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Feline Mammary Tumors – Literature Review and Case Study

Macskák emlődaganatai – irodalmi áttekintés és esettanulmányok

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Abstract

Feline mammary tumors represent the third most common type of neoplasm in female cats, with a proportion of 11-17%. The majority of these tumors are malignant, with an aggressive course of disease.

Even though, there are many treatment options like different surgical approaches or adjuvant therapies, the prognosis for this neoplastic disease is still very poor. With a survival time of less than one year in distinct cases.

In this study, a literature review was conducted in which various aspects were examined. This included an overview of the epidemiologic factors, like predispositions in older, non-neutered female cats, as well as risk factors, such as hormonal treatments.

The clinical presentation was also evaluated, with different opinions on the distribution of the tumor, in the thoracic or abdominal glands.

Diagnostic approaches, like hematology or diagnostic imaging were reviewed, with evaluation of their prognostic importance.

The therapeutic methods were also evaluated, including different forms of mastectomy, for example, regional or radical mastectomy. Various forms and treatment plans of chemotherapy were assessed as well. Prognosis was also reviewed, according to the most important factors such as tumor size, lymph node involvement and metastasis.

Furthermore, a case study consisting of 24 cases, from the years 2017-2024, was carried out. The study came to similar conclusions regarding the general characteristics of the cats, as those in previously reviewed studies. This included older (mean age: 10.2 years) female cats.

The OST was shown to be distinctly longer in neutered animals (mean: 721 days non-neutered vs 1123.9 days neutered), with no lymph node involvement (mean: 658.3 days with lymph node involvement vs 1633 days without lymph node involvement), no metastasis (mean: 686 days with distant metastasis vs 1198 days without distant metastasis) and with solitary (mean: 721 days with multiple tumors vs 835 days with solitary tumor) tumors.

As the case study consisted of only few cases, further studies are needed to better understand and approach this neoplastic disease.

Absztrakt

A macskák emlődaganatai a harmadik leggyakoribb daganattípust képviselik a nőstény macskáknál, 11-17%-os arányukkal. E daganatok többsége rosszindulatú, a betegség agresszív lefolyású.

Annak ellenére, hogy számos kezelési lehetőség létezik, mint például különböző sebészeti megközelítések vagy adjuváns terápiák, ennek a daganatos betegségnek a prognózisa még mindig nagyon rossz. A túlélési idő határozott esetekben kevesebb mint egy év.

Ebben a tanulmányban irodalmi áttekintést végeztünk, amelyben különböző szempontokat vizsgáltunk. Ez magában foglalta az epidemiológiai tényezők áttekintését, mint például az idősebb, nem ivartalanított nőstény macskák hajlamai, valamint a kockázati tényezők, mint például a hormonális kezelések.

A klinikai megjelenést is értékelték, a daganat eloszlásáról - a mellkasi vagy a hasi mirigyekben - eltérő véleményekkel. A diagnosztikai megközelítéseket, mint a hematológia vagy a képalkotó diagnosztika, áttekintették, és értékelték prognosztikai jelentőségüket.

A terápiás módszereket is értékelték, beleértve a masztektómia különböző formáit, például a regionális vagy radikális masztektómiát. A kemoterápia különböző formáit és kezelési terveit is értékelték. A prognózist is áttekintették a legfontosabb tényezők, például a daganat mérete, a nyirokcsomók érintettsége és az áttétképződés alapján.

Továbbá 24 esetből álló esettanulmányt végeztek, a 2017-2024 közötti évekből. A tanulmány a macskák általános jellemzőit illetően hasonló következtetésekre jutott, mint a korábban áttekintett tanulmányok. Ebben idősebb (átlagéletkor: 10.2 év) nőstény macskák szerepeltek.

Az OST egyértelműen hosszabbnak bizonyult az ivartalanított állatoknál (átlag: 721 nap nem ivartalanítva vs. 1123.9 nap ivartalanítva), nyirokcsomó érintettség nélkül (átlag: 658.3 nap nyirokcsomó érintettséggel vs. 1633 nap nyirokcsomó érintettség nélkül), metasztázis nélkül (átlag: 658.3 nap nyirokcsomó-érintettséggel vs 686 nap távoli metasztázissal vs 1198 nap távoli metasztázis nélkül) és szoliter (átlag: 721 nap többszörös daganattal vs 835 nap szoliter daganattal) daganattal.

Mivel az esettanulmány csak néhány esetből állt, további vizsgálatokra van szükség e daganatos betegség jobb megértéséhez és megközelítéséhez.

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List of abbreviations

A	
A1	Cranial Abdominal Gland
A2	Caudal Abdominal Gland
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
APP	Acute Phase Protein
B	
BCG	Bacille Galmette-Guérin
BSH	British Shorthair
C	
COX	Cyclooxygenase
CR	Complete Response
CT	Computed Tomography
D	
DFS	Disease Free Survival
DFP	Disease Free Period
DNA	Deoxyribonucleic Acid
E	
EDTA	Ethylene Diamine Tetraacetic Acid
EE	Elston and Ellis Grading System
ER	Estrogen Receptor
ESH	European Shorthair
F	
FAD	Fibroadenomatous Hyperplasia
FNA	Fine-Needle Aspiration
G	
GFR	Glomerular Filtration Rate
GH	Growth Hormone
GPT	Glutamate Pyruvate Transaminase

H	
Hb	Hemoglobin
Ht	Hematocrit
HR	Hormonal Receptor
I	
IGF	Insulin-like Growth Factor
IL	Interleukin
IV	Intravenous
L	
L-MTP-PE	Liposome-Enclosed-Muramyltripeptid-Phosphatidylethanolamin
Ln	Lymph Nodus
M	
MA	Megestrol Acetate
MCV	Mean Corpuscular Volume
MCHC	Mean Corpuscular Hemoglobin Concentration
MIB-1	Molecular Immunology Borstel-1
MMEE	Mitotic-Modified Elston and Ellis
MOST	Mean Overall Survival Time
MPA	Medroxyprogesteron Acetate
MPFS	Mean Progressive Free Survival Time
MPV	Mean Platelet Volume
MST	Mean Survival Time
N	
N	Nervus
NEU	Neutrophils
NLR	Neutrophile-to-Lymphatic Ratio
Nn	Nervi
NSAID	Non-Steroidal-Anti-Inflammatory-Drugs
O	
OS	Overall Survival
OST	Overall Survival Time

P

PCT	Plateletcrit
PCV	Packed Cell Volume
PD	Progressive Disease
PO	Per Os
PR	Progesterone Receptor
PRsp	Partial Response

R

RBC	Red Blood Cells
REE	Revised Elston and Ellis Grading System
RFP	Relapse Free Period
RNA	Ribonucleic Acid

S

SAA	Serum Amyloid A
SD	Standard Deviation
SERM	Selective Estrogen-Receptor-Modulators
StD	Stable Disease

T

T1	Cranial Thoracic Gland
T2	Caudal Thoracic Gland
TP	Total Protein
TSS	Tumor-Specific Survival

V

V	Vena

W

WBC	White Blood Cells

1. Introduction

Feline mammary tumors have a high clinical relevance with an incidence of 11-17% [1][2][3] and are the third most common neoplastic disease in female cats.

Due to their aggressive nature and rapid progression, they have a poor prognosis in most cases. Survival time can be less than one year in certain severe cases [4][5][6].

The rapid progression of the disease is, among other things, due to its distribution via the lymphatic vessels. The main lymphatic supply, to the eight mammary glands in cats, is via the axillary lymphatic center and inguinal lymph node. There is no evidence of lymphatic drainage between the glands.

Another potential route for the tumor spread is via the blood vessels, mainly the veins, as these run in large quantities across the midline and can thus pave the way for the tumor to reach the abdominal wall [7].

Some predisposing factors have already been researched and proven. These include the cat's age (more common in cats over 10 years old) [8][9][10] and sex (more common in females) [9].

In terms of breed, it can be said that oriental breeds, especially the Siamese cats, are predisposed. However, due to the clear majority in the number of the ESH breed, there are more overall cases [4][11].

The neutering status of the animals, as well as the timing of spaying, influences the likelihood of developing mammary tumors. Spaying at a young age (up to one year) significantly reduces the risk [12][13][14].

This is due to the hormonal influence, which cannot yet be fully understood, in relation to both endogenous and exogenous steroid hormones [15].

The tumors are distributed over the four pairs of glands, and here too, the studies are not entirely in agreement about their distribution [10][16][17].

Due to the aggressiveness of the tumor, the diagnostic and treatment approach is of particular importance.

The clinical examination includes palpation of the mammary glands, as well as the lymph nodes and assessment of the general condition [18].

Other diagnostic tools include blood tests, cytology and histopathology (after sampling during surgery), immunohistochemistry and diagnostic imaging.

One of the most important steps during the diagnostic process is the staging of the tumor according to the size of tumor, lymph node involvement and metastasis [19].

There are several different parameters and markers that can be used to determine the prognosis, but there are variations in cut-off values and interpretation of the results depending on the various authors [20][21].

Multiple treatment options are being investigated in relation to OST and RFP. This comprises methods like surgery, including regional or radical mastectomy, or adjuvant therapies such as chemotherapy, immunotherapy, hormone therapy or other therapeutic approaches.

This study analyzes publications on this topic and summarizes the different clinical, diagnostic, therapeutic and prognostic results.

In addition, a case study was conducted, consisting of n=24 cases from 2017-2024, collected from two small animal clinics.

Including evaluation of clinical characteristics, diagnosis and treatment methods.

Beyond that, correlations between different parameters are examined. These are, for example, the diagnostically important factors: tumor size, lymph node involvement and metastasis. The latter are correlated with various blood, and biochemical values. Furthermore, the OST is determined.

This study summarizes the most important results and discussions of various studies and compares it to its own results.

2. Dedication

I want to thank my supervisor, Prof. Dr. Vajdovich, for his help and guidance.

And I want to thank Budapest, for the 5 best years.

3. Literature Review

3.1. Feline Mammary Tumors

Mammary tumors are an important and common disease and account for around 11-17% of all cancers in female cats [3]. They are the third most common type of tumor in cats, preceded by lymphomas and skin tumors [11]. However they are still less common, than mammary tumors in dogs, with about half as frequent occurrence [8][9]. Most of the time, mammary tumors have an aggressive and fatal course of action.

3.1.1. Anatomy

Mammary glands are modified superficial apocrine sweat glands. Cats have four pairs of mammary glands that extend from axillar to inguinal. They are referred to as thoracic (T1 and T2) and abdominal (A1 and A2) glands. Some cats also have rudimentary/accessory glands in the inguinal area. The glands are slightly offset from each other to make it easier for the kittens to reach them. In the middle of the two chains lies the sulcus intermammarius [22].

The arterial blood supply of the thoracic mammary glands is provided by the internal thoracic artery (medial supply), the intercostal artery and the lateral thoracic artery (lateral supply). Supply to the abdominal glands happens through the cranial superficial epigastric artery and the caudal intercostal artery (A1), as well as the caudal superficial epigastric artery (A2).

The veins mainly run parallel to the arteries, with more veins in the midline area. This, as well as the flow of blood from the thoracic glands through the intercostal and internal thoracic veins, can lead to further distribution of the tumor, including to opposite glands or the thoracic wall.

The nervous innervation is provided by *N. genitofemoralis* and *Nn. intercostales* [23].

The lymphatic drainage is the major cause for the spread of tumor cells [7] (see **Figure 1**). Cranial drainage is to the axillary lymph center, including the *Ln. axillaris proprius, Ln. axillaris primae costae and Ln. axillaris accessories.* The caudal drainage is to the superficial inguinal lymph center [24].

T1 always has cranial flow to the axillary lymph center, while the caudal thoracic gland (T2) sometimes also has caudal drainage to the superficial inguinal lymph center. A1 has similar drainage as T2, flowing both in cranial and caudal direction.

The caudal abdominal gland only has continuous drainage to the superficial inguinal lymph center. A1, A2 and T1 also drain to the *Ln. sternales craniales.*

A study by Raharison and Sautet, 2006 [24] did not find any lymphatic drainage between consecutive or opposite lying mammary glands.

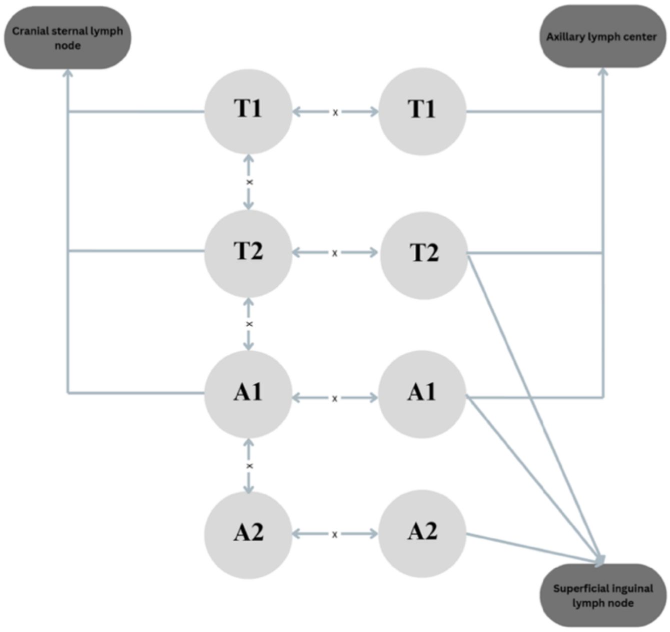


FIGURE 1: LYMPH DRAINAGE SYSTEM

3.1.2. Epidemiology and Risk Factors

Mammary tumors can occur in cats of all ages, but they are most commonly found in older animals. The average age is around 10-12 years [8][10]. The risk increases with age, but only becomes significant after 7-9 years of age [9].

Queens have a higher incidence than male cats. In particular, intact cats have a risk that is seven times higher than that of neutered animals [9].

A study by Overley et al., 2005 [12] proved, that the early spaying of cats has a high protective effect, if they are spayed before the age of six months.

The data of the study showed that the risk of feline mammary tumors could be reduced by 91%. A spaying at the age of less than one year has been shown to reduce the risk by 86%.

This means that animals spayed before the age of six months and animals spayed between 6 and 12 months, only had a risk of 9% and 14% compared to intact cats.

A spaying after 24 months of age, provides no additional benefit [25].

As discussed above, feline mammary tumors can also be found in tomcats, but only in around 1-5% [8][10][26].

Kessler, 2022 [8] had a proportion of 84 male cats in their study of 2324 cats, which corresponded to 3.6%. The neutering status was of no influence. However, one-third of the animals were known to have been previously treated with steroid hormones. A larger proportion of the males were Siamese cats. It was also shown, by the latter literature, that malignant tumors are less common in male cats (81%) than in females.

There are certain breed predispositions for this neoplastic disease. According to several studies Siamese and according to newer studies Oriental breeds in general, are more prone to mammary tumors than other breeds [11]. They have twice the risk of developing a mammary tumor than all other breeds together [4].

In the publication by Ito et al., 1996 [27] out of 53 cases of cats with malignant mammary tumors, 27 were Japanese domestic, 18 were Siamese and 4 were Persian. Which accounts for 92.45%.

Garcês et al., 2019 [28] showed, that in European shorthair cats, the most common tumors (372 out of 1788, which equals 42.2%) were of mammary origin. In Siamese cats, 45.3% (77 out of 170) of tumors were mammary neoplasms.

In these predisposed breeds, the mammary tumors will also occur at a younger age [29]. That points towards a genetic predisposition [8][27]. This is further confirmed by the fact that these breeds are predisposed to tumors in general [30]. It can result from a defect in the function of a tumor suppressor gene or alterations in a tumor susceptibility gene [25].

Kessler, 2022 [8] stated, that 92% of all tumors in domestic cats are malignant. Persian cats have a lower proportion of malignant tumors with 83%.

Another tendency is observed in Siamese cats, in which almost no benign tumors are found, which is why the author of the literature claims to always assume malignant mammary tumors in this breed.

The administration of hormones and its effect on the occurrence of mammary tumors is still not fully clear. However, it is known that growth hormones are a mediator of progestin-induced mammary tumorigenesis [15].

Mol et al., 1996 [13] stated: ”Progesterone stimulates directly or indirectly the expression of the gene encoding growth hormone (GH) which may stimulate the proliferation of epithelial cells directly or indirectly via induction of IGF release”.

The study by Tamada et al., 2003 [14] showed the effect of chlormadinone acetate, which is an agent with an anti-androgen and progestagene effect and is used to prevent the cycle. After 4 to 6 years of treatment with chlormadinone acetate, 2 out of 19 cats developed abnormal clinical signs like mammary vesicles or vaginal discharges.

In the case of a treatment period of 6 to 8 years, 6 out of 16 queens developed signs like mammary vesicles and/or mammary nodules, vaginal discharges or abdominal dropsy. One of the animals was diagnosed with pyometra.
After 8 to 10 years of treatment, 1 out of 4 animals showed clinical signs like mammary nodules and pyometra.

The research by Giménez et al., 2010 [7] also mentioned, as possible reason, the duration of estrogen and progesterone exposure.

Misdorp et al., 1991 [31] claimed that the elevated risk of either mammary carcinoma or a benign mammary lesion, was affiliated with regular administration of progestogens. However, this did not apply to irregular progestin administration, and it was also not related to an earlier appearance of tumors of the mammary glands.

There are many other studies with different opinions about the effect of hormones and the occurrence of mammary tumors in cats.

This study by Hayden et al., 1989 [32] compared cats treated with megestrol acetate (MA) with a group of cats that were not treated with steroid hormones. MA is a very potent antiovulatory agent, that can be used, for example, for the treatment of dermatological, behavioral or reproductive problems.

The result showed that out of the 17 treated animals, 9 developed fibroepithelial hyperplasia, 3 lobular hyperplasia, 2 duct ectasia, 1 tubular adenoma and 2 developed carcinomas. The two cats that developed carcinomas were a spayed female and a castrated male.

The control group of untreated cats consisted of 97 animals. Here, 71 cats were diagnosed with carcinomas and a total of 26 animals with benign lesions, such as fibroepithelial hyperplasia (n=11) or duct ectasia (n=12).

In summary, even though progestins like MA and MPA have been involved with mammary cancers, the results of this study neither proved nor disproved this association. Furthermore, it was said that, because of the occurrence of mammary tumors in male cats treated with steroid hormones, the administration of progestin in cats should be prudent.

The study by Jacobs et al., 2010 [15] documented the administration of medroxyprogesterone acetate (MPA) to three male housecats. The injection was done to treat aggressive behavior and urinary problems and administered in a time frame of 2.5-4 years. All three cats developed malignant mammary gland tumors and underwent surgery.

It was also noticed that an average of 2942 days passed from the first MAP injection until the first mammary tumor was found in any of the cats. The study stated that: "The possible association between MPA exposure and the development of mammary carcinoma in male cats is concerning and warrants further investigation".

Pregnancy was not proven to influence the development of mammary neoplasms [12][31].

3.1.3. Type

3.1.3.1. Malignant

Compared to other mammary tumors, feline mammary tumors are mostly of a malignant nature. Around 86-94% of the mammary tumors are malignant [33] (see **Table 1** [20]).

A publication by Cotchin, 1952 [34] stated that in cats, most tumors are malignant in comparison to dogs.

Similarly, Chen, 1968 [35] declared that contrary to dogs, most tumors in cats are carcinomas.

The malignant feline mammary tumors are most commonly adenocarcinomas, based on the publication by Kessler, 2022 [8] they account for over 90%. In this research, other carcinomas like squamous cell, solid or lipid-rich ones, made up for less than 1%.

The author stated:" Mesenchymal tumors (sarcomas) and carcinosarcomas (tumors with malignant components of both germ layers) are very rare". Investigation by the same writer found that sarcomas make up only 1.1% and carcinosarcomas 0.1%.

Weijer et al., 1972 [17] divided the tumors in the following divisions with their occurrence in 170 cats: 53 tubular adenocarcinomas, 52 papillary adenocarcinomas, 35 solid carcinomas, 34 compound carcinomas, 3 sarcomas and 2 mucoid carcinomas.

TABLE 1: HISTOLOGICAL CLASSIFICATION OF MALIGNANT TUMOR SUBTYPES INSPIRED BY CASALLI ET AL., 2020 [20]

CARCINOMAS	➢ **In situ carcinoma** o **Ductal *in situ* carcinoma** o **Lobular *in situ* carcinoma** ➢ **Tubulopapillary carcinoma** ➢ **Cribriform carcinoma** ➢ **Solid carcinoma** ➢ **Carcinoma in a mixed tumor** ➢ **Papillary carcinoma** ➢ **Tubular carcinoma**
SPECIAL TYPE CARCINOMAS	➢ Micropapillary carcinoma ➢ Pleomorphic lobular carcinoma ➢ Secretory carcinoma ➢ Mucinous carcinoma ➢ Lipid-rich carcinoma ➢ Glycogen-rich carcinoma ➢ Squamous cell carcinoma ➢ Spindle-cell carcinoma
MYOEPITHELIAL NEOPLASIAS	➢ Malignant adenomyoepithelioma ➢ Malignant myoepithelioma
SARCOMAS	➢ Fibrosarcoma ➢ Osteosarcoma ➢ Carcinosarcoma ➢ Sarcoma in mixed tumor
OTHER SARCOMAS	➢ Chondrosarcoma ➢ Liposarcoma ➢ Hemangiosarcoma

Seixas et al., 2011 [36] found that the most common type of invasive carcinoma was the solid carcinoma, with 39.1%. With 33.75% the mixed morphologic pattern carcinoma was less frequent. Tubulopapillary carcinomas ranked third, at 12% while invasive micropapillary and complex carcinomas were the least common with 7.6%.

Most of the mammary tumors are very invasive with rapid growth and will also infiltrate the lymphatic vessels and lymph nodes.

Metastasis is common (over 80%), according to Cassali et al., 2020 [20] it can be regional or distant.

The probability of metastases increases with the volume of the tumor. 79% of the cats with a tumor of less than 3cm did not have lymph node metastasis, whilst in animals with tumors bigger than 3cm, lymph node involvement was found in over 50% [37].

The latter publication also found from 37 case studies that they conducted, 64% of the tumors were categorized as stage 3. They also documented that in around 35% of the cases, there were metastases to the regional lymph nodes, at the time of the diagnosis.

Weijer et al., 1972 [17] conducted 129 necropsies, out of which 120 cases (93.02%) showed metastasis. The main location was in the lungs (83.6%), lymph nodes (82.8%), pleura (42.2%) or liver (23.6%). Other locations included the diaphragm, adrenal glands, spleen, kidney, uterus and ovaries.

Rarely metastasis can be found in the bones, usually in the distal parts of the limbs [38]. Invasive growth into the skin is more common than infiltration to the abdominal wall.

It was also found that because of infiltration into lymphatic vessels in the skin, an ulcerative rupture of the skin can be caused. This happens in one-fourth of the cases and is defined as lymphangiosis carcinomatosa [8].

3.1.3.2. Benign

Even though malignant lesions are much more common, the benign mammary lesions are important to know to determine the differential diagnosis.

Benign tumors account for about 10-20% of feline mammary lesions. A study over a period of 10 years by Cassali et al., 2020 [20] demonstrated, that 5 out of 55 mammary tumors in cats were benign.

They can be neoplastic or non-neoplastic. The neoplastic lesions can, among other things, be adenomas (simple or complex), duct papillomas, fibroadenomas or benign mixed tumors.

Fibroadenoma is the most common benign neoplastic mammary mass in cats [31]. It may affect one, several or all mammary glands. The neoplasm consists of different parts of luminal epithelial cells, stromal cells and sometimes myoepithelial cells.

The other benign neoplastic lesions, mentioned above, are less common.

Non-neoplastic lesions include cysts, duct ectasia, focal fibrosis as well as ductular and lobular hyperplasia. The latter can be further classified into 3 categories, which are epithelial, adenomatous and fibroadenomatous (FAD) hyperplasia.

According to Misdorp et al., 1991 [31] the duct ectasia, focal fibrosis and lobular hyperplasia are the most common mammary lesions in cats.

As claimed by Torrigiani et al., 2022 [39] fibroadenomatous hyperplasia, which can also be called feline mammahyperplasia, makes up 13-20% of mammary masses in cats. The cause of the disease is not known, but an involvement of sexual hormones has been confirmed.

It was most seen in young female cats, after the first or second heat or in pregnant cats.

In rarer cases, it was seen in older queens (after treatment with progesterone) or in tom cats (after treatment with gestagen). It can even appear after a single injection with steroid hormones [25].

FAD can be found in single or multiple mammary glands. It is characterized by an abnormal proliferation of the stroma (“fibro”) and duct epithel (“adeno”).

Clinical signs include circumscribed, often bilateral enlargements of the mammary glands, without milk production. They can be focal or diffuse and become so large that they restrict the movement of the animals [8].

The enlargement can even lead to ischemic lesions and necroses. They can become painful and edematous, sometimes there can be ulceration. The general state of the animals is normal, in severe cases they can develop systemic symptoms such as lethargy and anorexia.

In young cats, it is possible to resolve on its own [39]. Otherwise treatment with progesterone-antagonists like aglepristone, or spaying can be successful [8].

3.1.4. Clinical Presentation

During the physical exam, feline mammary tumors can normally be identified easily with palpation. They are firm, palpable and movable masses (see **Pictures 1** and **2** [5]).

The nipples often show signs of inflammation, like swelling or exudation of tan or yellow-colored fluid.

In some cases, a tumor thrombus in the femoral arteries or reduced vascular reflux from the femoral veins can lead to pain, swelling, edemas or decreased temperature in the hindlimbs.

Less commonly, in around 25%, tumor necrosis and ulceration can be found [7].

The primary location is discussed in different studies, where Hayden and Nielsen, 1971 [16] stated, that the masses most commonly occur in the thoracic mammary glands, whilst Weijer et al., 1972 [17] described them more often in the abdominal mammary glands.

The literature by Carpenter, 1987 [40] documented neoplastic changes more frequently in the caudal, thus the abdominal glands.

Other studies such as Viste et al., 2002 [10] found a similar occurrence in both the thoracic and abdominal glands. They documented 25 cases of which the different locations of the masses were put in a table.

This showed 7 cases where the tumor only involved the T1 mammary gland, 5 cases were only the T2 gland was involved, 1 case that affected the A1 gland and 6 cases that involved only the A2 gland. Out of these 25 documented cases, 6 involved more than one mammary gland.

Soares et al., 2016 [41] stated that it is common for the cats to have more than one mass at the time of the clinical presentation. In this study 54.1% of cats showed more than one mass at the time of the diagnosis.

The general state of the animal is often influenced by paraneoplastic changes. These are symptoms, influenced by the presence of a neoplastic disease, that occur systemic or at a distant location from the primary tumor.

They are a common and early signs in animals with tumors and are essential to recognize and act on. The most frequent paraneoplastic symptoms are hypercalcemia, hypoglycemia, cachexia and anemia [42].

PICTURE 1: CHERRY-SIZED MAMMARY TUMOR IN THE CAUDAL ABDOMINAL MAMMARY GLAND OF A 15-YEAR-OLD SPAYED QUEEN

PICTURE 2: ULCERATED A2 MAMMARY GLAND IN A NEUTERED, MALE CAT. BY MORRIS, 2013 [5]

3.1.5. Diagnosis

If mammary tumors are suspected, a complete diagnostic work-up should be performed. This includes clinical examination, hematology, cytology, urinalysis and diagnostic imaging.

3.1.5.1. Staging

The clinical staging of mammary tumors is an important step in the diagnostic process, in cats as well as in dogs. It is done locally and systemically.

The local staging is done by measuring the "local" size of the primary tumor, as well as the involvement of the regional lymph nodes (axillar, inguinal and sternal).

It should be assessed by palpation and radiographical measurement and the assessment of distant metastasis. These factors will lead to different treatment steps and influence the prognosis [19].

The staging process should be done according to the World Health Organization (WHO) – modified staging system (1999). It is also called the TNM system [43] (see **Table 2**). This stands for: T=tumor size; N=lymph node involvement; M=distant metastasis.

TABLE 2: TUMOR STAGING ACCORDING TO WHO – MODIFIED STAGING SYSTEM

TUMOR (T) CATEGORIES

T1	Tumor <1cm diameter
T2	Tumor 1-3cm diameter
T3	Tumor >3cm diameter T1-3a moveable T1-3b skin not moveable T1-3c not movable at the base
T4	Tumor with lymphangiosis carinomatosa (inflammatory carcinoma)

REGIONAL LYMPH NODES (N) CATEGORIES

N0	Lymph nodes not palpable
N1	Ipsilateral lymph node palpable N1a movable N1b not movable
N2	Bilateral lymph nodes palpable N2a movable N2b not movable

METASTASIS (M) CATEGORIES

M0	No indication of metastasis
M1	Distant metastasis (including not regional lymph nodes)

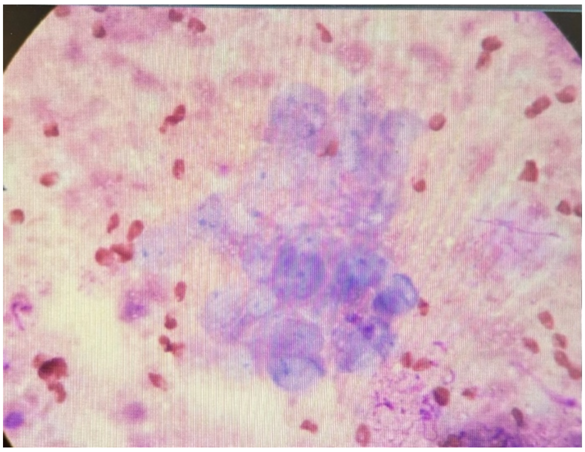
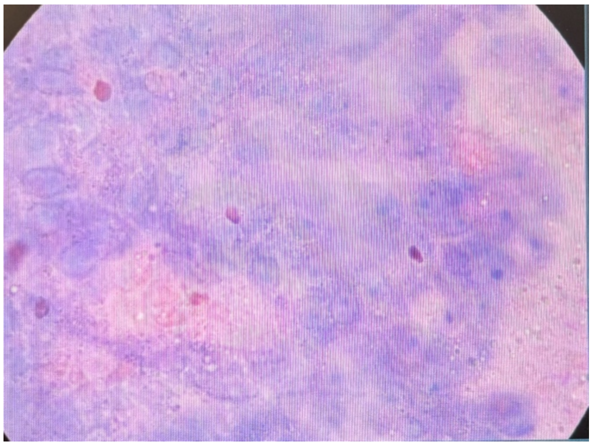
STAGING	T	N	M
1	T1a-c	N0	M0
2	T0, T1a-c	N1	M0
	T2a-c	N0, N1a	M0
3	All T3	All N	M0
	All T	All Nb	M0
4	All T	All N	M1

The systemic staging includes an x-ray of the thorax with 2 to 3 planes, in ventrodorsal, as well as right and left lateral views. It can also include an ultrasound of the abdominal area or a computerized tomography scan [19].

3.1.5.2. Cytology and Histology

Before the cells and tissues are microscopically analyzed and classified (see **Pictures 3** and **4**), a sample must be taken. This is most commonly done with fine-needle aspiration (FNA).

A 21-25-gauge needle is used which is inserted into the mass and then aspirated. It should be withdrawn and redirected several times [44].

PICTURE 3 & 4: CYTOLOGY SHOWS A LOT OF NECROTIC MATERIAL, CLUSTERS OF DYSPLASTIC EPITHELIAL CELLS IN BETWEEN, NO INFLAMMATION AND NO BACTERIA VISIBLE

For the grading of the histological samples, most commonly the Elston and Ellis (EE), grading system, is used (see **Table 3** [45]). This was originally invented for the diagnostic use in human medicine but is now also used for veterinary diagnostics [45].

First the structure of the tissue with tubule and gland formation is measured. The question arises, as to whether the tumor forms tubular tumor glands, or not. If it does, the amount is graded and scored.

Then the nuclear pleomorphism is evaluated and scored accordingly, and the same is done for the miotic count.

All three scores are added together and result in the grading system, that evaluates the invasiveness of the mammary tumor.

A score of 3-5 will result in a G1 grading, so the lowest and “least” malignant state. A value of 6-7 will be rated G2 and a score of 8-9 is evaluated as the highest grading G3, indicating a higher malignancy.

TABLE 3: INSPIRED BY ELSTON AND ELLIS., 1991 [45]

FEATURES		**SCORE**
TUBULE AND GLAND FORMATION	Majority of tumor (>75%)	1
	Moderate degree (10-75%)	2
	Little or none	3
NUCLEAR PLEOMORPHISM	Small, regular, uniform cells	1
	Moderate increase in size and variability	2
	Marked variation	3
MIOTIC COUNT	0-9	1
	10-19	2
	>20	3

Most studies like Seixas et al., 2011 [36] or Cassali et al., 2020 [20] found the grading system useful for the diagnosis and prognosis of both feline and canine mammary tumors.

However, the study of Mills et al., 2015 [46] stated, that the use of the EE-grading system as well as the revised Elston and Ellis grading system (REE) and the mitotic-modified Elston and Ellis (MMEE): "resulted in inferior discrimination between tumors and lacked overall association with OS", meaning, that the tumors had a tendency to be graded as score 2 (58.3%) or 3 (39.8%), which according to the study resulted in an lack of score 1.

Therefore, this study introduced and reviewed novel grading systems (see **Table 4** [46]), based on EE and REE. This new grading system included scores such as lymphovascular invasion, which could already be found in the REE system, as well as modified the miotic count and nuclear form scores.

TABLE 4: INSPIRED BY MILLS, ET AL., 2015 [46]

HISTOLOGICAL FEATURE	SCORE
LYMPHOVASCULAR INVASION	
ABSENT	0
PRESENT	1
NUCLEAR FORM	
<5% ABNORMAL	0
>5% ABNORMAL	1
MITOTIC COUNT	
≤62	0
≥62	1

The grading is then evaluated as follows: A total score of 0 equals a grade 1 tumor, whereas a score of 1 equates to grade 2. The highest scores of 2-3 equal a grade 3 tumor.

3.1.5.3. Immunohistochemistry

The immunohistochemistry diagnostic technique has been used in veterinary medicine as a valuable tool to determine prognostic and predictive factors for neoplasms.

According to the study by Cassali et al., 2020 [20] the professional consensus stated the following receptors/markers should be tested: Estrogen receptor (ER), Progesterone receptor (PR), Ki-67 and Cyclooxygenase-2 (COX-2) marker.

During the implementation it is important to use negative and positive controls, to validate the reaction.

Ki-67

Ki-67 is used for identification of the proliferation index. It is used in a 1:25 dilution. The antigen retrieval method is the use of pressurized heat (125°C/2min.) with citrate buffer (pH 6.0). The incubation time is 1h/room temperature [20].

Ki-67 is an antigen that is cell-cycle related and marks separating cells. It is found on the inside of the nucleus in growing or dividing cells and is good to determine the growth fraction and cell population in mammary tumors.

Frequently a MIB-1 (molecular immunology borstel) antibody is used. This method is often used for diagnosis of canine and feline mammary tumors according to Scholzen and Gerdes, 2000 [47].

It was shown by Cassali et al., 2020 [20] that an increase of the proliferation index could be seen in case of non-neoplastic lesions, benign tumors as well as in situ, metastatic and invasive carcinomas.

Morris et al., 2008 [48] showed the relation of a higher Ki-67 percentage with the type of mammary lesions. Meaning the score would get higher in case of non-neoplastic lesions (grade 1: 0-20%), benign (grade 2-4: 20-80%) or malignant (grade 3-5: 40-100%) tumors. Fibroadenomatous hyperplasia was the only exclusion with a score of 20-60%.

Soares et al., 2016 [49] found the mean Ki-67 index, in primary tumors, to be 26%. This would increase to 30.1% in case of regional lymph node metastasis and further increase to 48.2% in case of distant lymph node metastasis. According to this study, the ideal cut-off value for feline mammary tumors, was estimated to be 14%. This value was associated with larger tumor size, poorer differentiation, shorter OS and ER-negative receptor.

However, further studies are needed to find a more conclusive value, as the literature in general does not appear to be coherent on the Ki-67 cut-off point. This is recommended as cases above the cut-off value have a much worse prognosis.

Cyclooxygenase-2

Cyclooxygenase is an enzyme which has a key role in the conversion of arachidonic acid into prostaglandin and plays a role in inflammatory processes. Especially, COX-2 is elevated during inflammatory reactions and in tumors. COX-2 expression was also demonstrated in mammary tumors.

It is diluted in a ratio of 1:80 and retrieved in a water bath at 98°C/20min., together with citrate buffer (pH 6.0). The incubation time is 1hour/room temperature [20].

Millanta et al., 2006 [50] observed an elevated expression (80.9%) of the COX-2 protein in the case of feline mammary carcinomas.

Almost half of the cases (49%) showed a high expression. It was also documented that there is a relation between the elevated COX-2 expression and a negative ER value, as well as a correlation with a positive PR value.

Other studies, like Sayasith et al., 2009 [51] showed a lower percentage (37%), of cases with an intermediate or high expression. This was explained with the use of different antigen and staging systems.

Cassali et al., 2020 [20] also showed that an increased COX-2 expression is associated with a poorer prognosis. In this study, a 12-scale score was used, for both the primary tumor and the metastases. 0=absent; 1=weak; 2=moderate; 3=strong. Multiplication of distribution and intensity scores equals a low (0-5) or high (6-12) score.

To use COX-2 as a trustworthy prognostic tool for the diagnosis and prognosis of feline mammary tumors, studies agree, that there needs to be careful interpretation and evaluation of the distribution and/or intensity of the staining.

These criteria are already implemented in the studies about canine mammary tumors and have shown, that they are, in fact suitable for the diagnostic and prognostic classification of neoplasms in cats.

<u>Hormonal receptors</u>

The hormonal receptors (HR) for the diagnosis of feline mammary tumors include the estrogen receptor (ER) and progesterone receptor (PR).

They are both diluted at a ratio of 1:20 and the antigen retrieval method is done with pressurized heat (125°C/2min.) in an EDTA buffer (pH 9.0). The incubation time is the same as for the other markers mentioned before: 1hour/room temperature [20].

Kessler, 2022 [8] declared in his literature, that benign mammary tumors have more ERs than malignant, with 25-40% compared to 10-40%.

It was shown in a study by Brunetti et al., 2013 [52], that the positivity decreases with an increasing histological grade.

Multiple studies reported that most feline mammary tumors are ER and PR-negative [53][54].

In a release by Morris et al., 2008 [48] nuclear staining of >5% was regarded as positive. It had the following results: non-neoplastic cells and adenomas had more ER-positive cells, than carcinomas. And low-grade carcinomas had more positive cells than high-grade ones.

In another study by De Campos et al., 2016 [55], a value of >1% was classified as positive, but otherwise had similar conclusions as the study mentioned before.

The studies by Seixas et al., 2011 [36], Cassali et al., 2020 [20] and Chocteau et al., 2019 [56] determined a cut-off value of >10%. In the latter study, with 395 cats, 25.3% proved to be estrogen receptor positive and 9.4% progesterone receptor positive.

Altogether it can be said that more data is needed, for the estrogen and progesterone receptors to be used as trustful tools in the diagnostic process of feline mammary tumors.

3.1.5.4. Hematology

In the study by Kurban and Günay Uçmak, 2024 [21], the following results were found: The red blood cells (RBC) as well as the hemoglobin (Hb) and hematocrit (Ht; PCV%) values were reduced. This could result from the development of anemia, because of tumor hypoxia, which is a common paraneoplastic syndrome.

According to Varlotto and Stevenson, 2005 [18]: “approximately 40–64% of patients presenting for cancer therapy are anemic”.
A lower value in these parameters is linked to a worse grade of the tumor, as there is a disfunction in the oxygen supply of the cells. Meaning, a worse level of tumor hypoxia.

Another parameter, that can give an indication of mammary tumors is the mean corpuscular volume (MCV). This tends to be higher in cats with mammary tumors, than in healthy cats. It was also observed that cats with a higher staging/grading and a larger tumor, would have a higher MCV value, pointing towards a worse prognosis.

The white blood cells (WBC), were also shown to be elevated in diseased animals, compared to healthy ones.
Leukocytosis is a common abnormality described in animals with tumors, and it could come from the inflammation caused by the tumor.
It was also shown that cats with a lower staging had a smaller number of WBCs than animals with a higher staging score.

A further group of parameters that the study looked at were the thrombocytes and the platelet indices. Here the mean platelet volume (MPV) was much higher in the group of sick animals, compared to the control group.

Contradictory, another study by Günay Uçmak et al., 2021 [57] of hematological parameters in canines with mammary tumors, did not show an increase in these parameters in diseased animals. The previous study, that evaluated the parameters in feline mammary carcinomas [21], explained this with variations between species.

The plateletcrit (PCT) was also shown to be significantly elevated in diseased cats. Like the previous values, it was stated that the more the values are abnormal, the bigger the mass and the worse the prognosis.

The study by Da Silva Soares et al., 2023 [58] showed the changes of biochemical parameters in 18 cats. The alkaline phosphatase (ALP) increased in 22.22% and decreased in one cat (5.55%). The glutamate-pyruvate-transaminase (GPT) was increased in 27.78%.

Only one cat had a decreased creatinine value. Creatinine can be an individual marker for (overall survival) OS as well as (disease free survival) DFS.

The study also corelated the (overall survival time) OST and DFS with several blood parameters. Platelet count and pretreatment level of mean corpuscular hemoglobin concentration (MCHC) can be independent prognostic factors of survival.
The low MCHC values are related to a reduced oxygen transporting capacity of the erythrocytes. This hypoxic condition favors the growth of the tumor and implies a poorer prognosis.

Neutrophiles have an important part in cancer-related inflammations. They are the: "first line of innate immunity and a major source of cytokines and tumor growth promoting factors (...), contribute to angiogenesis, metastasis, inhibition of apoptosis and tumor progression" according to Da Silva Soares et al., 2023 [58].

Petrucci et al., 2021 [59] analyzed the neutrophile-to-lymphatic ratio (NLR) as an independent prognostic factor in 49 cats with stage 1 to 3 mammary carcinomas.

The latter study estimated the cut-off point for the white blood cells (WBC) at 8.49 x10^9/L (DFP and TSS), the neutrophile count (NEU) at 4.62 x10^9/L disease free period (DFP) and 6.65 x10^9/L tumor-specific survival (TSS) and for the NLR, it was estimated at 2.46.

It was also noted that higher values of these parameters are in relation with an increased risk of tumor recurrence and tumor-related death.

Altogether the research showed that the parameters mentioned above (WBC, NEU, NLR) are useful as prognostic factors for the DFP as well as the TSS. NLR was confirmed to be an independent prognostic factor for the survival time. This reflects the nature and severity of the inflammatory processes that occur in mammary carcinomas.

Serum Amyloid A (SAA) is another parameter to look at during the diagnostic process [60]. In mammals it is one of the most important acute phase proteins (APP). It is synthesized in the hepatocytes and adipocytes of the liver. In case of an inflammatory process, for example caused by a tumor. It releases secretions into the blood, which leads to an elevation of the SAA values in the blood serum. Because of that, it can be used as an inflammatory marker in the diagnosis of mammary tumors, as well as other inflammatory or infectious diseases [61].

It was also proven that, like the parameters above, a higher deviation from the normal value can be associated with higher metastisation. Because of that, SAA can be used as a tool to observe the progression of the neoplastic disease.

3.1.5.5. Diagnostic Imaging

3.1.5.5.1. Ultrasound

The normal visualization of the mammary glands during sonography in a mature, non-pregnant, non-lactating cat is almost not visible. With a thickness of less than 2mm, it may be less clear than the abdominal wall.

The mammary ducts are tight, and the low-density stroma and gland tissue are almost absent. The whole structure appears homogen, hypoechoic, with clear margins and no anechoic structures [62].

In case of a mammary gland mass, it is not possible to tell whether it is benign or malignant using only ultrasound.

The view can change with hormonal influence, for example during pregnancy or lactation.

With the Doppler-Mode, the caudal superficial epigastric artery and the hilar lymphatic vascularization can be viewed [63].

A study by Moraes and Borges, 2021 [63] analyzed abnormal mammary glands and the corresponding lymph nodes in 10 queens with mammary nodules. Both B-Mode and Doppler-Mode were used with longitudinal and transverse planes.

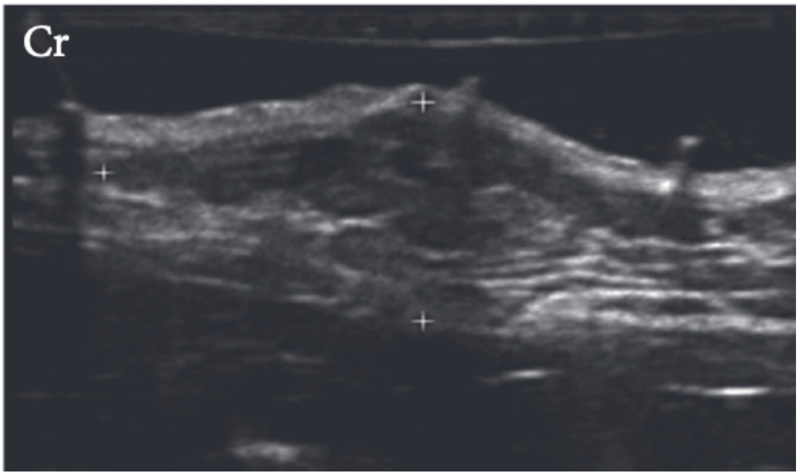
It was found that, in B-Mode, the changed mammary glands were enlarged with visible mammary ducts (33.33%). The structure was homogeneous in 54.17%, hypoechoic in 95.83% and had limited, regular margins in 52.08% (see **Picture 5** [63]).

Other abnormal changes like acoustic enhancement, intratumoral sedimentation or vacuoles were also found. Doppler-Mode showed changes such as intertwined vessels in 70%, peripheral vascularization in 20% or intratumoral-associated vascularization in 20%. Only one tenth of the mammary lesions showed no changes in the blood vessels.

According to Raskin and Meyer, 2016 [48] abnormal mammary glands seemed irregular, with mixed echogenicity and lesions of variable sizes.

In addition, the lymph nodes (axillar and inguinal) are also examined, which may be altered due to metastasis. There will be changes of the shape, size, echotexture, and echogenicity.

An abdominal sonographic examination is also recommended. It can show metastatic changes in the abdominal organs, most commonly in the liver, spleen and intraabdominal lymph nodes [63].



PICTURE 5: ULTRASOUND SHOWS RIGHT T1 GLAND, WITH HETEROGENOUS ECHOTEXTURE, UNEVEN SHAPE, UNCLEAR EDGES AND SEVERAL LOBULATIONS. BY MORAES AND BOGRES., 2021 [36]

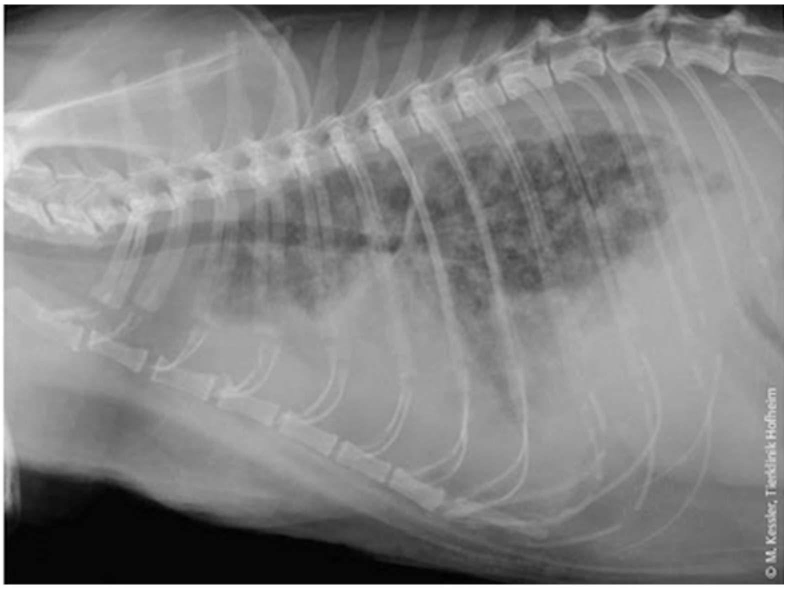
3.1.5.5.2. X-Ray

This examination is done in two to three planes, which are ventrodorsal, as well as right and left lateral views. In this context, it is mainly used to identify metastases in the lungs or rarely the bones.

Metastases in the lungs mostly appear as miliary pattern, and the pulmonary surfaces may also be involved.

They can also appear as interstitial, micro- or macronodular abnormalities (see **Picture 6** [8]). In some cases, metastases in the lungs can lead to pleural effusion [8].

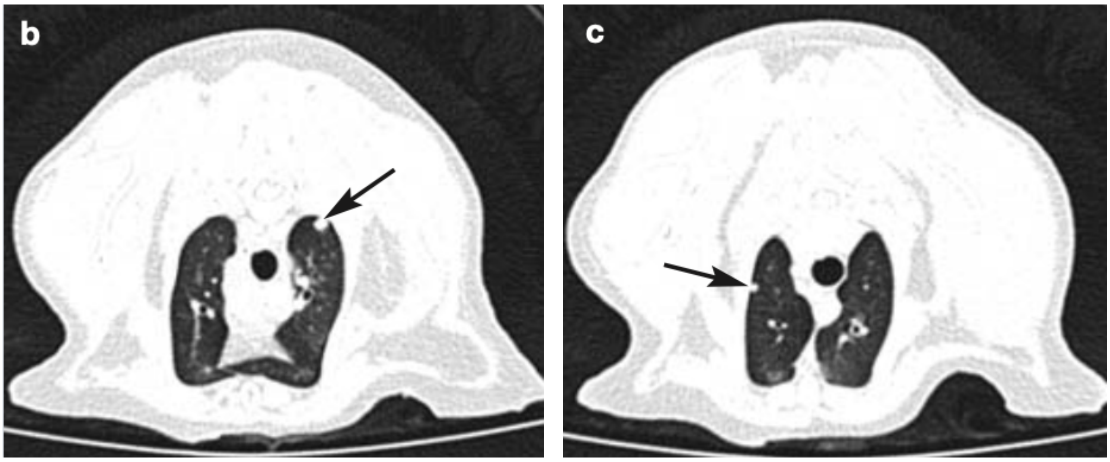
However, metastases smaller than 4-5 mm cannot be detected by most x-rays.



PICTURE 6: MICRONODULAR LUNG METASTASIS, WITH SECONDARY PLEURAL EFFUSION, IN A CAT WITH MAMMARY TUMOR. BY KESSLER, 2022 [8]

3.1.5.5.3. Computed Tomography

If x-rays reach their limits due to the size of the abnormal change, computed tomography (CT) is used. It can provide a more precise evaluation of metastases [65]. A high-resolution scan with a lung window field is used (see **Pictures 7** and **8** [5]).



PICTURE 7 & 8: CT SHOWS METASTASES IN THE RIGHT (B) AND LEFT (C) LUNG LOBE. BY MORRIS, 2013 [5]

3.1.6. Therapy

3.1.6.1. Surgery

The surgical approach is the preferred way of treatment of mammary tumors in cats. It can be a therapy on its own or combined with adjuvant therapy, according to Lana, 2007 [49].

It is not regarded as curative, because of the aggressive nature of feline mammary neoplasms to invade the surrounding and distant tissue.

A general rule is to remove everything in contact with or invaded by the tumor. For tumors with a high risk of recurrence, a margin of at least 2-3cm should be intended [44].

There are different approaches regarding the amount of glands/tissue to be removed.

The lumpectomy, where only the circumferential increase is removed without the surrounding mammary gland tissue.

The regional mastectomy where several parts of the mammary glands are removed.

And the radical approach (see **Picture 9** [66]), in which the whole mammary chain is removed, uni- or bilaterally, regardless of the size of the tumor and even in presence of unilateral growth.

In case of the bilateral removal, literature recommended doing this in two stages, with a 2-week interval between each procedure, because of an increased risk [67].

However, in animals with loose mammary tissue, it makes sense to perform the operation in one session [25].

A lot of studies introduced this as the preferred approach [25][68][69].

Cunha et al., 2018 [70] advised towards the radical chain mastectomy as it very much decreased the risk of local recurrence.

The lymphatic drainage also plays a role in the surgical approach, as to which lymph nodes need to be removed.

Some literature [6] recommended to always removing the inguinal lymph node with the A2 mammary gland, as it is embedded there.

The axillary lymph node, on the other hand, should not be routinely removed, but only if enlarged or diagnostically (FNA or biopsy) positive. It was claimed that the removal of the axillary lymph node has no therapeutic benefit, and it does not prolong the time of survival [7].

Other studies, like Cunha et al., 2018 [70] stated that regional lymph nodes (axillar or inguinal) should always be removed during mastectomy in cats, as regional metastasis at diagnosis is common. Even when not clinically palpable, as was observed in two cases.

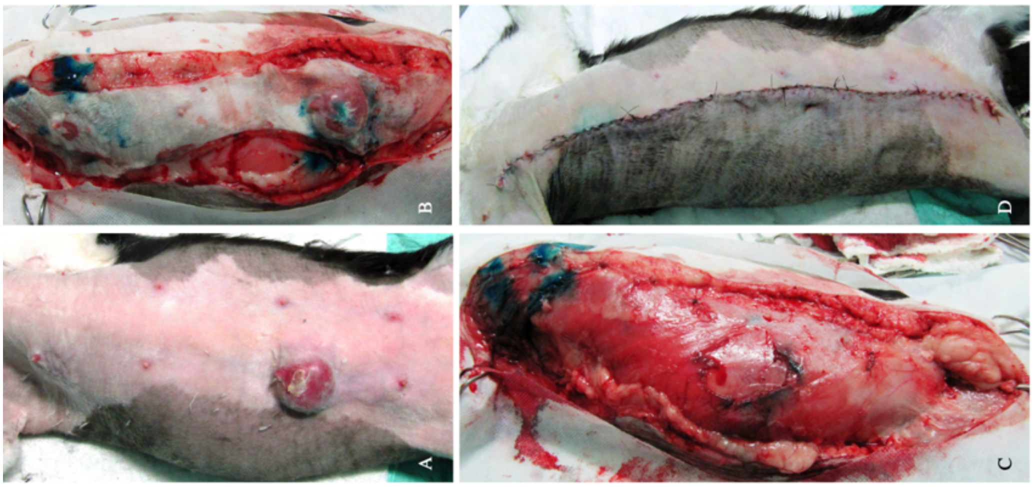
Cassali et al., 2020 [20] said, that regional axillary and inguinal lymph nodes should be removed during surgery, even if they are not clinically changed. This is due to the high incidence of regional metastasis.

For the lymphadenectomy of the axillary lymph node, it is recommended to intradermally applicate patent blue 2.5%, at a dose of 2mg/kg, but not more than 1ml per patient, to make identification and complete extraction easier.

During the radical approach, it is recommended to ligate the vessels early, as they are often invaded by the tumor [4].

It is also recommended, to flush the area where the tumor was removed plentiful, to eliminate any exfoliated neoplastic cells. Gentle handling of the tissues is also important, as the dissemination of tumor cells should be minimal.

And the entire removed mass should be histopathologically analyzed [44].



PICTURE 9: SHOWS STEPS OF UNILATERAL CHAIN MASTECTOMY. BY CUNHA ET AL., 2016 [66]

3.1.6.2. Adjuvant Therapy

3.1.6.2.1. Chemotherapy

Because feline mammary neoplasms are very aggressive, invasive and have such a high risk of metastasis, adjuvant chemotherapy is usually recommended. It is used to prolong the lifetime and increase the quality of life [71]. The full advantage of the therapy is still not clear, according to Morris, 2013 [5].

Cats with a late diagnosis, large primary tumor, metastasis in the local lymph nodes or vascular lymph invasion are recommended to receive chemotherapy [20]. The same applies for cats with unretractable tumors.

According to the literature by Kessler et al., 2014 [1] it was recommended to do adjuvant chemotherapy in all patients with tumors >1cm (stages 2 and 3), and/or lymph node metastases, as well as for recurrent neoplasms.

Before starting chemotherapy, it is important to have a full diagnostic workup and staging, as well as a stabile patient.

Maximum effect with minimal dose, toxicity and duration is the goal [71].

The therapy usually starts at the time of stitch removal, so around 2 weeks after the resection [72].

The most used agents are:

Doxorubicin

An antineoplastic agent, from the group of the antitumor antibiotics, used for the therapy of several malignant tumors [71]. It is obtained from *Streptomyces peucetius var. caesius.*

The mode of action is blocking the DNA and RNA synthesis, and with that invoke apoptosis. Activation happens in the liver, only 5% are excreted via the kidneys.

It is one of the most active agents and especially useful for advanced courses of disease. Administration can be on its own or together with other agents, for example cyclophosphamide.

In the publication by Kessler et al., 2014 [37] the product *Doxo-Cell* ® with a dosage of 1.1-1.2mg/kg, given intravenously over a period of 15-20 minutes, once every three weeks was used.

The study by Novosad et al., 2006 [73] also documented a treatment of 67 cats with doxorubicin. They received 1mg/kg intravenously, every three weeks for a maximum of 5 weeks, after surgery. Animals that were treated had an improved mean survival time (MST) of 448 days, and a DFP of 255 days.

Borrego et al. (2009) [74] conducted a study where after surgery, 23 cats were treated with doxorubicin and the COX-2 inhibitor meloxicam. The MST was 460 days and the DFP 269 days. This is not particularly different from other studies, so the combination is not supported by this study.

Cyclophosphamide

Is an alkylating agent, cytostatic and immunosuppressive. It is a prodrug which is activated by the phosphamidase in the liver. This agent can be used for auto-immune diseases or for the therapy of tumors.

It is excreted via the kidneys. The most common dose according to McKnight, 2003 [71] is 50mg/m^2, PO once daily for 4 days, or 200-250mg/m^2 IV once every 3 weeks.

In a publication by McNeill et al., 2009 [75], 76 cats did undergo surgery (33 radical resection) and received the first dose after an average of 27 days. 4 cats received only doxorubicin, whilst the rest (n=72) of the cats received a combination of doxorubicin and cyclophosphamide.

Doxorubicin was given in 5 (n=3), 4 (n=21), 3 (n=5), 2 (n=3) or 1 (n=4) dose(s). Cyclophosphamide was given in 5 (n=1), 4 (n=16), 3 (n=4), or 2 (n=4) doses. 81% received a treatment average of ≥ 3 cycles.

36 animals had a relapse and out of these, 21 received another treatment, consisting of either surgery alone (n=9), surgery together with chemotherapy (n=7), or rescue chemotherapy (n=5).

The MST, for cats that had only surgery, was 1406 and the DFP was 372 days. For animals that received additional treatment the MST was 848 days and the DFP was 372 days.

However, the study itself declared that the layout of the study had limitations, which could have influenced the outcome.

<u>Carboplatin</u>
Is a platinum compound and is administered as infusion [71]. Bonolo de Campos et al., 2014 [69] documented that the treatment with carboplatin was well-tolerated by the animals.

McKnight, 2003 [71] also noted, that the administration of this agent was well-tolerated and safe to use in cats.

Only few side effects were recorded, such as myelosuppression or gastrointestinal complications. As well as thrombocytopenia and neutropenia, with the lowest value at 14 to 21 days.

A common dose in cats is 200mg/m², intravenously once every 4 weeks. The administration should be slow with a duration of 5 to 10 minutes.

The study by Bonolo de Campos et al., 2014 [69] also compared the OS of surgery alone, or surgery followed by carboplatin therapy. 16 cats were treated out of which 9 only received surgery and 7 received chemotherapy after the surgery.

From the first group, 4 died due to further progress of the tumor, 3 died because of other reasons, and 2 survived. In the other group, 5 died because of progression of the tumor, one died because of other reasons, and one survived. The median survival time was 387 days in operated animals and 428 days in animals that also received adjuvant chemotherapy.

In conclusion, in this publication the OS was longer in animals that received carboplatin, but there was no significant, statistical difference regarding the OS.

Carboplatin can also be used as rescue therapy (alone or in combination with doxorubicin or mitoxantrone), or for cases with metastasis or recurrent tumor.

Mitoxantrone

The agent is part of the same group as doxorubicin. Its mode of action is the so-called intercalation. This means it forms hydrogen bonds that are attached to the DNA. Compared to doxorubicin, it is less effective but also less toxic [71].

Cunha et al., 2015 [76] did a study that reviewed 12 cases treated with mitoxantrone. Out of these 12 cats, 4 animals received 1 dose, another 4 cats received 3 doses and further 4 cats received 4 doses. The treatment was done with *Evomixan*® 20mg at a dose of 6mg/m², once every three weeks, administered slowly as intravenous injection.

During the study, the following side effects developed: 5 cats had azotemia, 4 had anorexia, 3 had leukopenia, and 1 vomitus.

Mitoxantrone is often used in cats with renal disorders, rather than doxorubicin. In this study 41.67% of animals had renal adverse effects. However, the authors of this study commented about the observed azotemia, which was not noted in previous studies, that it could be of pre-renal nature, resulting from anorexia and dehydration. Rather than secondary to the mitoxantrone.

Other agents include Vincristine and Methotrexate. However, Ito et al., 1996 [27] could not find the treatment with Vincristine (0.5mg/kg, weekly, 6-10 times) effective.

In case of adjuvant chemotherapy, it is very important to keep the many adverse effects in mind, that can be caused by this therapy. These include transient gastrointestinal symptoms like anorexia and vomitus, myelosuppression and nephrotoxicity. Because of that, the renal and hepatic function should be evaluated before the start of the treatment.

3.1.6.2.2. Immunotherapy

This is another type of adjuvant therapy, that requires the administration of an immunomodulator which stimulates an immune response towards the neoplastic cells.

These agents are for example the biological response modifier L-MTP-PE (liposome-enclosed muramyltripeptid-phosphatidylethanolamin). This is a synthetic derivate that is won from the cell wall of *Mycobacteria*. It has immune stimulating characteristics, by activating the immune cells, that then increase the expression of mediators like cytokines.

In this random, double-study by Fox et al., 1995 [77] 40 cats were treated with L-MTP-PE, with a dose of 2mg/m² weekly for 8 weeks, by slow intravenous administration. The treatment started one month after the surgical removal of the mammary tumor.

However, the study could not show a significant increase in DSF and neither in MST.

Another agent is the biological adjuvant Bacille Galmette-Guérin (BCG). It is a bacteria developed from *Mycobacteria bovis*. Most commonly, it is used for tuberculosis vaccination.

A study by Rutten et al., 1990 [78] showed no significant improvement of the DFP or MST.

The same study also investigated the effectiveness of *Corynebacterium parvum* for the immune therapy of dogs. Similar results were found. The last agent can be administered intratumorally or subcutaneously.

Levamisole is an anthelmintic, that nowadays is also used in cancer therapy because of its immunomodulating effect.

MacEwen et al., 1984 [79] and 1985 [80] documented, that the oral administration of 5mg/kg, 3 times a week, also did not have a significant effect on the MST or DFP. But it was shown that cats with a smaller tumor had a higher MST as well as a lower rate of recurrence in comparison to animals with larger tumors. It was also shown that there was a higher survival time in domestic shorthair cats compared to other breeds.

Another substance possible for adjuvant therapy is toceranib phosphate, which is an antineoplastic and -angiogenic agent. Its mode of action is the inhibition of tyrosine kinase, and it is most used for the treatment of mast cell tumors.

In a publication by Del Portillo Miguel et al., 2024 [81], a total of 17 cats were treated with an average quantity of 2,77mg/kg (2.3-3.2mg/kg). Administration would be done orally, every second day.

During the study eight cats developed adverse effects, the most common side effects were neutropenia (n=1), vomiting (n=1), elevated alanine aminotransferase (ALT) (n=2), and anorexia (n=2). Out of the 17 cats, 6 showed an overall response and 12 experienced clinical advantages.

The research worked with the so called "Response Evaluation Criteria In Solid Tumors (RECIST)" (see **Table 5**), to evaluate the clinical development of the cats. This criterion is divided into four classifications which are as follows:

TABLE 5: RESPONSE EVALUATION CRITERIA IN SOLID TUMORS (RECIST). INSPIRED BY DEL PORTILLO MIGUEL ET AL., 2024 [81]

Complete Response (CR)	All target lesions disappear or Normalization of lymph nodes
Partial Response (PRsp)	At least 30% decrease of the longest diameter of the target lesion
Stable Disease (StD)	<30% decrease of the longest diameter of the target lesion or 20% increase of the longest diameter of the target lesion
Progressive Disease (PD)	<20% increase of the longest diameter of the target lesion or ≥ 1 new lesion

One cat had a complete response, 5 cats had a partial response, 6 cats were categorized as StD (for a minimum of 2 months) and 5 as PD. 3 out of 5 animals from the PRsp category also showed an improvement of ulceration of the primary tumor.

The mean progressive free survival time (MPFS) was 91 days, and the mean overall survival time (MOST) was 145 days.

The study concluded that: "Toceranib is a reasonable therapeutic option in cats with advanced or recurrent mammary tumours, showing clinical benefit and a good toxicity profile".

3.1.6.2.3. Hormonal Therapy

In humans, the treatment of ER-positive breast cancer with antiestrogen therapy is widely used.

One of the agents used for the endocrine therapy is tamoxifen. This is a medicinal substance from the selective estrogen-receptor-modulators, short SERMs. It is a cytostatic agent that blocks the estrogen receptors.

Studies on the treatment of cats are very scarce. Kitchell and Fidel, 1992 [82] showed the effect of tamoxifen on 7 dogs with mammary tumors. A dose between 2,5-10mg was administered per os twice daily. 5 dogs were documented to have a response to the treatment.

Morris et al., 1993 [83] did a similar study, with a total of nine dogs. In this research no responses were shown. However, the research acknowledged that, in order to reach a conclusion about the potential preventative effects of tamoxifen, not enough animals were examined.

It is advisable to draw attention to the side effects before starting therapy with antiestrogenic agents. According to Novosad, 2003 [33] these include vulvar swelling, vaginal discharge as well as pyometra in intact cats, and stump pyometra in spayed animals.

3.1.6.2.4. Radiation Therapy

This way of therapy is rarely used, as there is no evidence that it has a beneficial effect in case of feline mammary tumors. It can be potentially used as local treatment, or in case of a nonresectable mammary neoplasms [33][84][85].

3.1.6.2.5. Supportive Therapy

It is advised to administer analgesia, such as non-steroidal-anti-inflammatory-drugs (NSAIDs) or opioids, to mammary tumor patients intra- and postoperative. They should also be given in case of ulcerating or large tumors and in case of metastases that causes the cat to be in pain [86].

Perioperative, prophylactic antibiotics should also be administered, as tumor patients often are at a higher risk of wound infection.

Aiken, 2003 [44] antibiotic of choice is Cephazolin administered intravenously with a dose of 20mg/kg every two hours.

Since the treatment of mammary tumors is associated with many side effects, it is also sensible to give medication that alleviates these. This includes for example antiemetics, appetite stimulants or supplemental feeding.

Because of the secondary effects of this therapy, for instance on the heart or kidney, it is also advised to treat this [87].

3.1.7. Prognosis

The prognosis for feline mammary tumors is mostly unfavorable this is equal for female and male cats. Multiple studies estimate the survival time to be less than one year [4][5][6].

There are several factors on which the prognosis can be based on, such as tumor size. This is regarded as the most important and the most reliable [27].

A study by MacEwen et al., 1984 [68] documented the mean survival time (MST) in animals with tumors of less than 2cm to be 4.5 years. In animals with a tumor size of 2 to 3cm the MST was 2 years. And in cats with tumors larger than 3cm it was 6 months.

In a release by Morris, 2013 [5] the MST in tumors of bigger size (>3cm), is proven to be shorter (4-12 months) compared to tumors with a smaller diameter (<3cm).

Ito et al., 1996 [27] stated a median survival of 9 months in tumors of less than 3cm diameter and a MST of 5 months in masses with a diameter of over 3cm.

Contrary to the other studies, Viste et al., 2002 [10] claimed no prognostic value of small tumors with a size of less than 3cm in diameter. This is explained with a too wide time frame from 3 to 54 months of the MST.

However, it is also said that tumors with a diameter of over 3cm provide a good value for prognosis. In this case the MST varied from 4-12 months.

The last research also determined its own limitations, with relatively few cases and follow-up data. The study was summarized with the following statement: “Therefore, the larger the proliferating mass, the greater the life-threatening risk” [10].

As discussed above, tumors size is also a part of the clinical staging of neoplasms. Staging in total can also provide prognostic insight.

Morris, 2013 [5] noted a mean survival time of 29 months in classified stage 1 patients, 12.5 months in stage 2 cases, 9 months in stage 3 and 1 month in stage 4.

Another part of the clinical staging is the lymph node involvement, this on its own can also be a good prognostic indicator.

Seixas et al., 2011 [36] stated that lymphovascular invasion and lymph node metastasis, were significant, independent prognostic parameters, for the prediction of OS and DFS. It was also noted that within 9 months, all cats with lymph node metastasis, at clinical examination, died.

Dagher et al., 2019 [2] came to a similar conclusion, with a MOST of 405 days without lymph node involvement and 234 days in animals where the lymph nodes were affected.

Cassali et al., 2020 [20] documented the quantity of affected lymph nodes, as well as the extracapsular expansion can be used to assess the prognosis.

Another prognostic tool is the differentiation of tumor types.

The last study found, that solid and micropapillary carcinomas had the shortest overall survival, while tubulopapillar and complex carcinomas had the longest.

A similar result was found by Dagher et al., 2019 [2]. Here the MOST in solid tumors was also the lowest with 272 days. Tubulopapillary tumors had a longer, average OS of 332 days.

Cassali et al., 2020 [20] stated that carcinomas in mixed tumors generally have a good prognosis, although in case of combined micropapillary or solid areas the survival time is shorter. Similar results were found by Dagher et al., 2019 [2].

A further prognostic value is the histological tumor grading (1-3). According to Zappulli et al., 2015 [88] it is a “highly reliable parameter”.

One limitation here, however, is the inconsistency in the classification of different studies, as already discussed above.

The type of surgery can also be a prognostic tool. Wypij et al., 2006 [86] documented that conservative surgery had a mean survival time of 500 days and DFP of 300 to 325 days. Radical surgery had a better prognosis with a mean survival time of 800 days and a DFP of 575 to 1300 days.

In this study by Cunha et al., 2018, all cats (n= 20) underwent a radical (either uni- or bilateral) mastectomy. The MST amounted to 768 days and at the time of the study, 12 cats were still alive. In conclusion, this study noted a longer survival of cats with malignant mammary tumors, after early and aggressive chain mastectomy.

Many more prognostic factors like age, breed or gender can be considered. However, they are far less reliable. And according to Zappulli et al., 2015 [88] a clear majority of studies saw no significance in these factors.

Other parameters, as diagnosed with immunohistochemistry or hematology, such as Ki-67 or ER and PR, are controversial as prognostic parameters, as discussed before.

4. Case Studies

4.1. Methods and Material

In this study a total of 24 cases of cats with feline mammary tumors were analyzed.

The cases were made available by the small animal clinics "Tierklinik Stuttgart-Plieningen" and the "Tiermedizinische Hochschule Hannover". The period of the cases ranged from 2017 to 2024.

Of the examined animals, the mean age was 10.2 ± 4.2 years. The median age was at 11 years.

All of the cats were female, of which 20 were neutered, and 4 were not.

The breeds of the examined cats were the following: European Shorthair (ESH) n=12, British Shorthair (BSH) n=3, Oriental Shorthair n=1, Maine Coon n=3, Siamese n=2, Turkish Van n=1, Neva Masquerade n=1, Mix n=1.

Diagnostic work-up was done according to a general scheme, including physical examination, hematology, diagnostic imaging, cytology and histopathology.

Blood was taken from the *V. cephalica antebrachii* or *V. saphena medials*. It was analyzed using the hematological analyzer "ProCyteOne" and the biochemical analyzer "CatalystOne" by IDEXX (see **Table 6**).

TABLE 6: MEAN VALUES AND STANDARD DEVIATION OF THE HEMATOLOGY AND BLOOD CHEMISTRY

	Hematocrit (PCV%)	***Leukocyte count ($x10^9/L$)***	***Lymphocyte count ($x10^9/L$)***	***Neutrophile count ($x10^9/L$)***	***Platelet Count ($x10^9/L$)***	***TP (g/L)***	***ALT (U/L)***	***Creatinine (µmol/L)***
Mean	31.85	12.98	4.63	6.87	318.67	61.34	43.02	103.20
± ***SD***	12.65	4.39	4.74	3.48	111.71	26.85	38.40	30.87

Of the total 24 cases, x-rays were taken in 14 cases, in 8 cases an ultrasound was made and in case of one cat, computed tomography was performed. The percentual breakdown is shown in **Figure 2**.

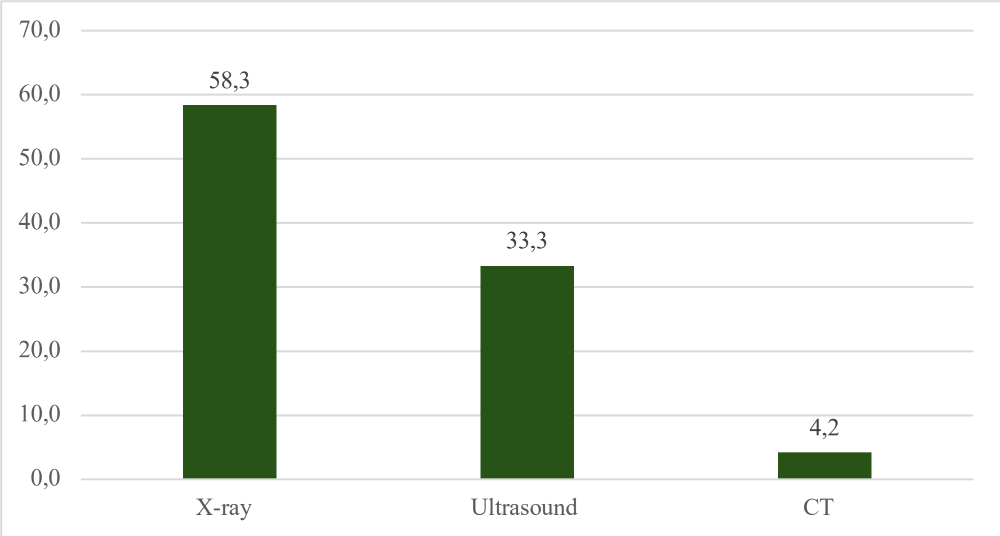


FIGURE 2: DISTRIBUTION OF DIAGNOSTIC IMAGING (%)

Cytology was done in 4 cases. And in 16 cases, histopathological examinations were carried out, with the tissue obtained during surgery.

Various tumor types were identified in this study, including squamous cell carcinoma n=10, adenocarcinoma (luminar carcinoma) n=4, as well as tubulopapillar carcinoma and carcinosarcoma with each n=1. The percentage distribution is illustrated in **Figure 3**.

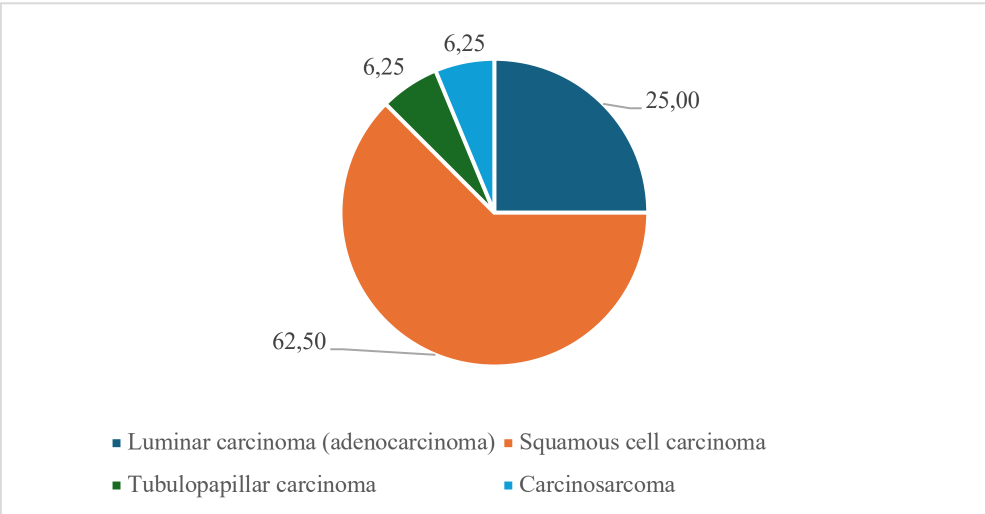


FIGURE 3: HISTOPATHOLOGICAL TYPES OF TUMORS (%) (N=16)

Surgeries were done in 17 of the 24 cases.

Anesthesia was performed as follows: For the pre-medication, the sedative midazolam (0.02-0.05ml), a benzodiazepine was administered, followed by the opioid methadone (0.02-0.05ml). The induction of anesthesia was performed with propofol. All agents were administered intravenously. The general anesthesia was maintained with isoflurane, as inhalation anesthesia.

The different approaches of surgery used in this study were: mastectomy n=9, regional mastectomy n=4 and uni- and bilateral mastectomy which were done in n=2 cases each. Lumpectomy was not performed in any case of this study. The percentages are visualized in **Figure 4**.
Reconstructive surgery was done in n=3 of the cases, which equals 17.65%.

The lymph nodes were always removed together with the tumor, during the surgeries.

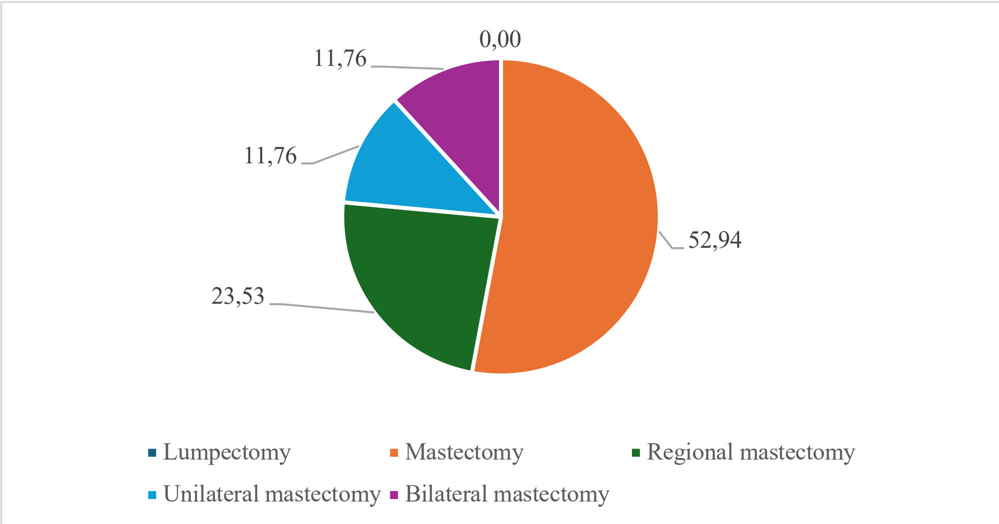


FIGURE 4: SURGICALLY TREATED CASES (N=17) DIVIDED BY THE SURGICAL METHOD (%)

Adjuvant, chemotherapy was done in 3 cases (12.5%). Either doxorubicin (n=1), or palladia (n=2) were administered.

Meloxicam was used in n=7 cases (29.17%) and prednisolone in n=1. For antibiotic treatment amoxicillin/clavulanic acid was given in n=4 cases.

Follow-up information was available in n=8 cases (see **Table 7**).

TABLE 7: FOLLOW-UP INFORMATION (N=8)

	Age (years) (n=24)	*Relapse free period (days) (n=8)*	*Overall survival time (days) (n=8)*
Mean	10.2	549.9	617.8
SD ±	4.2	472.3	431.4
Median	11.0	380.5	465.0

The study used the following tools for the statistical analysis: Khi-square test, regression analysis, Pearson correlation, F-test and T-test (with EXCEL), Kaplan-Meier curve, as well as Mann-Whitney U-test.

4.2. Results

This study tried to find a correlation between the age and the size of the tumor (at the largest diameter). A tendency towards a negative development of tumor size, with increasing age was observed, meaning the tumors became smaller with age (see **Figure 5**).

This was proven with the correlation coefficient being negative (r= -0.6379). The connection is statistically significant, with p= 0.0018.

The Pearson correlation had a significance in the two-tailed probability (p= 0.0014).

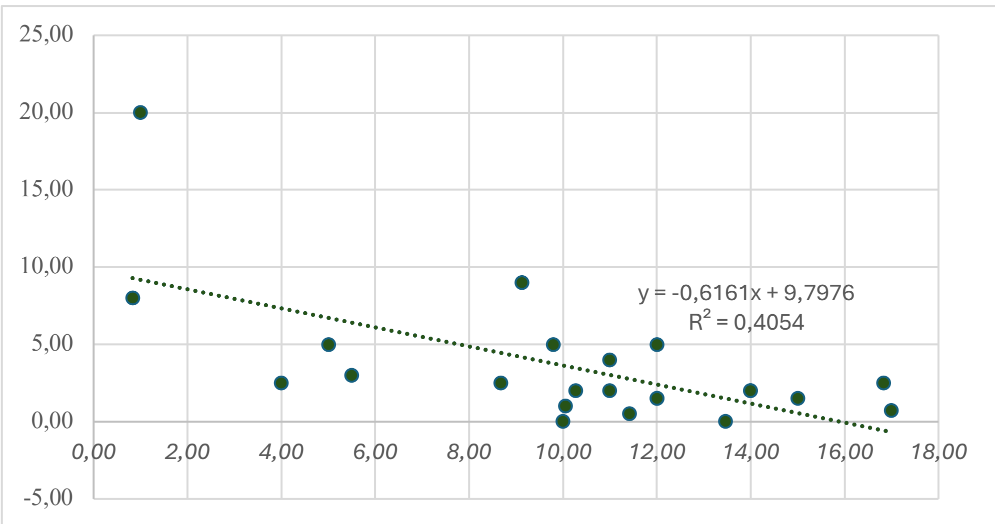


FIGURE 5: CORRELATION OF AGE (YEARS) VS SIZE OF TUMOR (LARGEST DIAMETER IN CM)

Another correlation that was looked at, was the correspondence of age with the creatinine value (µmol/L) (see **Figure 6**).

In this correlation, the two-tailed probability also showed a significance with p= 0.0074.

With the coefficient of determination (R^2) at 78.96%, it was possible to confirm the strong relationship of the two parameters, with a high amount of the creatinine changes being due to the age.

The creatinine would increase with the ascending age of the animal (r= 0.6810).

This statistic was also established to be significant, with a value of p= 0.0073.

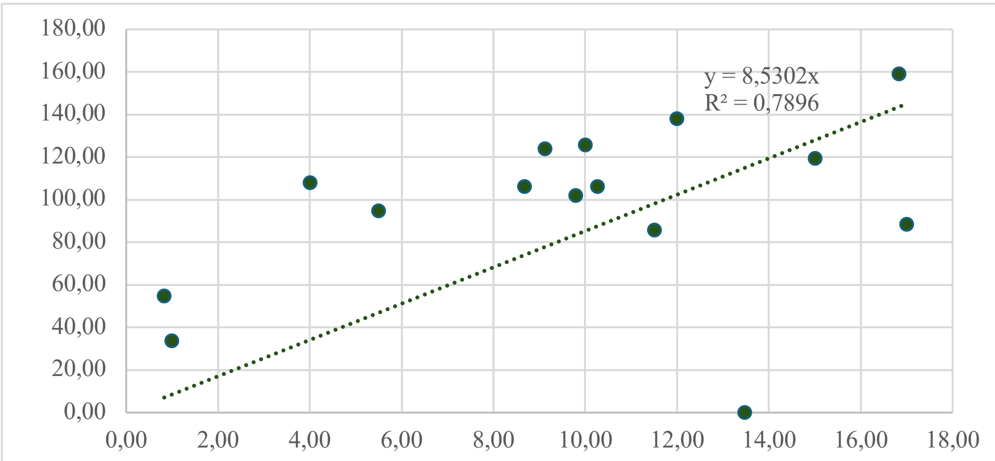


FIGURE 6: CORRELATION OF AGE (YEARS) VS SERUM CREATININE (µMOL/L)

A further association between age and a blood parameter was done, this time regarding the leucocyte count ($x10^9$/L).

In this case the one-tailed probability was of significance (p= 0.0467), however the two-tailed probability was of no importance (p= 0.0934).

The age and leukocyte count correlation (see **Figure 7**) showed an increase of y= 1.0124 of the leukocytes per year. Meaning, the leukocyte count would increase with the age of the animal. Which also aligns with the positive correlation coefficient (r= 0.4487).

The relation of white blood cells and the age of the animal was however not confirmed to be significant (p=0.0934) in this study.

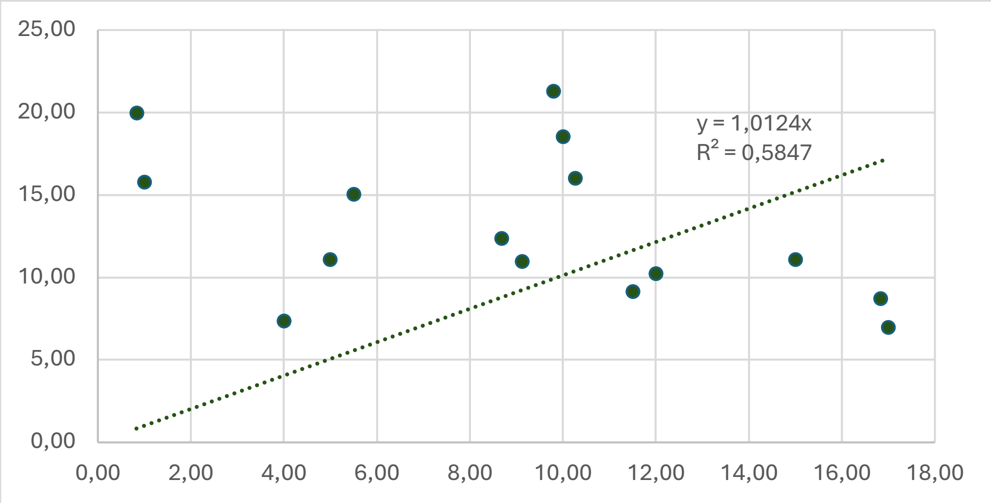


FIGURE 7: CORRELATION OF AGE (YEARS) VS LEUKOCYTE COUNT (X10^9/L)

The neutering status of the cats was also evaluated. Majority of the cats were neutered (n=20).

This was documented to have a positive influence on the OST. The mean estimate of non-neutered (n=4) animals was 721 days, whilst the mean OST of neutered animals was, with 1123.9 days, almost twice as long. The median value of the non-neutered animals was equal, for the neutered animals it was 1633.

However, statistically the Kaplan-Meier curve (see **Figure 8** and **Table 8**) showed no significance (p= 0.462).

TABLE 8: LOGRANK OF THE OST IN NON-NEUTERED (0) OR NEUTERED CATS (1)

	Khi-square	***df***	***p***
LogRank	*0.63*	*1*	*0.462*

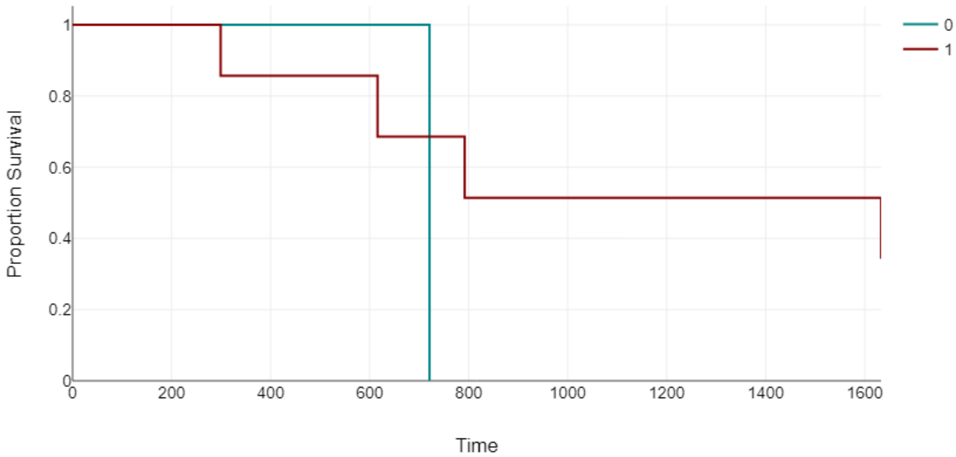


FIGURE 8: OST OF NON-NEUTERED (0) AND NEUTERED (1) CATS

Tumor location, size and quantity were also assessed.

The tumor size was related to various parameters in this study, one of them was the hematocrit value (PCV %).

A significance of the Pearson correlation in the one-tailed probability was found (p= 0.0350), but not with the two-tailed probability (p= 0.0700) (see **Figure 9**).

Overall, a tendency of decrease of the packed cell volume could be observed, as the size of the tumor decreased. This was demonstrated by a negative correlation coefficient value (r= -0.4979).

However, this study was not statistically significant (p= 0.0833).

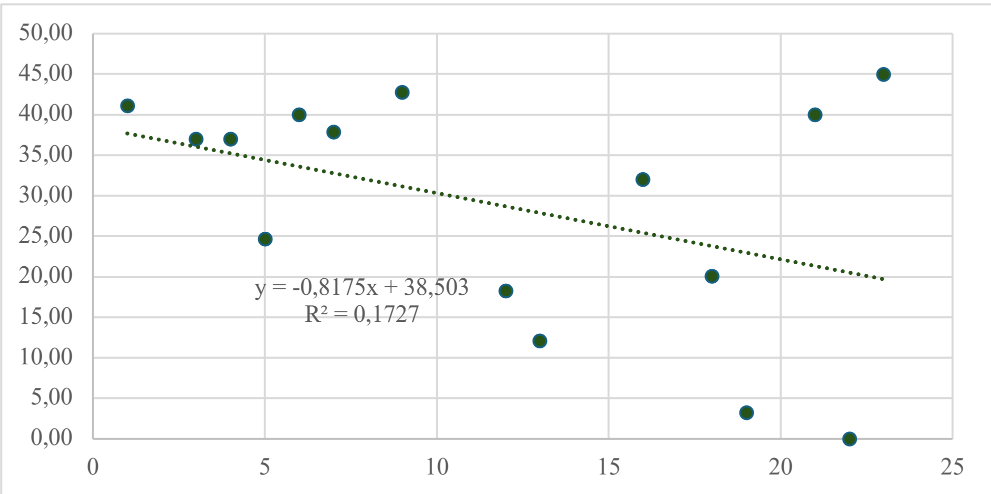


FIGURE 9: CORRELATION BETWEEN TUMOR SIZE (LARGEST DIAMETER IN CM) VS HT (PCV %)

A further parameter associated with the tumor size was the platelet count (x10^9/L) (see **Figure 10**).

It was shown that the number of thrombocytes increases, with the size of tumor (r= 0.5756).

The gradient is y= 12.83, meaning an increase of 12.83 x10^9/L per cm of tumor size.

Both one-tailed and two-tailed probability were of significance (p= 0.01562; p= 0.03125).

Altogether the study could be determined as statistically significant (p= 0.0395).

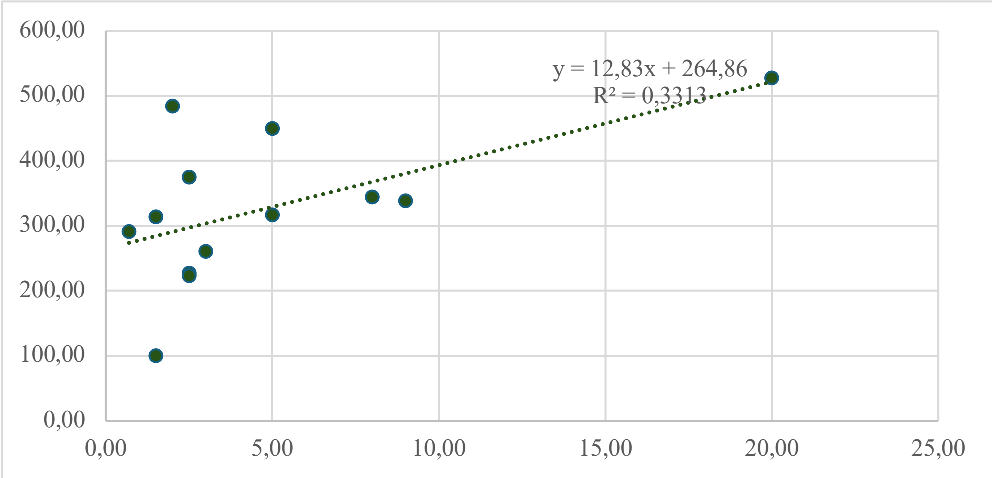


FIGURE 10: CORRELATION BETWEEN TUMOR SIZE (LARGEST DIAMETER IN CM) VS PLATELET COUNT ($x10^9$/L)

Lastly, the connection between creatinine (µmol/L) and the tumor size was established.

With the negative correlation coefficient of r= -0.6804, a decrease of creatinine with a diminishing tumor size could be recognized (see **Figure 11**).

Again, both the one-tailed and two-tailed probability (p= 0.00744; p= 0.01488) demonstrated to be significant and showed an association between the two parameters.

This statistical result proved to be of significance (p= 0.0148).

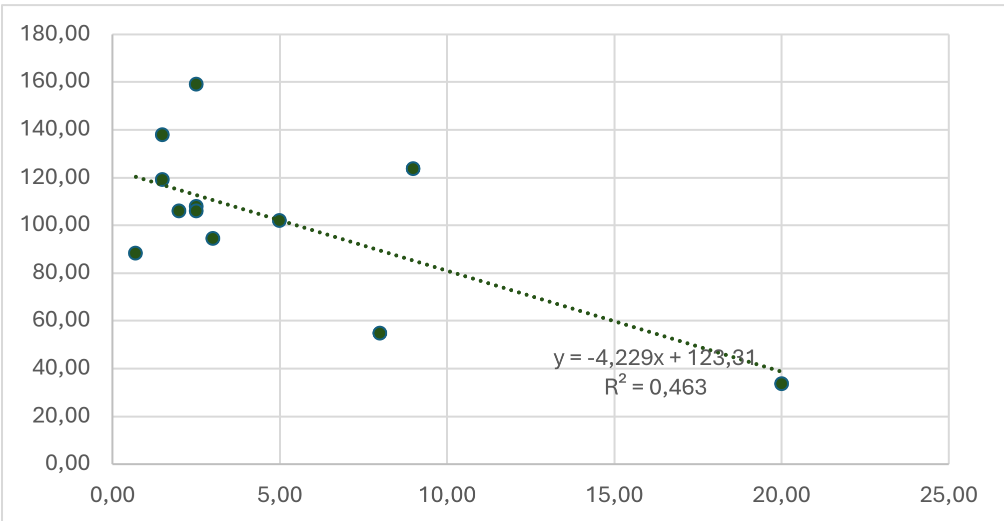


FIGURE 11: CORRELATION BETWEEN TUMOR SIZE (LARGEST DIAMETER IN CM) VS CREATININE (µMOL/L)

During the clinical presentation, different locations of the tumor were noted in 22 cases. In the T1 glands n=7 cats had nodules, n=4 in T2, n=7 cats had neoplastic enlargements in A1 and n=6 in A2. Multiple glands were involved in n=10 cats. The percentage spread is illustrated in **Figure 12**.

Except for somewhat less common nodules in T1, an equal distribution was noted in this study.

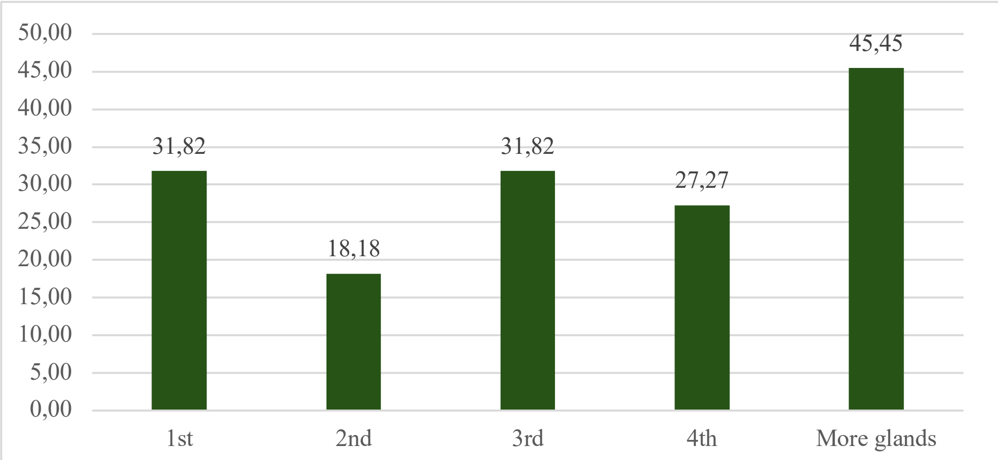


FIGURE 12: LOCATION OF TUMORS IN MAMMARY GLANDS (%) BASED ON AVAILABLE DATA (N=22)

There were also some differences in the distribution of the neoplasms on the two sides of the mammary chains. In 5 animals the tumor was only found on the left side and in 7 only on the right side.

Other animals were found with not exclusively unilateral enlargements on the left (n=15) and right (n=17) side. In 10 animals, the tumors were found on both sides. The percentage distribution is visible in **Figure 13**.

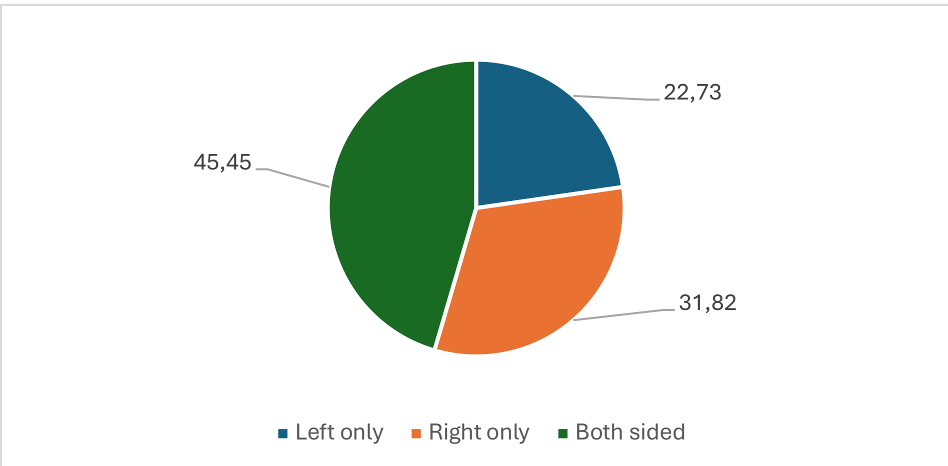


FIGURE 13: LOCATION OF TUMORS ACCORDING TO SIDE (%) BASED ON AVAILABLE DATA (N=22)

As already mentioned, in some cases multiple tumors were found. The relationship of OST and RFP with either single or multiple tumors was investigated.

The OST in animals with solitary tumors, was documented with a mean of 835 days. The median value was 792 days (see **Table 9** and **Figure 14**).

In cases with multiple tumors, the mean OST was shorter with 721 days.

The significance of this correlation was not given according to p= 0.109.

TABLE 9: LOGRANK OF OST WITH SOLITARY (0) OR MULTIPLE (1) TUMORS

	Khi-square	***df***	***p***
LogRank	2.57	1	0.109

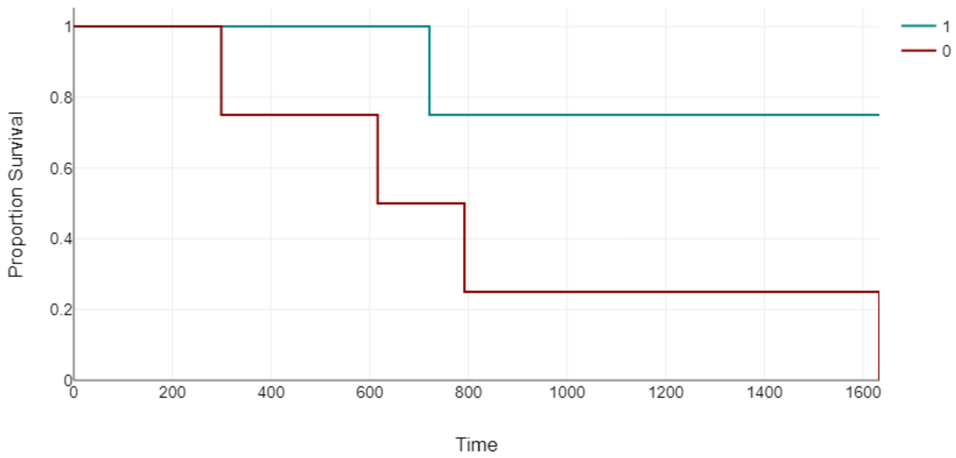


FIGURE 14: OST WITH SOLITARY (0) OR MULTIPLE (1) TUMORS

Similar results were found for the RFP. Here a mean of 462 days was recorded in cases with single tumors, whilst a shorter RFP was found in animals with multiple tumors (mean= 172 days, median= 196 days).

However, this was also not proven to be significant (p= 0.118) (see **Table 10** and **Figure 15**).

TABLE 10: LOGRANK OF RFP WITH SOLITARY (0) OR MULTIPLE (1) TUMORS

	Khi-square	***df***	***p***
LogRank	2.45	1	0.118

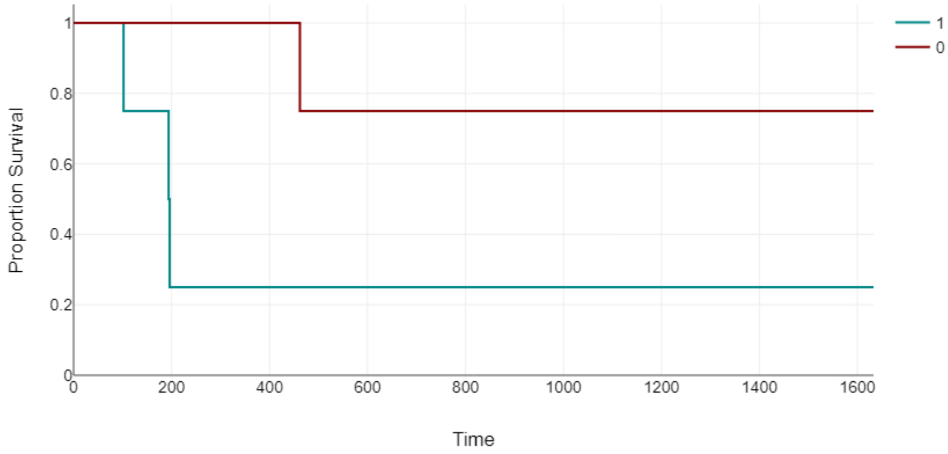


FIGURE 15: RFP WITH SOLITARY (0) OR MULTIPLE (1) TUMORS

Furthermore, several parameters, including age, size of tumor, time of relapse and death, as well as blood and biochemical parameters, were put in correlation with solitary or multiple neoplastic findings (see **Table 11** and **12**).

However, after applying the F-test and T-test, only a negligible significance was found, comparing the quantity of neoplastic changes with the evaluated parameters (see **Table 13**).

TABLE 11: MEAN AND STANDARD DEVIATION OF SEVERAL PARAMETERS IN CATS WITH SOLITARY TUMOR

	Age (years)	*Relapse (years)*	*Death (days)*	*Size of tumor (at the largest diameter in cm)*	*Ht (PCV%)*	*Leukocyte count ($x10^9$/L)*	*Lymphocyte count ($x10^9$/L)*	*Neutrophile count ($x10^9$/L)*	*Platelet count ($x10^9$/L)*	*TP (g/L)*	*ALT (U/L)*	*Creatinine (µmol/L)*
Mean	9.52	796.5	835	3.87	32.41	12.68	4.6	7.13	304.45	69.53	41.33	102.46
SD±	4.5889	514.5748	493.4344	4.6635	10.9559	3.9300	5.0722	2.9826	119.2578	11.2408	43.9621	35.6106

TABLE 12: MEAN AND STANDARD DEVIATION OF SEVERAL PARAMETERS IN CATS WITH MULTIPLE TUMORS

	Age (years)	*Relapse (years)*	*Death (days)*	*Size of tumor (at the largest diameter in cm)*	*Ht (PCV%)*	*Leukocyte count ($x10^9$/L)*	*Lymphocyte count ($x10^9$/L)*	*Neutrophile count ($x10^9$/L)*	*Platelet count ($x10^9$/L)*	*TP (g/L)*	*ALT (U/L)*	*Creatinine (µmol/L)*
Mean	11.95	303.25	400.5	3.87	30.34	13.81	4.73	6.14	357.75	46.6	46.8	105.06
SD±	2.4851	244.1591	185.4596	2.8294	16.3349	5.3792	3.6787	4.4971	74.8444	38.1240	20.5528	12.6262

TABLE 13: F-TEST AND T-TEST RESULTS OF CORRELATION OF VARIOUS PARAMETERS WITH SOLITARY OR MULTIPLE TUMOR

	Age (years)	*Relapse (years)*	*Death (days)*	*Size of tumor (at the largest diameter in cm)*	*Ht (PCV%)*	*Leukocyte count ($x10^9/L$)*	*Lymphocyte count ($x10^9/L$)*	*Neutrophile count ($x10^9/L$)*	*Platelet count ($x10^9/L$)*	*TP (g/L)*	*ALT (U/L)*	*Creatinine (µmol/L)*
F-Test	0.1683	0.2522	0.1422	0.3350	0.2050	0.2853	0.7844	0.1958	0.5898	**0.0029**	0.2940	0.1469
T-Test	0.1282	0.2033	0.2295	1	0.8473	0.7516	0.9634	0.7389	0.3801	0.2992	0.7852	0.8548

Of the 24 cats, the axillar lymph nodes were involved in n=6 of them. And another n=4 had alterations in the inguinal lymph nodes. Both axillar and inguinal lymph nodes were affected in n=2.

Lymph nodes on the left side were involved in n=3, on the right side in n=1, and on both sides in n=8. The percentual distribution is illustrated in **Figure 16**.

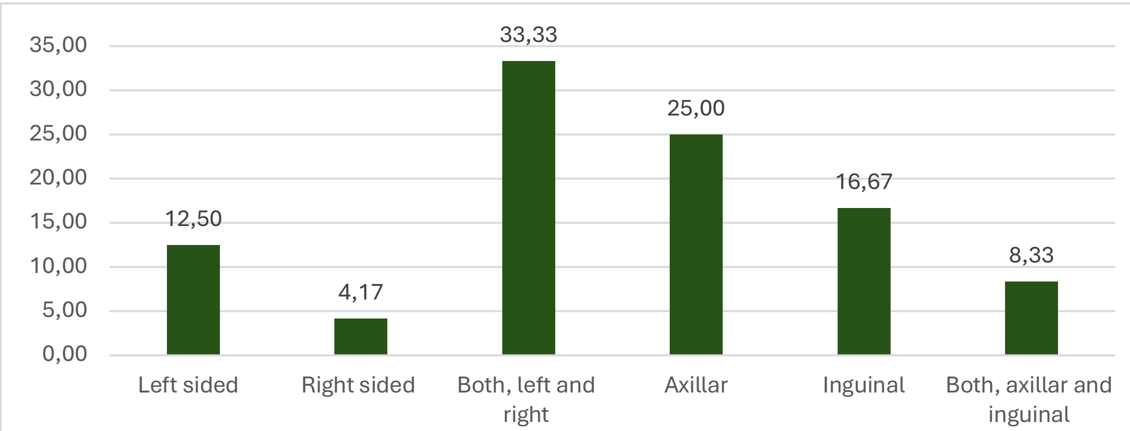


FIGURE 16: LOCATION OF LYMPH NODE INVOLVEMENT (%)

The involvement of the lymph nodes also correlated with the OST. This is depicted in the following **Figure 17**.

The mean OST of animals without lymph node involvement was 1633 days. In cases with lymph node involvement, the OST amounted to be not even half as long, with a mean of 658.3 days and a median of 721 days.

No statistical significance was seen according to the data of this study (p= 0.118) (see **Table 14**).

TABLE 14: LOGRANK OF OST WITHOUT LYMPH NODE INVOLVEMENT (0) OR WITH LYMPH NODE INVOLVEMENT (1)

	Khi-square	*df*	*p*
LogRank	2.45	1	0.118

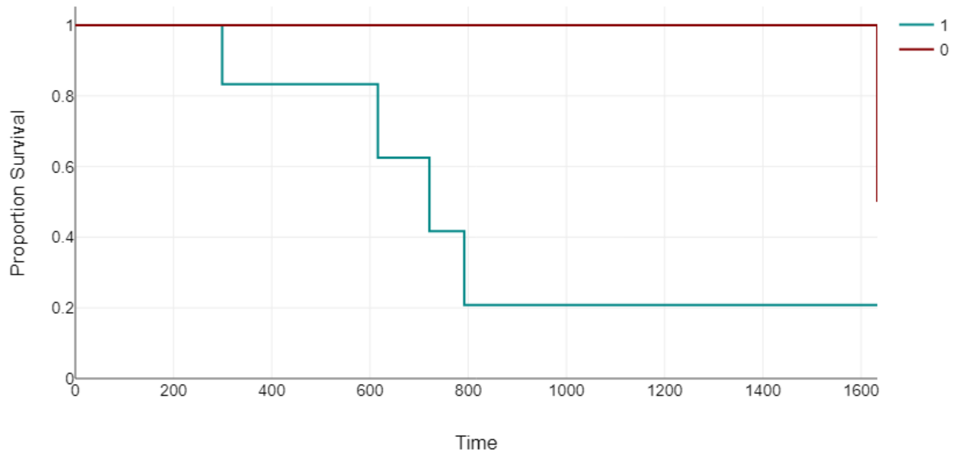


FIGURE 17: OST WITHOUT LYMPH NODE INVOLVEMENT (0) OR WITH LYMPH NODE INVOLVEMENT (1)

As with the quantity of tumors, several parameters, like size of tumor, as well as hematological and biochemical values, were put in relation with the lymph node involvement (see **Table 15** and **16**).

TABLE 15: MEAN AND STANDARD DEVIATION OF SEVERAL PARAMETERS IN CATS WITHOUT LYMPH NODE INVOLVEMENT

	Size of tumor (at the largest diameter in cm)	*Ht (PCV%)*	*Leukocyte count (x10⁹/L)*	*Lymphocyte count (x10⁹/L)*	*Neutrophile count (x10⁹/L)*	*Platelet count (x10⁹/L)*	*TP (g/L)*	*ALT (U/L)*	*Creatinine (µmol/L)*
Mean	5.38	24.19	12.89	5.83	5.2	351	67.79	31.43	100.65
SD±	5.2883	12.5040	4.3744	5.4843	2.1653	100.6727	11.1981	24.2419	40.8304

TABLE 16: MEAN AND STANDARD DEVIATION OF SEVERAL PARAMETERS IN CATS WITH LYMPH NODE INVOLVEMENT

	Size of tumor (at the largest diameter in cm)	***Ht (PCV%)***	***Leukocyte count (x10⁹/L)***	***Lymphocyte count (x10⁹/L)***	***Neutrophile count (x10⁹/L)***	***Platelet count (x10⁹/L)***	***TP (g/L)***	***ALT (U/L)***	***Creatinine (µmol/L)***
Mean	2.2	40.61	13.07	3.27	8.78	281.71	54.9	56.53	105.75
SD±	1.1661	4.5066	4.4112	3.2108	3.7079	112.2392	35.1242	46.5770	15.0110

TABLE 17: F-TEST AND T-TEST RESULTS OF CORRELATION OF VARIOUS PARAMETERS WITH OR WITHOUT LYMPH NODE INVOLVEMENT

	Size of tumor (at the largest diameter in cm)	***Ht (PCV%)***	***Leukocyte count (x10⁹/L)***	***Lymphocyte count (x10⁹/L)***	***Neutrophile count (x10⁹/L)***	***Platelet count (x10⁹/L)***	***TP (g/L)***	***ALT (U/L)***	***Creatinine (µmol/L)***
F-Test	**0.0001**	**0.0251**	0.9471	0.2217	0.1774	0.7547	**0.0136**	0.1351	**0.0280**
T-Test	0.0906	**0.0101**	0.9426	0.3186	0.0660	0.2670	0.4194	0.3113	0.7816

According to the F-test and T-test, several correlations could be detected (see **Table 17**).

There was a difference between the cats with lymph node involvement and the ones with no lymph node involvement, with respect to the dependent variable hematocrit (PCV%) (see **Table 18**).

This was further analyzed with a Mann-Whitney U-test, which is illustrated in **Figure 18**.

The results were proven to be significant (p= 0.009).

TABLE 18: CORRELATION OF LYMPH NODE INVOLVEMENT AND HT (PCV %)

	n	*Mean*	*Median*	*Standard Deviation ±*
No lymph node involvement (0)	8	24.19	22.38	13.37
Lymph node involvement (1)	7	40.61	40	4.87

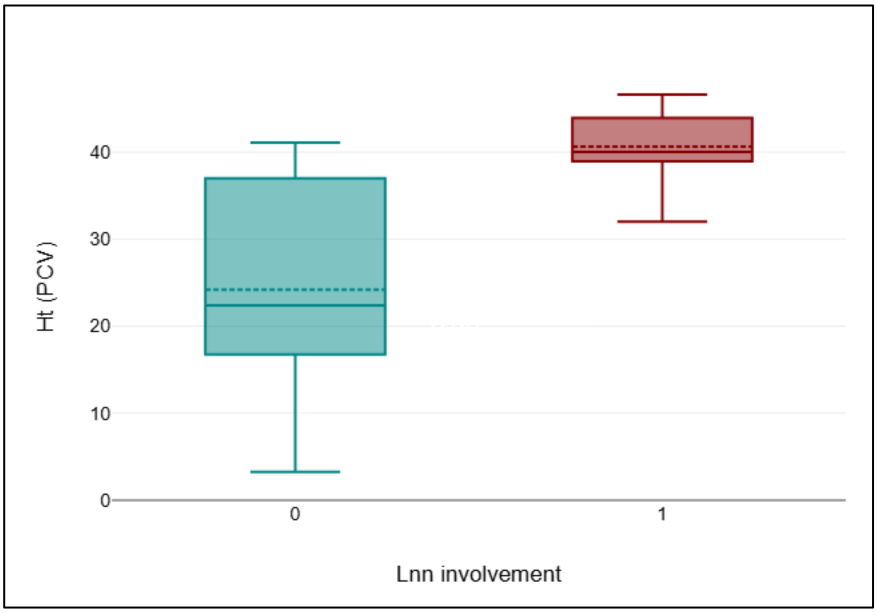


FIGURE 18: CORRELATION OF HT (PCV %) WITHOUT LYMPH NODE INVOLVEMENT (0) OR WITH LYMPH NODE INVOLVEMENT (1)

Metastases were not found in the abdominal lymph nodes; however, they were discovered in the chest (n=1) and different other locations (n=3). These locations included the lungs (n=2) as well as the abdominal cavity (n=1).

The correlation between the lymph node involvement and metastasis was as follows: In case of no lymph node involvement, there was also no distant metastasis in 12 cases.

None of the cases had distant metastasis without lymph node involvement (see **Figure 19**).

In cases where the lymph nodes were involved, there was distant metastasis in 4 cases, and no distant metastasis in 12 cats. A total of 8 cats had lymph node involvement without distant metastasis.

A statistical significance of this relation was proven (p= 0.000193).

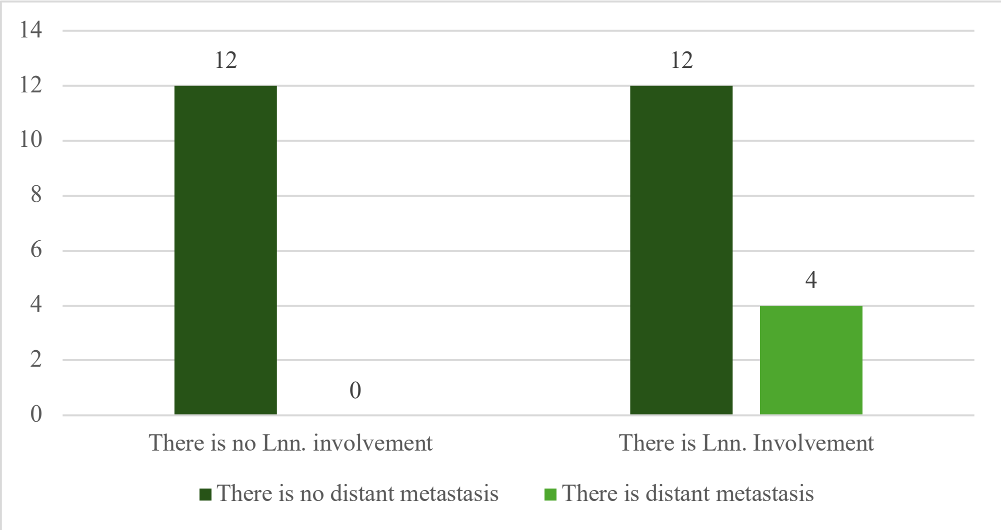


FIGURE 19: CORRELATION BETWEEN LYMPH NODE INVOLVEMENT AND DISTANT METASTASIS

Again, the correlation between various parameters was evaluated, comparing cases without distant metastasis, and cases with distant metastasis (see **Table 19** and **20**).

The F-test and T-test showed no significant results of note (see **Table 21**).

TABLE 19: MEAN AND STANDARD DEVIATION OF SEVERAL PARAMETERS IN CATS WITHOUT DISTANT METASTASIS

	Age (years)	*Relapse (years)*	*Death (days)*	*Size of tumor (at the largest diameter in cm)*	*Ht (PCV%)*	*Leukocyte count ($x10^9/L$)*	*Lymphocyte count ($x10^9/L$)*	*Neutrophile count ($x10^9/L$)*	*Platelet count ($x10^9/L$)*	*TP (g/L)*	*ALT (U/L)*	*Creatinine (µmol/L)*
Mean	10.64	622.8	658.2	4.07	29.16	12.51	4.8	6.37	320.82	63.53	40.2	102.28
SD±	2.4854	253.8245	172.5231	1.1726	4.6569	5.0857	3.9567	4.2289	105.7742	37.8535	30.2289	2.1478

Table 20: Mean and standard deviation of several parameters in cats with distant metastasis

	Age (years)	*Relapse (years)*	*Death (days)*	*Size of tumor (at the largest diameter in cm)*	*Ht (PCV%)*	*Leukocyte count (x10⁹/L)*	*Lymphocyte count (x10⁹/L)*	*Neutrophile count (x10⁹/L)*	*Platelet count (x10⁹/L)*	*TP (g/L)*	*ALT (U/L)*	*Creatinine (µmol/L)*
Mean	8.19	428.33	550.33	3	39.25	14.27	4.18	8.23	312.75	53.33	52.4	105.5
SD±	2.4854	253.8245	172.5231	1.1726	4.6569	5.0857	3.9567	4.2289	105.7742	37.8535	30.2289	2.1478

Table 21: F-Test and T-Test results of correlation of various parameters with or without distant metastasis

	Age (years)	*Relapse (years)*	*Death (days)*	*Size of tumor (at the largest diameter in cm)*	*Ht (PCV%)*	*Leukocyte count (x10⁹/L)*	*Lymphocyte count (x10⁹/L)*	*Neutrophile count (x10⁹/L)*	*Platelet count (x10⁹/L)*	*TP (g/L)*	*ALT (U/L)*	*Creatinine (µmol/L)*
F-Test	0.4985	0.4257	0.2360	0.0605	0.1369	0.37148	0.9200	0.2607	0.8262	0.1137	0.9849	**0.0007**
T-Test	0.2088	0.5761	0.7235	0.4354	0.0677	0.6113	0.8305	0.5190	0.9135	0.7440	0.6553	0.7977

The OST of cases with or without distant metastasis was also estimated (see **Figure 20**).

In cases with no distant metastasis, the mean OST was 1198 days, and the median was documented at 1633 days. The cases in which distant metastasis was present, had a distinctly shorter mean and median OST (mean= 686 days; median= 721 days).

However, this result had no statistical significance, as indicated by p= 0.164 (see **Table 22**).

Table 22: LogRank of OST without distant metastasis (0) or with distant metastasis (1)

	Khi-square	***df***	***p***
LogRank	1.93	1	0.164

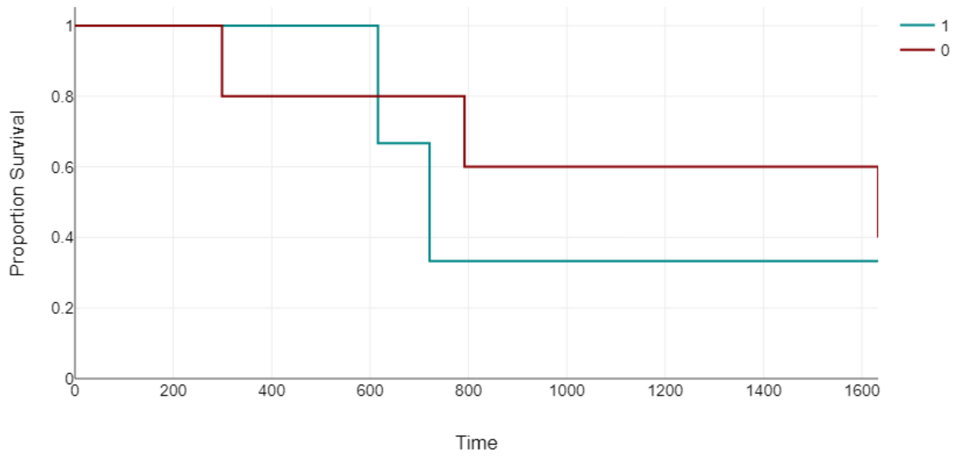


FIGURE 20: OST WITHOUT DISTANT METASTASIS (0) OR WITH DISTANT METASTASIS (1)

4.3. Discussion

The study was able to show a significant relationship (p= 0.0018) between the increasing age of the animal and the decreasing size of the tumor. This could have a list of reasons.

One of them is the reduced hormonal activity in older animals, which was discussed during the literature review, which potentially has an effect on the growth of the tumor [13][15].

Another reason could be the more regular examination of the animals at an older age, which can lead to an earlier detection of the neoplasm, therefore at a smaller size.

A further significant (p= 0.0073) correlation with the age of the animal was found with higher creatinine (µmol/L) values. However, this is most likely due to the physiological process, connected with the growing older of the cat, rather than because of the tumor.

Older cats are prone to chronic kidney insufficiencies, reduction of the glomerular filtration rate (GFR) and general deterioration of the kidney function. All these factors may lead to a higher creatinine value.

A higher leukocyte count can be, as mentioned in the literature review, a guide for the increased risk of tumor recurrence and tumor-related death [59].

As part of the immune defense, the leukocytes migrate to the site of inflammation, in this case the mammary tumor, to target it. Contradictory, this can also promote tumor growth, as it supplies nutrients and growth factors.

This reaction can also be an explanation of the results of this study, even though they were not statistically significant ($p = 0.0934$).

It should however not be forgotten that the high leukocyte count can also be caused by other inflammatory processes in the body, especially at an older age.

The tumor size also showed some significance when put in correlation with different parameters.

Even if the statistical significance ($p = 0.0833$) was not clear, there was a connection between the declining hematocrit (PCV%) value and the decreasing size of the tumor.

As in the studies mentioned above during the literature review, the hematocrit value drops because of the tumor. This is due to the development of anemia from the hypoxia caused by the neoplastic processes [18]. However, there is a contradiction, compared to the previous studies. As in those, a larger tumor or worse tumor grade is associated with a lower hematocrit value [21].

The results of this study can be explained by the low significance of the values and the small number of cases.

Another significance ($p = 0.0395$) was found in the relationship of tumor size with the platelet count (µmol/L). The studies discussed in the literature review, showed a correlation of increased platelet values with tumor masses and poorer prognosis [21].

In this study, it was also demonstrated, that an increase in tumor size correlates with a higher thrombocyte count.

This can be explained with various reactions of the tumor towards the platelets.

For one thing, it can be caused by the release of growth factors like thrombopoietin or other inflammatory factors like interleukins (for example IL-6), both of which might lead to increased production of platelets.

Another factor are the inflammatory processes caused by the tumor, which can also lead to a higher number of thrombocytes.

Similarly to leukocytes, thrombocytes might also indirectly aid the tumor growth, in this case through angiogenesis.

The last parameter of significance (p= 0.0148) in connection with the tumor size in this study, was the creatinine. This value decreased with an increasing tumor size.

A possible explanation for this is the worse general condition of the affected cat. A larger tumor size indicates more pronounced paraneoplastic symptoms, which was also mentioned during the literature review [42]. These oftentimes encompass cachexia. Tumor cachexia also includes a lower muscle mass, which can be an explanation for the lower creatinine values.

The distribution of the tumor location in the different glands is much discussed in several publications, some of which were also mentioned in the literature review at the beginning of the study [10][16][17].

The data from the cases discussed here, documented a similar distribution of the tumor masses within the thoracic and abdominal glands. This can probably be explained by a homogenous lymphatic drainage, to both the axillar and inguinal lymph nodes.

The correlation of (distant) metastasis and lymph node involvement was also proven significant (p= 0.00019).

Often, when the lymph nodes are involved, it indicates a possible metastisation of the tumor. This is also an important part of the staging during the diagnostic work-up.

It was shown that there is no distant metastasis without any lymph node involvement. This may be an indication that the metastisation mainly spreads via the lymphatic pathways.

However, metastases were not found in every case of affected lymph nodes. Which, for example, could be a sign of a lower staging of the tumors in this study.

These findings also emphasis the importance of the examination of the lymph nodes during the clinical anamnesis.

Another significant (p= 0.009) correlation, with an effect on the lymph nodes, was found looking at the hematocrit (PCV%) value. A higher value was found with a higher proportion of lymph node involvement.

This does not quite match the previous results of decreasing hematocrit with increasing tumor size. It could, however, be related to the connection of the age and lymph node involvement of the cats. This was demonstrated to be significantly (p= 0.004) higher in older animals, with a mean age of 11.7 years. The mean age of animals without lymph node involvement was 8.7 years.

These results can be an explanation for the higher hematocrit values, as older animals exhibit a physiologically higher packed cell volume. The fact that older animals have more frequently affected lymph nodes can therefore also explain the increased hematocrit.

Another reason for an elevated packed cell volume could be the tumor related dehydration of the animals. However, this is more likely to be an accidental finding.

A distinct difference of mean and median OST was found when looking at neutered and non-neutered cats. With cats that were spayed having a longer survival time.

This can be attributed to the hormonal influence before spaying, which has been proven in several studies, that were also discussed in the literature review [9][12].

Even though, in this study a clear distinction in the different number of days could be documented, the result had no statistical significance (p= 0.462).

The mean and median OST and RFP of multiple, compared to solitary tumors was also clearly distinctive, with both parameters being shorter in animals with multiple tumors. But they were not proven to be statistically significant (p= 0.109; p= 0.118).

A shorter survival and worse prognosis can be the result of a higher tumor load and aggressiveness of the neoplasm, indicated by the multiple tumor masses.

The OST in cases of lymph node involvement was also estimated and noted to be more than half as short, as in animals without lymph node involvement. However, this difference was again not statistically significant (p= 0.118).

A similar explanation applies, as for the previous factor, with more rapid tumor progression. Additionally, lymph node involvement factors a higher staging of the tumor, and therefore higher malignancy and worse prognosis.

The same reasoning can be used for the difference in OST, of cases with or without distant metastasis. An almost twice as long OST was seen in cases where no distant metastasis was present, however, also not statistically significant (p= 0.164)

Tumors with metastasis are staged as grade 4 and the prognosis in general is poor, which correlates with the findings of this case study.

4.4. Conclusion

The study showed similar results regarding the general characteristics of cats affected by mammary tumors like other, previous studies, mentioned in the literature review. This includes older age of the animals, mostly (in this study exclusively) females and the typical breeds such as ESH and different oriental breeds.

Most of the animals in this study were neutered, nevertheless it aligns with the consensus, as a distinct difference in the OST of non-neutered and neutered cats could be shown.

The OST in general could be traced back to the most important, influential factors, which were: tumor size, lymph node involvement and metastases.

For the diagnosis, various hematological parameters were identified as useful, like platelet and leukocyte count, which both increase with growing tumor size. Or the creatinine value, which decreases with an increasing tumor size.

As the study consists of only few cases, it is difficult to draw any further significant conclusions.

5. Summary

Mammary tumors in cats are of high clinical relevance due to their frequent occurrence and fatal course.

The predisposing factors are consistent in most studies and could also be confirmed with this case study.

In summary, the majority are female cats, around 10 years old, of the ESH or oriental breed, predominantly Siamese cats.

Steroid hormones have, in several studies, also been found to enhance the development of mammary tumors. This applies to exogenous hormones such as progestin or endogenous hormones. For the latter, a significant reduction in the risk of mammary tumors has been observed with early spaying.

The majority of mammary tumors in cats are malignant (80-90%) and, by histopathological differentiation, mainly adenocarcinomas. In the case of benign changes, which can also be influenced by steroid treatments, they can be non-neoplastic or neoplastic. The most common are fibroadenoma or fibroadenomatous hyperplasia.

The distribution of malignant tumors is mainly via the lymphatic system, and less frequently via the blood vessels, in that case mostly through the veins.

Metastases are mainly in the lungs, lymph nodes and organs of the abdominal cavity, such as the liver or spleen.

The clinical presentation of the disease and localization of the tumors in the various glands, could not be determined uniformly. However, in the majority of cases multiple glands were affected.

Early detection and treatment of the disease is important, as early treatment leads to better results.

The diagnosis can be made by various means. The most important step is the initial staging of the tumor, according to the system by the WHO. Here the size of the tumor, involvement of the lymph nodes and metastases are analyzed, brought together and graded. This grading is one of the most important steps in predicting the prognosis.

Further diagnostic steps include blood and biochemical testing, immunohistochemistry, cytology or histopathology and diagnostic imaging.

The studies discussed, as well as this case study, come to different prognoses depending on the interpretation of the markers, values or image material, and so standardization of the cut-off values and the associated result is necessary in future studies.

Even as this is the case, the general consensus is, that the prognosis for the course of the disease is usually poor.

There are also various approaches to treatment, with radical mastectomy being the preferred method. Despite the higher risk of wound healing in patients with neoplastic changes, this method provides the best conditions for recovery and less frequent relapse.

There are different opinions on chemotherapy as an additional treatment, with the active substance playing a major role. It is mainly used in severe cases such as high-grade or inoperable tumors. The most used agent is doxorubicin, although research has come to different conclusions, regarding its effectiveness in terms of DFS and MST.

Other possible treatment options include radiotherapy, immunotherapy or hormone therapy. There have been only few proven effective cases, and further research is needed in this area.

To conclude, mammary tumors in cats are an important and frequent disease, which is why further research is needed as key, for better understanding and approaching the disease.

6. Literature references

1. Kessler M, Kühnel S, Kurz K, Wimmer N (2014) KTP- Mammakarzinome der Katze – klinische Befunde und Therapieverlauf bei 34 Patienten. Kleintierpraxis 245–251. https://doi.org/10.2377/0023-2076-59-245

2. Dagher E, Abadie J, Loussouarn D, Campone M, Nguyen F (2019) Feline Invasive Mammary Carcinomas: Prognostic Value of Histological Grading. Vet Pathol 56:660–670. https://doi.org/10.1177/0300985819846870

3. Hughes K, Dobson JM (2012) Prognostic histopathological and molecular markers in feline mammary neoplasia. Vet J 194:19–26. https://doi.org/10.1016/j.tvjl.2012.05.008

4. Hahn K (1997) Feline mammary neoplasia: Biological behavior, diagnosis, and treatment alternatives. Feline Pract 25:

5. Morris J (2013) Mammary Tumours in the Cat: Size matters, so early intervention saves lives. J Feline Med Surg 15:391–400. https://doi.org/10.1177/1098612X13483237

6. Lana S (2007) Tumors of the Mammary Gland. In: Withrow & MacEwen's Small Animal Clinical Oncology. Elsevier, pp 619–636

7. Giménez F, Hecht S, Craig LE, Legendre AM (2010) Early Detection, Aggressive Therapy: Optimizing the Management of Feline Mammary Masses. J Feline Med Surg 12:214–224. https://doi.org/10.1016/j.jfms.2010.01.004

8. Kessler M (2022) Kleintieronkologie: Diagnose und Therapie von Tumorerkrankungen bei Hund und Katze, 4., vollständig überarbeitete Auflage. Thieme, Stuttgart

9. Dorn CR, Taylor DO, Schneider R, Hibbard HH, Klauber MR (1968) Survey of animal neoplasms in Alameda and Contra Costa Counties, California. II. Cancer morbidity in dogs and cats from Alameda County. J Natl Cancer Inst 40:307–318

10. Viste JR, Myers SL, Singh B, Simko E (2002) Feline mammary adenocarcinoma: tumor size as a prognostic indicator. Can Vet J Rev Veterinaire Can 43:33–37

11. Graf R, Grüntzig K, Hässig M, Axhausen KW, Fabrikant S, Welle M, Meier D, Guscetti F, Folkers G, Otto V, Pospischil A (2015) Swiss Feline Cancer Registry: A Retrospective

Study of the Occurrence of Tumours in Cats in Switzerland from 1965 to 2008. J Comp Pathol 153:266–277. https://doi.org/10.1016/j.jcpa.2015.08.007

12. Overley B, Shofer FS, Goldschmidt MH, Sherer D, Sorenmo KU (2005) Association between Ovarihysterectomy and Feline Mammary Carcinoma. J Vet Intern Med 19:560–563. https://doi.org/10.1111/j.1939-1676.2005.tb02727.x

13. Mol JA, Van Garderen E, Rutteman GR, Rijnberk A (1996) New insights in the molecular mechanism of progestin-induced proliferation of mammary epithelium: Induction of the local biosynthesis of growth hormone (GH) in the mammary gland of dogs, cats and humans. J Steroid Biochem Mol Biol 57:67–71. https://doi.org/10.1016/0960-0760(95)00251-0

14. Tamada H, Kawate N, Inaba T, Sawada T (2003) Long-term prevention of estrus in the bitch and queen using chlormadinone acetate. Can Vet J Rev Veterinaire Can 44:416–417

15. Jacobs TM, Hoppe BR, Poehlmann CE, Ferracone JD, Sorenmo KU (2010) Mammary adenocarcinomas in three male cats exposed to medroxyprogesterone acetate (1990-2006). J Feline Med Surg 12:169–174. https://doi.org/10.1016/j.jfms.2009.08.004

16. Hayden DW, Nielsen SW (1971) Feline mammary tumours. J Small Anim Pract 12:687–698. https://doi.org/10.1111/j.1748-5827.1971.tb06197.x

17. Weijer K, Head KW, Misdorp W, Hampe JF (1972) Feline Malignant Mammary Tumors. I. Morphology and Biology: Some Comparisons With Human and Canine Mammary Carcinomas2. JNCI J Natl Cancer Inst 49:1697–1704. https://doi.org/10.1093/jnci/49.6.1697

18. Varlotto J, Stevenson MA (2005) Anemia, tumor hypoxemia, and the cancer patient. Int J Radiat Oncol 63:25–36. https://doi.org/10.1016/j.ijrobp.2005.04.049

19. Lautscham E (2017) Feline Mammatumoren. Veterinär Spieg 27:9–14. https://doi.org/10.1055/s-0042-122352

20. Cassali G, Jark P, Gamba C, Damasceno K, Estrela-Lima A, Nardi, A, Ferreira E, Horta R, Firmo B, Sueiro F, Rodrigues L, Nakagaki K (2020) Consensus Regarding the Diagnosis, Prognosis and Treatment of Canine and Feline Mammary Tumors - 2019. Braz J Vet Pathol 13:555–574. https://doi.org/10.24070/bjvp.1983-0246.v13i3p555-574

21. Kurban İ, Günay Uçmak Z (2024) Evaluation of Erythrocytes Indices, Platelet Indices and Complete Blood Count in Feline Mammary Carcinomas. Kocatepe Vet J. https://doi.org/10.30607/kvj.1479289

22. König HE, Liebich H-G (2005) Anatomie der Haussäugetiere: Lehrbuch und Farbatlas für Studium und Praxis ; Ausgabe in einem Band, 3., überarb. und erw. Aufl. Schattauer, Stuttgart

23. Habermehl K-H (2005) Lehrbuch der Anatomie der Haustiere. 3: Kreislaufsystem, Haut und Hautorgane, 4., unveränd. Aufl. Parey, Berlin Hamburg

24. Raharison F, Sautet J (2006) Lymph drainage of the mammary glands in female cats. J Morphol 267:292–299. https://doi.org/10.1002/jmor.10403

25. Withrow SJ (2013) Withrow and MacEwen's small animal clinical oncology, 5. ed. Saunders, Philadelphia, Pa.

26. Skorupski KA, Overley B, Shofer FS, Goldschmidt MH, Miller CA, Sørenmo KU (2005) Clinical Characteristics of Mammary Carcinoma in Male Cats. J Vet Intern Med 19:52–55. https://doi.org/10.1111/j.1939-1676.2005.tb02658.x

27. Ito T, Kadosawa T, Mochizuki M, Matsunaga S, Nishimura R, Sasaki N (1996) Prognosis of Malignant Mammary Tumor in 53 Cats. J Vet Med Sci 58:723–726. https://doi.org/10.1292/jvms.58.723

28. Garcês A, Delgado L, Brilhante Simões P, Pires I, Queiroga F, Prada J (2019) Most Frequent Feline Tumours-A Breed Approach 10-Year Retrospective Study

29. Hayes H, Milne K, Mandell C (1981) Epidemiological features of feline mammary carcinoma. Vet Rec 108:476–479. https://doi.org/10.1136/vr.108.22.476

30. Rissetto K, Villamil JA, Selting KA, Tyler J, Henry CJ (2011) Recent Trends in Feline Intestinal Neoplasia: an Epidemiologic Study of 1,129 Cases in the Veterinary Medical Database from 1964 to 2004. J Am Anim Hosp Assoc 47:28–36. https://doi.org/10.5326/JAAHA-MS-5554

31. Misdorp W, Romijn A, Hart AA (1991) Feline mammary tumors: a case-control study of hormonal factors. Anticancer Res 11:1793–1797

32. Hayden DW, Barnes DM, Johnson KH (1989) Morphologic Changes in the Mammary Gland of Megestrol Acetate-treated and Untreated Cats: A Retrospective Study. Vet Pathol 26:104–113. https://doi.org/10.1177/030098588902600202

33. Novosad CA (2003) Principles of treatment for mammary gland tumors. Clin Tech Small Anim Pract 18:107–109. https://doi.org/10.1053/svms.2003.36625

34. Cotchin E (1952) Neoplasms in cats. Proc R Soc Med 45:671–674

35. Chen HC (1968) A case of feline papilliferous mammary adenocarcinoma with widespread metastases. Can J Comp Med Rev Can Med Comp 32:465–467

36. Seixas F, Palmeira C, Pires MA, Bento MJ, Lopes C (2011) Grade is an independent prognostic factor for feline mammary carcinomas: A clinicopathological and survival analysis. Vet J 187:65–71. https://doi.org/10.1016/j.tvjl.2009.10.030

37. Kessler M, Sandra Kühnel, Johanna Kurz, Nina Wimmer (2014) Mammakarzinome der Katze – klinische Befunde und Therapieverlauf bei 34 Patienten. Kleintierpraxis 59:245–251

38. Waters D, Honeckman A, Cooley D, DeNicola D (1998) Skeletal metastasis in feline mammary carcinoma: case report and literature review. J Am Anim Hosp Assoc 34:103–108. https://doi.org/10.5326/15473317-34-2-103

39. Torrigiani F, Moccia V, Brunetti B, Millanta F, Valdivia G, Peña L, Cavicchioli L, Zappulli V (2022) Mammary Fibroadenoma in Cats: A Matter of Classification. Vet Sci 9:253. https://doi.org/10.3390/vetsci9060253

40. Carpenter JA LK; Holzworth, J; Holzworth, J (1987) Tumors and tumor-like lesions. Saunders, Philadelphia

41. Soares M, Correia J, Peleteiro MC, Ferreira F (2016) St Gallen molecular subtypes in feline mammary carcinoma and paired metastases—disease progression and clinical implications from a 3-year follow-up study. Tumor Biol 37:4053–4064. https://doi.org/10.1007/s13277-015-4251-z

42. Finora K (2003) Common paraneoplastic syndromes. Clin Tech Small Anim Pract 18:123–126. https://doi.org/10.1053/svms.2003.36629

43. Owen LN, World Health Organization. Veterinary Public Health Unit, WHO Collaborating Center for Comparative Oncology (1980) TNM Classification of Tumours in Domestic Animals/ edited by L.N. Owen

44. Aiken SW (2003) Principles of surgery for the cancer patient. Clin Tech Small Anim Pract 18:75–81. https://doi.org/10.1053/svms.2003.36619

45. Elston CW, Ellis IO (1991) pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 19:403–410. https://doi.org/10.1111/j.1365-2559.1991.tb00229.x

46. Mills SW, Musil KM, Davies JL, Hendrick S, Duncan C, Jackson ML, Kidney B, Philibert H, Wobeser BK, Simko E (2015) Prognostic Value of Histologic Grading for Feline Mammary Carcinoma: A Retrospective Survival Analysis. Vet Pathol 52:238–249. https://doi.org/10.1177/0300985814543198

47. Scholzen T, Gerdes J (2000) The Ki-67 protein: from the known and the unknown. J Cell Physiol 182:311–322. https://doi.org/10.1002/(SICI)1097-4652(200003)182:3<311::AID-JCP1>3.0.CO;2-9

48. Morris JS, Nixon C, Bruck A, Nasir L, Morgan IM, Philbey AW (2008) Immunohistochemical expression of TopBP1 in feline mammary neoplasia in relation to histological grade, Ki67, ERα and p53. Vet J 175:218–226. https://doi.org/10.1016/j.tvjl.2007.01.006

49. Soares M, Ribeiro R, Carvalho S, Peleteiro M, Correia J, Ferreira F (2016) Ki-67 as a Prognostic Factor in Feline Mammary Carcinoma: What Is the Optimal Cutoff Value? Vet Pathol 53:37–43. https://doi.org/10.1177/0300985815588606

50. Millanta F, Citi S, Della Santa D, Porciani M, Poli A (2006) COX-2 expression in canine and feline invasive mammary carcinomas: correlation with clinicopathological features and prognostic fmolecular markers. Breast Cancer Res Treat 98:115–120. https://doi.org/10.1007/s10549-005-9138-z

51. Sayasith K, Sirois J, DorÉ M (2009) Molecular Characterization of Feline COX-2 and Expression in Feline Mammary Carcinomas. Vet Pathol 46:423–429. https://doi.org/10.1354/vp.08-VP-0161-D-FL

52. Brunetti B, Asproni P, Beha G, Muscatello LV, Millanta F, Poli A, Benazzi C, Sarli G (2013) Molecular Phenotype in Mammary Tumours of Queens: Correlation between Primary Tumour and Lymph Node Metastasis. J Comp Pathol 148:206–213. https://doi.org/10.1016/j.jcpa.2012.05.012

53. Martín De Las Mulas J, Niel MV, Millán Y, Ordás J, Blankenstein MA, Mil FV, Misdorp W (2002) Progesterone receptors in normal, dysplastic and tumourous feline mammary glands. Comparison with oestrogen receptors status. Res Vet Sci 72:153–161. https://doi.org/10.1053/rvsc.2001.0542

54. Hamilton JM, Else RW, Forshaw P (1976) Oestrogen receptors in feline mammary carcinomas. Vet Rec 99:477–479. https://doi.org/10.1136/vr.99.24.477

55. De Campos CB, Damasceno KA, Gamba CO, Ribeiro AM, Machado CJ, Lavalle GE, Cassali GD (2016) Evaluation of prognostic factors and survival rates in malignant feline mammary gland neoplasms. J Feline Med Surg 18:1003–1012. https://doi.org/10.1177/1098612X15610367

56. Chocteau F, Abadie J, Loussouarn D, Nguyen F (2019) Proposal for a Histological Staging System of Mammary Carcinomas in Dogs and Cats. Part 1: Canine Mammary Carcinomas. Front Vet Sci 6:388. https://doi.org/10.3389/fvets.2019.00388

57. Günay Uçmak Z, Koenhemsi L, Uçmak M, Or ME, Erdoğan Bamaç Ö, Öztürk Gürgen H, Parkan Yaramış Ç (2021) Evaluation of Platelet Indices and Complete Blood Count in Canine Mammary Tumors. Acta Sci Vet 49:. https://doi.org/10.22456/1679-9216.114293

58. Da Silva Soares E, Rocha CC, Valente FL, Dos Anjos LRA, De Oliveira FLD, De Oliveira Loures C, Rocha PT, Castro VR, Sarandy TB, Borges APB (2023) Platelet count and MCHC as independent prognostic markers for feline mammary carcinomas. Res Vet Sci 164:105024. https://doi.org/10.1016/j.rvsc.2023.105024

59. Petrucci GN, Lobo L, Queiroga F, Martins J, Prada J, Pires I, Henriques J (2021) Neutrophil-to-lymphocyte ratio is an independent prognostic marker for feline mammary carcinomas. Vet Comp Oncol 19:482–491. https://doi.org/10.1111/vco.12686

60. Tamamoto T, Ohno K, Goto-Koshino Y, Tsujimoto H (2014) Serum Amyloid A Promotes Invasion of Feline Mammary Carcinoma Cells. J Vet Med Sci 76:1183–1188. https://doi.org/10.1292/jvms.14-0108

61. Tamamoto T, Ohno K, Ohmi A, Seki I, Tsujimoto H (2009) Time–course monitoring of serum amyloid A in a cat with pancreatitis. Vet Clin Pathol 38:83–86. https://doi.org/10.1111/j.1939-165X.2008.00082.x

62. Payan-Carreira R, Martins-Bessa AC (2008) Ultrasonographic assessment of the feline mammary gland. J Feline Med Surg 10:466–471. https://doi.org/10.1016/j.jfms.2008.03.006

63. Moraes NS, Borges NC (2021) Sonographic Assessment of the Normal and Abnormal Feline Mammary Glands and Axillary and Inguinal Lymph Nodes. Vet Med Int 2021:1–7. https://doi.org/10.1155/2021/9998025

64. Raskin RE, Meyer DJ (2016) Canine and feline cytology: a color atlas and interpretation guide, 3rd edition. Elsevier, St. Louis, Missouri

65. Matos AJF, Baptista CS, Gärtner MF, Rutteman GR (2012) Prognostic studies of canine and feline mammary tumours: The need for standardized procedures. Vet J 193:24–31. https://doi.org/10.1016/j.tvjl.2011.12.019

66. Cunha S, Corgozinho K, Justen H, Silva K, Leite J, Ferreira AM (2016) Survival and Disease-free Interval of Cats with Mammary Carcinoma Treated with Chain Mastectomy. Acta Sci Vet 44:8. https://doi.org/10.22456/1679-9216.80900

67. Gemignani F, Mayhew PD, Giuffrida MA, Palaigos J, Runge JJ, Holt DE, Robertson NA, Seguin B, Walker M, Singh A, Liptak JM, Romanelli G, Martano M, Boston SE, Lux C, Busetto R, Culp WTN, Skorupski KA, Burton JH (2018) Association of surgical approach with complication rate, progression-free survival time, and disease-specific survival time in cats with mammary adenocarcinoma: 107 cases (1991–2014). J Am Vet Med Assoc 252:1393–1402. https://doi.org/10.2460/javma.252.11.1393

68. MacEwen EG, Hayes AA, Harvey HJ, Patnaik AK, Mooney S, Passe S (1984) Prognostic factors for feline mammary tumors. J Am Vet Med Assoc 185:201–204

69. Bonolo de Campos C, Nunes F, Lavalle G, Cassali G (2014) Use of Surgery and Carboplatin in Feline Malignant Mammary Gland Neoplasms with Advanced Clinical Staging. Vivo Athens Greece 28:863–866

70. Cunha S, Corgozinho K, Justen H, Silva K, Leite J, Ferreira A (2018) Survival and Disease-free Interval of Cats with Mammary Carcinoma Treated with Chain Mastectomy. Acta Sci Vet 44:8. https://doi.org/10.22456/1679-9216.80900

71. McKnight JA (2003) Principles of chemotherapy. Clin Tech Small Anim Pract 18:67–72. https://doi.org/10.1053/svms.2003.36617

72. Mauldin GN, Matus RE, Patnaik AK, Bond BR, Mooney SC (1988) Efficacy and Toxicity of Doxorubicin and Cyclophosphamide Used in the Treatment of Selected Malignant Tumors in 23 Cats. J Vet Intern Med 2:60–65. https://doi.org/10.1111/j.1939-1676.1988.tb02794.x

73. Novosad CA, Bergman PJ, O'Brien MG, McKnight JA, Charney SC, Selting KA, Graham JC, Correa SS, Rosenberg MP, Gieger TL (2006) Retrospective Evaluation of Adjunctive Doxorubicin for the Treatment of Feline Mammary Gland Adenocarcinoma: 67 Cases. J Am Anim Hosp Assoc 42:110–120. https://doi.org/10.5326/0420110

74. Borrego JF, Cartagena JC, Engel J (2009) Treatment of feline mammary tumours using chemotherapy, surgery and a COX-2 inhibitor drug (meloxicam): a retrospective study of 23 cases (2002-2007)*. Vet Comp Oncol 7:213–221. https://doi.org/10.1111/j.1476-5829.2009.00194.x

75. McNeill CJ, Sorenmo KU, Shofer FS, Gibeon L, Durham AC, Barber LG, Baez JL, Overley B (2009) Evaluation of Adjuvant Doxorubicin-Based Chemotherapy for the Treatment of Feline Mammary Carcinoma. J Vet Intern Med 23:123–129. https://doi.org/10.1111/j.1939-1676.2008.0244.x

76. Cunha SCS, Corgozinho KB, Souza HJM, Silva KVGC, Ferreira AMR (2015) Adjuvant chemotherapy with mitoxantrone for cats with mammary carcinomas treated with radical mastectomy. J Feline Med Surg 17:1000–1004. https://doi.org/10.1177/1098612X14567159

77. Fox LE, MacEwen EG, Kurzman RD, Dubielzig RR, Helfand SC, Vail DM, Kisseberth W, London C, Madewell BR, Rodriguez CO, Jeglum KA, Rosenberg M, Rosenthal RC (1995) Liposome-encapsulated Muramyl Tripeptide Phosphatidylethanolamine for the Treatment of Feline Mammary Adenocarcinoma-A Multicenter Randomized Double-blind Study. Cancer Biother 10:125–130. https://doi.org/10.1089/cbr.1995.10.125

78. Rutten VPMG, Misdorp W, Gauthier A, Estrada M, Mialot JP, Parodi AL, Rutteman GR, Weyer K (1990) Immunological aspects of mammary tumors in dogs and cats: a survey including own studies and pertinent literature. Vet Immunol Immunopathol 26:211–225. https://doi.org/10.1016/0165-2427(90)90092-7

79. MacEwen GE, Hayes AA, Mooney S, Patnaik A, Kurzman I, Hardy WDJr (1985) Levamisole as Adjuvant to Chemotherapy for Canine Lymphosarcoma. J Immunother 4:

80. MacEwen GE, Hayes AA, Mooney S, Patnaik AK, Harvey JH, Passe S, Hardy WDJr (1984) Evaluation of Effect of Levamisole on Feline Mammary Cancer. J Immunother 3:

81. Del Portillo Miguel I, Blackwood L, Maiques E, Pérez Roger I, Poch Jiménez E, Borrego J (2024) Evaluation of the efficacy and safety of toceranib phosphate in cats with macroscopic mammary adenocarcinoma. J Feline Med Surg 26:1098612X241256473. https://doi.org/10.1177/1098612X241256473

82. Kitchell B, Fidel J (1992) Tamoxifen as a potential therapy for canine mammary carcinoma. Proc Vet Can Soc 91:91–4

83. Morris J, Dobson J, Bostock D (1993) Use of tamoxifen in the control of canine mammary neoplasia. Vet Rec 133:539–542. https://doi.org/10.1136/vr.133.22.539

84. Dobson JM, Lascelles BDX (2011) BSAVA manual of canine and feline oncology, Third edition. British Small Animal Veterinary Association, Quedgeley, Gloucester

85. Kitchell B, LaRue SM, Rooks R (2000) Veterinary cancer therapy handbook: chemotherapy, radiation therapy, and surgical oncology for the practicing veterinarian, 2nd ed. AAHA Press, Lakewood, Colo.

86. Wypij J, Fan T, de Lorimier L-P (2006) Malignant mammary tumors: Biologic behavior, prognostic factors, and therapeutic approach in cats. Vet Med 101:

87. Moore A (2006) Advances in the treatment of mammary neoplasia

88. Zappulli V, Rasotto R, Caliari D, Mainenti M, Peña L, Goldschmidt MH, Kiupel M (2015) Prognostic Evaluation of Feline Mammary Carcinomas: A Review of the Literature. Vet Pathol 52:46–60. https://doi.org/10.1177/0300985814528221