-needle aspiration (FNA) for LNs that are difficult to palpate, and concurrent inguinal or axillary lymphadenectomy during chain mastectomy. Additionally, the intradermal application of patent blue (2.5%) at a dose of 2 mg/kg (not exceeding 1 ml per patient) is recommended to facilitate the identification and removal of axillary lymph nodes. However, cats with delayed diagnosis, large primary tumours, or metastatic local LNs do not respond well to surgery alone. (Cassali et al., 2020; Vail et al., 2020)

In cats, MGT typically demonstrates a suboptimal response to chemotherapy once metastases have occurred. The indication for antineoplastic chemotherapy is guided by several clinical, histopathological, and immunohistochemical parameters. In clinical practice, adjuvant chemotherapy is recommended for patients with aggressive histological types, malignant tumours larger than 3 cm, and evidence of metastasis in regional or distant lymph nodes. In terms of histopathology, the following criteria are considered: the presence of neoplastic cells in lymphatic or blood vessels, histological grade III, and the presence of aggressive histological types, including micropapillary carcinoma, solid carcinoma, cribriform carcinoma, and carcinosarcoma. In addition, an

immunohistochemical Ki-67 index greater than 14% also indicates the necessity for postoperative chemotherapy. (Cassali et al., 2020; Giménez et al., 2010)

Previous research has explored doxorubicin as an adjuvant therapy in feline mammary neoplasms, sometimes combined with COX-2 inhibitors like meloxicam. However, the lack of control populations limits these findings. Additionally, doxorubicin's nephrotoxicity in cats necessitates careful monitoring of renal function. Another non-selective COX-2 inhibitor, piroxicam, is also used at a dose of 0.3 mg/kg every 48 hours.(Cassali et al., 2020; Giménez et al., 2010)

Chemotherapy protocols typically involve the use of doxorubicin, either alone or in combination with cyclophosphamide; carboplatin, either alone or in combination with doxorubicin; and mitoxantrone, either alone or in combination with cyclophosphamide. Further studies are necessary to determine the most effective doses and combinations. Carboplatin has been proposed as a potential rescue therapy, either alone or in combination with other antineoplastic agents, such as mitoxantrone and doxorubicin. (Cassali et al., 2020; Giménez et al., 2010)

Despite the frequent occurrence of metastasis following surgery, there have been limited advancements in identifying effective adjuvant systemic treatments (Cassali et al., 2020)

Due to the high aggressiveness of feline mammary tumours, chemotherapy is recommended for aggressive histological types and grades or advanced clinical staging, like protocols in women and dogs. The choice of the best protocol requires further research, considering patient comorbidities, cost, and treatment logistics. Despite the lack of robust evidence in the literature, veterinary oncologists continue to recommend chemotherapy for some cats with MGTs. (Cassali et al., 2020; Vail et al., 2020)

1.7. Comparative oncology

The use of rat models in MGT research is of great importance, but it is important to acknowledge the inherent limitations of this approach. Rat MGT rarely metastasise, which renders them unsuitable for the study of this critical aspect of the disease. Moreover, in comparison to mice, the limited availability of genetic engineering techniques, such as nuclear transfer for gene knockouts, has impeded the widespread adoption of rats as genetic models for breast cancer. Although a mutagenesis approach has been devised to produce breast cancer-associated gene mutants such as BRCA1 and BRCA2, more efficient technologies are required for achieving complete gene knockouts in breast cancer investigations. (Abdelmegeed & Mohammed, 2018)

The striking similarities in epidemiological and clinicopathological features between spontaneous tumours in companion animals and humans serve to highlight the significance of these animals as pertinent models for human cancer research. The accelerated progression of cancer in dogs and cats, coupled with their comparatively shorter lifespans, allows for the acquisition of data at a

more rapid pace than in human studies. Furthermore, the analogous health consequences resulting from exposure to environmental hazards, including cancer, emphasise the utility of dogs and cats as reliable indicators for human malignancies. Consequently, comparative oncology—the study of naturally occurring cancers in animals as models for human disease—has become an increasingly significant field of study in recent decades.(Vilhena et al., 2020)

In recent years, a growing body of research has highlighted the significance of comparative oncology in understanding the similarities between human breast cancer and mammary tumours in companion animals, particularly cats and dogs. Feline mammary tumours have been subjected to extensive study to elucidate their parallels with human disease. Previous investigations have revealed that feline mammary intraepithelial lesions share several characteristics with human premalignant lesions, including spontaneous onset, high prevalence, and morphological features. Notably, these lesions also demonstrate a loss of PR and ER expression, suggesting that cats can serve as models for pre-invasive ER- and PR-negative breast tumours. Moreover, studies have demonstrated that tumour lesion size, growth method, and histological grade in feline mammary carcinomas are associated with disease prognosis, in a manner analogous to findings observed in humans. Consequently, feline mammary carcinomas provide a valuable platform for comparative studies on therapeutic interventions and serve as an intermediate model for investigating mammary tumour histogenesis and aetiology. (Abdelmegeed & Mohammed, 2018; Adega et al., 2016)

Similarly, canine MGTs have emerged as important models for human breast cancer research, underscoring the relevance of comparative oncology in this field. Human breast cancer is one of the most prevalent and well-known types of cancer among women worldwide. It is noteworthy that significant clinical and molecular parallels have been identified between human breast cancer and canine mammary tumours. Clinically, both diseases exhibit similarities, including spontaneous tumour incidence, onset age, hormonal aetiology, and disease progression. Furthermore, factors influencing clinical outcomes, including tumour size, clinical stage, and lymph node invasion, demonstrate remarkable parallels between the two species. Molecularly, canine mammary tumours share similarities with human breast cancer in terms of steroid receptor expression, epidermal growth factor (EGF) signalling, proliferation markers, metalloproteinase and cyclooxygenase overexpression, and p53 mutation. These findings emphasise the significance of comparative oncology in utilising insights from companion animals to enhance our comprehension and treatment of human breast cancer. (Abdelmegeed & Mohammed, 2018; Adega et al., 2016)

The human breast shares homology with the canine mamma, comprising a mammary gland, connective tissue, skin, and a nipple. Women typically possess two breasts situated laterally to the median sagittal plane on the anterior thoracic wall. Dogs, on the other hand, have two mammary

chains - left and right - each with five mammae. These include two thoracic pairs (M1 and M2), two abdominal pairs (M3 and M4), and one inguinal pair (M5).(Ferreira et al., 2023)

In women, primary lymphatic drainage occurs in the axillary and internal mammary lymph nodes, with approximately 75% of mammary gland lymph directed to the axillary nodes. Female dogs exhibit similar drainage patterns, with cranial (M1) and caudal thoracic (M2) mammary glands primarily draining into the axillary lymph centre. The drainage of the cranial abdominal gland (M3) varies, flowing into either the axillary lymph nodes or the superficial inguinal lymph nodes, depending on its connection to the caudal abdominal gland lymphatics. The caudal abdominal (M4) and inguinal (M5) mammary glands drain into the superficial inguinal lymph nodes and medial iliac nodes (secondary). Notably, there are no direct connections between the left and right mammary lymphatic vessels.(Ferreira et al., 2023)

Understanding these anatomical details is crucial for both veterinarians and human doctors. Aside from the imperative of early metastasis detection in animals, the concept of "one health" underscores the interconnectedness between human and animal health. Given that dogs often inhabit the same environments as their owners and may share similar health conditions, they are viewed by many researchers as an appealing model for studying spontaneous breast cancer in humans.(Ferreira et al., 2023)

2. DIAGNOSTIC IMAGING IN MAMMARY GLAND ONCOLOGY

2.1. Relevance of oncologic imaging

In the field of oncology, a multidisciplinary approach has been recommended and progressively implemented to optimise the management of cancer patients. Among the various methodologies employed in the diagnosis and monitoring of cancer in dogs and cats, diagnostic imaging techniques play a pivotal role. Indeed, the sensitive detection and characterisation of lesions are essential for determining the most appropriate local or systemic therapy and for managing the therapeutic outcomes.(Sánchez et al., 2019)

Imaging modalities are available for the evaluation of mammary gland abnormalities in domestic animals, with the dual purpose of aiding both research and clinical practice. This is achieved by assisting in the differential diagnosis of masses. The most employed imaging modalities for the evaluation of mammary gland abnormalities include radiology, computed tomography (CT), positron emission tomography (PET), magnetic resonance imaging (MRI), and ultrasonography (US). Among these, radiology and ultrasound are the most commonly accessible imaging techniques, offering widespread availability. The utilisation of various imaging modalities allows for gaining unique insights into anatomical features, structures, and physiological processes. Both CT and MRI are highly effective in providing detailed structural information about organs. In contrast, PET primarily elucidates organ function, offering valuable insights into physiological processes. (Barbagianni & Gouletsou, 2023)

2.2. Radiology

Radiology is routinely used to obtain baseline information on the thorax and musculoskeletal system. Like in humans, thoracic radiographs are the mainstay for pulmonary metastasis monitoring. Radiography complements ultrasound by providing information about the relative size and position of organs and skeletal metastasis. (Mattoon & Bryan, 2013)

2.3. Ultrasound

In veterinary medicine, ultrasound (US) is the primary imaging modality for abdominal evaluations, with computed tomography (CT) being the preferred alternative. This is due to several factors, including the smaller patient size, the absence of the need for general anaesthesia, the lack of ionising radiation, and the lower cost. Furthermore, the US is particularly adept at delineating the organ-specific location of lesions. However, the main limitation of ultrasound is the restricted cross-sectional field of view in comparison to CT or MRI, which impedes the accurate measurement of large lesions. In the context of mammary gland abnormalities, ultrasonography plays a pivotal role. B-mode ultrasonography is frequently the initial step in the evaluation process, employed to assess parameters

such as gland dimensions, echotexture, and echogenicity. Studies of canine mastitis have demonstrated that inflammation alters the echogenicity of tissue, thereby obscuring the distinct layers that are characteristic of healthy mammary tissue. Despite these observations, there are currently no definitive ultrasonographic criteria for distinguishing between inflammatory and neoplastic conditions. Doppler ultrasonography provides further insight into the evaluation of mammary gland health by assessing blood flow dynamics. Power Doppler imaging detects blood movement without indicating direction, while colour Doppler imaging, often combined with grayscale images, provides duplex images that visualise blood velocity on a colour scale. These techniques collectively offer a comprehensive insight into mammary gland health and pathology. (Barbagianni & Gouletsou, 2023; Mattoon & Bryan, 2013)

An emerging modality for tumour assessment is contrast-enhanced ultrasound (CEUS), which allows real-time evaluation of blood flow and vasculature using US contrast agents composed of tiny gas bubbles. These agents remain within the intravascular space, providing detailed blood flow imaging. While CEUS has been used to monitor vascular therapies and pharmacological effects on tumour perfusion in dogs, its diagnostic value for superficial neoplasms remains underexplored due to limited studies in veterinary medicine. Ultrasonographic characteristics identified by B-mode US and CEUS, such as border definition, echogenicity, echotexture, and enhancement patterns at wash-in, are potential indicators of malignancy. Benign neoplasms typically exhibit an ovoid shape, well-defined borders, hypoechoic echogenicity, homogeneous echotexture, and a centripetal enhancement pattern. In contrast, malignant neoplasms often present a multilobulated or ovoid shape, mixed echogenicity, heterogeneous echotexture, and a chaotic enhancement pattern. B-mode US provides useful initial evaluations by correlating with histopathological findings, although it cannot replace cytology or histopathology. Malignancy prediction is also based on vascularity, with neoplastic blood vessels showing abnormal function and structure compared to normal vascularization. Doppler US and CEUS enable the non-invasive assessment of vascular patterns. However, Doppler US is limited in detecting smaller blood vessels, a limitation that CEUS overcomes. In human medicine, CEUS has shown promise in differentiating benign from malignant superficial lesions, but few studies have evaluated its use in dogs. Echotexture has been particularly useful in distinguishing between benign and malignant mammary masses, with heterogeneous echotexture often indicating malignancy. The perfusion pattern at wash-in, another significant parameter, also differs between benign and malignant neoplasms. In conclusion, B-mode US and CEUS show significant potential in predicting malignancy in superficial neoplasms in dogs. Important parameters include border definition, echogenicity, echotexture, and enhancement pattern at wash-in, with the quantitative CEUS parameter WoAUC at the tumour centre also showing promise. Despite the relatively small sample size, these findings suggest a significant role for the US in evaluating superficial neoplasms in dogs.

Further studies with larger sample sizes are needed to refine the use of US in cancer evaluation and explore the combined use of ultrasonographic characteristics for malignancy prediction. (Hillaert et al., 2022)

2.4. Computed Tomography

The CT is the examination of choice in cases of mammary tumours because it can provide anatomical details, accurate depiction of calcification, visualization of soft tissue components of tumours and high sensitivity in the detection of potential metastases. CT stands out as the most sensitive imaging modality for identifying and evaluating pulmonary lesions in both human and veterinary medicine. (Mattoon & Bryan, 2013) The disadvantages of this technique include the low soft tissue contrast, the little information regarding the function of the organs, the use of ionizing radiation, the reduced availability of equipment, and the high cost. A study investigating sentinel LN metastasis in canine mammary tumours found that contrast-enhanced CT imaging exhibited notable sensitivity and specificity, reaching 87.5% and 89.3%, respectively. (Barbagianni & Gouletsou, 2023)

CT demonstrated superior precision compared to plain radiographic examination, particularly in detecting metastases. Previous research has consistently shown CT to be more sensitive in identifying small lesions. For instance, in a retrospective study involving 18 dogs with pulmonary metastatic neoplasia, CT imaging revealed nodules as small as 1 mm, whereas radiographs only detected lesions ranging from 7 to 9 mm. (Otoni et al., 2010)

2.5. Positron Emission Tomography (PET)

PET is a relatively recent imaging modality that offers insights into functional processes in three dimensions by administering intravenous radiolabelled tracers. (Barbagianni & Gouletsou, 2023)

In companion animals, PET imaging has found utility in the detection of mammary tumours. While PET may not be as effective in diagnosing small primary mammary gland tumours or well-differentiated mammary gland tumours or in identifying regional lymph node involvement, it surpasses conventional imaging methods in detecting distant metastases and recurrences, as well as in monitoring therapeutic responses.(Lim et al., 2017)

Sanchez et al. established a standardized maximum glucose uptake value (mean: 1.1) and its correlation with tumour size and the nature of lesions (benign vs. malignant). They determined that a minimum tumour size of 1.5 cm was necessary to differentiate malignant lesions based on the maximum glucose uptake value, with a glucose uptake value exceeding 2 exhibiting 100% sensitivity for malignancy. However, no significant association was noted between the maximum glucose uptake value and histologic subtype or grade. Conversely, in human medicine, PET/CT has shown relatively low sensitivity (52.2%) but high specificity (91.6%) in detecting axillary lymph node metastasis in

patients with breast cancer. Drawbacks of this technique include limited anatomical detail, the high cost and low sensitivity of equipment, restricted availability of equipment, and the requirement for radioactive tracers. (Barbagianni & Gouletsou, 2023)

2.6. Magnetic Resonance Imaging

MRI provides detailed images with better soft tissue contrast when compared to CT. In companion animals, conventional static MRI was applied for the examination of the morphological characteristics of mammary tumours in dogs, such as their size, shape, and tissue structure, while dynamic contrast-enhanced MRI provided information about the physiological properties of tumours. The advantages of MRI include the high spatial resolution achieved during the examination, the excellent soft tissue contrast, and the lack of requirement to use X-rays or radioactive tracers. Furthermore, within human medicine, studies have proved that MRI offers substantial sensitivity (94.6%) and specificity (74.2%) for diagnosing breast cancer, surpassing ultrasound, or mammography alone. The sensitivity is further augmented when combining multiple imaging modalities, such as ultrasonography, mammography, and MRI. The disadvantages of this technique include the need for high-cost equipment and the reduced availability of equipment. (Barbagianni & Gouletsou, 2023)

3. UNCOMMON SITES OF MAMMARY GLAND TUMOR METASTASIS

There is a noticeable increase in reported cases of mammary gland neoplasia. However, studies regarding the prevalence of metastases in uncommon sites are rare. Existing literature highlights the lungs as the primary site for mammary tumour metastasis, accounting for 60-80% of cases. Nevertheless, metastases can also occur in other organs such as lymph nodes, adrenal glands, kidneys, heart, bones, liver, brain, eyes, nose, spleen, uterus, and serous membranes. In rare instances, canine mammary tumours may metastasize to the skin, leading to cutaneous carcinomatosis. (Soare et al., 2013) This work will only review heart metastasis, liver metastasis, eye metastasis, and bone metastasis, with a particular emphasis on liver metastasis and bone metastasis due to its significance., All information is focused on companion animals, dogs and cats, and where reference is made to other species this will be indicated.

3.1 Heart metastasis

3.1.1. Prevalence of heart metastasis

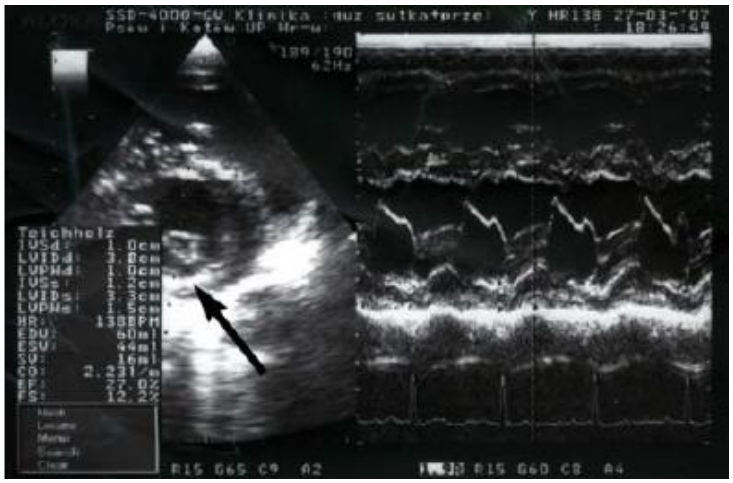

Figure 1: Dog echography of the heart. Projection in the short birefringent axis. A Hyperechogenic element is noted in the vicinity of the papillary muscle of the left ventricle (Vignoli et al., 2013)

Heart metastasis as well as primary cardiac neoplasms are very rare but in general secondary heart tumours occur more frequently than primary neoplasms. (Aupperle et al., 2007) Approximately 7.12% of heart tumours originate from mammary gland carcinomas. (Walter & Rudolph, 1996)

In humans, cardiac metastases exhibit a relatively even distribution, with approximately 30% occurring in the right ventricular wall, 30% in the left free ventricular wall, and 30% in both free ventricular walls. In contrast, in dogs, cardiac metastases are frequently localized to the left free ventricular wall.(Aupperle et al., 2007)

3.1.2. Clinical Signs

Symptoms of cardiac metastasis typically correlate with the size and location of the tumour. Electrocardiographic abnormalities are often a result of myocardial infiltration by neoplastic cells, leading to conduction system damage (Grieco et al., n.d.) In dogs, signs may include right-sided heart failure due to cardiac tamponade, congestive left heart failure linked to atrial fibrillation, and, in some instances, pulmonary hypertension. Owners may observe reduced appetite, lethargy, weakness, neurological symptoms, vomiting, and abdominal distention.(Aupperle et al., 2007)

3.1.3. Imaging characteristics of heart metastasis

In humans, cardiac metastases often manifest as small, multiple lesions, although single large tumour masses can also occur. Carcinomas may cause diffuse thickening of the pericardium due to metastatic deposits. These metastases can reach the heart through lymphatic or hematogenous routes, or via direct or transvenous extension. (Reynen et al., 2004).

Transthoracic echocardiography has been described as the modality of choice for cardiac lesions. (*fig.1*) (Vignoli et al., 2013) Echocardiography is generally considered moderately accurate, with an agreement of approximately 86% regarding location and 65% regarding tissue-type diagnosis when compared with necropsy findings. (Liza S. Köster, 2021) In cases of metastatic disease, however, MRI and CT offer significant advantages. Both imaging modalities provide a comprehensive evaluation of thoracic conditions due to their wide field of view. They permit the assessment of lung tissue, the pleura surrounding the mediastinum, and the vascular structures of the heart. Furthermore, MRI offers superior contrast resolution in comparison to ultrasonography or CT, which enables clear differentiation between tumour and myocardial tissue. In certain instances, the signal intensities observed on MRI scans can assist in the characterisation of tissue. Cardiac tumours, for instance, typically exhibit low signal intensity on T1-weighted images and appear brighter on T2-weighted images. Furthermore, most malignant conditions demonstrate enhancement following the administration of contrast material. (Chiles et al., 2001)

Pericardial effusion and thickening are common findings on CT scans of patients with MGTs. Malignant involvement is suggested by enhancement of the pericardium or pericardial nodularity, although pericardiocentesis is necessary for a definitive diagnosis. (Chiles et al., 2001)

3.2 Liver metastasis

3.2.1. Prevalence of liver metastasis

Primary hepatic tumours are relatively uncommon, whereas metastatic disease to the liver is a highly prevalent phenomenon. Nevertheless, among all metastases, liver metastasis occurs in approximately 25% of feline mammary carcinoma patients.(Chang et al., 2023)

In dogs, liver metastasis is most associated with primary tumours of the mammary gland. (Vilkovyskiy et al., 2020) The liver is a common site for metastasis due to its dual blood supply. Approximately 20-30% of blood enters the liver from the hepatic artery, while 70-80% enters from the portal vein. This renders the liver frequently the initial site of metastasis, whereby cancerous cells are disseminated through the bloodstream. (Nyman et al., 2004)

3.2.2. Clinical signs

Hepatobiliary tumours are associated with symptoms in approximately 50% of cats and 75% of dogs. The most common initial symptoms are non-specific and may include loss of appetite, weight loss, lethargy, vomiting, increased thirst and urination, and ascites. (M. Liptak et al., 2004). The median survival of dogs with liver metastasis of MGT is approximately 480 days. (Vilkovyskiy et al., 2020)

3.2.3. Imaging characteristics of liver metastasis

B-mode ultrasound is the primary diagnostic imaging method for liver assessment in both human and veterinary medicine. While B-mode ultrasound is commonly employed for detecting focal liver lesions, it frequently lacks the definitive features required for histological characterisation. Consequently, certain lesions may mimic liver parenchyma, thus evading detection. Consequently, additional modalities such as MRI, CT, CEUS, and histological assessments are indispensable for determining the histotype of each lesion. Despite the growing body of research on the use of CEUS in the evaluation of liver lesions in dogs and cats, the veterinary literature remains limited, with most studies comprising small-scale case reports. (Burti et al., 2020)

Most liver neoplasms are in the left medial or left lateral lobes, a distribution that can be attributed to the liver's blood circulation. These lobes receive a substantial portion of hepatic blood flow from both the hepatic artery and the portal vein, which increases their exposure to circulating carcinogens and metastatic cells. This makes them more susceptible to tumour development. (Vilkovyskiy et al., 2020)

In humans, a variety of techniques are employed to enhance the detection of small liver metastases, with contrast studies and alterations in liver blood flow being the primary focus. These occur shortly after the metastatic cells settle in the liver. The overall accuracy rates of ultrasound imaging in detecting liver lesions smaller than 2 cm have been found to vary considerably, with rates ranging from 53% to 84%. These assessments have been compared to those obtained using CT, MRI,

and intraoperative ultrasound, which have been established as the gold standard techniques. Magnetic resonance imaging (MRI) is becoming the imaging modality of choice for identifying and characterising liver lesions in humans due to its high specificity, achieved through optimal contrast between lesions and liver tissue, and without subjecting patients to radiation exposure. (Namasivayam, 2007; Nyman et al., 2004)

Helical CT has been employed in dogs, providing images of the entire liver during peak parenchymal enhancement. This enables the optimal detection of focal hepatic lesions and facilitates preoperative planning, thanks to the three-dimensional (3D) reconstruction capabilities of triple-phase helical CT. (*fig.2*) (Kutara et al., 2014)

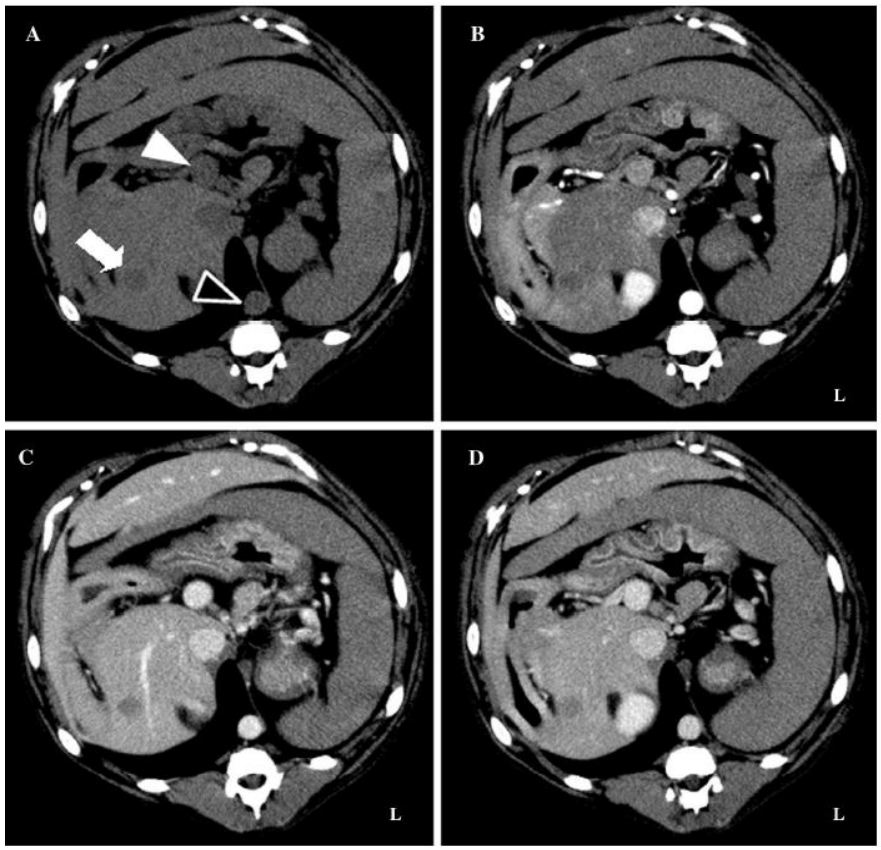

Figure 2: A-D Triple phase CT characteristics in a dog with a hepatic metastatic tumour. (A) Transverse precontrast CT image showing a mass in the right lobe (arrow). (B) Homogeneous tumour hypoenhancement in the arterial phase. (C) Homogeneous hypoenhancement in the portal venous phase. (D) Homogeneous hypoenhancement in the delayed phase. White arrow: tumour. Black arrowhead: aorta. White arrowhead: portal vein. (Kutara et al., 2014)

In dogs with hepatic metastatic tumours, common CT findings include homogeneously hypoenhanced lesions in both the arterial and portal venous phases. Furthermore, the presence of hypoattenuating lesions within the hepatic parenchyma in post-bolus contrast CT, such as the delayed phase, is indicative of probable hepatic metastatic tumours, with a reported sensitivity of 73%, specificity of 79%, and Positive predictive value (PPV) of 73%. (*fig.3*) (Kutara et al., 2014).

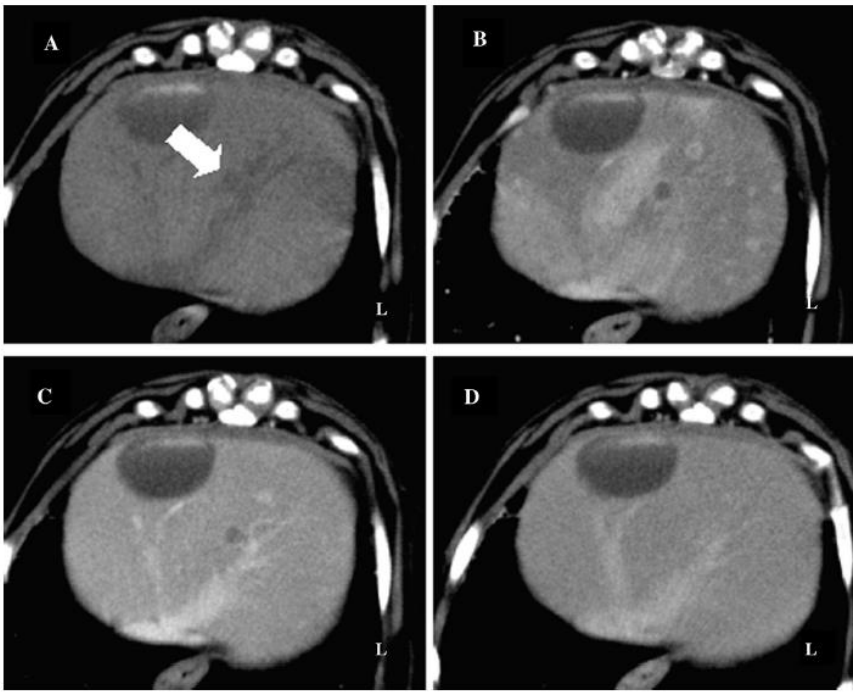

Figure 3: A-D Triple phase CT characteristics in a dog with a hepatic metastatic tumour. (A) Transverse precontrast CT image showing a mass in the right lobe (arrow). (B) Homogeneous tumour hypoenhancement in the arterial phase. (C) Homogeneous hypoenhancement in the portal venous phase. (D) Homogeneous isoenhancement in the delayed phase. White arrow: tumour. (Kutara et al., 2014)

A recent study evaluated the efficacy of CT and ultrasonography in distinguishing benign from malignant hepatic masses in dogs. The lowest region of absolute enhancement during the delayed phase of triphasic CT scans was found to accurately predict the classification of hepatic masses as benign or malignant, with an accuracy of 91.3%. This was accompanied by a sensitivity of 93.3% and a specificity of 87.5%. When combined with the highest venous-phase mass conspicuity, this variable increased the accuracy to 93.5%, with sensitivity and specificity reaching 96.7% and 87.5%, respectively. It is noteworthy that the presence or absence of cavitations within masses observed during B-mode ultrasonography significantly contributed to the classification of masses as malignant, with an accuracy of 80.4%, sensitivity of 76.6%, and specificity of 87.5% (*see cavitations on Fig. 4*). However, neither CT nor ultrasonography was able to identify the specific cause of each liver mass. In general, CT exhibited superior diagnostic accuracy compared to ultrasonography. This is likely due to its ability to offer dynamic contrast uptake information and conduct quantitative measurements. (Griebie et al., 2017)

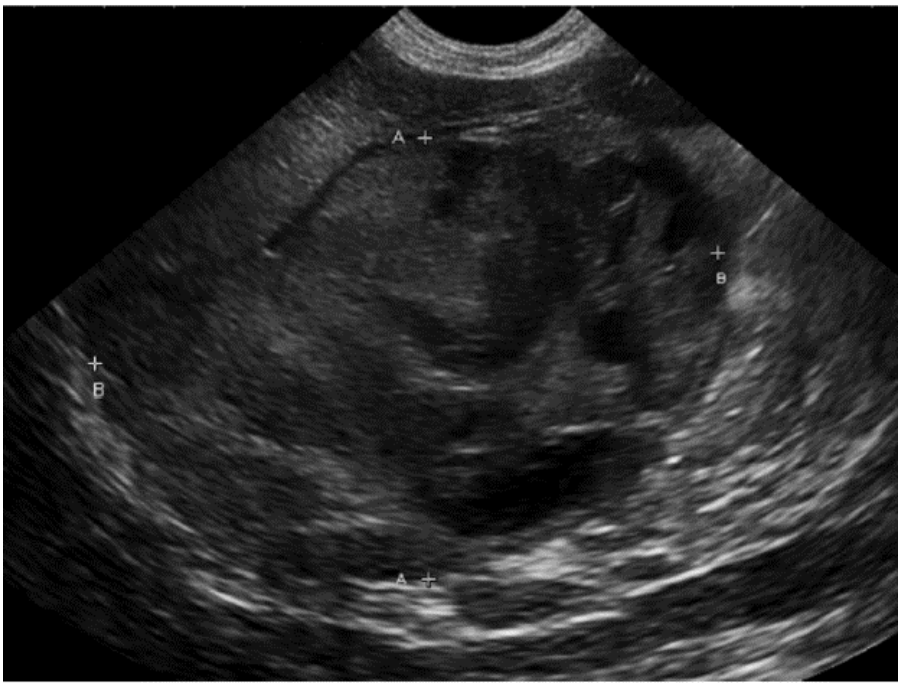

Figure 4: The B-mode ultrasonographic image of a hepatocellular carcinoma (long-axis view) with cavitations throughout the mass. Height (electronic callipers [plus signs] A to A, 6.3 cm) and length (electronic callipers B to B, 9.0 cm) are depicted. (Griebie et al., 2017)

3.3 Eye metastasis

3.3.1. Prevalence of eye metastasis

In dogs and cats, the eyes are not typically a common site for metastasis. However, excluding lymphomas that affect the eyes of dogs, mammary gland carcinoma is a notable secondary ocular disease in female dogs, like its occurrence in women and female cats.(Bandinelli et al., 2020).

In dogs, metastatic neoplasia is more frequently bilateral and predominantly affects the anterior uvea, whereas in cats, it is more commonly observed in the choroid (Eördögh et al., 2019). No cases of ocular metastases have been reported in the last 10 years.

3.3.2. Clinical signs

The most reported clinical signs associated with orbital neoplasia include slowly progressive exophthalmos, strabismus, protrusion of the third eyelid, resistance to retropulsion, periocular swelling, absence of pain, exposure keratitis, and either normal vision or vision loss.(Bandinelli et al., 2020)

3.3.3. Imaging characteristics of eye metastasis

Lesions in the eyes commonly affect multiple anatomical sites, with a higher frequency observed at the ciliary body, iris, and choroid (uveal tract - 56.8%), followed by the sclera, limbus, and

cornea (fibrous layer - 32%), and the meninges, optic nerve, and retina (nervous layer - 11.2%). (Bandinelli et al., 2020).

Ocular ultrasound is becoming increasingly crucial in veterinary ophthalmology diagnostics. Utilizing the B-scan mode (two-dimensional), it enables a topographical assessment of the eye, evaluating the location, size, shape, sound attenuation, extension, and consistency of any tumour mass. Additionally, it allows for kinetic assessment of parameters such as mobility and vascularity of intraocular masses. A-mode studies of intraocular masses assess their acoustic characteristics, internal reflectivity, sound attenuation, vascularity, and mobility. (Baptista et al., 2006)

The US evaluation of a metastatic ocular mammary gland carcinoma was described as a large, irregular, and heterogeneous mass. A-scan echogram displayed prominent acoustic interfaces, high internal reflectivity, reduced sound attenuation, irregular spikes, and limited vascularization. (*fig.5)* (Baptista et al., 2006)

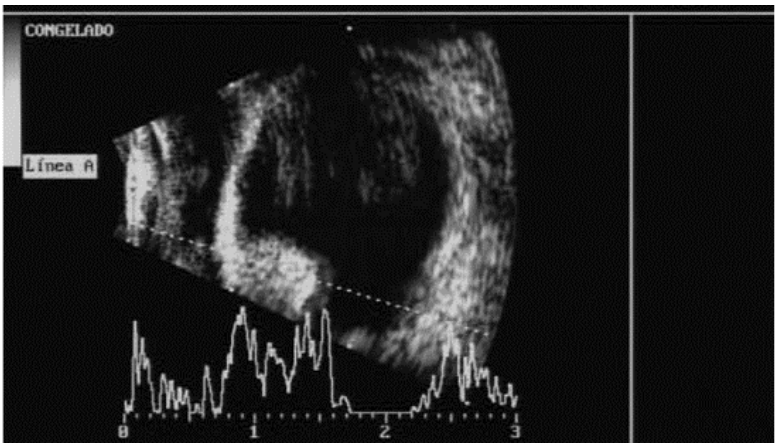

Figure 5: Metastatic ocular mammary gland carcinoma. A large, irregular, heterogeneous anterior uveal mass is present. The A-scan echogram shows large acoustic interfaces, high internal reflectivity, low sound attenuation, irregular spikes, and poor vascularization (Baptista et al., 2006)

3.4 Bone metastasis

3.4.1. Prevalence of bone metastasis

In humans, skeletal metastases are more common than primary bone sarcomas. Notably, carcinomas from the prostate and breast exhibit a strong propensity to spread to the skeleton, a well-established fact.(Cooley & Waters, 1998) Remarkably, in humans, up to 70% of breast and prostate cancer cases progress to bone metastasis. (Zhang et al., 2010)

In contrast to humans, bone metastases are less common in dogs than primary bone tumours. Dogs presenting with bone metastases tend to be an older and heterogeneous group, with metastases most commonly occurring in the axial skeleton and proximal bones of the appendicular skeleton. A

prevailing hypothesis is that the lower reported incidence of bone metastases in dogs may be due to the infrequency of skeletal radiographs and necropsy examinations. (Zuchi et al., 2020)

Bone metastasis is a challenging and poorly comprehended aspect of Veterinary Medicine, commonly associated with a bad prognosis among metastatic sites. (Grisoni Sanchez et al., 2023) Bone metastasis in canine medicine involves complex interactions, including remodelling of the tissue matrix and perturbation of the local immune system, endothelial cells, fibroblasts, osteoblasts and osteoclasts. Among the few studies investigating the molecular roles in this process, osteopontin (OPN), also known as secreted phosphoprotein 1, is emerging as a significant player. OPN has been implicated in the control and regulation of local inflammation and immunity, with links to metastatic cancer prognosis and overall survival. In addition, OPN contributes to tumour growth as a paracrine and autocrine mediator produced by macrophages and fibroblasts, promoting tumour invasion and angiogenesis, both of which are associated with poor prognosis. This glycoprotein belongs to a family of small integrin-binding proteins strongly implicated in the progression of bone metastasis. OPN actively promotes an environment conducive to cancer cell proliferation in metastatic bone regions. By promoting the degradation of bone tissue through interactions with osteoclasts, it facilitates the creation of a favourable environment for cancer cells to invade and thrive at the metastatic site. Despite studies in female dogs with MGTs, no association has been found between OPN expression and bone metastasis or prognosis. In contrast, human studies have highlighted the importance of OPN in determining prognosis in women and its potential as a marker for breast neoplasia. (Grisoni Sanchez et al., 2023)

Metastasis frequently target the lumbar vertebrae, femur, humerus, ribs, and pelvis, likely due to their susceptibility as preferred sites for bone metastasis originating from urogenital malignancies such as prostate, bladder, urethral, and mammary cancer. Metastatic lesions in long bones frequently affect the diaphysis, likely because of the proximity to a nutrient foramen.(Vail et al., 2020)

3.4.2. Clinical Signs

Metastatic neoplasia lesions in the skeleton frequently remain clinically silent, devoid of noticeable signs of pain or swelling, posing a significant factor in treatment decisions. Moreover, animals might display lameness absent of accompanying pain or swelling. While uncommon, pathologic fractures associated with metastasis or neoplasm infiltration into the bone can occasionally serve as initial indicators. Neoplasms causing vertebral destruction may result in neurological symptoms due to compression of the spinal cord. (Russell & Walker, 1983; Zuchi et al., 2020)

3.4.3. Imaging characteristics of bone metastasis

In Humans and in veterinary medicine radiographs are suggested for evaluating abnormal radionuclide uptake or assessing the risk of pathological fracture, serving as the first imaging modality in patients experiencing bone pain. (Costelloe et al., 2009).

Radiographs remain the primary imaging modality for the evaluation of bone tumours in veterinary medicine. The radiographic appearance of bone tumours can vary widely, from bone lysis to osteoblastic or osteosclerotic changes, with many intermediate forms. Common features include cortical lysis severe enough to cause pathological fractures and new bone formation in a palisading pattern perpendicular to the cortical bone (sunburst effect). In addition, tumour proliferation often causes elevation and rupture of the periosteum at the periphery of the lesion, resulting in Codman's triangle. Some tumours cause an increase in the size of the affected part of the long bone, known as an expansile lesion, which is often associated with soft tissue swelling. Radiographs typically show an unclear demarcation between the tumour and adjacent normal bone. For definitive diagnosis, bone biopsies are usually performed using ultrasound, fluoroscopy or CT guidance to obtain needle core biopsy samples. In human medicine, CT and MRI are commonly used and considered reference modalities for the assessment of bone tumours. In veterinary medicine, however, these modalities require general anaesthesia or deep sedation, which increases both the risk and cost of the procedure. CT is particularly useful for anatomical assessment of bone destruction and sclerosis, especially in axial sites. MRI is valuable in assessing the extent of the tumour and its relationship to surrounding structures, which is particularly important for axial sites close to the vertebral canal. Commonly used sequences include T1- and T2-weighted images. T1-weighted images can easily distinguish the hypointense intramedullary tumour from the hyperintense medullary fat. If the tumour is extraosseous, T2-weighted sequences can assess tumour margins by differentiating signal intensities between the tumour and normal muscle. (Vanel et al., 2013)

Bone scintigraphy, also known as bone scanning, is an invaluable tool for detecting bone metastases and assessing the skeletal system. This imaging technique is based on the principle that actively metabolizing bone incorporates specific bone "tracers," with their distribution influenced by the rate of bone turnover and blood flow. The resulting images show the relative distribution of these tracers throughout the skeletal system. Because bone scintigraphy primarily reflects changes in bone metabolism rather than bone structure, it is used as an adjunct to plain radiography (X-ray) rather than a replacement. Typically, metabolic changes detected by bone scintigraphy precede structural changes visible on X-rays, as changes in bone metabolism occur before changes in bone structure. (*Bone Scintigraphy*, n.d.)

In conclusion, while radiographs remain the mainstay of bone tumour assessment in veterinary medicine, advanced imaging modalities such as CT and MRI offer significant advantages in detailed

assessment and diagnosis, albeit at increased risk and cost. Nuclear scintigraphy is a highly sensitive method for detecting bone metastasis. When there's suspicion of metastatic bone neoplasia, it's recommended to perform a whole-body bone scan or PET/CT imaging. This approach is crucial because multiple metastatic sites may be present, even if symptoms are localized to one bone (Vail et al., 2020) (*fig.6*)

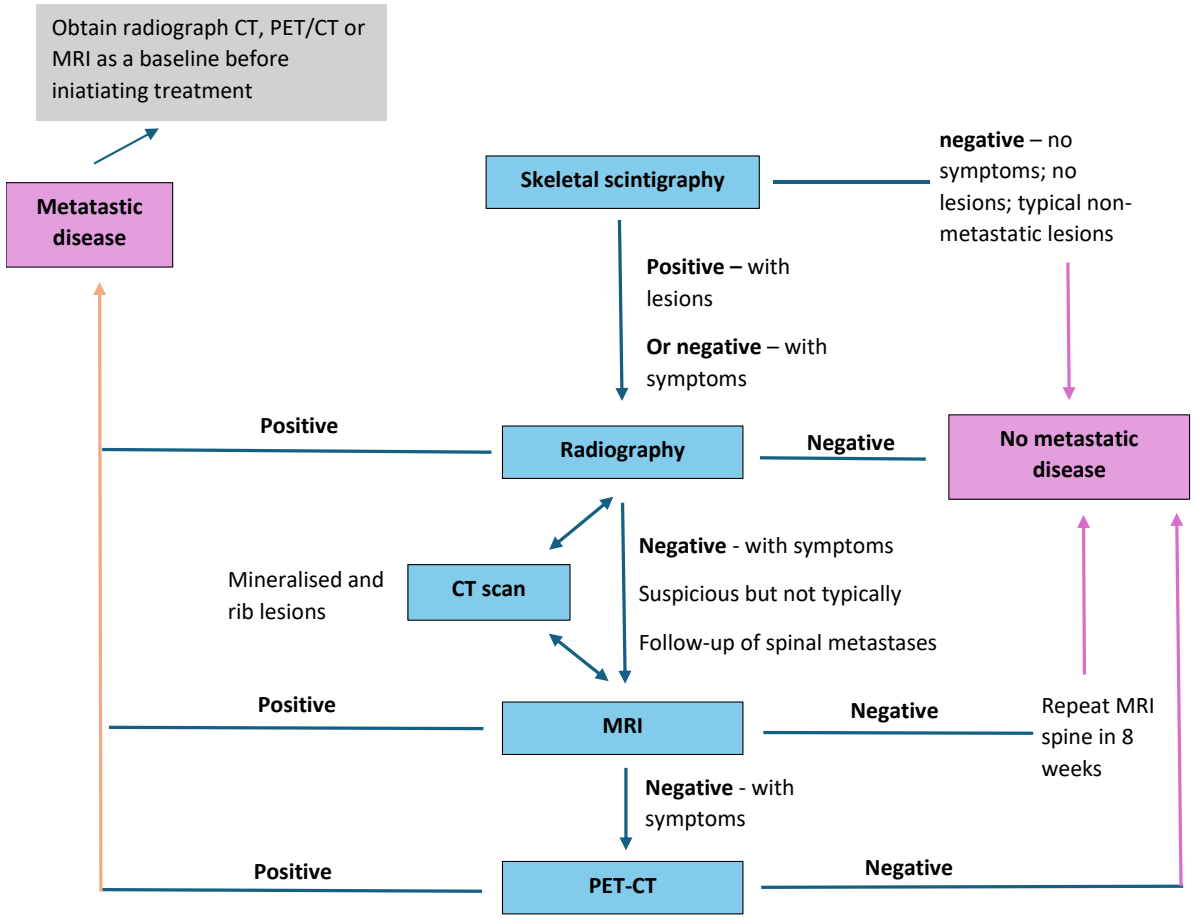

Figure 6: Algorithm of imaging studies for the detection of osseous metastases based on (Costelloe et al., 2009)

4. CLINICAL CASE

4.1. Patient characterisation and reason for consultation

Lola was a sterilised 11-year-old female canine patient, Chihuahua breed, with 4.7kg. She was admitted to the Anicura Valencia Sur Veterinary Hospital due to anorexia, apathy with thrombocytopenia and bilious vomiting.

4.2. Anamnesis

Lola had no cohabitants and was an indoor dog with a private outdoor area. She was fed with dry food and had water *ad libitum*. She had no access to rubbish or toxins. Her vaccinations and deworming were up to date. She was spayed at the age of 3 years old due to pyometra. She was referred to the hospital due to anorexia, apathy and the onset of weakness and bilious vomiting. Her tutor also mentioned that she doesn't drink water or urinate.

4.3. Physical examination

A normal attitude. Alert mental state with a nervous temperament. **BC:** 5/9; **Breathing movements:** Costoabdominal, deep, rhythmic, and regular, 40 rpm; **Metatarsal pulse:** strong, regular, bilateral, symmetrical, synchronous, and rhythmic. **Temperature:** 38.3ºC; **Mucous membranes:** pink, moist and shiny with CRT< 2 seconds; **Degree of dehydration:** <5%; **Lymph nodes:** enlargement of the left medial iliac and superficial inguinal lymphadenopathy; **Abdominal palpation:** Hyperesthesia in the cranial abdominal area; **Cardiac and pulmonary auscultation:** both normal with a heart rate of 160. Inflammation at left M5 with associated erythema and haematoma. Mammary lump on left M3 and right M2.

4.4. List of problems

Anorexia, bilious vomiting, hyperesthesia in the cranial abdominal area, inflammation at the left M5 with associated erythema and haematoma, mammary lump on left M3 and right M2

4.5. Differential diagnoses

Peritonitis, Pancreatitis, Benign mammary gland hyperplasia (Fibroadenomatous hyperplasia; Ductal hyperplasia); Benign mammary gland tumours (mammary gland adenoma; Fibroadenoma; Intraductal papilloma); Malignant mammary gland Tumours (carcinoma; adenocarcinoma; Inflammatory carcinoma; Malignant mixed tumour; Sarcoma); Mastitis; mammary abscess; mammary cysts; Lipoma; Lobular or ductal hyperplasia.

4.5. Additional diagnostic examinations

Blood analysis

TBC: Thrombocytopenia 46k/µL; Serum biochemistry: Azotaemia; Clotting times: no changes (*see Annex B for blood count and serum biochemistry*).

Ultrasound

An abdominal ultrasound was then carried out using a Canon ultrasound scanner to accurately assess the pancreas (*Fig.7*), liver (*Fig 12: A-D*), spleen (*Fig.11: A-B*), LN (*Fig.8 and Fig. 9*) and mammary nodules (*Fig.10*).

The described changes in the pancreas are compatible with acute pancreatitis or may be artefactual secondary to peritoneal reaction. The nodules in the caudal aspect of the left mammary chain are compatible with breast neoplasia and are associated with subcutaneous/breast haemorrhage. In the context of the other findings, these nodules may also be consistent with mastitis or neoplastic infiltration. There is metastatic/neoplastic left medial iliac and superficial inguinal lymphadenopathy which may be secondary to breast neoplasia or other unrecognised neoplasia, although reactive lymphadenitis/lymphadenopathy is less likely. Hepatic nodules and masses are compatible with a metastatic neoplasm, possibly secondary to a breast neoplasm or other unrecognised neoplasm or may indicate a primary malignancy such as hepatocellular carcinoma. A benign process such as haematoma, granuloma, adenoma, nodular hyperplasia or haematopoietic nodules from extramedullary haematopoiesis is less likely. Splenic nodules and masses are compatible with either metastatic or primary neoplasia, such as hemangiosarcoma, other sarcomas or round cell neoplasia. Again, a benign process such as haematoma, granuloma, nodular hyperplasia or extramedullary haematopoiesis is less likely.

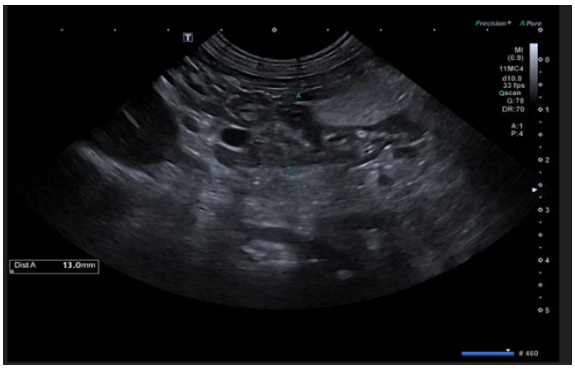

Figure 7: The pancreatic parenchyma is questionably thickened A-B (up to a maximum of 1.3 cm) and hypoechoic. The image was kindly provided by AniCura Valencia Sur Hospital.

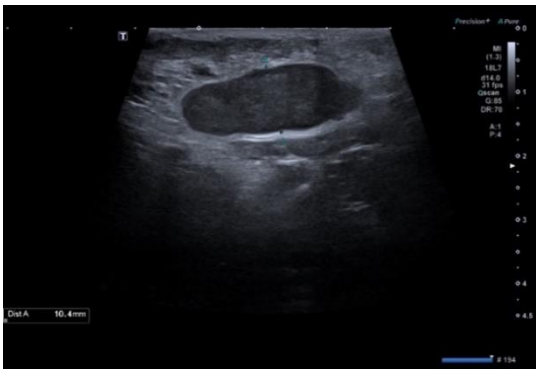

Figure 8: Left medial iliac LN moderately enlarged 10.4 mm hypoechoic, with marked reaction of adjacent fat. The image was kindly provided by AniCura Valencia Sur Hospital.

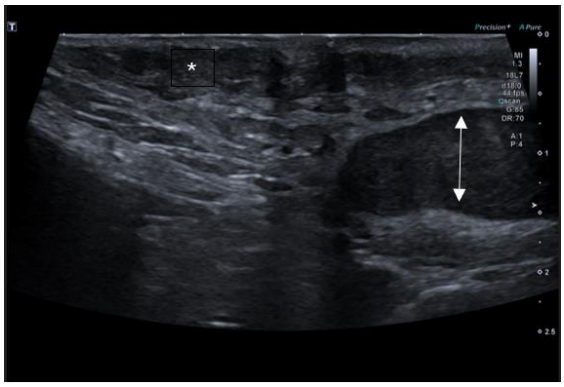

Figure 9: Mammary gland chain (*) and caudally left superficial inguinal LN (arrow) moderately enlarged and 9 mm thick. The image was kindly provided by AniCura Valencia Sur Hospital.

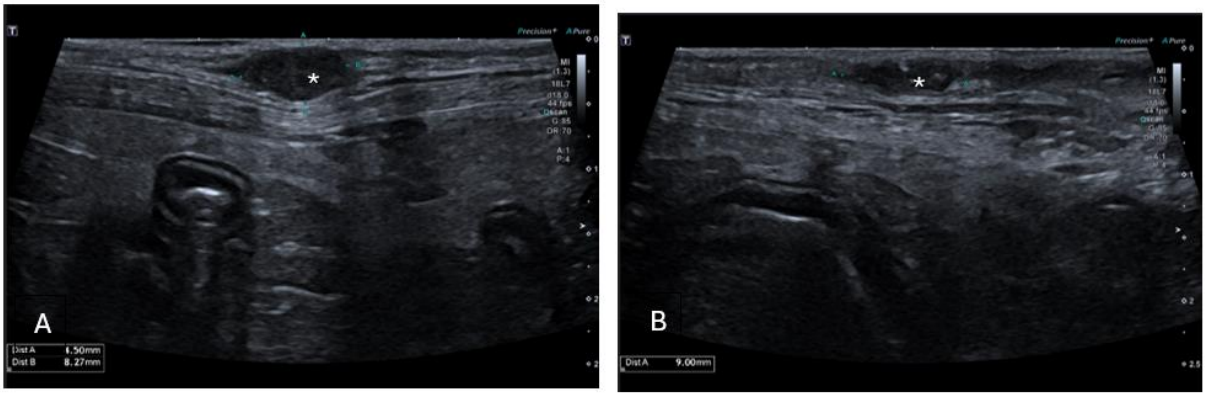

Figure 10: A-B Two nodules (*) in the caudal mammary gland of the left mammary chain, hypoattenuating, irregular, poorly defined, < 1cm of maximum dimension, well delimited. The image was kindly provided by AniCura Valencia Sur Hospital.

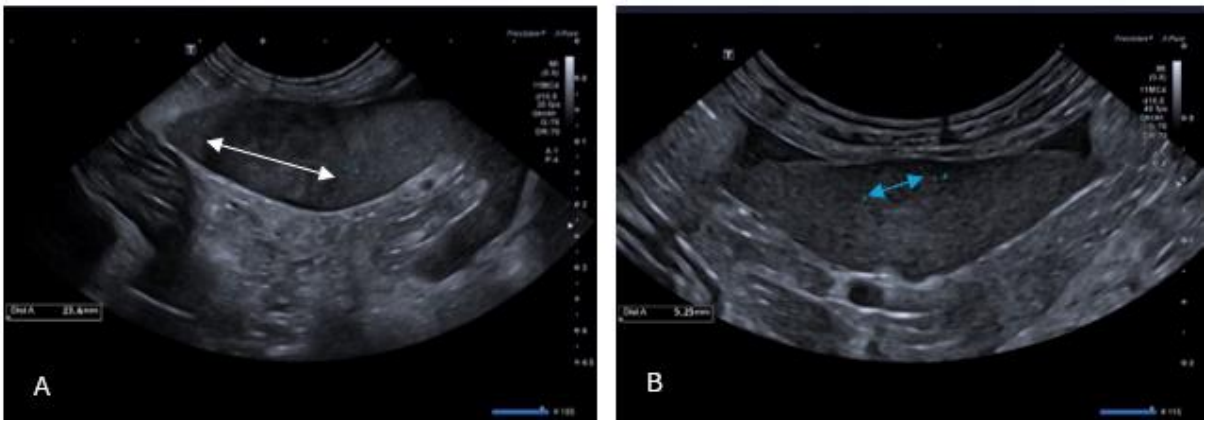

Figure 11: A-B Spleen - Parenchyma normal size, with the presence of heterogeneous, hypoechoic, ill-defined, irregular masses (10A – white arrow) and nodules (10B – blue arrow), with a maximum dimension of up to 2.3 cm. The image was kindly provided by AniCura Valencia Sur Hospital.

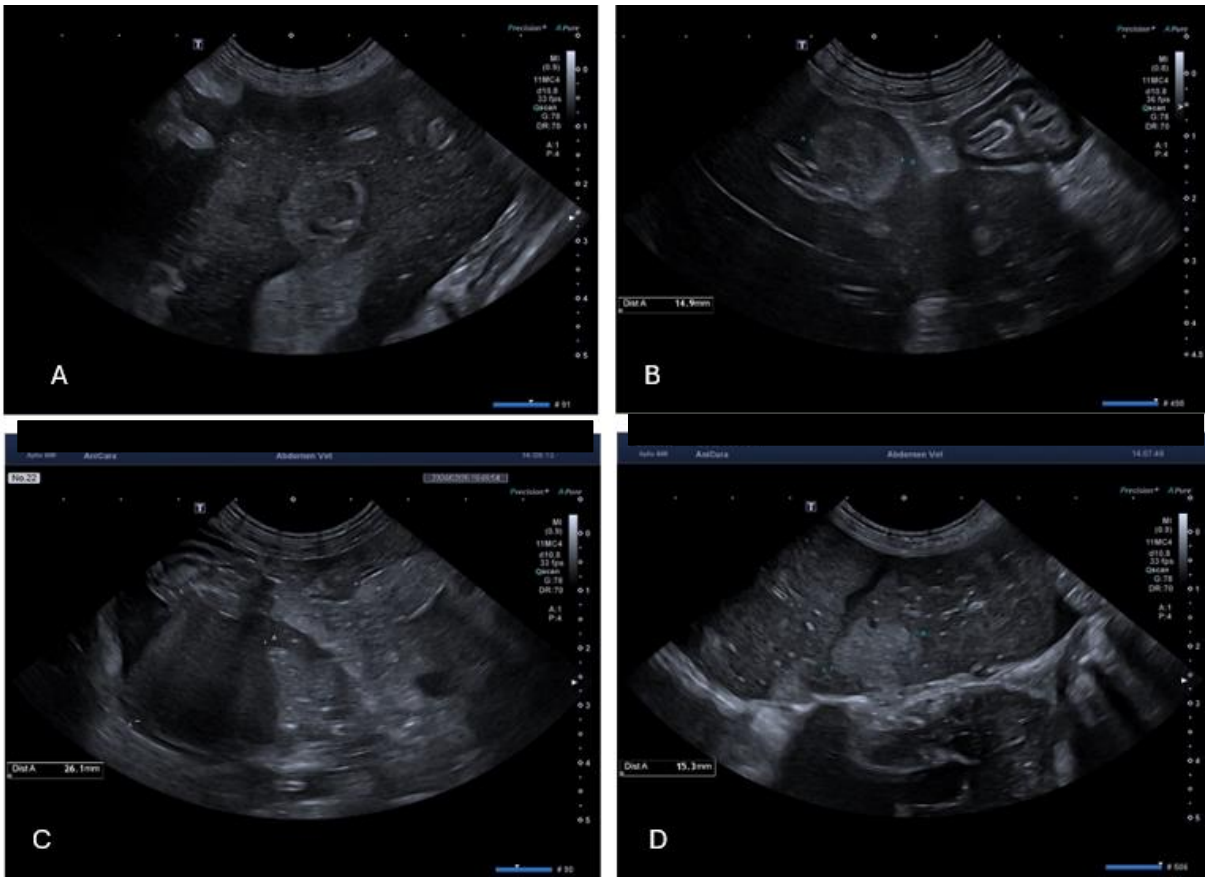

Figure 12: A-D Liver parenchyma of normal size and echogenicity, with multiple hyperechoic hepatic nodules and masses, several of them heterogeneous, well-defined, irregular and with a maximum dimension of up to 2.6 cm (11C). The image was kindly provided by AniCura Valencia Sur Hospital.

Cytology

Several fine needle aspiration biopsies (FNABs) were performed, namely of the breast nodule, left iliac lymph node, left inguinal lymph node, splenic nodule, splenic mass and liver mass.

Mammary nodule:

- Preparations with moderate to high cellularity, with moderate preservation of cell morphology. The background is pink (proteinaceous), with abundant blood, ruptured cells, and necrosis. There is a population of epithelial cells with moderate atypia, which are distributed in cohesive aggregates of varying size, sometimes with a papillary, pseudoacinar or impaled arrangement. They are rounded to polygonal, with well-defined borders and a scarce to moderate amount of medium blue cytoplasm with occasional clear vacuoles. The nuclei are round and oval and sometimes irregular, with finely punctate to heterogeneous chromatin and one or more visible nucleoli, sometimes prominent. Anisocytosis and mitosis are isolated. The nucleus: cytoplasm ratio is high. There is a moderate number of macrophages with a foamy appearance and phagocytised cellular detritus. There are numerous neutrophils, most of them non-degenerate. *(see fig.13)*

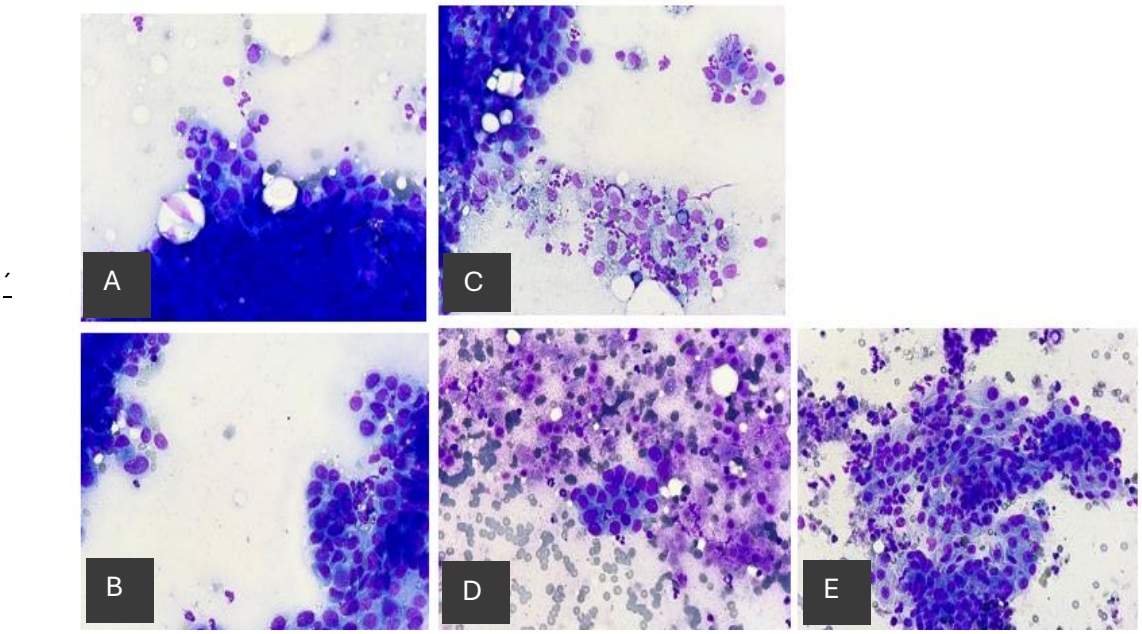

Figure 13: A-E Cytology of mammary node. The image was kindly provided by AniCura Valencia Sur Hospital.

Left iliac and inguinal lymph nodes:

- Described together as showing similar cytological characteristics. The preparations are of moderate cellularity with poor to moderate preservation of cell morphology. The background is pink (proteinaceous) with a moderate amount of blood, frequent ruptured cells and abundant necrosis. There is an epithelial population with similar characteristics to those described in the nodule-breast although less well preserved or necrotic. A moderate number of non-degenerate neutrophils and macrophages are found. *(fig.14 and fig.15)*

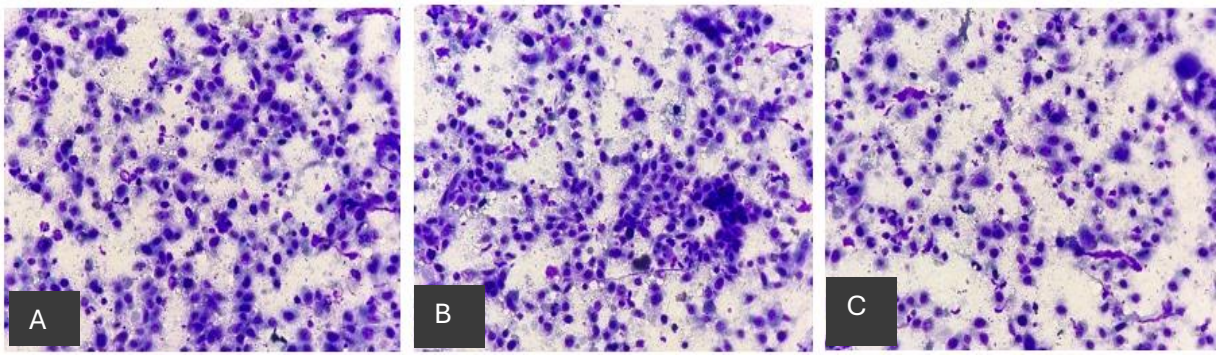

Figure 14: A-C Cytology of left iliac LN. The image was kindly provided by AniCura Valencia Sur Hospital.

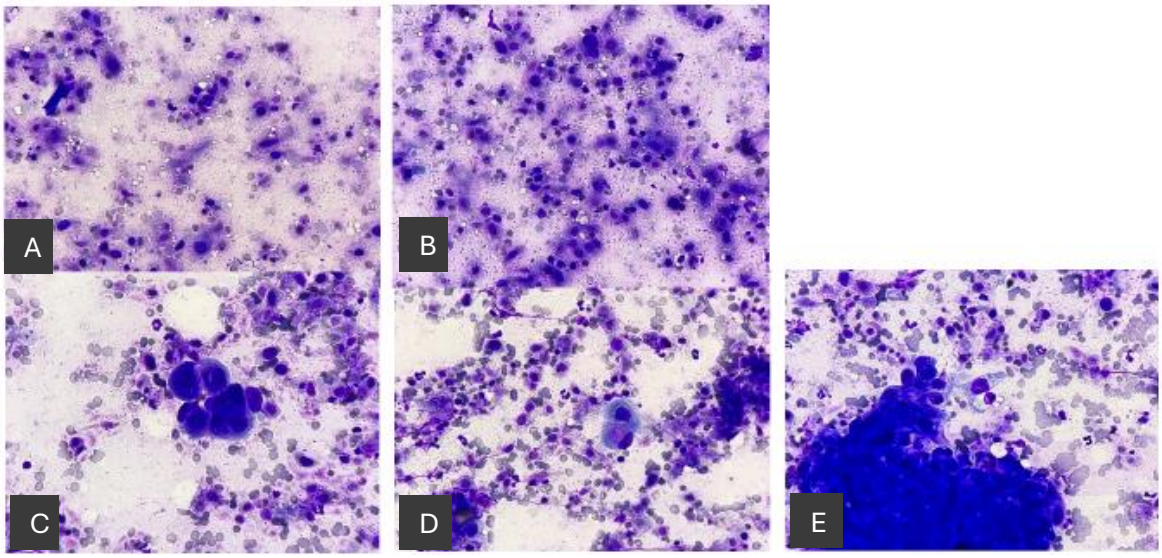

Figure 15: A-E Cytology of left inguinal LN. The image was kindly provided by AniCura Valencia Sur Hospital.

Splenic nodule:

-The preparations are of low to moderate cellularity, with poor to moderate preservation of cell morphology. The background is clear, with abundant blood, frequently ruptured cells and necrosis. Aggregates of splenic stroma with isolated iron deposits are seen. There is a heterogeneous lymphocyte population with a predominance of small forms - there is a slight increase in intermixed and large forms and isolated plasma cells. Erythroid, myeloid/granulocytic precursors and megakaryocytes are seen in moderate numbers. In one of the preparations, there are epithelial cells with similar characteristics to those described in nodule-breast, arranged singly or in small cohesive groups, although generally less well preserved. *(fig. 16)*

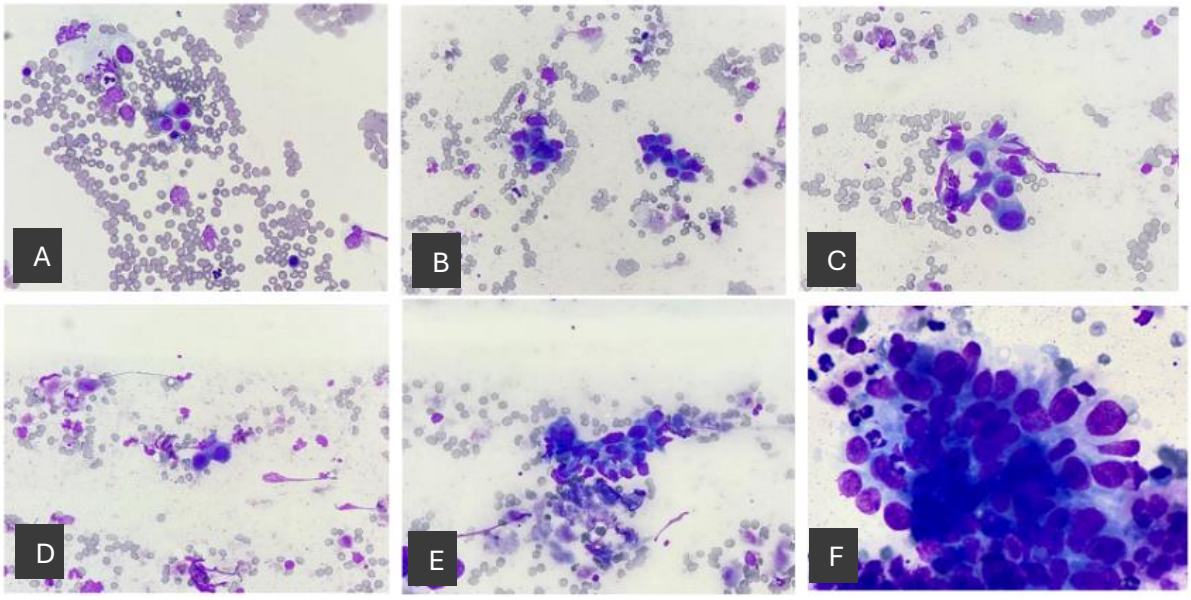

Figure 16: A-F Cytology of spleen node. The image was kindly provided by AniCura Valencia Sur Hospital.

Splenic mass:

- Preparations are of moderate cellularity with moderate preservation of cell morphology. The background is clear, with abundant blood and frequently ruptured cells. Aggregates of splenic stroma with isolated iron deposits are seen. There is a heterogeneous lymphocyte population with a predominance of small forms and a slight increase in intermediate forms. Plasma cells are found in small numbers. Moderate numbers of erythroid, myeloid/granulocytic precursors and megakaryocytes are also seen. There are occasional macrophages, with vacuolated cytoplasm and/or phagocytosed cellular detritus. *(see Fig. 17)*

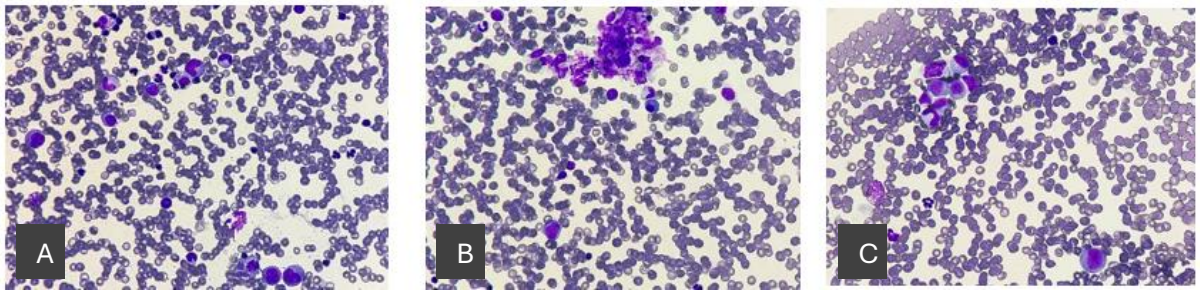

Figure 17: A-C Cytology of spleen mass. The image was kindly provided by AniCura Valencia Sur Hospital.

Liver mass:

- Preparations are low cellularity, with poor to moderate preservation of cell morphology. The background is clear with abundant blood and frequently ruptured cells. Few clusters of hepatocytes are seen. Some have a small number of small, well-defined, clear vacuoles. Anisocytosis and anisokaryosis are mild. Isolated binucleated hepatocytes are seen. The leukocytes found, by number and distribution, are of peripheral blood origin. *(see Fig. 18)*

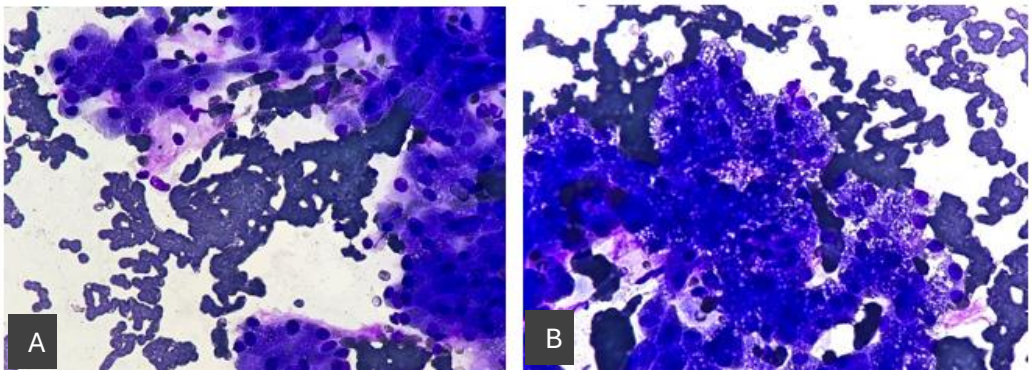

Figure 18: A-B Cytology of liver mass. The image was kindly provided by AniCura Valencia Sur Hospital.

Cytology interpretation:

- Breast lump: malignant epithelial neoplasm, mixed inflammation (neutrophilic and macrophagic) and necrosis.

- Left iliac and inguinal lymph nodes: malignant epithelial neoplasm, mixed inflammation (neutrophilic and macrophagic) and necrosis.

- Spleen nodule: compatible with malignant epithelial neoplasm, reactive lymphoid hyperplasia, extramedullary haematopoiesis and necrosis.

- Spleen mass: moderate extramedullary haematopoiesis and mild reactive lymphoid hyperplasia.

- Hepatic mass: Mild lipid vacuolar degeneration (lipidosis) of hepatocytes

Interpretation comments:

The cytological findings showed a malignant epithelial neoplasm (carcinoma/adenocarcinoma) in the mammary, iliac and inguinal lymph nodes and probably in the splenic node. The cytological origin cannot be determined, although it is most likely a mammary neoplasm with metastases in various sites. It should be remembered that the main criteria for malignancy in mammary neoplasms are invasive growth and invasion of lymphatic vessels, which can only be assessed by histology. In this case, as the neoplastic population was found in the LNs and spleen, this would confirm malignancy. The inflammation and necrosis seen in these sites are secondary to the neoplastic growth. Reactive hyperplasia in the LNs and splenic mass is a non-specific response of the lymphoid tissue secondary to antigenic stimulation by inflammation, infection or neoplasia. Extramedullary haematopoiesis is mild and in most cases of uncertain clinical relevance. In the liver, lipid vacuolar degeneration of hepatocytes has been reported. This may reflect a disorder of lipid/carbohydrate metabolism (e.g. endocrinopathies such as diabetes mellitus or hypothyroidism) or be a consequence of inflammatory processes, exposure to hepatotoxic substances or hypoxia. These findings should be treated with caution due to the low cellularity of the preparations and the suboptimal preservation of cell morphology.

Recommendations:

- Oncological management of the patient is recommended and, if clinically indicated, biopsy and histopathological study of the mammary lesion and lymph nodes for a better characterisation of the lesions.

4.6. Diagnosis

Malignant epithelial neoplasia of the mammary gland with distant metastases (lymph nodes and spleen) with associated hemoabdomen.

4.7. Treatment and Evolution

Lola was hospitalised for symptomatic treatment (methadone 0.1 mg/Kg IV q4h; Maropitant 1mg/Kg IV SID; Ethamsylate 12,5 mg/Kg QID) and fluid therapy with Ringer's lactate at a maintenance rate with correction of 5% dehydration in 24h (19.6 ml/h). She was only hospitalised for two days to control the hemoabdomen and then went to her veterinary clinic for monitoring. After she was no longer hospitalised, Lola didn't receive any oncological care due to her tutor´s decision.

4.8. Follow-Up

Any current records of Lola's case are not available, but until April Lola hadn't undergone any kind of treatment for the breast cancer, but she had stabilised from the hemoabdomen and pancreatitis and remained stable until then.

4.9. Prognosis

Reserved due to being a malignant epithelial neoplasm with metastases in the lymph nodes and spleen.

4.10. Discussion

Lola is an 11-year-old dog who was neutered at the age of 3 due to pyometra. Considering all the information reported in this literature review, Lola has three risk factors for mammary neoplasia. i) Age (11 years) - The risk of MGTs in dogs increases with age and becomes clinically significant at 7 or 8 years of age. This risk continues to increase until the age of 11-13 years; ii) Hormonal exposure - she was not neutered until after the age of 3 years - the protective effect of OHE diminishes over the first few oestrous cycles and some studies suggest that there is no significant benefit after the age of 4 years. Iii) Breed - MGTs are more common in smaller breeds, such as Chihuahuas. (Vail et al., 2020) At the time of hospitalisation, Lola presented with a peritoneal effusion consistent with a hemoabdomen, presumably due to the liver and/or splenic lesions. Considering the characteristics of Lola's mammary neoplasm, which has already metastasised to the lymph nodes and spleen, with possible metastasis to the liver, we can stage it as stage V according to Table A4. (Vail et al., 2020) Regarding the cytological results of the liver mass, it should be borne in mind that they must be interpreted with caution due to the low cellularity of the preparations and the suboptimal preservation of the cell morphology. In addition, ultrasound images of the liver showed the presence of nodules and a liver mass, most likely consistent with metastatic neoplasia. Therefore, it cannot be excluded that in addition to metastasis in the spleen, there is also metastasis in the liver. In this case, it would be interesting to perform an MRI to better identify and characterise the liver lesions. However, the treatment and prognosis will not differ depending on whether there is only metastasis in the lymph nodes and spleen or also in the liver. An important part of the staging of mammary neoplasms is the three-view thoracic radiographs,

which did not reveal any lung metastases. A biopsy and histopathological study of the mammary lesion and lymph nodes (Diagnostic gold standard) should be carried out for an accurate characterisation of the neoplasia. (Vail et al., 2020)

Surgery remains the gold standard treatment for breast tumours. Lola has 2 nodules, one on the left M3 and one on the right M2, and she also has inflammation on the left M5 with associated erythema and haematoma. In animals with multiple MGTs, more extensive resections such as regional mastectomy, unilateral chain mastectomy or bilateral mastectomy may be considered. The concept of regional mastectomy is based on the lymphatic drainage patterns of the mammary glands. Glands 1-3 drain into the cranial sternal and axillary lymph nodes, whereas glands 4 and 5 drain into the superficial inguinal and medial iliac lymph nodes. Therefore, glands 1-3 or 4 and 5 should be removed together. In addition, the associated regional lymph nodes should be removed and examined histopathologically. (Klopfleisch, 2016)

Unilateral or bilateral radical mastectomy involves the removal of all five mammary glands in one block. The benefits of this procedure in improving survival are controversial. It creates a large wound, which may increase the risk of delayed postoperative healing. However, due to the frequent occurrence of multiple tumours in dogs, radical mastectomy may be necessary. Bilateral mastectomy is only recommended in dogs with pendulous mammary glands as it allows tension-free wound closure. For staged bilateral mastectomies, a minimum interval of 2 weeks is recommended. Lola could therefore undergo a radical mastectomy on the left and a regional mastectomy on the right, but Lola is an elderly patient with metastases already identified in her spleen and lymph nodes. Therefore, surgery is not the best recommendation due to the post-operative period and the need for general anaesthesia, and successful surgical outcomes are achievable in cases without lymphatic or distant metastasis involvement or those with less aggressive histological types. (Cassali et al., 2020; Vail et al., 2020)

Palliative treatment is recommended. Chemotherapy can be used as a palliative treatment but the criteria for selecting and implementing the type of chemotherapy are not standardized. It is noteworthy that NSAIDs, either alone or in combination with chemotherapy, have been observed to effectively extend survival in dogs with high-risk (grade III and/or advanced clinical stage) or inflammatory carcinomas, as indicated by various retrospective and prospective studies. According to Table A8, the most proper treatment for this case would be a protocol with Carboplatin + NSAIDs (Firocoxib). There is a study that did 3 cycles of carboplatin (300 mg/m^2 or 10 mg/kg IV as a slow bolus every 3 weeks) plus firocoxib (5 mg/kg, every 24 hours during six months) selectively inhibits COX-2 without affecting Cox-1, making it the preferred non-steroidal anti-inflammatory drug (NSAID) for long-term use in dogs and the outcome was favourable having a median survival of 570 days. (Eunice Lavalle et al., 2012; Vail et al., 2020). Although the survival time in this study was good, Lola is a patient with a poor prognosis

and even with this treatment the survival time would be shorter, and the quality of life would not improve significantly.

During Lola's hospitalisation, the main aim of the treatment was to improve her well-being and comfort (methadone 0.1mg/Kg IV q4h; Maropitant 1mg/Kg IV SID;) and to stop the bleeding that was leading to the hemoabdomen (Ethamsylate 12,5 mg/Kg QID). This brings up an extremely critical issue which is pain management in cancer patients. Drug treatment is central to pain management in veterinary oncology, and following the WHO cancer pain ladder is a logical strategy. For mild pain, prescribing an NSAID with or without an adjuvant like gabapentin is advised. (Biller et al., 2016). Long-term use of oral opioids is discouraged for chronic pain management in dogs, due to concerns about potential human abuse and poor uptake in canines, attributed to enterohepatic recirculation and elimination. Opioid-type drugs, including those combined with acetaminophen, have shown inadequate analgesia for chronic pain due to unreliable drug levels after oral administration. On the other hand, several NSAIDs are approved for chronic pain management in dogs, with potential risks of renal, hepatic, and gastrointestinal toxicity, albeit likely low and poorly understood. Studies suggest no increased organ-based toxicity with longer NSAID treatment, but a positive trend towards increased efficacy. (Gruen et al., 2022).

5. CONCLUSION

A literature review was undertaken to document the imaging features of mammary gland tumour metastases in dogs and cats to unusual sites. From this work, it can be concluded that the lungs and lymph nodes are the most common sites of MGT metastasis, followed by the heart, bone and liver. Liver metastases are present in 25% of feline mammary carcinoma patients and in dogs, when metastases occur in the liver, they are usually from breast cancer. This information is important when approaching a patient with breast cancer, as liver metastases may occur, further reinforcing the importance of abdominal ultrasound in the staging of this neoplasia. Although MRI is the preferred imaging method in the search for liver metastases, it is an expensive method and there is still little availability in the current clinical context in Portugal, so abdominal ultrasound becomes the most relevant imaging method, especially Doppler, because even small metastases can disrupt hepatic blood flow dynamics, leading to alterations in perfusion indices. CEUS is also a very interesting method that can be applied in veterinary oncology due to its ability to predict malignancy.

In contrast to human medicine, bone metastases from mammary neoplasia are extremely rare in dogs and cats. This is extremely interesting and deserves to be studied in more detail. We need to understand why this happens, whether it's because of the different anatomy, the different biological components involved, the lack of access to diagnostic imaging, or because these animals don't live long enough to reach the stage where bone metastases are evident. It is therefore important to encourage research in this area to try to understand why there is a difference in the prevalence of bone metastases in MGT between humans and dogs. It should be done a large-scale study in dogs and cats in which mammary neoplasms were examined for possible bone metastases and, where possible, necropsies were performed for future confirmation, so that we could systematically assess the prevalence of bone metastases in MGT.

MGTs are the most common type of tumour in uncastrated females, making them an important and necessary focus of research. In addition, the anatomical, histological and behavioural similarities with human breast cancer make it an essential point in the concept of one health and should be a starting point for action on prevention, diagnosis and treatment. Throughout this endeavour, we encountered a conspicuous absence of scientific literature on the subject. This underscored the critical importance of conducting such studies, as they play a pivotal role in advancing our collective knowledge and improving both the scientific and clinical communities.

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ANNEXE A

Classification and Grading of Canine and feline Mammary Tumors (Goldschmidt et al., 2011)

1974 HISTOLOGIC CLASSIFICATION[7]	1999 HISTOLOGIC CLASSIFICATION[13]
I. Carcinoma	1 Malignant tumors
A. Adenocarcinoma	1.1 Noninfiltrating (in situ) carcinoma
1. Tubular	1.2 Complex carcinoma
(a) Simple type	1.3 Simple carcinoma
(b) Complex type	1.3.1 Tubulopapillary carcinoma
2. Papillary	1.3.2 Solid carcinoma
(a) simple type	1.3.3 Anaplastic carcinoma
(b) complex type	1.4 Special type of carcinomas
3. Papillary cystic	1.4.1 Spindle cell carcinoma
(a) simple type	1.4.2 Squamous cell carcinoma
(b) complex type	1.4.3 Mucinous carcinoma
B. Solid carcinoma	1.4.4 Lipid-rich carcinoma
(a) simple type	1.5 Sarcoma
(b) complex type	1.5.1 Fibrosarcoma
C. Spindle cell carcinoma	1.5.2 Osteosarcoma
(a) simple type	1.5.3 Other sarcomas
(b) complex type	1.6 Carcinosarcoma
D. Anaplastic carcinoma	1.7 Carcinoma or sarcoma in benign tumor
E. Squamous cell carcinoma	2 Benign tumors
F. Mucinous carcinoma	2.1 Adenoma
II. Sarcoma	2.1.1 Simple adenoma
A. Osteosarcoma	2.1.2 Complex adenoma
B. Fibrosarcoma	2.1.3 Basaloid adenoma
C. Combined sarcoma	
D. Other sarcomas	2.2 Fibroadenoma
III. Carcinosarcoma (malignant mixed tumor)	2.2.1 Low-cellularity fibroadenoma
IV. Benign	2.2.2 High-cellularity fibroadenoma
A. Adenoma	2.3 Benign mixed tumor
B. Papilloma	2.4 Duct papilloma
1. Duct papilloma	3 Unclassified Tumors
2. Duct papillomatosis	4 Mammary hyperplasia and dysplasia
C. Fibroadenoma	4.1 Ductal hyperplasia
1. Pericanilicular	4.2 Lobular hyperplasia
2. Intracanilicular	4.2.1 Epithelial hyperplasia
(a) noncellular type	4.2.2 Adenosis
(b) cellular type	4.3 Cysts
3. Benign mixed tumor	4.4 Duct ectasia
4. Total fibroadenomatous change	4.5 Focal fibrosis (fibrosclerosis)
D. Benign soft tissue tumor	4.6 Gynecomastia
V. Unclassified Tumors	
VI. Dysplasias	
A. Cyst	
1. Nonpapillary	
2. Papillary	
B. Adenosis	
C. Epitheliosis	
D. Duct ectasia	
E. Fibrosclerosis	
F. Gynecomastia	
G. Other nonneoplastic proliferative lesions	
1. Noninflammatory lobular hyperplasia	
2. Inflammatory lobular hyperplasia	

ANNEXE B

Total blood count and serum biochemistry of clinical case

Table B1: Total Blood Count, kindly provided by AniCura Valencia Sur Hospital.

Parameter	Reference Interval (IDEXX)	21/02/2024
Erythrocytes (10^6/µL)	5.64 – 8.87	6.20
Haemoglobin (g/dL)	13.1 – 20.5	13.0
Haematocrit (%)	37.3 – 61.7	38.7
VCM (fL)	61.6 – 73.5	62.
HCM (pg)	21.2 – 25.9	21.0
CHCM (g/L)	32.0 – 37.9	33.6
RDW (%)	13.6 – 21.7	15.5
Reticulocytes (10^3/µL)	10.0 – 110.0	70.7
Reticulocytes (%)		1.1
Platelets (10^3/µL)	148 – 484	46
MPV (fL)	8.7 – 13.2	20.1
Plateletcrit (%)	0.14 – 0.46	0.09
Leukocytes (10^3/µL)	5.05 – 16.76	14.88
Segmented neutrophils (10^3/µL)	2.95 – 11.64	8.72
Lymphocytes (10^3/µL)	1.05 – 5.10	4.57
Monocytes (10^3/µL)	0.16 – 1.12	1.51
Eosinophils (10^3/µL)	0.06 – 1.23	0.08
Basophils (10^3/µL)	0.00 – 0.10	0.00

Table B2: Serum biochemistry, kindly provided by AniCura Valencia Sur Hospital.

Parameter	Reference Interval (IDEXX)	20/02/2024	21/02/2024
Glucose (mg/dL)	74 – 143	119	124
Albumin (g/dL)	2.3 – 4.0	3.3	----
Globulin (g/dL)	2.5 – 4.5	4.0	---
Albumin/Globulin		0.8	---
Total proteins (g/dL)	5.2 – 8.2	7.3	---
ALP (U/L)	23 – 212	540	---
AST (U/L)	0 – 50 U/L	102	---
ALT (U/L)	10 – 125	>1000	---
GGT (U/L)	0 – 11	0	---
Total bilirubin (mg/dL)	0.0 – 0.9	0.8	----
Creatinine (mg/dL)	0.5 – 1.8	3.8	0.8
BUN (mg/dL)	7 – 27	79	14
BUN/CRE		21	18
Sodium (mmol/L)	144 – 160	140	---
Potassium (mmol/L)	3.5 – 5.8	5.5	---
Chlorine (mmol/L)	109 – 122	106	---
Na/K		25	----
Osmolarity (mmol/kg)		305	----

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