ESCOLA UNIVERSITÁRIA VASCO DA GAMA

MASTER'S DEGREE

FELINE MAMMARY TUMOURS: A LITERATURE REVIEW

Sandra Cristina da Silva Antunes

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Author

Sandra Cristina da Silva Antunes

Supervisors

Professor Anália do Carmo

(Escola Universitária Vasco da Gama)

Dr. Hugo Vilhena

(Escola Universitária Vasco da Gama)

(Hospital Veterinário do Baixo Vouga)

“Thesis to obtain the Master Degree in Veterinary Medicine in Escola Universitária Vasco da Gama”

ABSTRACT

Mammary tumours (MTs) are among the most common tumours in the cat, 17% of the tumours among female cats. These tumours are highly infiltrative tumours, frequently associated to lymph node metastasis at the time of the initial diagnosis and have a mean survival rate from the time of diagnosis between 6 to 12 months. The aetiology of MTs is not clearly understood but some risk factors have been pointed, namely, age, breed, reproductive status and exposure to oestrogen and progesterone.

In order to establish an adequate treatment it is important to obtain the histological classification, the grading and the staging of the tumour, since most of the MTs present local and distant metastasis at the time of the diagnosis. Tumours of the feline mammary gland are histologically classified according to the diagnostic criteria proposed by the World Health Organization (WHO). This classification is based on descriptive morphology and divides MTs into four main groups: mammary hyperplasia/dysplasia; benign tumours, malignant tumours, and unclassified tumours. The histological grade is determined according to an adaptation of the classification used by Elston and Ellis and is based on the degree of tubule formation, degree of nuclear and cellular pleomorphism, and mitotic count. The staging of feline MT is based on a modified WHO clinical staging system.

Surgery is the most widely used treatment for mammary tumours in the cat; it is used alone or in combination with chemotherapy. Radical mastectomy is the surgical method of choice because it significantly reduces the chance of local tumour recurrence. The inguinal lymph node is virtually always removed with the mammary gland, while the axillary lymph nodes are removed only if enlarged and cytological positive for neoplastic cells.

Combination chemotherapy using doxorubicin and cyclophosphamide has been shown to induce short-term responses in about half of the cats with metastatic or nonresectable local disease, and is recommended after complete excision of the tumour to prolong disease-free period. However, controversial results due to the effect of the adjuvant therapy have been reported and therefore further studies are needed to better understand the role of doxorubicin and cyclophosphamide as adjuvant therapy.

The most significant prognostic factors affecting recurrence and survival times for cats with malignant mammary tumours are tumour size, extent of surgery, and histologic grading. Tumour size been considered the most important prognostic factor. Recently, efforts have been made to determine molecular markers in order to determine their role as prognostic factor or therapeutic targets but until the moment there are no consistent results.

Keywords: Mammary tumours; Cats; Histological grading; Staging; Prognostic factors.

RESUMO

Os tumores mamários (TMs) estão entre os tumores mais comuns no gato, representando 17% dos tumores nas gatas. Estes tumores são altamente infiltrativos, apresentando frequentemente metástases no momento do diagnóstico, apresentando uma taxa média de sobrevivência a partir do momento do diagnóstico, que varia entre 6 a 12 meses. A etiologia dos TMs não é claramente compreendida, mas alguns fatores de risco têm sido apontados, tais como a idade, a raça, o estado reprodutivo e a exposição aos estrogénios e progesterona.

A fim de estabelecer um tratamento adequado é importante obter a classificação histológica, o grau histológico e o estadiamento do tumor, pois a maioria dos TMs apresenta metástases locais e distantes, no momento do diagnóstico. Os tumores da glândula mamária felina são histologicamente classificados, de acordo com os critérios de diagnóstico, propostos pela Organização Mundial da Saúde (OMS). Esta classificação é baseada em características morfológicas, encontrando-se os TMs organizados em quatro grupos principais: hiperplasia mamária / displasia, tumores benignos, tumores malignos e tumores não classificados. O grau histológico é determinado de acordo com uma adaptação da classificação utilizada por Elston e Ellis e baseia-se na formação de túbulos, grau de pleomorfismo nuclear e celular, e na atividade mitótica. O estadiamento dos TMs felinos baseia-se no sistema de estadiamento clínico da OMS modificado.

A cirurgia é o tratamento mais utilizado para as neoplasias mamárias no gato; pode ser efetuada de forma isolada ou em combinação com quimioterapia. A mastectomia radical é o método cirúrgico de escolha, pois reduz significativamente a recorrência local do tumor. Os gânglios linfáticos inguinais são quase sempre removidos com a glândula mamária, enquanto que, os linfonodos axilares são removidos somente quando apresentam tamanho aumentado e células neoplásicas na citologia.

A quimioterapia com doxorrubicina e ciclofosfamida como terapia adjuvante, após a excisão completa do tumor é recomendada para prolongar o período livre de doença. No entanto, os resultados obtidos com a quimioterapia adjuvante têm sido controversos, sendo necessários mais estudos para entender melhor o papel de doxorrubicina e da ciclofosfamida como terapia adjuvante.

Os fatores prognósticos mais importantes que afetam a recorrência e sobrevivência dos gatos com tumores mamários malignos são o tamanho do tumor, a extensão da cirurgia e a classificação histológica. O tamanho do tumor é o fator de prognóstico mais importante. Recentemente, têm sido efetuados esforços para determinar novos marcadores moleculares a fim de determinar o seu papel como fator prognóstico ou como alvos terapêuticos, mas até ao momento não há resultados consistentes.

Palavras-chave: Tumores mamários; Gatas; Grau histológico; Estadiamento; Fatores de prognóstico.

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“To my parents, Alberto and Ismália…”

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LIST OF ABBREVIATIONS

A1 - First abdominal mammary gland
A2 - Second abdominal mammary gland
AgNORs - Argyrophilic nucleolar organiser regions
BCG - Bacillus Calmette-Guerin
BRCA-2 - Breast cancer 2
c-erB-2/ HER-2/neu - Human epidermal growth factor receptor type-2
COX-2 - Cyclooxygenase -2
DFI - Disease-free interval
DNA - Desoxyribonucleic acid
eNOS - Endothelial nitric oxide synthase
ER- Oestrogen receptors
FNA - Fine needle aspirate
HIF-1-α - Hypoxia inducible factor -1 -α
iNOS - Inducible nitric oxide synthase
LMTPE-PE - Liposome encapsulated muramyl tripeptide phosphatidylethanolamine
mg/kg - milligrams per kilograms
MST - Median survival time
MTs - Mammary Tumours
NOS - Nitric oxide synthase
NSAID - Non-steroidal anti-inflammatory drugs
OHE - Ovarihysterectomy
PCNA - Proliferating cell nuclear antigen
PR - Progesterone receptors
PKC - Protein kinase c
PTEN - Phosphatase and tensin homolog
RTKI's - Receptor tyrosine kinase inhibitors
ST - Survival time
STK - Stem cell-derived tyrosine kinase
T1 - First thoracic mammary gland
T2 - Second thoracic mammary gland
TNM - Tumour-node-metastases
TK – Tyrosine kinase
TopBP1 – Topoisomerase II β binding protein 1
VEGF - Vascular endothelial growth factor
WHO - World Health Organization

1. INTRODUCTION

The mammary tumours (MTs) are a very important cause of morbidity among cats.[1] In a study performed in the United States of America these tumours were considered the third most diagnosed tumours after skin tumours and hematopoietic neoplasms.[2,3] However, in a study performed, in Brazil, by Togni *et al.* (2013) MTs were the second most diagnosed, after skin tumours and before the hematopoietic neoplasm.[4] These discrepancies among the prevalence of feline MT are due to differences in the inclusion criteria, tumour diagnosis and classification.[2,3,4,5,6]

The MTs represent 17% of tumours affecting female cats, being less frequent in males (1 to 5%) [7,8,9,10] and at least 85% to 95% of feline MTs are malignant.[2,11] Histologically, most malignant MT are adenocarcinomas, being the tubular and papillary types more common than the solid or mucoid types.[12,13] Feline mammary carcinoma are highly infiltrative tumours, frequently associated with lymph node metastases at the time of the initial diagnosis and have a mean survival rate from the time of diagnosis that ranges between 6 to 12 months.[14,15] Fibroadenomatous hyperplasia (fibroepithelial hypertrophy, feline mammary hypertrophy) is the most common histopathologic lesions in cats.[15] It is a consequence of an exaggerated proliferative response of mammary glandular tissue to several situations, namely pregnant or pseudopregnant queens, cats receiving prolonged megestrol acetate or medroxyprogesterone acetate therapy, cats receiving progestational compound when endogenous levels of oestrogen are increased.[16]

The aetiology of MTs remains poorly understood, however some factors have been pointed as risk factors for MTs namely age, breed, reproductive status and exposure to oestrogen and progesterone.[15] In fact, MTs are more frequent in middle-aged to older cats, with the mean age of diagnosis between 10-12 years.[8,17,18,19] The risk increases with age, presenting a peak between 7 to 9 years and continuing to increase until 12 to 14 years.[17]

The Domestic Shorthair cat is the breed more frequently affected.[2,8,18] Nevertheless, when MTs are diagnosed at younger age, Siamese cats and other Oriental breeds are the most affected.[7,8] Since genetic predisposition for a disease is often associated with a younger age of diagnosis,[15] and young Siamese cats have an increased risk for many types of tumour, it is possible that Siamese cats present breed-associated germ line alterations that confer increase risk for many different malignancies.[15]

Relatively to reproductive and hormonal status, it is considered that oestrogen and progesterone play an important role in mammary tumorigenesis, and also that exposure to these hormones at an early age seems to be crucial for MTs development.[15,20] In fact, some studies revealed that queens spayed before 1 year of age have a decreased risk of developing MTs and that sexually intact queens have a sevenfold higher risk of feline mammary carcinoma than spayed cats.[8,11,17,20] Consistently with these results, other studies indicated that ovarihysterectomy (OHE) may be protective against MTs development.[2,20] Nevertheless, according to Overley *et al.* (2005), the protective effect of OHE diminishes quickly over the first years; risk reductions of 91%, 86%, and 11% were achieved in cats that were ovariohysterectomized before 6 months, between 7 and 12 months, or between 13 and 24 months,

respectively, and no benefit was found after 24 months.[20] According to the same study, cats spayed after 2 years of age had a statistically significant increased risk to develop MTs compared with intact cats, and also that a gradual loss of protection occurred with increasing hormone exposure prior to OHE.[20] In addition to endogenous ovarian hormonal influence, the long-term exposure to exogenous progestins or to oestrogen-progestin to prevent pregnancy or control of aggressive behaviour, increases the risk of both benign and malignant MTs development, while intermittent or occasional progestin administration has no effect.[8,15] According to several studies, these effects were associated to the fact that a prolonged steroid exposure induces proliferation of mammary epithelial cells and facilitates the accumulation of genetic errors, contributing to the development of MTs.[11,20]

As already mentioned, MTs are rare in males, but in a report of 22 cases, 8 (36%) had a history of progestin administration.[9] In other study, performed by Jacobs *et al.* (2010), cats with repeatedly injections of medroxyprogesterone acetate for inter-cat aggression and urinary house soiling, also demonstrated development of mammary adenocarcinoma.[21]

Obesity and an unbalanced diet have been linked to increased risk of tumours of the mammary gland.[22] Nunes *et al.* (2011) found that sixteen cats (66,7%) with neoplasia were fed by a mixed diet (homemade food and feed industrialized), while five cats (20,8%) received only animal feed industries, and only three cats (12,5%) ate homemade diet.[22] An unbalanced diet with high caloric levels could predisposed to an occurrence of MTs analysed in cats but the correlation between diet and MTs needs to be clarified.[22]

Other breeding-related factors such as age at first pregnancy, number of pregnancies, pseudopregnancy and changes in the oestrous cycle were suggested as risk factors, but there is no consensus regarding their contribution for the development of MTs.[20]

Taking in consideration that MTs are very aggressive tumours, their aetiology and prognosis is poorly understood, it seems important to perform a bibliographic revision in order to clarify some of the discrepancies.

2. CLINICAL PRESENTATION AND DIAGNOSIS OF FELINE MAMMARY TUMOURS

Cats have four pairs of mammary glands, two thoracic pairs (T1; T2) and two abdominal pairs (A1; A2), (Figure 1) and in some cats additional glands may be present at the inguinal region.[16,23] The secretory unit of the mammary gland is the alveolus, which is constituted by an inner layer of cuboidal secretory epithelial cells and an outer layer of myoepithelial cells. In the stroma of the alveoli, there are plasma cells that secrete immunoglobulin A.[16,23] Secretory alveoli drain into collecting alveoli, which drain into intralobular ducts, then into interlobular ducts that continue into lobar lactiferous ducts, and finally open into lactiferous sinus. The lactiferous sinus is continuous with the teat sinus.[16,23] The arterial supply to the thoracic glands is provided by the lateral thoracic vessels, the intercostal and the internal thoracic vessels.[16,23] The arterial supply of the abdominal glands occurs through cranial superficial epigastric

artery.[16,23] The venous drainage occurs through vessels similar to the arteries and also through small veins that cross midline and establish communication between contralateral glands.[16,23] Regarding lymph drainage it is generally considered that mammary glands drain cranially towards the axillary lymph center and caudally towards superficial inguinal lymph center.[16,23] Malignant MTs may occur in any gland, but are more frequent in the abdominal glands.[16] Due to its aggressivity MTs may spread to other glands, to ipsilateral regional lymph nodes and also to other organs.[8,15,16]

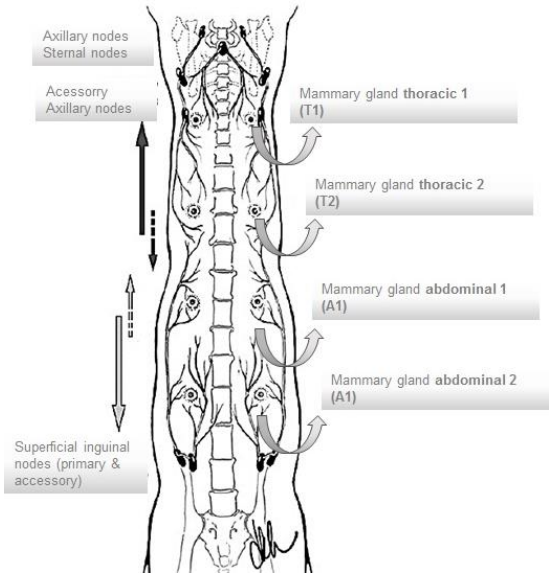

Figure 1- The four pairs of mammary glands in the cat, with their associated lymph nodes and lymphatic drainage. Adapted from dkhaines, 2009, The University of Tennessee

The development of malignant MTs is usually characterized by a nodular mass in one or more mammary glands. Cats tend to lick and groom the area excessively, and a strong odour can result as the tumour becomes ulcerated, necrotic and infected. General signs of illness such as anorexia or depression are often seen as the disease progresses.[16,24]

A detailed history should be collected, giving particular attention to breed, age, reproduction status, age at ovariohysterectomy and the use of progestins or oestrogens as well as the duration of clinical signs.[25] Physical examination should include examination of both mammary chains and evaluation of the health condition of the patient,[14] aiming to determine the number of affected glands, the tumour size, texture and location, evidence of tumour ulceration and fixation to underlying tissues and evidence of distant metastases.[25,26] Generally, palpation of subcutaneous nodule(s) or mass(es) within one or many glands simultaneously is highly suggestive of MTs.[27] When evaluating a cat with a prior history of mammary tumours, especially if treated with simple mastectomy, a careful examination of the remaining mammary glands is mandatory, because new primary tumours are common.[15] Nodules may be discrete, well defined, firm or mobile and attached to skin or underlying tissues (Figures 2 and 3). A few nodules may

Figure 3 - Subcutaneous nodules in mammary gland. (Kindly provided by Dr. Hugo Vilhena, Hospital Veterinário do Baixo Vouga)

Figure 2 – Subcutaneous nodules in mammary gland. (Kindly provided by Dr. Hugo Vilhena, Hospital Veterinário do Baixo Vouga)

appear cystic, inflamed, infected and ulcerated mainly if associated to an extensive tumoural necrosis.[7,8,15,27]

In general, nodules are located in adjacent ipsilateral glands and are often considered to be caused by lymphogenous spread of a single primary tumour. Contralateral concomitant neoplastic nodules are observed less frequently and may be associated with haematogenous involvement.[27] In fact, until recently, it was thought that spread into contralateral mammary glands occurred through lymphatic vessels but no interglandular lymphatic connections were identified.[23] Recent studies reported that metastization to contralateral glands probably occur through venous system that is shared by the glands.[16]

In case of inflammatory mammary carcinomas, the glands may be swollen, hot and painful, with extensive lymphatic involvement. This presentation may be difficult to differentiate from fibroadenomatous hyperplasia (fibroepithelial hypertrophy, feline mammary hypertrophy) (Figure 4), although the latter is more common in young cats[8,15] and is usually characterized by a more typical presentation with one or multiple enlarged mammary glands and without milk production.[16] In a case of feline primary inflammatory carcinoma reported by Millanta *et al.* (2012), this was characterized with rapid onset of oedema, severe erythema, local pain and warmth of the inguinal region, with a pustular-to-nodular cutaneous lesion in association with an ill-defined underlying mass.[28]

Perez-Alenza *et al.* (2004) has described the same characteristics (rapid onset of erythema, severe oedema, extreme local pain and firmness, absence of subjacent mammary nodules, and involvement of extremities) in three cases after surgical excision of a malignant mammary tumour, and they were classified as secondary postsurgical inflammatory mammary carcinoma.[29]

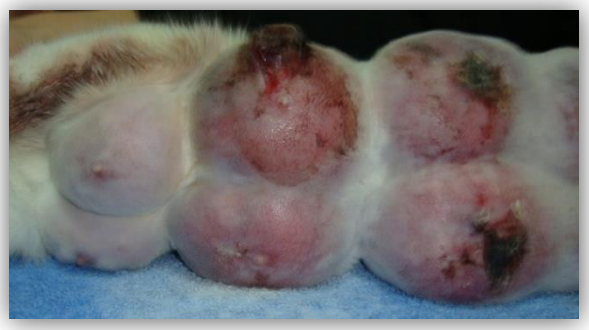

Figure 4 – Fibroadenomatous hyperplasia (Kindly provided by Dr. Hugo Vilhena, Hospital Veterinário do Baixo Vouga

Some nonspecific clinical signs may appear such as weight loss, inappetence and lethargy, and less commonly, exercise intolerance. Mammary tumours have a high metastatic potential not only to the regional lymph nodes but also into lungs, pleura, liver, skeleton, spleen, kidneys and to distant lymph nodes.[27] In cats, pulmonary metastases are more frequent than regional metastases.[7,8,14,30] Respiratory distress (dyspnea or cyanosis) and pleural effusion caused by diffuse pulmonary metastases is common due to pleural carcinomatosis and extensive pulmonary involvement.[25,27,31,32]

Due to distant metastization, the sternal, inguinal and axillary lymph nodes should be examined for size, consistency and, if suspicious, should be analysed by fine needle aspirate (FNA) or biopsy for detection of tumour infiltration. Paraneoplastic syndromes are uncommon.[7,8,12,14,15]

In addition to the collection of a detailed history and to the physical examination the diagnosis of MTs should also include a complete blood count, serum biochemistry, urinalysis, determination of serum tiroxine (T4) concentration and Feline Immunodeficiency Virus/Feline Leukaemia Virus infection status.[14,15,30] A coagulation profile is indicated if an inflammatory mammary carcinoma is suspected, due to the high association with disseminated intravascular coagulation.[33]

To evaluate the presence of distant metastases, three-view thoracic radiographs (including ventrodorsal and right and lateral views, preferably performed under anaesthesia with inflated lungs) and abdominal ultrasonography should be performed.[8,16] Pulmonary metastases may appear as a small to large nodular opacities on thoracic radiographs.[7,8,14,30] Advanced imaging computed tomography, when available, should also be performed since it provides more accurate assessment of lung metastases clarifying doubts on the radiographic exams.[8]

As previously reported, FNA of lymph nodes as well as scraping of ulcerated lesion and cytology of fluids from affected glands or pleural effusion should also be performed to rule out skin and subcutaneous non mammary malignancies and/or to differentiate mammary carcinoma from fibroadenomatous hyperplasia.[16] Particular attention should be given to the cytologic examination since it may yield false negative results.[25,30] Cytology could be essential to differentiate tumours from inflammatory processes, but the distinction between benign and malignant MTs may be difficult. Therefore, if cytology do not allow a definitive diagnosis, excisional biopsy with subsequent

histopathologic examination is recommended in order to determine the malignancy of the tumour prior to define the adequate treatment.[8,16] Histopathologic examination of the excisional biopsy is considered the gold standard for the diagnosis of feline MTs.[12,27]

3. HISTOPATHOLOGY AND BIOLOGICAL BEHAVIOUR OF MAMMARY TUMOURS

Tumours of the mammary gland of felines are histologically classified according to the diagnostic criteria proposed by the World Health Organization (WHO) as summarized in Table 1.[34] These tumours are mainly classified based on descriptive morphology and divided into four main groups: mammary hyperplasia/dysplasia; benign tumours, malignant tumours, and unclassified tumours.[14,27,34]

3.1 Hyperplasia and Dysplasia

Fibroadenomatous change (fibroepithelial hyperplasia, fibroepithelial hypertrophy, mammary hypertrophy) is characterized by a rapid and abnormal proliferation of interlobular ducts and periductal stromal cells of one or more mammary glands, table 1.[15,16] This alteration is more commonly detected in the cat than in the dog.[15,16]

3.2 Benign mammary tumours

Benign fibroadenoma is the most frequently reported benign mammary tumour in cats, with simple adenoma or duct papilloma rarely seen, table 1.[15]

3.3 Malignant mammary tumours

There are some criteria that help to differentiate adenocarcinomas from benign MTs, such as atipia in the epithelial component of the tumour growth, infiltrative growth, high mitotic activity and cellularity, and/or necrosis.[12]

The most frequent histological type of malignant tumour is the simple adenocarcinoma. This carcinoma is derived from the luminal epithelium of the mammary ducts and alveoli. Other types of malignant mammary tumours are very rare in queens, namely squamous cell carcinoma, mucinous carcinoma and carcinosarcomas, table 1.[8,14,34]

Complex carcinoma (biphasic epithelial myoepithelial) is a different and unusual histological type in queens that was described by Seixas *et al.* (2007). This type of carcinoma shows histopathological features distinctive from other tumours, being characterized by the presence of two cell populations, epithelial luminal cells and mioepithelial cells.[35] These tumours have low grade of malignancy and it appears to have a better prognosis and disease-free-survival rates than other carcinomas on queens.[35,36] In addition to these carcinomas it was also reported in a cat, a very rare type of carcinoma, a lipid-rich carcinoma of the mammary gland, which seems to be a variant of mammary carcinoma in cats.[37]

Table 1- Histological Classification of Mammary Tumours of the cat, defined by WHO.

Mammary Hyperplasias/Dysplasias	Ductal hyperplasia
	Lobular hyperplasia
	Epithelial hyperplasia
	Adenosis
	Fibroadenomatous change (feline mammary hypertrophy, fibroepithelial hypertrophy)
	Cysts
	Duct ectasia
	Focal fibrosis
Benign tumours	Adenoma
	Simple Adenoma
	Complex Adenoma
	Fibroadenoma
	Low-cellularity fibroadenoma
	High-cellularity fibroadenoma
	Benign mixed tumour
	Duct papilloma
Malignant Tumours	Noninflitration (*in situ*) carcinoma
	Tubulopapillary carcinoma
	Solid carcinoma
	Cribriform carcinoma
	Squamous cell carcinoma
	Mucinous carcinoma
	Carcinosarcoma
	Carcinoma or sarcoma in benign tumour
Unclassified Tumours	

Adapted from Misdorp, 1999.

4. HISTOLOGICAL GRADING SYSTEM

The histological grading of the feline MT is adapted from the human grading systems and takes into account several morphological criteria.[38,39] In a large number of studies, the histological grade is determined according to an adaptation of the classification used by Elston and Ellis. This, histological grading system is based on the degree of tubule formation, degree of nuclear and cellular pleomorphism, and mitoses activity.[12,13,19, 39,40] The histological grade is defined by the sum of individual scores for these features and a score of 1 to 3 is given for each, as it is described on table 2.[12,38]

Table 2- Individual scores for assessing histological grade in feline mammary carcinoma.

Criteria	***Score***
Tubule Formation	
Majority of tumour (>75)	1
Moderate degree (10-75%)	2
Little or none (<10%)	3
Nuclear pleomorphism	
Small, regular uniform cells	1
Moderate increase in size and variability	2
Marked variation	3
Mitotic counts	
Dependent on microscope field area	1-3

Adapted from Elston and Ellis, 1998.

For degree of tubular formation, a score of one point is given when more than 75% of the examined area exhibited definite tubular formation; two points are for tumours in which the tubules occupy about 10 to 75% of the area; and three points are when the tubules occupy 10% or less of the area.[38,41]
Nuclear pleomorphism is scored qualitatively. One point is given when nuclei are small, with minimal increase or with variation in size, regular outlines and uniform chromatin; when nuclei is larger than normal with vesicular nuclei and single visible nucleoli, and moderate variation in shape and size is scored two points; for marked variation in size and shape, with very large, bizarre, and vesicular nuclei with prominent and multiple nucleoli is given three points.[38,41]

Regarding, mitotic count, this is evaluate quantitatively and the count of this feature depends on microscope field area.[38,41]

The overall tumour grade results from the sum of the individual scores. For a sum between three to five points the tumour is considered a well-differentiated carcinoma (grade I); for a sum between six and seven points the tumour is considered a moderately differentiated carcinoma (grade II); and the sum is between eight and nine points the tumour is considered a poorly differentiated carcinoma (grade III).[38,41]

Recently, it was evaluated the possible correlation between the histological grade, using the Elston and Ellis method, and the post-surgery survival of queens with mammary carcinomas. The results demonstrated that tumour grade using this method way be considered an independent prognostic factor.[42]

According to this study, the histological grading system is a good parameter to stratify tumour according to their morphological characteristics and aggressive biological behaviour, in order to establish a correlation between the morphological structure of tumours and their clinical behaviour.[12,42] Histological grade may also provide useful information to predict the response to chemotherapy and, therefore, be a predictive factor.[42]

5. STAGING

In order to plan the adequate therapeutic approach and prognosis, it is essential to determine the local extent and the degree of spread throughout the body.[8,16]

The WHO's TNM (tumour-node-metastases) classification system is usually applied to staging tumours in domestic animals and takes in consideration criteria related with the size of the primary tumour, the presence of lymph node and distant visceral metastases.[8,16,27] A modified WHO clinical staging system established from the original system published by Owens is applied to the feline mammary tumours, except to the mammary gland sarcomas.[15,43]

The extent of the primary tumour (size and clinical evidence of local invasion, such as fixation to skin or fascia) is represented by "T".[16,43] As the size of the tumour is important to the prognosis, it should be measured with callipers: those 3 cm diameter are associated with better survival rates than those >3 cm.[8,43] The "N" is associated to the presence of tumour cells at the regional lymph nodes. Normal-sized lymph nodes may still contain tumour cells, thus, the regional lymph nodes (axillary and inguinal) should be examined and FNA or surgical removal may be necessary to help categorize N status.[16] The "M" designates the presence or absence of distant metastases.[8,16] By using these criteria, tumours are classified in stage I to IV, as summarized on table 3 and 4.[16]

Table 3- TNM Classification system for mammary tumours of domestic animals

Tumour size (diameter) (T)	***Classification***	***Regional Lymph nodes Metastases (N)***	***Classification***	***Distant Metastases (M)***	***Classification***
<2cm	T1	Without metastases	N0	Without metastases	M0
2-3cm	T2	With metastases	N1	With metastases	M1
>3cm	T3				

Adapted from Owen, 1980.

Table 4- Staging of Feline Mammary Tumours

Stage	***T Classification***	***N Classification***	***M Classification***
I	*T1*	*N0*	*M0*
II	*T2*	*N0*	*M0*
III	*T1, T2*	*N1*	*M0*
	T3	*Any N*	*M0*
IV	*Any T*	*Any N*	*M1*

Adapted from Owen, 1980

6. MOLECULAR CHARACTERIZATION

The molecular characterization of mammary tumours represents a new stage in the development of effective predictive models and targeted therapies.[44,45,46] A molecular marker could be deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) based or a protein directly measured in tissues, serum, or other organic fluids that can be used for tumour detection, tumour staging, monitoring of response to therapy, and prediction of patient prognosis.[47] Considering that tumour cells are characterized by increased proliferation rate, resistance to death, increased ability to invade surround and distant tissues and increased ability to promote angiogenesis, the search for markers is orientated to signalling pathways that control these characteristics.[44,45] In cats, there are several molecules that are being investigated in order to understand their role in the development of MTs, such as oestrogen receptors (ER), human epidermal growth factor receptor-2 (c-erbB-2/HER2/neu), and the vascular endothelial growth factor (VEGF).[24, 48] Others molecules such as cadherins, integrins, p53, topoisomerase IIβ binding protein 1 (TopBP1), PI3K/AKT, phosphatase and tensin homolog (PTEN), c-kit are also under

investigation.[40,49,50,51,52] However, until now there are no consistent results regarding their contribution to the tumorigenesis and there are no studies regarding their utility as prognostic markers. Additional studies are needed to clarify the role of these molecules in feline MTs.[8]

7. TREATMENT

Treatment options depend on tumour staging and can include surgery, radiation therapy, chemotherapy, or a combination of treatments. Palliative care is also important in oncologic patient especially in tumours that at the moment of diagnosis already present metastases, such as, what frequently occurs in the malignant MT.[8,15]

7.1 Surgery

Surgical excision of MTs remains the most widely accepted treatment option for feline MTs, however it is usually not curative due to the ability of these tumours to invade surround and distant tissues.[16,27]
Complete removal of neoplastic tissue is hampered by the degree of invasion and ulceration.[16] Due to the invasive nature of malignant MT and to the tendency of these tumours to early metastases, the treatment of choice for tumours without signs of distant metastases is radical mastectomy of the affected side, regardless of tumour size.[53] After surgery, the entire specimen with the surgical margins inked should be submitted for histological review to ensure that complete margins have been obtained.[16]
Malignant mammary neoplasms in the cat often invade lymphatics and veins. The lymphatic drainage in mammary gland of queen influences the extent of surgery, as tumour cells spread readily beyond the primary site.[8] Therefore, the recommended approach based on drainage studies is to perform chain mastectomy with removal of draining lymph nodes: unilateral mastectomy if the tumour is confined to one side, or a two staged bilateral chain mastectomy (with a two week interval between surgeries) for cats with bilateral tumours.[8,15,16] According previous studies, the extent of surgery could influence the development of local recurrence as well as the disease-free interval (DFI) and the survival time (ST).[8,54,55] Taking into the account the invasion of veins, early vessel ligation is essential, when performing radical unilateral or bilateral chain mastectomy.[16]
In addition to the removal of the draining lymph nodes, the inguinal lymph node is always removed with the A2 (Figure 1**)**, whereas the axillary lymph node is removed, only if it is enlarged or if it is positive for tumour spread on FNA or biopsy.[8,16] Prophylactic removal of axillary lymph nodes is unlikely to have a therapeutic benefit and extends survival.[8,16]
Tumour fixation to skin, muscular fascia or portions of the body wall should be included with en bloc resections.[15,16]
The question of perform OHE at the same time of radical mastectomy, has been long discussed.[27,56] There is no evidence that OHE has any benefit in prevention of the development of metastases [56], neither on survival or tumour recurrence and has no effect on development of new tumours in the

remaining mammary glands or tumour progression.[8] However, OHE may be beneficial in order to reduce the use of progestin therapy.[8]

7.2 Chemotherapy

Early detection and aggressive surgery can result in long-term survival in cats with early stage MTs, however because of the high rate of metastases after surgery, adjuvant chemotherapy is commonly recommended for the treatment of feline mammary carcinomas.[3,15] The benefit of using chemotherapy as an adjuvant therapy to surgical excision is still not clear [8], but cats with delayed diagnosis, large primary tumours, metastatic local lymph nodes or vascular or lymphatic invasion are usually submitted to adjuvant chemotherapy in order to reduce the occurrence of recurrences and the development of the metastases.[15, 33] In addition, the systemic adjuvant chemotherapy may be useful, for providing pain relief in cats with unresectable tumours.[7] Some antineoplastic drugs are used, alone or in combination, as adjuvant therapy in malignant MTs, namely doxorubicin, carboplatin, cyclophosphamide, vincristine, methotrexate and mitoxantrone.[7,27,30]

Doxorubicin is considered one of the most active agents for patients with advanced disease and chemotherapy protocols commonly used for feline mammary carcinoma include doxorubicin as a single agent or in combination with cyclophosphamide.[3,33] In a large retrospective multicentre study performed in 2006 with 67 cats, mammary adenocarcinomas, received adjunctive single-agent doxorubicin at a dose of 1 mg/kg intravenously every three weeks for a maximum of five treatments, or until the cat develop progressive disease or concurrent illness. The results from this study showed that cats that completed the protocol had significantly improved survival with a median survival time (MST) of 448 days and a DFI of 255 days.[57] Similar results were obtained by Borrego *et al.* (2009) who also evaluated the efficacy of treatment with a Cyclooxygenase – 2 (COX-2) inhibitor (meloxicam), a doxorubicin-based chemotherapy protocol and surgery in 23 cats with mammary adenocarcinomas. In the study of Borrego *et al.*, which did not have a control group it reported a MSTs of 460 days and DFI of 269 days.[58]

The effect of adjuvant doxorubicin-based chemotherapy in the outcome of feline mammary carcinoma compared with surgery alone has been also accessed, in study of McNeill *et al.* (2009).[3] Adjunctive chemotherapy was defined as chemotherapy treatment after definitive surgery to remove the primary tumour and before any recurrence or progression.[3] Seventy-three cats were evaluated, of which 37 were only submitted to surgery (MST=1406 days; DFI=372 days) and 36 were submitted to surgery and then to chemotherapy group (MST=848 days; DFI=676 days). In the spite of the differences, the statistical analysis of the MST and DFI obtained in both groups did not identify an overall benefit of adjuvant chemotherapy.[3] There were no significant differences in clinical data (surgical procedures, tumour size, stage, or histopathologic parameters).[3]

The combination of doxorubicin with cyclophosphamide may shrink tumour in 35 to 50% on cats with unresectable locally disease or distant metastatic disease, with complete remission in 21% of patients, and thus increase ST.[8,25,27,33] In addition, cats that responded to this combination therapy had a longer MST (150 to 180 days) than did those patients unresponsive to therapy (75 to 86 days).[58,59] The

relatively high response rate in the macroscopic setting suggests that this may be an effective protocol in patients with microscopic minimal residual disease (i.e., following surgical cytoreduction). However, results from adjuvant studies do not reflect this, albeit none of the studies were prospective or randomized.[15]

Despite the apparent efficacy, chemotherapy has several side effects that should be considered. Treatment based on doxorubicin protocol can cause transient gastrointestinal side effects and other adverse complications such as anorexia, nephrotoxicity and myelosuppression.[25,27,58,60] Furthermore, it is also important to evaluate the toxic effect of the chemotherapeutic drugs among liver and kidneys. Cyclophosphamide is activated by the liver and excreted by the kidneys, whereas doxorubicin is metabolised by the liver with only 5% excreted in urine. Therefore, the hepatic and renal functions should be assessed before using any of the protocols.[14]

In summary, given the high metastatic potential of feline mammary carcinomas, the adjuvant use of systemic chemotherapy after radical mastectomy to maximize survival, could be considered upon the evaluation of the hepatic and renal function.[25] Nevertheless, more studies are needed to clarify the actual effect of doxorubicin as well as to identify the malignant MTs that are more sensitive to doxorubicin.

Alternatively, a different approach with anti-angiogenic metronomic (low dose) chemotherapy could be considered, although low dose chemotherapy using vincristine, cyclophosphamide and methotrexate was reported not to prevent recurrence or metastases.[8]

7.3 Immunotherapy

This is a method of cancer treatment that requires administration of immunomodulators to stimulate the host immune response to neoplastic cells.[16]

The immunomodulators like bacillus Calmette-Guerin (BCG), *Corynebacterium parvum,* liposome-encapsulated muramyl tripeptide phosphatidylethanolamine (LMTP-PE) and levamisole have been used as adjuvants to surgical excision of feline mammary tumours.[54,55,61]

The administration of *Corynebacterium parvum* vaccine, either intratumorously or subcutaneously, in combination with surgery was been showed not to be successful.[55] In addition, the LMTP-PE used to stimulate monocyte cytotoxic activity following radical mastectomy also did not improve the DFI or ST of cats when compared with placebo-treated cats.[54] McEwen *et al.* (1984) reported that treatment of feline malignant MTS with oral levamisole (5mg/kg, 3 days a week) as an adjuvant to surgery was ineffective in altering the recurrence rate of disease and did not increase the ST of treated cats compared with control groups.[61]

The small molecule inhibitors that target receptor tyrosine kinases (receptor tyrosine kinase inhibitors or RTKIs) are effective in the management of some types of veterinary cancers, particularly those with altered tyrosine kinase activity. Imatinib and masitinib are well tolerated in cats; however, there is no information about their efficacy against feline mammary tumours.[8]

Santana *et al.* (2014) reported a case of a cat with a highly malignant MT treated with radical mastectomy and toceranib phosphate (3.33 mg/kg every 72 hours) and metronomic adjunctive chemotherapy with cyclophosphamide (10mg/m2 every 48 hours).[62] This was considered a good option to treat a feline patient after surgery.[62]

7.4 Radiotherapy

Radiotherapy is a local treatment used to prevent recurrences.[30] However, there is no evidence that radiotherapy is beneficial in improving clinical outcome in feline MTs compared with surgery alone and is seldom used in the treatment due to lack of evidence to support increased survival rates in this species.[7,16]

7.5 Hormonal therapy

Endocrine therapy, namely tamoxifen, is widely used to treat oestrogen-receptor positive breast tumours in humans, but there are no reports of using anti-oestrogens in cats, probably because most malignant feline MTs lack oestrogen receptors and expected benefits would, therefore, appear to be minimal.[8,27]

7.6 Supportive care

Analgesia is essential during and after surgical removal of any MTs.[30,63] Analgesics should be considered for patients suffering from large or ulcerated tumours, or painful metastases, such as to bone metastases. Nonsteroidal anti-inflammatory drugs (NSAID) and opioids may be beneficial for the supportive management of these advanced-stage patients.[25]

Anti-emetics can be helpful at reducing the adverse effects of chemotherapy, and supplemental feeding methods and appetite stimulants must be considered in all patients to facilitate healing and prevent weight loss during therapy.[30,63]

In addition, treatment of underlying secondary problems such as renal or heart disease is important.[63]

8. PROGNOSTIC FACTORS

One of the most important prognostic factors in cats with mammary gland neoplasia is tumour size or volume, which is associated to an increased risk for metastases and a reduced of both DFI and ST.[16,39] Furthermore, tumour size is an important factor to determine the need for adjuvant treatments.[15,27]

Tumour size correlates with number of tumour cell divisions and higher chance for the progression to a more malignant behaviour due to accumulation of mutations.[39] Three size categories have shown prognostic significance at initial presentation: (1) tumours smaller than 8 cm^3 or smaller than 2 cm diameter; (2) tumours larger than 8 to 27 cm^3 or larger than 2 to 3 cm diameter; and (3) tumours larger than 27 cm^3 or larger than 3 cm diameter.[8]

Tumour size appears to have higher prognostic relevance in mammary carcinomas larger than 3 cm in diameter.[64] In fact, cats with tumours larger than 3 cm have a poor prognosis with a short ST (4–12

months). Cats with smaller tumours (<2 cm) can be effectively treated with surgery alone, with a mean ST higher than 3 years.[8,15,64,65] On the other hand, cats with tumours of 2 to 3 cm in diameter had a mean ST of 2 years.[15] Given this association between tumour size and ST, early diagnosis and treatment have an important bearing on the prognosis for cats with malignant MTs.[16]

Another prognostic factor is the degree of dissemination at initial presentation. The presence of regional lymph nodes and pulmonary metastases at the moment of the diagnostic are associated with a worst prognosis.[8,25,65]

In addition to the existence of metastases, the location of the metastases is also a prognostic factor.[9,25] In fact, Novosad *et al.* (2006) showed that in cats with metastatic disease in regional lymph node, lungs and pleura have a MST of 1543, 332, and 188 days, respectively.[57]

Another factor that is important to evaluate prognostic is the surgical technique. Radical mastectomy had a significantly reduced rate of local recurrence and produced a significantly longer DFI than conservative surgery.[8,16] Weijer *et al.* (1983) reported that cats treated by mastectomy had a better prognosis than that cats treated by block dissection, because block-dissected cats showed more regional lymph node involvement.[66]

Relatively to breed, a recent retrospective study showed that Siamese cats have a statistically worse prognosis with decreased median DFI and MST compared with Domestic shorthair cats.[58,61]

Older cats have a worse prognosis,[15] however, a prospective randomized trial found no difference according to age when comparing cats that were younger or older than 10 years.[61]

As previously referred tumour size and lymph node involvement together with metastases at distant sites are used to stage MTs and to give a significant prognostic indication (TNM staging).[27] In addition, clinical stage at presentation of feline mammary carcinomas and some of their anaplastic features have been reported to be useful prognostic indicators and has been shown to be associated with survival time.[64] Median survival time of cats with stage I, II, III and IV were 29, 12.5, 9 and 1 month(s), respectively.[67]

Histological subtypes of breast cancer have been described as prognostic in humans.[34] However, Weijer *et al.* (1983), showed that in cats, the histologic type has not been found to be related to prognosis.[66] On the other hand, Novosad *et al.* (2006) reports that histological subtype is prognostic for DFI, with papillary or tubular, ductal, and anaplastic tumours having a DFI greater than 1131, 306, and 95 days, respectively.[57] In addition, Castagnaro *et al.* (1998) reported that histologic grade was inversely correlated with prognosis and ST.[13] In its study it was reported that the rate of death, one year after surgery was 0% in cats with grade I, and 100% in those with grade III.[13] Yet, there was not a good correlation between moderated differentiation and ST.[16]

The number of mitotic figures found in tumour tissue is an independent prognostic indicator.[44] It correlates with ST and is significantly different between feline mammary carcinomas with various histological stage of invasion.[44] Longer ST were seen in animals with tumour exhibiting fewer than two mitotic figures per high power field.[66]

Other markers of cell proliferation, including Ki-67, proliferating cell nuclear antigen (PCNA) and argyrophilic nucleolar organizer regions (AgNORs) have been used to determine high histological grade in feline MTs, and have been evaluated individually, but they have not been shown to be independent prognostic markers.[8,25,44]

Ki-67 antigen is a non-histone nuclear protein expressed at various levels during the different phases of the cell cycle. Therefore the relative number of Ki-67 positive cells indicates the growth fraction.[44,68] Ki-67 generally measured on paraffin sections by immunohistochemical.[44] The staining is higher in mammary carcinomas than in benign lesions, and has also been correlated with histological grade of feline mammary carcinomas, with higher grade tumours exhibiting more positive cells.[44] It was suggested that Ki-67 was correlated with the post-surgical ST, but controversial results have been described for this index , table 5.[44,68]

Proliferating cell nuclear antigen is an auxiliary subunit of DNA polymerase delta and is involved in DNA repair.[44] Expression is maximal during the G1/S phase of the cell cycle.[44] PCNA in feline mammary carcinoma has significant related to malignant tumours, reflecting greater mitotic activity, but has no correlation with degree of nuclear pleomorphism.[25,27,44] Preziosi *et al.* (1995) reported that the PCNA value is correlated with mitotic index in mammary carcinomas when typical and atypical mitotic figures are considered together, table 5.[69]

Argyrophilic nucleolar organiser regions are structural and functional nucleolar components associated with argyrophilic proteins involved in ribosomal ribonucleic acid transcription and processing.[44] The AgNOR size and number might correlate with cell proliferation, for that reason some studies have evaluated its role in the malignant MTs.[44] However the results obtained were controversial[44,70,71] with no correlation between AgNOR size and tumour morphological sub-type, tumour stage, nuclear atipia, or mitotic index [72], whereas, other study relate AgNOR index to survival in univariate analysis and has an independent prognostic value in multiparametric survival test , table 5.[70]

Metallothioneins are metal binding proteins which also have a role in proliferation, and apoptosis.[44] In queens, immunohistochemical metallothionein was detected only in mammary carcinomas and no positivity in benign lesions and cases of fibroadenomatous hyperplasia.[73] However, its role in feline MTs needs further investigation , table 5.[73]

Other factors associated with malignant transformation and clinical outcome have been evaluated, including various molecular markers.[25] The vascular endothelial growth factor is an angiogenic factor involved in new blood vessel formation and tumour microvessel density and is routinely used to assess neoplastic angiogenesis.[25] Tumour progression is usually associated with hypoxia which induces the production of nitric oxide, with subsequent stabilization of the hypoxia inducible factor-1-α (HIF-1-α), that in turn stimulates the production of the pro-angiogenic VEGF.[74, 75] In human breast cancer, retrospective studies suggest that VEGF plays a relevant biological role in their progression also that high levels of VEGF is associated to poor prognosis, and who may benefit from validated anti-VEGF treatments.[76] In feline mammary carcinomas, studies performed by Millanta *et al.* (2006) indicated that an increased

VEGF expression is correlated to tumour anaplasia and shorter overall survival time, indicating that VEGF could be considered a prognostic indicator in feline MTs, table 5.[16,25,41,44,77]

Considering that the development of a tumour is usually associated to an increased production of nitric oxide, that together with HIF-1-α stimulate the production of VEGF, several studies determined the expression of nitric oxide synthase (NOS) isoforms, like the endothelial and inducible NOS (e/iNOS) and VEGF in feline MTs in order to clarify the correlations between these proteins and their relationship with angiogenesis and microvessel density.[74] iNOS immunoreactivity was localised in tumour cells and sporadically in stromal myofibroblasts, whereas eNOS and VEGF were localised in the cytoplasm of tumour epithelial cells and endothelium. Expression of iNOS was increased in malignant tumours and was significantly higher in grade III and grade II when compared with grade I cases.[74] Furthermore, expression of iNOS was positively correlated with VEGF and microvessel density of feline MTs and both measures were significantly greater in less differentiated phenotypes. However, increasing eNOS expression was limited to hyperplastic lesions and showed no significant with histological grade.[74] With this study was possible to conclude that the expression of NOS isoforms depends on tumour grade and positive correlations between iNOS and angiogenic markers suggests that iNOS synthesised by tumour cells promotes tumour growth, table 5.[74]

In human breast cancer, mutations in the tumour suppressor gene p53 confer a worse prognosis independently of other risk factors.[44] To evaluate the role of p53 in feline MTs it was performed a study, that showed that 17,3 % of feline mammary carcinoma express this protein, while there was no p53 expression in benign lesions.[27] Other study, reported that the expression of p53 vary from 18.9% to 33% in feline carcinomas, and there was no expression in adenosis and fibroadenoma.[44] However, until now there is no persuasive evidence that assessment of p53 expression by immunohistochemistry holds great prognostic potential for feline MTs , table 5.[44]

Another molecule that has been studied in feline MTs is COX-2. Millanta *et al.* (2006), found COX-2 expression in 96% of the feline MTs and associated this with a poor prognosis, table 5. However, Beam *et al.* (2003) reported the absence of COX-2 expression in all adenocarcinomas of feline mammary gland, table 5.[78,79] In addition, Sayasith, *et al.* (2009), showed that a large proportion (87%) of feline MTs expressed COX-2 and this has also been linked to poor prognosis, table 5.[80] However these results from Sayasith, *et al.* (2009) were complicated by the fact that it also correlates with expression of the angiogenic factor VEGF, which itself is significantly correlated with overall survival, table 5.[80] The COX-2 expression, also has been correlated with negativity for oestrogen receptors (ER) and increased expression of progesterone receptors (PR).[24]

In order to understand the role of steroid hormones in MTs tumorigenesis, the expression of oestrogen receptors α (ERα) and PR were studied by immunohistochemistry and biochemical methods.[24,48,81,82,83] The results demonstrated that in the *in situ* carcinomas the expression of ERα and of PR varies between 7% and 17% respectively, while in invasive carcinomas the expression of ERα and of PR ranges from

15% to 40%, respectively.[48,81] Some studies pointed progesterone as the main contributor to tumorigenesis, since expression of PR in malignant tumours is significantly higher than the expression of ERα.[24,48,81] Moreover, several studies reported a statistically significant decrease in ER positivity in invasive carcinomas when compared with normal and dysplastic tissues[48,81,82], and in the study performed by Maniscalco *et al.* (2013), there was a positive correlation between ERα expression and benign neoplasia.[84] Due to these results, it was suggested that MTs loose steroid dependence during malignant progression.[48,82]

In contrast, a statistically significant increase in the PR expression was reported in neoplastic lesions when compared with healthy and dysplastic tissues.[48] In a study performed by Millanta *et al.* 2006, the PR expression reached the maximum expression in the *in situ* carcinomas, and a progressive decrease in invasive tumours; nonetheless, 61,7% of the samples were still PR positive. Therefore, it is considered that oestrogen exposure may constitute a risk for the development of tumours, but the expression of ER and of PR in invasive carcinomas do not correlate with histological parameters or with overall ST.[24]

The human epidermal growth factor receptor-2 is one of the best characterized breast cancer marker in human medicine.[85,86] This receptor is composed by an intracellular tyrosine kinase domain and once activated through a set of ligands (endothelial growth factor or transforming growth factor-α) will activate downstream signal transduction pathways that control cell growth, regulate cell survival and apoptosis through the Ras/Raf/mitogen-activated protein kinase pathway, the PI3K/AKT pathway, protein kinase C (PKC) among others.[87] The overexpression of HER-2 is caused by the amplification of the HER-2/neu proto-oncogene.[88,89] In humans, it has been demonstrated that gene amplification and/or overexpression of the c-erbB-2/HER2/neu tyrosine kinase is associated with poor prognosis in breast cancer, , table 5.[89,90]

In cats, the role of c-erbB-2/HER2/neu in MTs is controversial since the results vary significantly. According to Rasotto the incidence of c-erbB-2/HER2/neu overexpression in feline MTs is of 5,5%.[91] However, in a study performed by Maniscalco *et al.* (2013) the incidence of c-erbB-2/HER2/neu overexpression was 33,33%, in a study performed by Ordás *et al.* (2007) the incidence was 40%, and in a study performed by Millanta *et al.* (2005) the incidence was 59,6%, table 5.[24,84,88,92] Besides the controversies, some studies consider that HER-2 overexpression did not show significant correlation with histologic type, tumour grading, or presence of lymphatic invasion, but its expression seems to correlate with shorter overall survival, table 5.[84,88,91]

Topoisomerase IIβ binding protein 1 is a nuclear protein that has structural and functional similarities with gene BRCA2.[93] In feline MTs, TopBP1 expression has been positively correlated with histological grade, and in high grade carcinomas there was convincing correlation with ERα negative status.[44] The expression of TopBP1 in feline MTs increases with increasing grade of malignancy but until now there is not clear evidence that this molecule to use this molecule could be used as a prognostic marker, table 5.[93]

Another important molecule is the tyrosine kinase receptor gene (RON) which is involved in the activation of the signalling cascade responsible for invasive properties of neoplastic cells.[94] The study

of the expression of RON stem cell-derived tyrosine kinase (STK) homologue gene in cats, showed that its expression is significantly increased in feline mammary carcinoma.[95] In fact, RON/STK was found to be expressed at a very high level in 20% of feline mammary carcinomas, however further studies to evaluate its possible contribution as a prognostic marker are needed, table 5.[95]

Cadherins are cell adhesion molecules important in the morphogenesis and maintenance of the normal tissue architecture.[96] The reduction or absence of E-cadherin expression and abnormalities in the pattern of immunostaining were evidenced in a subgroup of feline mammary carcinoma but its role as a prognostic factor is not fully understood, table 5.[97]

The AKT, also known as protein kinase B, is a serine/threonine protein kinase which, when activated by phosphorylation (p-AKT), promotes growth factor-mediated cell growth, proliferation, migration and survival.[52] The expression of AKT was correlated with malignancy and tumour differentiation, and was associated with a shorter DFI but further studies are needed to better understand its role in feline MTs, table 5.[52]

Phosphatase and tensin homolog belongs to the group of gatekeeper tumour suppressor genes and is involved in multiple mechanisms leading to cellular defence against neoplastic transformation and progression.[51] In a recent study, loss of PTEN expression as revealed by immunohistochemistry was found in 76% of feline mammary carcinomas. Furthermore, the female cats with PTEN-negative tumours had a worse prognosis, and the loss of PTEN expression was significantly correlated with lymphatic vessels invasion on feline mammary carcinoma.[51] In spite of these results larger studies are needed to clarify the importance of PTEN to the prognostic of MTs, table 5.[51]

Cyclin A is a gene that regulates the cell cycle and it is commonly targeted in feline MTS.[98,99] Over-expression of this gene were found in mammary carcinoma.[98,99] The results suggest that cyclin A may be associated with tumorigenesis in cases of feline mammary carcinomas but there is no information regarding its role as prognostic factor, table 5.[98,99]

Another factor that can constitute a marker for diagnosis and also a marker of prognosis is the presence of cancer stem cells (CSC). It is generally accepted that CSCs are involved in the tumorigenesis process due to its self-renewal ability, differentiation potential and tumour-initiating ability. In an experimental study performed by Pang *et al.* 2013 it was demonstrated that CSC from feline mammary tumours contribute to invasiveness and resistance to radiation and chemotherapy, table 5.[50]

Table 5 – Molecular markers and their prognostic value

Molecule	***Expression***	***Prognostic value***
Ki-67	Increased in malignant MT and higher grade	Controversial results
AgNOR	Increased in malignant in MT	Further investigations
PCNA	Increased in malignant MT	Controversial results
Metallothioneins	Increased in malignant MT	Further investigations
VEGF	Increased in malignant MT	Shorter overall survival time
iNOS	Increased in malignant MT and Grade II and III	Poor prognostic
eNOS	Increased in hyperplastic lesions	Further investigations
p53	Increased in malignant MT	Poor prognostic
COX-2	Malignant MT (96% - Millanta *et al.* (2006)	Poor prognostic
	Malignant MT (87 % - Sayasith *et al.* (2009))	Poor prognostic
	Absence in Malignant MT (Beam *et al.* (2003))	
HER-2/neu	Malignant MT (5,5% - Rasotto *et al.* (2011))	Poor prognostic
	Malignant MT (33,3% - Maniscalco *et al.* (2013))	Short overall survival
	Malignant MT (40%- Ordás *et al.* (2007))	Short overall survival
	Malignant MT (59,6% - Millanta *et al.* (2005))	Short overall survival
TopBP1	Increased in high grade of malignant MT	Further investigations
RON	Increased in malignant MT	Further investigations
E – Cadherin	Reduction or absence in malignant MT	Further investigations
AKT	Malignant MT and tumour differentiation	Shorter DFI/ Further investigations
PTEN	Loss in malignant MT	Poor prognosis/Further investigations
Cyclin A	Increased in malignant MT	Further investigations
Cancer Stem Cells	Increased in malignant MT	Poor prognosis

9. CONCLUSION

Feline MTs comprise approximately 12% of the feline tumours. Approximately 85% to 95% of the MT are malignant and have a poor prognosis due to a high ability of proliferation, high probability of local and distant invasion at the time of diagnostic and due to the high recurrence ratio.

In spite of the prevalence and aggressiveness of these tumours, its aetiology remains poorly understood. Some studies pointed several risk factors for MT namely age, breed, reproductive status and exposure to oestrogen and progesterone but the clear contribution of each one to the tumorigenesis of the MT is still poorly understood.

Regarding tumour classification, most of the studies use the criteria proposed by the World Health Organization (WHO) which facilitates the interpretation of the results. The histological grade is considered a reliable prognostic parameters, however, there is a lack of uniformity among techniques used to obtain the tumour grade which introduce a bias in the interpretation of the results.

The literature also evidence a clear lack of uniformity among the evaluation of the prognostic factors. Most of the studies are retrospective which difficult the accurate collection of information, do not use similar methodology in the design of the studies, use different inclusion criteria of the patients, most of them are non-randomized studies and include a reduced number of patients. Together, these variables turn difficult the comparison of the results and the establishment of an adequate conclusion regarding the role of each molecule. Due to these discrepancies is still difficult to establish a prognostic and also to understand the mechanisms involved in recurrences.

Furthermore, due to the lack of uniformity among studies it is difficult to understand the role of adjuvant chemotherapy in the feline MTs and therefore it is difficult to establish an adequate therapeutic strategy.

The most recent studies are studying the molecular characterization of feline MTs which may represent a new stage in the characterization of the tumours and also in the development of effective targeted therapies. These studies may contribute to the understanding of the aggressiveness of the MTs and therefore to the establishment of individual therapeutic strategies, opening a new era in the study of the feline MTs.

10. REFERENCES

1. Egenvall, A.; Bonnett, B. N.; Haggstrom, J.; Strom Holst, B.; Moller, L.; Nodtvedt, A., Morbidity of insured Swedish cats during 1999-2006 by age, breed, sex, and diagnosis. *J Feline Med Surg* **2010,** *12* (12), 948-59.

2. Hayes, H. M., Jr.; Milne, K. L.; Mandell, C. P., Epidemiological features of feline mammary carcinoma. *Vet Rec* **1981,** *108* (22), 476-9.

3. McNeill, C. J.; Sorenmo, K. U.; Shofer, F. S.; Gibeon, L.; Durham, A. C.; Barber, L. G.; Baez, J. L. a.; Overley, B., Evaluation of Adjuvant Doxorubicin-Based Chemotherapy for the Treatment of Feline Mammary Carcinoma. *J Vet Intern Med* **2009,** *23*, 123-129.

4. Togni, M.; Masuda, E. K.; Kommers, G. D.; Fighera, R. A.; Irigoyen, L. F., Estudo retrospectivo de 207 casos de tumores mamários em gatas. *Pesq. Vet. Bras.* **2013,** *33* (3), 353-358.

5. Vascellari, M.; Baioni, E.; Ru, G.; Carminato, A.; Mutinelli, F., Animal tumour registry of two provinces in northern Italy: incidence of spontaneous tumours in dogs and cats. *BMC veterinary research* **2009,** *5*, 39.

6. Egenvall, A.; Nodtvedt, A.; Haggstrom, J.; Strom Holst, B.; Moller, L.; Bonnett, B. N., Mortality of Life-Insured Swedish Cats during 1999 –2006: Age, Breed, Sex, and Diagnosis. *J Vet Intern Med* **2009,** *23*, 1175-1183.

7. von Euler, H., Tumours of the mammary glands. In *BSAVA Manual of Canine and Feline Oncology*, 3rd ed.; Dobson, J.; Lascelles, B. D. X., Eds. 2011; pp 243-247.

8. Morris, J., Mammary tumours in the cat - Size matter, so early intervention saves lives. *Journal of Feline Medicine and Surgery* **2013,** *15* (5), 391-400.

9. Skorupski, K. A.; Overley, B.; Shofer, F. S.; Goldschmidt, M. H.; Miller, C. A.; Sorenmo, K. U., Clinical characteristics of mammary carcinoma in male cats. *J Vet Int Med* **2005,** *19*, 52-55.

10. Simeonov, R.; Simeonova, G., Nuclear cytomorphometry in feline mammary gland epithelial tumours. *Veterinary journal* **2009,** *179* (2), 296-300.

11. Misdorp, W.; Romijn, A.; Hart, A. A., Feline mammary tumors: a case-control study of hormonal factors. *Anticancer research* **1991,** *11* (5), 1793-7.

12. Shafiee, R.; Javanbakht, J.; Atyabi, N.; Bahrami, A.; Kheradmand, D.; Safaei, R.; Khadivar, F.; Hosseini, E., Comparative value of clinical, cytological, and histopathological features in feline mammary gland tumors; an experimental model for the study of human breast cancer. *Diagn Pathol* **2013,** *8*, 136.

13. Castagnaro, M.; Casalone, C.; Bozzetta, E.; al., e., Tumour grading and the one-year post-surgical prognosis in feline mammary carcinomas. *J Comp Pathol* **1998,** *119*, 263-275.

14. Pinto, R. Neoplasias Mamárias em Cadelas e Gatas. UNIVERSIDADE TÉCNICA DE LISBOA - Faculdade de Medicina Veterinária, Lisboa, 2009.

15. Sorenmo, K.; Worley, D.; Goldschmidt, M., Tumors of the mammary gland. In *Withrow & MacEwen's Small Animal Clinical Oncology*, 5th ed.; Withrow, S.; Vail, D.; Page, R., Eds. Saunders Elsevier: St. Louis, Missouri, 2013; pp 548-556.

16. Gimenez, F.; Hecht, S.; Craig, L. E.; Legendre, A. M., Early detection, aggressive therapy: optimizing the management of feline mammary masses. *J Feline Med Surg* **2010,** *12* (3), 214-24.

17. Dorn, C. R.; Taylor, D. O.; Schneider, R.; Hibbard, H. H.; Klauber, M. R., Survey of animal neoplasms in Alameda and Contra Costa Counties, California. II. Cancer morbidity in dogs and cats from Alameda County. *Journal of the National Cancer Institute* **1968,** *40* (2), 307-18.

18. Hayden, D. W.; Nielsen, S. W., Feline mammary tumours. *J. small Anim. Pract.* **1971,** *12*, 687-697.

19. Weijer, K.; Head, K. W.; Misdorp, W.; Hampe, J. F., Feline Malignant Mammary Tumors. I.Morphology and Biology: Some Comparisons With Human and Canine Mammary Carcinomas. *JNCI* **1972,** *49* (6), 1697-1704.

20. Overley, B.; Shofer, F. S.; Goldschmidt, M. H.; Sherer, D.; Sorenmo, K. U., Association between Ovarihysterectomy and Feline mammary carcinoma. *J Vet Int Med* **2005,** *19*, 560-563.

21. Jacobs, T. M.; Hoppe, B. R.; Poehlmann, C. E.; Ferracone, J. D.; Sorenmo, K. U., Mammary adenocarcinomas in three male cats exposed to medroxyprogesterone acetate (1990-2006). *J Feline Med Surg* **2010,** *12* (2), 169-74.

22. Nunes, G.; Filgueira, F.; de Paula, V.; Reis, P.; Filgueira, K., Neoplasias Mamárias em Gatas Domésticas Possível Influência da Dieta na Etiologia. *Revista Brasileira de Higiene e Sanidade Animal* **2011,** *05* (02), 30 - 37.

23. Raharison, F.; Sautet, J., Lymph drainage of the mammary glands in female cats. *Journal of morphology* **2006,** *267* (3), 292-9.

24. Millanta, F.; Calandrella, M.; Bari, G.; Niccolini, M.; Vannozzi, I.; Poli, A., Comparison of steroid receptor expression in normal, dysplastic, and neoplastic canine and feline mammary tissues. *Research in veterinary science* **2005,** *79* (3), 225-32.

25. Wypij, J.; Fan, T. M.; Lorimier, L., Malignant mammary tumors: Biologic behavior, prognostic factors, and therapeutic approach in cats. *Veterinary Medicine* **2006**.

26. Pastor, J. In *Tumores da Mama em Cadelas e Gatas*, Oncologia, Dermatologia e Oftalmologia e Pequenos Animais, XXIX Jornadas Médico-Veterinárias da AEMFV, XXIX Jornadas Médico-Veterinárias da AEMFV, 2005.

27. Zappulli, V.; De Zan, G.; Cardazzo, B.; Bargelloni, L.; Castagnaro, M., Feline mammary tumours in comparative oncology. *Journal of Dairy Research* **2005,** *72* (S1), 98.

28. Millanta, F.; Verin, R.; Asproni, P.; Giannetti, G.; Poli, A., A case of feline primary inflammatory mammary carcinoma: clinicopathological and immunohistochemical findings. *J Feline Med Surg* **2012,** *14* (6), 420-3.

29. Perez-Alenza, M. D.; Jimenez, A.; Nieto, A. I.; Pena, L., First description of feline inflammatory mammary carcinoma: clinicopathological and immunohistochemical characteristics of three cases. *Breast cancer research : BCR* **2004,** *6* (4), R300-7.

30. Albertus, J. C. C., *Oncología veterinaria. Manuales clínicos por especialidades*. Servet: 2011.

31. Blackwood, L., Cats with the cancer: where to start. *Journal of Feline Medicine and Surgery* **2013,** *15* (5), 366-377.

32. Morris, J.; Dobson, J., Mammary Gland. In *Small Animal Oncology*, 2011.

33. Novosad, C. A., Principles of treatment for mammary gland tumors. *Clinical Techniques in Small Animal Practice* **2003,** *18* (2), 107-109.

34. Misdorp, W.; Else, R. W.; Hellmén, E.; Lipscomb, T. P., *Histological Classification of Mammary Tumors of the Dog and the Cat*. 2nd ed.; Armed Forces Institute of Pathology in cooperation with the American Registry of Pathology and The World Health Organization Colaborating Center for Worldwide Reference on Comparative Oncology: Washington, 1999; Vol. VII.

35. Seixas, F.; Pires, M. A.; Lopes, C. A., Complex carcinomas of the mammary gland in cats: pathological and immunohistochemical features. *Veterinary journal* **2008,** *176* (2), 210-5.

36. Seixas, F.; Palmeira, C.; Pires, M. A.; Lopes, C., Are complex carcinoma of the feline mammary gland and other invasive mammary carcinoma identical tumours? Comparison of clinicopathologic features, DNA ploidy and follow up. *Research in veterinary science* **2008,** *84* (3), 428-33.

37. Kamstock, D. A.; Fredrickson, R.; Ehrhart, E. J., Lipid-rich carcinoma of the mammary gland in a cat. *Veterinary pathology* **2005,** *42* (3), 360-2.

38. Elston, E. W.; Ellis, I. O., Assessment of histological grade. In *The Breast*, Elston, E. W.; Ellis, I. O., Eds. Curchill Livingstone: 1998; Vol. 13, pp 365-382.

39. Matos, A. J.; Baptista, C. S.; Gartner, M. F.; Rutteman, G. R., Prognostic studies of canine and feline mammary tumours: the need for standardized procedures. *Veterinary journal* **2012,** *193* (1), 24-31.

40. Figueira, A. C.; Teodosio, A. S.; Carvalheira, J.; Lacerda, M.; de Matos, A.; Gartner, F., P-cadherin expression in feline mammary tissues. *Veterinary medicine international* **2012,** *2012*, 687424.

41. Millanta, F.; Lazzeri, G.; Mazzei, M.; Vannozzi, I.; Poli, A., MIB-1 labeling index in feline dysplastic and neoplastic mammary lesions and its relationship with postsurgical prognosis. *Veterinary Pathology* **2002,** *39*, 120-126.

42. Seixas, F.; Palmeira, C.; Pires, M. A.; Bento, M. J.; Lopes, C., Grade is an independent prognostic factor for feline mammary carcinomas: a clinicopathological and survival analysis. *Veterinary journal* **2011,** *187* (1), 65-71.

43. Owens, L., *TNM: Classification of tumours in domestic animals*. Geneva, 1980.

44. Hughes, K.; Dobson, J. M., Prognostic histopathological and molecular markers in feline mammary neoplasia. *Veterinary journal* **2012,** *194* (1), 19-26.

45. Hanahan, D.; Weinberg, R. A., The Hallmarks of Cancer. *Cell* **2000,** *100*, 57-70.

46. Hanahan, D.; Weinberg, R. A., Hallmarks of cancer: the next generation. *Cell* **2011,** *144* (5), 646-74.

47. Madu, C.; Lu, Y., Novel diagnostic biomarkers for prostate cancer. *Journal of Cancer* **2010,** *1*, 150-177.

48. de las Mulas, J. M.; van, N. M.; Millan, Y.; Ordas, J.; Blankstein, M. A.; Van Mil, F.; Misdorp, W., Progesterone receptors in normal, dysplastic and tumourous feline mammary glands. Comparison with oestrogen receptors status. *Research in veterinary science* **2002,** *72*, 153-161.

49. Mayr, B.; Blauensteiner, J.; Edlinger, A.; Reifinger, M.; Alton, K.; Schaffner, G.; Brem, G., Presence of p53 mutations in feline neoplasms. *Research in veterinary science* **2000,** *68* (1), 63-70.

50. Pang, L. Y.; Blacking, T. M.; Else, R. W.; Sherman, A.; Sang, H. M.; Whitelaw, B. A.; Hupp, T. R.; Argyle, D. J., Feline mammary carcinoma stem cells are tumorigenic, radioresistant, chemoresistant and defective in activation of the ATM/p53 DNA damage pathway. *Veterinary journal* **2013,** *196* (3), 414-23.

51. Ressel, L.; Millanta, F.; Caleri, E.; Innocenti, V. M.; Poli, A., Reduced PTEN protein expression and its prognostic implications in canine and feline mammary tumors. *Veterinary pathology* **2009,** *46* (5), 860-8.

52. Maniscalco, L.; Iussich, S.; de Las Mulas, J. M.; Millan, Y.; Biolatti, B.; Sasaki, N.; Nakagawa, T.; De Maria, R., Activation of AKT in feline mammary carcinoma: a new prognostic factor for feline mammary tumours. *Veterinary journal* **2012,** *191* (1), 65-71.

53. Van Nimwegen, S.; Kirpensteijn, J., Specific Disorders - Neoplastic Skin Disorders. In *Veterinary Surgery Small Animal*, Tobias, K. M.; Johnston, S. A., Eds. Elsevier Saunders: 2012; Vol. 2.

54. Fox, L. E.; MacEwen, E. G.; Kurzman, I. D.; Dubielzig, R. R.; Helfand, S. C.; Vail, D. M.; Kisseberth, W.; London, C.; Madewell, B. R.; Rodriguez, C. O. J., Liposome-encapsulated Muramyl Tripeptide Phosphatidylethanolamine for the Treatment of Feline Mammary Adenocarcinoma-A Multicenter Randomized Double-blind Study. *CANCER BIOTHERAPY* **1995,** *10* (2).

55. Rutten, V. P. M. G.; Misdorp, W.; Gauthier, A.; Estrada, M.; Mialo, J. P., Immunological aspects of mammary tumors in dog. *Veterinary Immunology and Immunopathology* **1990,** *26* (3).

56. Rutteman, G. R.; Misdorp, W., Hormonal background of canine and feline mammary tumours. *J. Reprod. Fert. Suppl.* **1993,** *47*, 483-487.

57. Novosad, C. A.; Bergman, P. J.; O'Brien, M. G.; McKnight, J. A.; Charney, S. C.; Selting, K. A.; Graham, J. C.; Correa, S. S.; Rosenberg, M. P.; Gieger, T. L., Retrospective evaluation of adjunctive doxorubicin for the treatment of feline mammary gland adenocarcinoma: 67 cases. *Journal of the American Animal Hospital Association* **2006,** *42* (2), 110-120.

58. Borrego, J. F.; Cartagena, J. C.; Engel, J., Treatment of feline mammary tumours using chemotherapy, surgery and a COX-2 inhibitor drug (meloxicam): a retrospective study of 23 cases (2002-2007)*. *Veterinary and comparative oncology* **2009,** *7* (4), 213-21.

59. Jeglum, K. A.; deGuzman, E.; Young, K. M., Chemotherapy of advanced mammary adenocarcinoma in 14 cats. *Journal of the American Veterinary Medical Association* **1985,** *187* (2), 157-60.

60. Mauldin, G. N.; Matus, R. E.; Patnaik, A. K.; Bond, B. R.; Mooney, S. C., Efficacy and toxicity of doxorubicin and cyclophosphamide used in the treatment of selected malignant tumors in 23 cats. *J Vet Intern Med* **1988,** *2* (2), 60-5.

61. MacEwen, E. G.; Hayes, A. A.; Mooney, S.; Patnaik, A. K.; Harvey, H. J.; Passe, S.; Hardy, W. D., Jr., Evaluation of effect of levamisole on feline mammary cancer. *Journal of biological response modifiers* **1984,** *3* (5), 541-6.

62. Santana, C. M. S.; Ramos-Vega, S.; Rizkallal, C.; Roiz-Martin, S.; Herráez, P.; Grau-Bassas, E. In *Adjuvant Treatment with Toceranib Phosphate and Metronomic Chemotherapy on a Cat with Aggressive Mammary tumour*, European Society of Veterinary Oncology, PALAIS NIEDERÖSTERREICH - Vienna, Austria: 2014; p 136.

63. Moore, A. In *Advances in the treatment of mammary neoplasia*, World Congress WSAVA/FECAVA/CSAVA, 2006; pp 562-565.

64. Viste, J. R.; Myers, S. L.; B., S.; al., e., Feline mammary adenocarcinoma: tumor size as a prognostic indicator. *Can Vet J* **2002,** *43*, 33-37.

65. de las Mulas J.M.; Reymundo, C., Animal models of humam breast carcinoma: canine and feline neoplasms. *Rev Oncologia* **2000,** *2*, 274-281.

66. Weijer, K.; Hart, A. A. M., Prognostic Factors in Feline Mammary Carcinoma. *JNCI* **1983,** *70* (4), 709-716.

67. Ito, T.; Kadosawa, T.; Mochizuki, M.; Matsunaga, S.; Nishimura, R.; Sasaki, N., Prognosis of malignant mammary tumor in 53 cats. *J Vet Med Sci* **1996,** *58* (8), 723-6.

68. Castagnaro, M.; De Maria, R.; Bozzetta, E.; Ru, G.; Casalone, C.; Biolatti, B.; Caramelli, M., Ki-67 index as indicator of the post-surgical prognosis in feline mammary carcinomas. *Research in veterinary science* **1998,** *65* (3), 223-226.

69. Preziosi, R.; Sarli, G.; Benazzi, C.; Marcato, P. S., Detection of proliferating cell nuclear antigen (PCNA) in canine and feline mammary tumours. *J Comp Pathol* **1995,** *113* (4), 301-13.

70. Preziosi, R.; Sarli, G.; Benazzi, C.; Mandrioli, L.; Marcato, P. S., Multiparametric survival analysis of histological stage and proliferative activity in feline mammary carcinomas. *Research in veterinary science* **2002,** *73* (1), 53-60.

71. Castagnaro, M.; Casalone, C.; Ru, G.; Nervi, G. C.; Bozzetta, E.; Caramelli, M., Argyrophilic nucleolar organiser regions (AgNORs) count as indicator of post-surgical prognosis in feline mammary carcinomas. *Veterinary Science* **1998,** *64* (2), 97-100.

72. De Vico, G.; Maiolino, P.; Restucci, B., Silver-stained nucleolar (Ag-NOR) cluster size in feline mammary carcinomas: lack of correlation with histological appearance, mitotic activity, tumour stage, and degree of nuclear atypia. *J Comp Pathol* **1995,** *113* (1), 69-73.

73. Dincer, Z.; Jasani, B.; Haywood, S.; Mullins, J. E.; Fuentealba, I. C., Metallothionein expression in canine and feline mammary and melanotic tumours. *J Comp Pathol* **2001,** *125* (2-3), 130-6.

74. Islam, M. S.; Matsumoto, M.; Hidaka, R.; Miyoshi, N.; Yasuda, N., Expression of NOS and VEGF in feline mammary tumours and their correlation with angiogenesis. *Veterinary journal* **2012,** *192* (3), 338-44.

75. Furuya, M.; Nishiyama, M.; Kasuya, Y.; Kimura, S.; Ishikura, H., Pathophysiology of tumor neovascularization. *Vascular Health and Risk Management* **2005,** *1* (4), 277-290.

76. Gasparini, G., Prognostic value of vascular endothelial growth factor in breast cancer. *The oncologist* **2000,** *5 Suppl 1*, 37-44.

77. Millanta, F.; Silvestri, G.; Vaselli, C.; Citi, S.; Pisani, G.; Lorenzi, D.; Poli, A., The role of vascular endothelial growth factor and its receptor Flk-1/KDR in promoting tumour angiogenesis in feline and canine mammary carcinomas: a preliminary study of autocrine and paracrine loops. *Research in veterinary science* **2006,** *81* (3), 350-7.

78. Millanta, F.; Citi, S.; Della Santa, D.; Porciani, M.; Poli, A., COX-2 expression in canine and feline invasive mammary carcinomas: correlation with clinicopathological features and prognostic molecular markers. *Breast Cancer Res Treat*

2006, *98*, 115-120.

79. Beam, S. L.; Rassnick, K. M.; Moore, A. S.; McDonough, S. P., An Immunohistochemical Study of Cyclooxygenase-2 Expression in Various Feline Neoplasms. *Veterinary pathology* **2003,** *40* (5), 496-500.

80. Sayasith, K.; Sirois, J.; Dore, M., Molecular characterization of feline COX-2 and expression in feline mammary carcinomas. *Veterinary pathology* **2009,** *46* (3), 423-9.

81. J.M., d. l. M.; van, N. M.; Millan, Y.; Ordas, J.; Blankstein, M. A.; Van Mil, F.; Misdorp, W., Immunohistochemical analysis of estrogen receptors in feline mammary gland benign and malignant lesions: comparison with biochemical assay. *Domestic Animal Endocrinology* **2000,** *18*, 111-125.

82. Millanta, F.; Calandrella, M.; Vannozzi, I.; Poli, A., Steroid hormone receptors in normal, dysplastic and neoplastic feline mammary tissues and their prognostic significance. *Vet Rec* **2006,** *158* (24), 821-4.

83. Burrai, G. P.; Mohammed, S. I.; Miller, M. A.; Marras, V.; Pirino, S.; Addis, M. F.; Uzzau, S.; Antuofermo, E., Spontaneous feline mammary intraepithelial lesions as a model for human estrogen receptor- and progesterone receptor-negative breast lesions. *BMC cancer* **2010,** *10*, 156.

84. Maniscalco, L.; Y., M.; Iussich, S.; Denina, M.; Sánchez-Céspedes, R.; Gattino, F.; Biolatti, B.; Sasaki, N.; Nakagawa, T.; Di Renzo, M.; de las Mulas, J. M.; De Maria, R., Activation of mammalian target of rapamycin (mTOR) in triple negative feline mammary carcinomas. *BMC veterinary research* **2013,** *9* (80).

85. Howe, L. R.; Brown, P. H., Targeting the HER/EGFR/ErbB family to prevent breast cancer. *Cancer prevention research* **2011,** *4* (8), 1149-57.

86. Johnson, E.; Seachrist, D. D.; DeLeon-Rodriguez, C. M.; Lozada, K. L.; Miedler, J.; Abdul-Karim, F. W.; Keri, R. A., HER2/ErbB2-induced breast cancer cell migration and invasion require p120 catenin activation of Rac1 and Cdc42. *The Journal of biological chemistry* **2010,** *285* (38), 29491-501.

87. Arora, A.; Scholar, E. M., Role of tyrosine kinase inhibitors in cancer therapy. *J Pharmacol Exp Ther* **2005,** *315* (3), 971-9.

88. Millanta, F.; Calandrella, M.; Citi, S.; Della Santa, D.; Poli, A., Overexpression of HER-2 in feline invasive mammary carcinomas: an immunohistochemical survey and evaluation of its prognostic potential. *Veterinary pathology* **2005,** *42* (1), 30-4.

89. Shah, S.; Chen, B., Testing for HER2 in Breast Cancer: A Continuing Evolution. *Pathology research international* **2011,** *2011*, 903202.

90. Issa, A.; Gill, J. W.; Heideman, M. R.; Sahin, O.; Wiemann, S.; Dey, J. H.; Hynes, N. E., Combinatorial targeting of FGF and ErbB receptors blocks growth and metastatic spread of breast cancer models. *Breast cancer research : BCR* **2013,** *15* (1), R8.

91. Rasotto, R.; Caliari, D.; Castagnaro, M.; Zanetti, R.; Zappulli, V., An Immunohistochemical study of HER-2 expression in feline mammary tumours. *J Comp Pathol* **2011,** *144* (2-3), 170-9.

92. Ordas, J.; Millan, Y.; Dios, R.; Reymundo, C.; de Las Mulas, J. M., Proto-oncogene HER-2 in normal, dysplastic and tumorous feline mammary glands: an immunohistochemical and chromogenic in situ hybridization study. *BMC cancer* **2007,** *7*, 179.

93. Morris, J. S.; Nixon, C.; Bruck, A.; Nasir, L.; Morgan, I. M.; Philbey, A. W., Immunohistochemical expression of TopBP1 in feline mammary neoplasia in relation to histological grade, Ki67, ERalpha and p53. *Veterinary journal* **2008,** *175* (2), 218-26.

94. Maggiora, P.; Marchio, S.; Stella, M. C.; Giai, M.; Belfiore, A.; De Bortoli, M.; Di Renzo, M. F.; Costantino, A.; Sismondi, P.; Comoglio, P. M., Overexpression of the RON gene in human breast carcinoma. *Oncogene* **1998,** *16* (22), 2927-33.

95. De Maria, R.; Maggiora, P.; Biolatti, B.; Prat, M.; Comoglio, P. M.; Castagnaro, M.; Di Renzo, M. F., Feline STK gene expression in mammary carcinomas. *Oncogene* **2002,** *21* (11), 1785-90.

96. (a) Angst, B. D.; Marcozzi, C.; Magee, A. I., The cadherin superfamily: diversity in form and function. *Journal of cell science* **2001,** *114* (Pt 4), 629-41; (b) Takeichi, M., Cadherin cell adhesion receptors as a morphogenetic regulator. *Science (New York, N.Y.)* **1991,** *251* (5000), 1451-5.

97. Scibelli, A.; d'Angelo, D.; Pelagalli, A.; Tafuri, S.; Avallone, L.; Della Morte, R.; Staiano, N., Expression levels of the focal adhesion-associated proteins paxillin and p130CAS in canine and feline mammary tumors. *Vet Res.* **2003,** *34* (2), 193-202.

98. Murakami, Y.; Tateyama, S.; Rungsipipat, A.; Uchida, K.; Yamaguchi, R., Immunohistochemical analysis of cyclin A, cyclin D1 and P53 in mammary tumors, squamous cell carcinomas and basal cell tumors of dogs and cats. *J Vet Med Sci* **2000,** *62* (7), 743-50.

99. Murakami, Y.; Tateyama, S.; Rungsipipat, A.; Uchida, K.; Yamaguchi, R., Amplification of the cyclin A gene in canine and feline mammary tumors. *J Vet Med Sci* **2000,** *62* (7), 783-7.