ESCOLA UNIVERSITÁRIA VASCO DA GAMA

MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

CHARACTERIZATION AND EVALUATION OF PROGNOSTIC FACTORS OF FELINE SPONTANEOUS MALIGNANT MAMMARY TUMORS

Joana Rodrigues de Jesus

Coimbra, julho 2020

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Characterization and evaluation of prognostic factors of feline spontaneous malignant mammary tumors

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EUVG – Escola Universitária Vasco da Gama

ICBAS-UP – Instituto de Ciências Biomédicas Abel Salazar-Universidade do Porto

WHO – World Health Organization

SD – Standard deviation

DFI – Disease-free interval

OS – Overall survival

Characterization and evaluation of prognostic factors of feline spontaneous malignant mammary tumors

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Resumo

Os tumores da glândula mamária são uma das neoplasias mais comuns em felinos. Estão associados a elevadas taxas de malignidade e mau prognóstico, resultando numa morbilidade e mortalidade elevadas. Nesse sentido, a definição de fatores de risco e de prognóstico são essenciais, no sentido de auxiliar a estabelecer um plano terapêutico e a prever a sua evolução.

Os objetivos principais deste estudo retrospetivo consistiram em fazer uma caraterização dos casos de lesões mamárias espontâneas em gatas domésticas que se apresentaram à consulta em três centros de atendimento médico veterinário de Portugal entre 2010 e 2020, e avaliar potenciais fatores de prognóstico de tumores mamários malignos espontâneos.

Foram recolhidos dados clínicos de 116 gatas que foram apresentadas à consulta devido à presença de massas mamárias. Destas, 95 apresentavam tumores mamários malignos (num total de 114 tumores malignos), três gatas foram diagnosticadas com tumores mamários benignos e 18 tinham alterações mamárias não neoplásicas.

Este estudo proporcionou uma caracterização detalhada dos tumores mamários de gatas. Sessenta e sete gatas foram incluídas na análise estatística de acompanhamento dos animais, que envolveu um total de 21 parâmetros. Na análise univariada, 11 fatores analisados mostraram uma correlação negativa estatisticamente significativa com o tempo de sobrevida, incluindo o tamanho do tumor, invasão de linfonodos, metástases distantes, estadio clínico, tipo de cirurgia, margens cirúrgicas, tipo de crescimento do tumor, necrose do tumor, invasão linfovascular, pleomorfismo nuclear e grau histológico. Sete destes fatores tiveram também uma correlação negativa significativa com o tempo livre de doença, nomeadamente o tamanho do tumor, estadio clínico, margens cirúrgicas, tipo de crescimento do tumor, invasão linfovascular, pleomorfismo nuclear e grau histológico. A regressão multivariada de avaliação da influência global dos diferentes fatores sobre o tempo livre de doença apresentou um valor elevado (R^2 ajustado de 0,90).

Palavras-chave: felino, glândula mamária, prognóstico, tumores

Abstract

Tumors of the mammary gland are one of the most common neoplasms in cats. They are associated with high rates of malignancy and poor prognosis, resulting in high mortality. Therefore, detection of risk and prognostic factors are important in order to define therapeutic strategies and to predict the evolution of the disease.

The main objectives of this retrospective study were to characterize the spontaneous mammary lesions presented by domestic cats that were evaluated at three veterinary medical centers in Portugal between 2010 and 2020, and evaluate potential prognostic factors of spontaneous malignant mammary tumors.

Clinical data were collected from 116 cats that were presented due to the presence of mammary gland masses. Of these, 95 cats had malignant mammary tumors (in a total of 114 malignant tumors), three were diagnosed with benign breast tumors and 18 had non-neoplastic mammary lesions.

This study provided a detailed characterization of the feline mammary tumors. Sixty-seven cats were included in the follow-up statistical analysis, which involved a total of 21 parameters. Eleven prognostic factors showed a statistically significant negative correlation with the overall survival in univariate analysis, including tumor size, lymph node invasion, distant metastasis, clinical stage, type of surgery, surgical margins, type of tumor growth, tumor necrosis, lymphovascular invasion, nuclear pleomorphism and histologic grade. Seven of these factors also showed a significant negative correlation with the disease-free interval, namely, tumor size, clinical stage, surgical margins, type of tumor growth, lymphovascular invasion, nuclear pleomorphism and histologic grade. Multiple regression of combined contribution of the different parameters analyzed in the overall survival presented a good correlation coefficient (adjusted R^2 of 0,90).

Keywords: feline, mammary gland, prognosis, tumors

Introduction

Mammary neoplasms are among the most frequent tumors in cats (Hayes & Mooney, 1985; Togni, Masuda, Kommers, Fighera, & Irigoyen, 2013). In felines most mammary tumors are malignant, with malignancy rates reported to be over 86%, and usually associated with an aggressive biologic behavior (Amorim, Souza, Ferreira, & Fonseca, 2006; Hahn & Adams, 1997; Hayes & Mooney, 1985). Moreover, due to the rapid growth and progression of these tumors, animals are usually presented in advanced stages of the disease (Zappulli, De Zan, Cardazzo, Bargelloni, & Castagnaro, 2005). Therefore, an early detection and diagnosis is paramount in order to start aggressive treatment and achieve a longer survival time (Amorim et al., 2006; Giménez, Hecht, Craig, & Legendre, 2010; Hayes & Mooney, 1985; Morris, 2013). Given the high malignancy rates and aggressive behavior, every mammary mass should be treated as a potential malignant tumor, and complete staging should be performed (Morris, 2013).

Age, breed and hormonal exposure have been recognized as risk factors for feline mammary gland development (Sorenmo, Worley, & Zappulli, 2020). Risk progressively increases, peaking at 11 years of age (Dorn, Taylor, Schneider, Hibbard, & Klauber, 1968). Siamese cats have been reported as having a higher incidence of the disease and to develop mammary gland tumors at a younger age, (Hayes & Mooney, 1985; Ito et al., 1996).

Early ovariohysterectomy has been documented to have a protective effect on feline mammary neoplasms development. In female cats spayed before six months of age, the risk is reduced by 91%, and in those spayed before one year the risk is reduced by 86% when compared to intact cats (Overley, Shofer, Goldschmidt, Sherer, & Sorenmo, 2005). Regular administration of exogenous progestogens, commonly used as contraceptives, also increases the risk of mammary tumor development (Misdorp, 1991; Misdorp, Romijn, & Hart, 1991).

Feline mammary gland tumors appear as discrete, palpable single or multiple masses, that may be mobile or attached to subjacent tissues (Giménez et al., 2010; Morris, 2013). Multiple nodules are frequent, and are usually found in adjacent ipsilateral glands (Hayes & Mooney, 1985; Zappulli et al., 2005). Ulceration can be a common finding, and is usually associated with extensive tumor necrosis (Novosad et al., 2006; Weijer & Hart, 1983). Involved mammary glands might present inflamed, with swelling and erythema of the affected tissues (Giménez et al., 2010).

Identification of prognostic markers is of major importance to assess the clinical outcome and evaluate possible treatment results (Webster et al., 2011). Several prognostic factors have been studied for feline mammary gland tumors, namely subject-related parameters, clinical, histopathological and immunohistochemical parameters (Zappulli et al., 2015). Tumor size, clinical stage, lymph node metastasis, lymphovascular neoplastic invasion, and mitotic counts are the main markers on which most authors agree as having a prognostic significance (Dagher, Abadie, Loussouarn, Campone, & Nguyen, 2019; Gemignani et al., 2018; Ito et al., 1996; Mills et al., 2015; Seixas, Palmeira, Pires, Bento, & Lopes, 2011; Weijer & Hart, 1983; Weijer, Head, Misdorp, & Hampe, 1972). Other tumor features have also

been reported as having prognostic significance, however, results are not always consistent among authors.

Given the advances in veterinary medicine concerning diagnosis and treatment of oncologic diseases, and the increasing interest of tutors in veterinary care (Webster et al., 2011; Zappulli et al., 2005), accurate prognostic and predictive markers are needed, as they are a helpful tool to manage expectations and devise treatment plans (Cassali et al., 2018; Webster et al., 2011). For these reasons, the main objectives of this retrospective study were to characterize the spontaneous mammary lesions presented by a cohort of domestic cats that were evaluated at three veterinary medical care centers in Portugal between 2010 and 2020, and to evaluate potential prognostic factors of feline spontaneous malignant mammary tumors.

Materials and methods

The present study was approved by the Scientific Council of Escola Universitária Vasco da Gama (EUVG), Coimbra, Portugal.

Female cats presented to three veterinary medical centers from the center region of Portugal (Hospital Veterinário do Baixo Vouga, Hospital Veterinário Universitário de Coimbra, Policlínica Veterinária de Aveiro), between July 2010 and February 2020, due to the presence of mammary lesions were recruited for this retrospective study. In all animals included in the study, mammary gland lesions were biopsied or excised by surgery or necropsy and submitted to histopathology evaluation at the Laboratory of Veterinary Pathology of the Instituto de Ciências Biomédicas Abel Salazar (ICBAS – UP). Data from the cohort of queens included in the study were retrieved directly from the medical records. Queens with previous history of mammary gland tumors or without histopathology evaluation were excluded from the research.

Subject-related information was collected in all cats studied, and included breed, age, weight, reproductive status at presentation (spayed or intact), ovariohysterectomy timing/age and history of progestogens administration.

Clinical data of the queens with mammary tumors included tumor size (largest diameter) before surgery, number of nodules, affected mammary glands location, presence or absence of ulceration, presence or absence of distant metastasis (in malignant tumors) and clinical stage (in malignant tumors). When available, disease-free interval (DFI), recurrence site and survival time were also recorded. Disease-free interval was considered the time period between surgery and recurrence of local or distant tumors. Overall survival (OS) was defined as the interval between diagnosis and date of death. Queens submitted to adjuvant chemotherapy, those who died due to post-surgical complications and queens with multiple malignant tumors with different histological subtypes were also excluded from both OS and DFI analysis.

Queens with malignant tumors were submitted to a complete physical and clinical examination, including hematology, serum biochemistry profile, lymph node evaluation, thoracic radiology and abdominal ultrasonography, at admission and in control visits when clinically indicated and at tutors' consent. Recommended post-surgical protocol included clinical reevaluations at one and three months after surgery, and then every three months until death of the animal, or until local disease or distant metastasis were detected. Clinical stage at diagnosis was assessed according to the modified World Health Organization (WHO) criteria, based on tumor diameter, presence of regional lymph node metastasis and evidence of distant metastasis (McNeill et al., 2009).

Information regarding treatments was also retrieved from the medical records of each case, and included evaluation of the type of surgical procedure (nodulectomy, regional, unilateral or bilateral mastectomy), number of surgeries, and administration of adjuvant chemotherapy.

Tissue samples were collected by surgery or necropsy, fixed in 10% neutral buffered formalin, routinely processed, cut into 2 µm sections and stained with hematoxylin and eosin for histological diagnosis. Histopathology evaluation was performed by two Pathologists in all cases. Histopathological features evaluated included histologic subtype, histologic grading, type of tumor growth (infiltrative or expansive), mitotic count, nuclear pleomorphism (mild, moderate or marked), surgical margins (complete, narrow or incomplete), presence or absence of necrosis, lymph node invasion and lymphovascular invasion. Surgical margins were considered complete when there was over a 2 mm distance to the tumor, narrow when that distance was between 1-2 mm, and incomplete when neoplastic cells were detected in the excision's margin. Neoplasms were classified according to the World Health Organization histological criteria for canine and feline mammary tumors (Misdorp, Else, Hellmen, & Lipscomb, 1999). Grading of malignant tumors was performed according with Elston and Ellis, based on tubule formation, nuclear pleomorphism and mitotic index. Neoplasms were assigned to grade I (well differentiated), grade II (moderately differentiated) or grade III (poorly differentiated) (Elston & Ellis, 1991). Mitotic figures were counted in ten high power fields (Nikon® E600 microscope with a field diameter of 0,55 mm, high power field area of 0,237 mm^2). Nuclear pleomorphism was assessed in high power fields throughout the entire tumor sections and classified as mild, moderate or marked based on nuclei size and shape variation, vesiculation and nucleoli appearance (Elston & Ellis, 1991; Mills et al., 2015).

Data retrieved from the medical records of the animals included in the study was compiled in Excel Professional Plus 2016 (Microsoft Office, Washington, USA). Descriptive statistics were also performed using Excel Professional Plus 2016 (Microsoft Office, Washington, USA). Spearman's rho correlation coefficient was applied to assess the relationships between variables in a text-book case of "non-conforming to assumptions" dataset using the software package XLSTAT (version 2018.7; Addinsoft, Paris, France). Multiple regression was also applied to determine the combined contribution of the selected predictors to explain the computed total variance, using the software package XLSTAT (version 2018.7; Addinsoft, Paris, France). A *P* value <0.05 was used to determine the level of statistical significance.

Results

One hundred and sixteen female cats were included in the study, of which 95 (81,9%) presented malignant mammary tumors, three (2,6%) had benign mammary tumors (fibroadenomas) and 18 (15,5%) had mammary lesions of non-neoplastic origin, four of which with more than one concurrent lesion (mammary cysts, n=9; fibroadenomatous hyperplasia, n=8; mammary hyperplasia, n=4; ductal ectasia, n=1). Only three out of 98 neoplastic lesions were benign.

Most queens included in the study were Domestic Shorthair cats (n=109; 94%), and most were intact (n=78; 67,4%) at diagnosis. Information regarding progestogens administration was available in 71 cases, of which 77,5% (n=55) had progestogens administered at some point in their lives. Characterization of the population included in the study is presented in Table 1.

Table 1. Subject-related characterization of the 116 animals included in the study.

	Mammary malignant tumors n=95 (%)	**Mammary benign tumors** n=3 (%)	**Mammary non-neoplastic lesions** n=18 (%)
Breed			
Domestic shorthair	90 (94,7%)	3 (100%)	16 (88,9%)
Persian	5 (5,3%)	-	2 (11,1%)
Age (years)			
Mean (SD)	11,7 (2,9)	6,7 (4,0)	6,7 (2,8)
Range	3 - 19	1 - 10	2 - 11
Weight (kg)			
Mean (SD)	3,9 (0,9)	4,0 (1,1)	3,7 (0,8)
Range	2 - 7,9	2,5 – 5,0	2,2 - 5,1
Reproductive status (n=113)			
Intact	59 (62,1%)	3 (100%)	16 (88,9%)
Spayed	33 (34,7%)	-	2 (11,1%)
Age at ovariohysterectomy (n=22)			
≤ 1 year	1 (3,0%)	-	-
1-2 years	1 (3,0%)	-	-
≥ 2 years	19 (57,6%)	-	1 (5,6%)
Contraceptives (n=71)			
Yes	44 (46,3%)	2 (66,7%)	9 (50,0%)
No	12 (12,6%)	-	4 (22,2%)

Out of the 95 animals with malignant tumors, 58 (61,1%) had one solitary mass, 23 (24,2%) had two nodules and 14 (14,7%) had three or more palpable nodules. Overall, a total of 114 malignant mammary

tumors were evaluated. Among the queens with multiple malignant masses, 17 were diagnosed with two (n=15) and three (n=2) concomitant different subtypes. Due to the presence of large, multicentric tumors that affected several mammary glands simultaneously, a total of 132 mammary glands were affected by malignant mammary tumors. Clinical features of malignant tumors are shown in Table 2. The three cats diagnosed with fibroadenoma had only one palpable nodule.

The majority of the queens were submitted to at least one surgery over the course of the disease (84 out of 95; 88,4%). Eleven out of 95 queens with malignant tumors were presented with advanced disease at diagnosis (clinical stage IV) and, consequently, surgery was not recommended. In these queens, mammary tumors were collected at necropsy.

Table 2. Clinical characterization of animals with malignant tumors.

	No. of cases	**%**
Affected mammary gland [a] (n=132)		
Thoracic 1	24	18,2
Thoracic 2	33	25,0
Abdominal 1	38	28,8
Abdominal 2	37	28,0
Ulceration (n=94)		
Yes	34	36,2
No	60	63,8
Tumor size before surgery [b] (n=94)		
< 2 cm	30	31,6
2-3 cm	21	22,1
> 3 cm	44	46,3
Mean (SD)	3,2 (1,9)	-
Range	0,3 - 9	-
Distant metastasis (n=94)		
Yes	11	11,7
No	82	87,2
Dubious	1	1,1
Clinical stage (n=93)		
I	15	16,1
II	10	10,8
III	57	61,3
IV	11	11,8
No. of surgeries (n=84)		
1	61	72,6
2	10	11,9

3	8	9,5
4	2	2,4
5	3	3,6
Type of surgery (n=84)		
Unilateral	54	64,3
Nodulectomy	14	16,7
Regional	12	14,3
Bilateral	4	4,8
Recurrence (n=53)		
Yes	50	94,3
No	3	5,7
Recurrence site (n=50)		
Local	26	52,0
Lungs	11	22,0
Local and lungs	10	20,0
Local, abdominal lymph nodes and lungs	2	4,0
Intra-abdominal	1	2,0

[a] All affected mammary glands were considered in cases in which large nodules affected more than one gland. Mammary tumors located between two adjacent glands were not included. [b] In queens with multiple nodules, the largest diameter was considered.

Reliable blood count data at diagnosis was only available for 45 diseased queens. Twelve (26,7%) cats presented anemia, five of which had marked anemia, with hematocrit results equal or below 15%. Leukocytosis and leukopenia were observed in 20% (n=9) and 8,9% (n=4) of the cats, respectively.

Most malignant carcinomas had an infiltrative behavior (97 out of 114; 85,1%). Histopathologic characterization of the different malignant tumors analyzed is presented in Table 3. Most queens with malignant mammary tumors presented high mitotic counts (≥ 19) and marked nuclear pleomorphism.

Table 3. Histopathologic characteristics of the different malignant tumors.

	No. of cases	**%**
Histological classification (n=114)		
Tubulopapillary carcinoma	55	48,2
Solid carcinoma	38	33,3
Cribriform carcinoma	15	13,2
Mucinous carcinoma	2	1,8
Carcinosarcoma	2	1,8
Squamous cell carcinoma	1	0,9
Undifferentiated malignant tumor	1	0,9

Necrosis (n=114)		
Yes	80	70,2
No	34	29,8
Lymphovascular invasion (n=114)		
Yes	43	37,7
No	71	62,3
Regional lymph node invasion (n=95)		
Yes	41	43,2
No	54	56,8
Surgical margins [a] (n=100)		
Complete	47	47,0
Narrow	31	31,0
Incomplete	22	22,0
Pleomorphism score [b] (n=77)		
Mild	3	3,9
Moderate	18	23,4
Marked	56	72,7
Mitotic count (n=110)		
0-8	26	23,9
9-18	21	19,3
≥ 19	62	56,9
Mean (SD)	23,5 (15,5)	-
Histologic grading [d] (n=108)		
Grade I	13	12,0
Grade II	39	36,1
Grade III	56	51,9

[b] Queens with dubious pleomorphism scores (i.e. classified between two pleomorphism scores) were not included.

Histological grading according with each histologic type is presented in Table 4. Histological grading was not attributed in carcinosarcomas, mucinous carcinomas and undifferentiated malignant tumors (Elston & Ellis, 1991).

Table 4. Histologic grading of different malignant carcinomas evaluated according to Elston and Ellis (1991).

Histologic type	**No. of cases**	**Grade I** n=13 (%)	**Grade II** n=39 (%)	**Grade III** n=56 (%)
Tubulopapillary carcinoma	54	12 (22,2%)	21 (38,9%)	21 (38,9%)
Solid carcinoma	38	1 (2,6%)	9 (23,7%)	28 (73,7%)

Cribriform carcinoma	15	-	8 (53,3%)	7 (46,6%)
Squamous cell carcinoma	1	-	1 (100%)	-

Adjuvant chemotherapy was administered to 10 (10,5%) queens with malignant mammary tumors, namely doxorubicin (n=2; 18,2%), carboplatin (n=2; 18,2%), doxorubicin and carboplatin (n=2; 15,4%), cyclophosphamide and toceranib (n=1; 7,7%), long-term administration of meloxicam (n=1; 7,7%), and metronomic chemotherapy with cyclophosphamide and meloxicam (n=1; 7,7%), and chlorambucil and meloxicam (n=1; 7,7%).

Overall follow-up data including all cats with malignant tumors are summarized in Table 5. Survival time was possible to determine in 83 queens, while DFS was available in 75. Sixty diseased queens died to tumor-related causes, while 17 died from causes unrelated to the mammary carcinoma. Six queens were still alive by the end of the present study. Overall survival ranged from 0,5 to 54 months (mean; 13,8 months; SD 12,7 months), while DFI ranged from 0,3 to 52 months (mean 9,1 months; SD 10,3 months).

Table 5. Follow-up data including all queens with malignant tumors (follow-up of three years after diagnosis).

Follow-up (months)	**Alive after diagnosis** n=83 (%)	**Free of disease** n=75 (%)
3	72 (86,7%)	56 (74,7%)
6	59 (71,1%)	39 (52,0%)
9	47 (56,6%)	27 (36,0%)
12	35 (42,2%)	17 (22,7%)
18	23 (27,7%)	12 (16,0%)
24	13 (15,7%)	5 (6,7%)
30	9 (10,8%)	3 (4,0%)
36	8 (9,6%)	3 (4,0%)

Out of the 95 queens with malignant mammary tumors, 67 were eligible for follow-up statistical analysis. Twenty-two queens were excluded due to death related with post-surgery complications (n=6), administrations of adjuvant chemotherapy (n=10) and presence of multiple mammary carcinomas of different histological subtypes (n=17).

Twenty-one potential prognostic factors were evaluated using Spearman's rho correlation univariate analysis, including age, reproductive status, age of spay surgery, progestogen administration, number of tumors, location of affected mammary glands, tumor ulceration, tumor size, presence of distant metastasis, clinical stage, type of surgery, surgical margins, location of tumor recurrence, type of tumor growth, tumor necrosis, histologic subtype, lymph node invasion, lymphovascular invasion, mitotic counts, nuclear pleomorphism and histologic grade. Influence of different features of malignant mammary tumors in DFI and OS are shown in Table 6.

Table 6. Influence of different features of malignant mammary tumors in disease-free interval and overall survival.

Factor	Survival time	Disease-free survival
	p value (r [a])	p value (r [a])
Tumor size	**.007** (-.349)	**.011** (-.378)
Lymph node invasion	**.035** (-.258)	.128 (-.223)
Distant metastasis	**< .0001** (-.585)	**-**
Clinical stage	**< .0001** (-.535)	**.006** (-.397)
Type of surgery	**.003** (-.361)	.720 (-.053)
Surgical margins	**.002** (-.401)	**.032** (-.310)
Tumor growth	**.029** (-.267)	**.004** (-.411)
Necrosis	**.036** (-.258)	.117 (-.229)
Lymphovascular invasion	**.001** (-.399)	**.030** (-.315)
Pleomorphism	**.008** (-.385)	**.001** (-.561)
Histologic grade	**.000** (-.436)	**.004** (-.418)

[a] Correlation coefficient. Significant results are presented in bold.

Multiple regression was determined to evaluate the combined contribution of the different parameters analyzed on DFI and OS. Correlation coefficient (R^2) and adjusted R^2 on DFI were 0.929 and 0.607, respectively (Figure 1). Correlation coefficient (R^2) and adjusted R^2 on OS were 0.981 and 0.895, respectively (Figure 2).

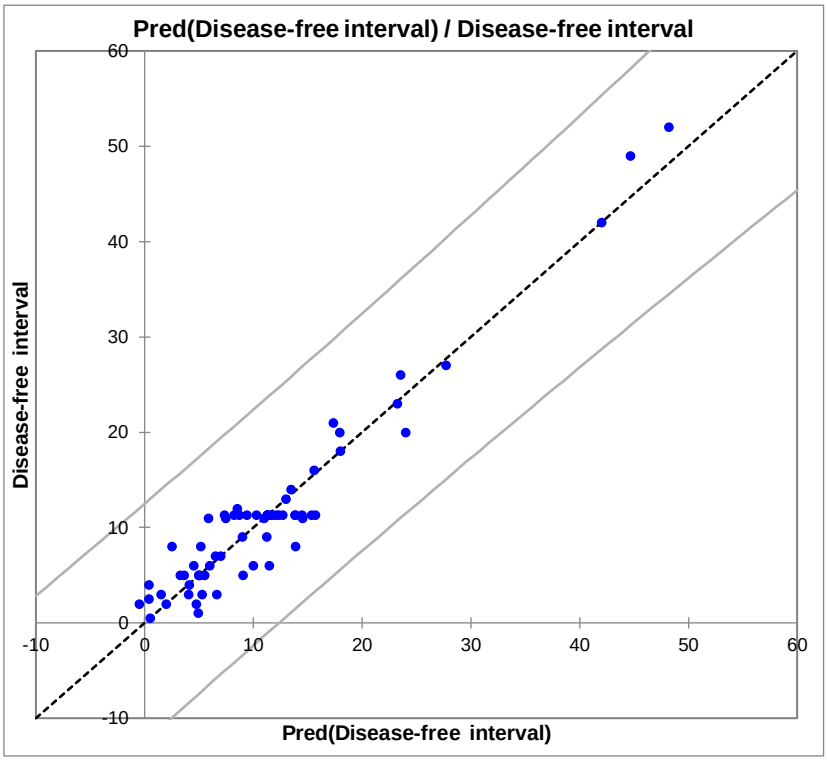

Figure 1. Multiple regression of disease-free survival.

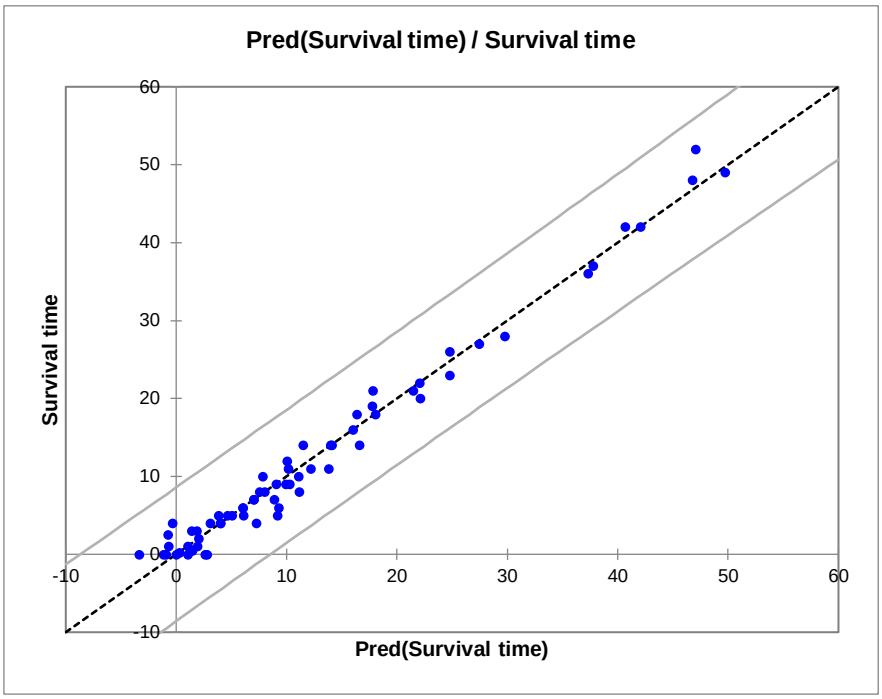

Figure 2. Multiple regression of overall survival.

Discussion

In the present study, 116 queens presented to veterinary medical care centers due to the presence of mammary gland masses were evaluated. Of these, 98 cats had neoplastic mammary lesions, including 95 (97,9%) with malignant mammary tumors. This rate is slightly higher than those found in previous studies, that also reported a predominance of malignant over benign mammary tumors, with malignancy rates ranging from 86 to 95% (Amorim et al., 2006; Hayes & Mooney, 1985; Togni et al., 2013).

Previous works described Siamese cats as having an increased risk of developing malignant mammary neoplasms (Hayes & Mooney, 1985; Ito et al., 1996), as well as developing the disease at an earlier age (Hayes & Mooney, 1985). However, there might be an overrepresentation of the breed in the aforementioned studies; Siamese cats have also been described as predisposed to other types of tumors (Sorenmo et al., 2020). Another study observed that, despite the small sample of Siamese cats, these tended to have a worse prognosis (Borrego, Cartagena, & Engel, 2009). No Siamese cats were included in this study, and Domestic Shorthair cats were overrepresented. Therefore, the previous associations were not established. Similar to results in former reports, feline mammary tumors were more common in middle-aged to older cats in our study (Castagnaro et al., 1998; Dagher et al., 2019; Gemignani et al., 2018; Ito et al., 1996; McNeill et al., 2009; Mills et al., 2015; Novosad et al., 2006; Seixas et al., 2011; Viste, Myers, Singh, & Simko, 2002; Weijer et al., 1972). Moreover, queens with malignant tumors tended to be older than queens with benign mammary tumors and non-neoplastic mammary lesions. Although feline mammary carcinomas have been reported in males, this is a rare finding (Gemignani et al., 2018; Ito et al., 1996; McNeill et al., 2009; Novosad et al., 2006; Viste et al., 2002). None of the veterinary medical centers included in the present study registered the occurrence of malignant mammary tumors in male cats betweenmid-2010 and early-2020.

Early spaying has been described as having a significant protective effect on mammary tumor development in felines when performed under one year of age (Overley et al., 2005). In the present study, the evaluation of this protective effect was not performed due to the lack of a control group, and because most of the females with known reproductive history were either intact or spayed at an older age. Nevertheless, one of the queens with a malignant mammary carcinoma was spayed before one year of age. Contraception with progestogens has been reported as a risk factor for mammary tumor development in queens (Misdorp, 1991; Misdorp et al., 1991). In our study, the risk of development of mammary tumors associated with the administration of progestogens was not evaluated, also due to a lack of a control group. However, in this research, most cats with available information had been exposed to these drugs, suggesting a tumorigenic effect of progestogens in mammary cancer in cats. Although neutering queens alongside mammary tumor excision is a common practice, there are no documented benefits with regards to tumor recurrence and survival (Morris, 2013). Nevertheless, this practice leads to cessation of contraceptives use, and avoids development of pyometra and reproductive tumors. In the present study, five queens submitted to ovariohysterectomy and mastectomy had

concurrent pyometra and six had concomitant uterine tumors, most of them diagnosed as accidental findings on histopathology.

Multiple mammary nodules were present in 38,9% of the diseased queens, suggesting that this is a common presentation, as supported by previous evidence (Hayes & Mooney, 1985; Mills et al., 2015). Abdominal mammary glands were more frequently affected in our cohort, as has been shown in prior reports (Amorim et al., 2006; Gemignani et al., 2018; Weijer & Hart, 1983). However, on the contrary, thoracic glands were found to be more frequently affected in other studies (Viste et al., 2002; Weijer et al., 1972).

At diagnosis, over 60% of the diseased queens were in clinical stage III, while other animals were evenly distributed through stages I, II and IV. Although most of the cats had no evidence of distant metastasis at presentation, many had already evidence of regional lymph node invasion and tumors measuring over 3 cm of diameter, which explains the higher number of cats included in clinical stage III. Even though similar studies have reported higher frequencies of animals in clinical stage III, the difference between stage III and stages I, II and IV was not as marked as in the present study (Borrego et al., 2009; Gemignani et al., 2018; Ito et al., 1996; Novosad et al., 2006). Conversely, in one study, stage I was the most frequently observed clinical stage (Mills et al., 2015).

In the univariate analysis, clinical stage showed a significant influence in both OS and DFI. Similar results have been reported by several authors regarding OS (Dagher et al., 2019; Gemignani et al., 2018; Ito et al., 1996; Mills et al., 2015; Seixas et al., 2011) and DFI (Novosad et al., 2006; Seixas et al., 2011). On the other hand, regional lymph node neoplastic invasion status had a significant impact only in OS; queens with no signs of metastasis survived longer. This evidence is supported by several previous studies (Dagher et al., 2019; Gemignani et al., 2018; Mills et al., 2015; Seixas et al., 2011; Weijer & Hart, 1983). A couple of studies also observed an association between regional lymph node metastasis and DFI, and identified this factor as an independent prognostic predictor for both OS (Seixas et al., 2011) and DFI (Gemignani et al., 2018). Presence of distant metastasis at presentation also significantly influenced OS, which confirms findings of earlier studies (Novosad et al., 2006; Weijer & Hart, 1983). In the present study, queens with distant metastasis at diagnosis survived, on average, less than one month.

Tumor size is one of the main well documented prognostic factors in feline malignant mammary tumors. Although several studies evaluated the tumor volume (MacEwen et al., 1984; Novosad et al., 2006; Weijer et al., 1972), most studies assessed tumor diameter as a measure for tumor size (Borrego et al., 2009; Dagher et al., 2019; Ito et al., 1996; Mills et al., 2015; Seixas et al., 2011; Weijer & Hart, 1983), and the results are consensual among authors. Weijer and Hart (1983) reported a better association between tumor diameter and survival when compared to tumor volume. In the present study, a significant influence of tumor diameter in OS and DFI was found, leading to shorter mean OS and DFI in queens with larger tumors. While cutoff values for tumor size have yet to be statistically established

(Zappulli et al., 2015), most authors agree that tumors with a diameter above 3 cm are associated with a worse prognosis (Ito et al., 1996; Mills et al., 2015; Viste et al., 2002).

Most diseased queens (n=84, 88,4%) were submitted to surgical treatment, considered the treatment of choice in most cases (Cassali et al., 2018; De Campos et al., 2015; Hayes & Mooney, 1985; Morris, 2013). Unilateral mastectomy was the most frequently performed procedure. Considering that 53,8% of the tumors affected the second thoracic gland and/or the first abdominal gland, the main type of surgery performed was in agreement with recommendations based on lymphatic drainage of the feline mammary glands (Raharison & Sautet, 2006). In the present study, a significant influence of the type of surgery on OS, but not on DFI. Similar results were reported in one study's subgroup (Novosad et al., 2006).On the contrary, a couple of studies found a significant association between the type of surgery procedure and DFI, but not OS (Hayes & Mooney, 1985; MacEwen et al., 1984). One study observed a significant association between type of mastectomy and both OS and DFI, and reported it as an independent prognostic factor for DFI. Nevertheless, it should be taken into account that only unilateral and bilateral mastectomies were included in the aforementioned study (Gemignani et al., 2018). Despite the high number of radical mastectomies performed, margins were still incomplete or narrow in 53,0% of the cases. This might be due to the highly infiltrative behavior showed by these tumors, as well as the size of the tumor which precluded a surgical excision with adequate free margins. The present study found a significant impact of the surgical margins in both OS and DFI; the larger the excision margins, the longer mean OS and DFI. Only a few studies mention the analysis of surgical margins, one of which found an association with OS (Weijer & Hart, 1983), and one found an association with DFI but not with OS (Gemignani et al., 2018). Recurrence occurred in most cats, mainly locally and in the lungs, which has also been reported in other works (McNeill et al., 2009; Togni et al., 2013).

Tubulopapillary carcinomas were most frequently diagnosed, followed by solid and cribriform carcinomas. A similar study also reported tubulopapillary, cribriform and solid carcinomas as being the most common patterns (Togni et al., 2013). Nevertheless, according with our results, the subtype of mammary carcinoma does not influence DFI or OS in cats. Despite the presence of necrotic areas in the majority of the tumors, most of these showed no evidence of ulceration. Necrosis was significantly associated to OS, confirming results of one earlier study (Weijer & Hart, 1983); queens with tumor necrosis presented, on average, shorter survival times. One work considered tumor ulceration to be an independent prognostic factor for DFI (Gemignani et al., 2018). The present study did not show any influence of tumor ulceration on OS or DFI in the univariate analysis. Type of tumor growth had a statistically significant influence on both OS and DFI; queens with non-infiltrative tumors survived longer and free of disease than queens with infiltrative tumors. Weijer and Hart (1983) have reported a significant association between an infiltrating growth and worse survival, but it was not considered an independent factor.

Lymphovascular invasion is generally considered a negative prognostic factor on OS and DFI (Dagher et al., 2019; Gemignani et al., 2018; Mills et al., 2015; Seixas et al., 2011; Weijer et al., 1972), which

was also observed in the present study. Queens with neoplastic emboli had shorter mean OS and DFI times. This parameter has been considered an independent prognostic predictor for DFI (Seixas et al., 2011) and OS (Mills et al., 2015).

Histologic grade, determined by Elston and Ellis (1991) criteria, was significantly associated with OS and DFI. Queens with higher histologic grades had a progressively lower survival and shorter DFI. These results are in accordance to Seixas and colleagues' observations, who also reported histological grading as an independent prognostic factor for both OS and DFI (Seixas et al., 2011). Although the Elston and Ellis (1991) grading system has been generally accepted and reported as having significant prognostic value in survival, this often is not found in all tumor grades (Castagnaro et al., 1998; Dagher et al., 2019; De Campos et al., 2015; Millanta, Lazzeri, Mazzei, Vannozzi, & Poli, 2002). Conversely, one work compared the prognostic value of different histologic grading systems, and reported a lack of association between any of the histological grades and OS (Mills et al., 2015). These inconsistencies among authors might reflect the subjectivity of histologic grading, which might result in different classifications among pathologists (Castagnaro et al., 1998; Dagher et al., 2019; Mills et al., 2015). Furthermore, some studies documented an underrepresentation of grade I tumors (Dagher et al., 2019; Mills et al., 2015), which was also observed in the present study; and others reported no predictive value of grade II (Castagnaro et al., 1998). Interestingly, in our study, solid carcinomas presented a higher proportion of grade III tumors than other carcinoma subtypes, which might suggest a more aggressive behavior of solid tumors. On the other hand, tubular formation is lost in solid carcinomas, therefore higher histologic grades are a common finding in these tumors. However, as stated above, no significant differences on DFI or OS were found between queens with different tumor subtypes.

Most tumors had both high mitotic counts and pleomorphism scores, reflecting the aggressive behavior of feline mammary gland neoplasms. Contrary to previously published studies (Mills et al., 2015; Seixas et al., 2011), in the present study a significant influence of mitotic counts on OS and DFI was not observed. Nonetheless, nuclear pleomorphism showed a significant influence on both OS and DFI. Similar results regarding nuclear pleomorphism were demonstrated by Mills and colleagues (2015), with both mitotic counts and nuclear form also presenting as independent prognostic factors. The lack of statistical significance regarding mitotic counts found in our study might have been due to an underrepresentation of tumors with low mitotic counts.

Multiple regression of combined contribution of the different parameters analyzed in OS presented a good correlation coefficient, showing the important influence of these potential prognostic factors in survival times of diseased queens. The lower value obtained for DFI might be attributed, at least in part, to the lack of compliance of owners with follow-up controls, which might have contributed to a delay in diagnosis of recurrences.

Over 50% of the queens were alive and free of disease six months post-surgery. However, these numbers dropped and were below 50% at one year after diagnosis. After 24 months, most queens had

died and/or already presented evidence of tumor progression. These data are in accordance with previous published studies (Morris, 2013; Sorenmo et al., 2020).

One of the biggest handicaps of these retrospective studies is the lack of complete data, especially clinical and follow-up data, which leads to the exclusion and censoring of many cats in the survival analysis. More studies with multivariable analysis are required in order to confirm whether reported prognostic factors are independent. Additionally, prospective studies with standardized clinical staging and histological grading methods, as well as an appropriate follow-up of the cats might help getting more reliable of the results.

Conclusions

The present study allowed a detailed characterization of feline malignant mammary tumors. Tumor size, lymph node invasion, distant metastasis, clinical stage, type of surgery, surgical margins, type of tumor growth, tumor necrosis, lymphovascular neoplastic invasion, nuclear pleomorphism and histologic grade significantly influenced OS in the univariate analysis. Additionally, DFI was significantly influenced by tumor size, clinical stage, surgical margins, type of tumor growth, lymphovascular invasion, nuclear pleomorphism and histologic grade. Multiple regression of combined contribution of the different parameters analyzed in OS presented a good correlation coefficient.

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