A retrospective study and survival analysis on bitches with mammary tumours spayed at the same time of mastectomy

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Spaying bitches with mammary tumors

ABSTRACT

The aim of the present study was to retrospectively assess whether spaying at the same time of mastectomy increased disease-free survival (DFS) in bitches with mammary tumors and to investigate the utility of clinical data when designing a surgical plan that includes gonadectomy. Data from 225 bitches were retrieved. Only 116 were surgically treated. Among these, 52 bitches underwent mastectomy and ovariectomy and 46 bitches underwent mastectomy alone. Survival analysis by Kaplan-Meier and in-between groups comparisons using Student's T, Chi-square, and one-way ANOVA tests were performed. Eighteen bitches were already spayed. DFS was longer for bitches that underwent ovariectomy and mastectomy compared to those that were left intact ($P=0.00064$) or were already spayed ($P=0.0098$). Spaying status affected the tumor size (spayed: 2.75 cm±2.72; intact: 1.76 cm±2.04; $P=0.039$), but not malignancy ($P>0.05$). Differences in age were detected between animals with benign and malignant tumors (9.1±2.8 and 10±2.3; $P=0.004$), with multiple and single tumors (10.18±2.6 and 9.3±2.8; $P=0.007$), and between purebred and mixed breed bitches (10.46 years ±1.78 and 9.27 years ±2.68; $P = 0.005$). Malignant tumors were larger than benign ones (2.17 years ±2.31 and 1.34 years ±1.82; $P = 0.005$) and size increased according to the degree of malignancy. DFS was shorter for animals presenting tumors >2 cm in size ($P<0.006$) and with tumors in the first pair of thoracic mammary glands ($P=0.00009$). Gonadectomy should be suggested to owners of intact bitches carrying mammary tumors and age, size of the tumor, and location should be carefully considered when performing surgery.

Key words: Dog | mammary tumor | mastectomy | ovariectomy | gonadectomy

1. INTRODUCTION

Canine mammary tumors (CMTs) are the most common reproductive neoplastic disease in dogs and, generally, the most reported tumor in intact bitches[1]. Surgery is the standard treatment, with good prognosis in animals with benign-to-low grade non metastatic tumors.

The role of ovarian steroids on carcinogenesis of the mammary gland has been the object of several studies in bitches. Sexual steroids act both under physiological and pathological conditions due to the presence of hormone receptors in mammary tissue[2-4], and they may have an autocrine/paracrine role in the growth of mammary tumors and in the maintenance of the disease[5]. Ductal growth is promoted by estrogens, whereas progesterone causes development and hyperplasia of lobulo-alveolar tissue[6]. Progesterone might be involved in the upregulation of growth hormone (GH) production within the mammary tissue, leading to proliferation of mammary stem cells that could have a primary role in carcinogenesis [1, 7]. Hormonal stimulation of mammary tissue occurs at every estrous cycle, so that the reduction of risk of mammary cancer development has been calculated in relation to age (i.e., number of estrous cycles) at gonadal removal [8-10]. A systematic review of the literature on the effect of spaying on the risk of benign and malignant mammary tumors in the canine species, concluded that scientific evidence is too weak to serve as a basis for firm recommendation of spaying as a preventive measure[11]. Nevertheless, epidemiological studies suggest that in countries where dogs are routinely spayed at an early age, the incidence of mammary neoplasms is lower (e.g., United States) when compared to countries where spaying is not routinely performed (e.g., Norway)[12, 13]. On the other hand, associations between gonadectomy and other pathological conditions, such as urinary incontinence, cranial cruciate ligament rupture, hip dysplasia, osteosarcoma, and hemangiosarcoma have been recognized[14]. Hormonal deprivation following gonadal removal has also an impact on future health and longevity[15]. Therefore, surgical spaying of young healthy bitches should be performed based on a patient-specific approach, considering breed, age, surgical risk, and behavioral characteristics of the animal[16].

Gonadectomy has also been suggested as an adjuvant treatment to mastectomy: bitches with benign mammary tumors and hyperplastic lesions that underwent both mastectomy and gonadal removal at the same time, were seen to have a 50% decrease in recurrence of disease[17], whereas bitches with mammary carcinomas variably responded to neutering at the time of mastectomy[6].

As literature data are not univocal and seem to suggest that gonadal removal in association with mastectomy can be beneficial mainly when hormone receptors are expressed by tumors, it would be very useful to re-evaluate this observation that is crucial for a clinician when suggesting the best treatment option for a patient.

Some history data, such as the reproductive condition, and some clinically assessable factors, such as age, tumor size and tumor number, have been described for risk of CMTs development and for their value in predicting malignancy. CMTs are typically diagnosed in older animals and the median age of occurrence ranges from 8 to 10 years[18-20]. A correlation exists between tumor size and malignancy, with larger masses having higher risk of malignancy[18, 21]. On the contrary, the presence of multiple tumors does not necessarily indicate a high degree of malignancy or a bad prognosis, because each neoplasm can belong to a different subtype[21, 22].

This study is a retrospective investigation aiming to assess whether spaying at the time of mastectomy should be suggested to owners based on parameters collected in the contest of the clinical examination and on the analysis of disease-free survival.

2. MATERIALS AND METHODS

2.1 Data collection

The database of the *** was searched for records of bitches that had been presented because of CMTs and that underwent mastectomy between January 2011 and January 2020. Each dog was counted only once, irrespective of the number of visits, and records were evaluated retrospectively. Only bitches with no previous history of mammary tumor were included. Data from animals that did not undergo surgery were included only in the descriptive analysis. Proper informed consent had been signed by the owners prior to surgery, allowing for surgical treatment and data collection for research purposes.

Age, breed and spaying status of the patients, previous hormonal treatments, previous pregnancies, pseudo-pregnancies, previous reproductive conditions, clinical tumor features (number, location, and size), and evaluation of regional lymph nodes were retrieved from the records.

The database contained also the standard pre-surgical diagnostics, such as blood exams, thoracic radiographies, cardiological assessment and, in some cases, abdominal ultrasounds and cytologic exams. All these preliminary exams had led to the decision of performing surgery.

Surgery type, either mastectomy alone or mastectomy and gonadectomy (ovariectomy or ovariohysterectomy) had been recorded, together with the surgical approach for mastectomy and the histological diagnosis. Histological classification and grading were based on criteria defined by Zappulli (2019) and Peña (2019).

Follow-up data were obtained by the clinical records or by contacting the owners for a check-up clinical examination at >365 days from surgery.

2.2 Analysis of data

Descriptive statistics was carried out considering data extracted from all retrieved clinical records and data are presented as mean and standard deviation (SD) for continuous parameters or as frequency for categories. Normality for continuous parameters was assessed by Shapiro-Wilk test.

Survival analysis was carried out using Kaplan-Meier method with log-rank tests and Bonferroni's post hoc test to estimate differences in disease-free survival (DFS) among spayed bitches, intact bitches that were subjected to mastectomy alone, and bitches that underwent mastectomy and gonadectomy at the same time. Only bitches that underwent surgery and had the surgically excised mammary tumor histologically evaluated were included. The same analysis was carried out to estimate differences in disease-free survival (DFS) according to tumor size, malignancy, and tumor location. Tumor size was considered as continuous; however, data were grouped in five categories for the survival analysis (A < 1 cm, B = 1 to <2 cm, C = 2 to <3 cm, D = 3 to <5 cm, E > 5 cm)[10, 21].

Disease-free survival (DFS) was calculated from the time of surgery to the time of diagnosis of a new mammary tumor. Bitches lost to follow-up and animals that died or that were euthanized for causes unrelated to mammary tumors were censored at the time of death. Animals lost to follow-up were censored at the time of their last contact with the clinician.

Student's T test for continuous normally distributed variables, Chi-square test, and one-way ANOVA followed by Bonferroni's post hoc test for categories, were used to point out differences based on age, breed, spaying status, tumor size, and malignancy of tumors in bitches that underwent surgery.

Significance was considered for $P < 0.05$. Statistical analyses were performed with the software *R version 3.2.2.*

3. RESULTS

Two-hundred and twenty-five bitches with a total number of 489 tumors were retrieved from the database. Characteristics of the animals (age, purebred or mixed breed, and spaying status) and characteristics of the tumors (size, number, location) are reported in *Table 1* and in *Table 2*. The frequency of the different breeds is reported in Supplementary material (S1).
None of the included bitches had ever received any hormonal treatment during its lifetime or had ever presented with any reproductive disease, according to information reported by owners. Nevertheless, eight bitches had previous pregnancies (0.03%, five bitches had one previous pregnancy, whereas three bitches had two previous pregnancies) and three bitches had previous pseudo-pregnancies (0.01%). At clinical examination, 13 bitches (5.8%) presented altered regional lymph nodes. Cytology was performed and they were included in the study only when the node was not metastatic. Nine of these patients were deemed as node-positive after histology (69.2%), whereas two of them presented just lymphadenitis (30.8%). The number of bitches that underwent mastectomy and that were diagnosed with CMTs based on the histological examination was 116, carrying a total number of 298 tumors. Frequencies of benign and malignant tumors are reported in

Table 3. Surgical margins were clear in all the bitches according to histological examination. Histological types are reported in *Table 4*.

Tumor removal was carried out with different approaches, more frequently with a regional mastectomy or with a combination of different techniques (i.e., regional mastectomy and simple mastectomy), when tumors were present on both sides (*Table 5*).

Only 15.6% of the bitches that underwent surgery (n = 18) was already spayed and the gonadectomy happened at least two years before mammary tumors occurrence. Fifty-two out of 98 intact bitches were spayed at the same time of mastectomy. Survival analysis showed a statistically significant difference in DFS depending on spaying status (P = 0.0007). Specifically, bitches that were subjected to spaying at the time of mastectomy showed longer DFS when compared with both bitches that were already spayed (P = 0.0098) and bitches that remained intact (P = 0.00064). However, median DFS for bitches that were subjected to spaying at the time of mastectomy was not available because recurrence was < 50% in both intact bitches and bitches that were spayed at the time of mastectomy (n = 9/64, 14% and n = 2/52, 3%, respectively). Recurrence in bitches that were already spayed was 27.8% (n = 5/18) and their median DFS was 757 days (95% CI, 369-1026).

Statistically significant differences in mean age were detected between animals with benign and malignant tumors, as shown in *Table 6*. Animals with multiple neoplasms were older than the ones with single tumors (10.18 ± SD 2.6 and 9.3 ± SD 2.8, respectively), with statistically significant results (P = 0.004).

No differences between the incidence of benign and malignant tumors between purebred and mixed breed animals were detected (P > 0.05), although purebred bitches had the tendency to develop mammary tumors at a younger age (mean 10.46 years ± SD 1.78) if compared to mixed breed ones (mean 9.27 years ± SD 2.68; P = 0.005).
Being already spayed did not affect the frequency of benign and malignant tumors (P > 0.05), nor the degree of malignancy, I, II, or III (P > 0.05). However, intact bitches had smaller tumors when compared to spayed ones (mean 1.76 cm, ± SD 2.04 and 2.75 cm ± SD 2.72, respectively; P=0.003), although they showed a higher tendency to multiple tumors (P = 0.039).
Tumor size was statistically different between benign and malignant neoplasms (*Table 5*) and differences in size were also detected based on the tumor grade, with grade III tumors being larger than grades I and II (P = 0.05 and P = 0.003, respectively). Grade I malignant tumors had a mean

size of 2.1 cm (± SD 2.3), grade II malignant tumors had a mean size of 1.64 cm (± SD 1.1), and grade III malignant tumors had a mean size of 3.6 cm (± SD 2.2).

Survival analysis showed a statistically significant difference in DFS depending on the size of mammary tumors (P = 0.003), considering the five classes mentioned in subsection 2.2. Specifically, smaller tumors belonging to classes A and B had a longer DFS when compared to larger tumors belonging to class E (P = 0.002 and P = 0.006, respectively; A: median DFS 2102 days, 95% CI 1143-2385; B: median DFS 1148 days, 95% CI 1076-2267; D: median DFS 669 days, 95% CI 434-669; E: median DFS 359, 95% CI 72-811). Class C included a low number of data, that were insufficient to the purpose of Kaplan-Meier analysis.

Survival analysis showed also a statistically significant difference in DFS depending on location of mammary tumors (P = 0.00009). Animals presenting with neoplasms located in the cranial thoracic mammary glands (I pair), had a worse prognosis for mammary tumors recurrence (I: median DFS 434 days, 95% CI 188-434; II: median DFS 1143 days, 95% CI 659-1143; III: median DFS 1502 CI 811-2385; IV: median DFS 1259 days, 95% CI 1096-2385; V: median DFS 1148, 95% CI 759-2385). No differences in DFS were detected between bitches presenting with single and multiple tumors (P > 0.05).

4. DISCUSSION

The effect on time free of disease of OHE at the same time of mastectomy was evaluated in a mixed population of bitches affected by mammary tumors at different stages. The population included in the present study shared some common characteristics to those included in previous studies in terms of age, breed, spaying status, and mean size of benign and malignant tumors[18-21, 25] and additional factors such as location and number of tumors were assessed. The typical presentation for the diagnosis of canine mammary tumor is middle-aged non-spayed purebred bitches, however younger and mixed breed animals can be affected.
Spaying status effect on canine mammary tumors has been widely investigated, with contradictory results[11]. It is commonly known that spaying before the first estrus comes with a lower risk of mammary tumors development[8], and this confirms the involvement of ovarian steroids in mammary tissue carcinogenesis. Accordingly, our data showed that the number of spayed bitches presenting with CMTs was consistently lower than the number of intact ones. However, this might be also the consequence of a smaller general population of spayed animals in Italy, compared to the one of intact bitches. There is no data in the literature about the population of ovariectomized bitches, although spaying is a rather diffuse practice in Italy. Nevertheless, early spaying is becoming less

popular when balancing benefits and possible adverse effects.
Some owners decided upon mastectomy alone, notwithstanding the fact that gonadectomy was always recommended to owners of intact bitches presenting with CMTs, when overall clinical conditions made it advisable. The recommendation was based on the higher risk of uterine and ovarian disease in middle-aged and old bitches[26] and on the higher risk of new malignant CMTs in bitches with a previous history of malignant CMT[27]. The reasons underneath this increased risk of CMTs might be well explained by the hormonal effect to which the whole mammary tissue is exposed to[1]. Furthermore, the positive effect of gonadectomy at the time of mastectomy as an adjuvant therapy has been investigated, with encouraging results especially on hyperplastic/dysplastic and benign mammary diseases[17] and bitches with grade II carcinomas presenting estrogen receptors or with increased peri-surgical serum concentrations of 17β-estradiol[6]. However, to classify a tumor as hormonally dependent, receptors for sexual steroids need to be detected on neoplastic tissue. Some authors relate a decrease in receptors for ovarian steroids with a worse prognosis[28, 29]. Therefore, including the search of receptors for both estrogens and progesterone in post-surgical investigations in intact bitches, could represent a very useful tool to improve prognostic precision and treatment protocols[1].
The observation on hormone receptors in the removed tumors could not be included because it was not available in the database, and this represents an important limitation. However, when the clinician suggests a treatment option, he cannot rely on this information and focuses on general findings only. Results on DFS and rate of recurrence of CMTs in bitches that were spayed at the time of mastectomy were encouraging. Patients that remained intact had higher recurrence of CMTs. The fact that recurrence was even higher in already spayed bitches should be furtherly investigated in order to point out factors influencing mammary tissue carcinogenesis in the absence of hormonal stimulation. In addition, our results agree with those of Burrai *et al.* (2020), showing that spaying status had no significant influence on whether tumors were benign or malignant. The limited number of spayed bitches included does not allow us to consider malignancy responsible for higher recurrence rates in spayed bitches.

The decisional process of the clinician should start with a complete evaluation of the patient, in order to assess its suitability for mastectomy and to decide the appropriate surgical technique and whether to include gonadectomy in its surgical plan. Patients presenting with mammary tumors should be carefully checked for evidence of metastatic disease[30], starting with the evaluation of regional lymph nodes. These organs are difficult to assess when normal, and the easily palpable ones should be checked, possibly indicating regional metastasis[1, 31], to be confirmed through cytological examination. There is evidence that disease-free survival is shorter and survival rate is

lower in node positive patients[32]. Other clinical parameters are related to malignancy and prognosis. Age is a risk factor for neoplastic disease in general[33], and the median age of occurrence of CMTs ranges from 8 to 10 years[18-20], in accordance with our results, that also agree on the fact that median age of bitches with benign tumors is lower than age of animals with malignant ones[21]. Incidence of CMTs in purebred animals was higher than in mixed breed bitches and frequencies are coherent with information reported in studies that indicate a higher risk of CMTs in breeds such as Poodles, English Springer Spaniels, Brittany Spaniels, German Shepherds, Maltese terries, Yorkshire Terriers, Dachshunds, Doberman Pinschers, Leonbergers, and Boxers[1, 34-35]. However, few studies investigate the genetic predisposition of specific breeds towards mammary subtypes[36, 37] and further studies should be conducted.
Majority of patients carried multiple nodules and had malignant neoplasms, although a lower degree of malignancy was more common than higher ones. In general, older animals have the tendency to carry multiple nodules and are expected to be diagnosed with malignant neoplasms. The presence of multiple nodules does not necessarily indicate a high degree of malignancy or a bad prognosis, because each neoplasm can belong to a different subtype[21, 22].
Some studies indicate that tumor location is not associated with tumor type[38] nor with survival time[38], whereas a more recent paper[39] indicates tumor location as predictive of malignancy, with a significantly higher proportion of malignant tumors developing in the inguinal mammary glands. We found that incidence of nodules progressively increased from cranial to caudal mammary glands, probably because caudal abdominal and inguinal mammary glands physiologically have more abundant parenchyma[40]. In contrast with Ariyarathna *et al.* (2018), no difference in malignancy occurred according to tumor location, although a lower DFS was pointed out for bitches presented with nodules located in the first thoracic pair of mammary glands. This should be kept in mind by the surgeon, because more invasive surgery could be considered in these cases, although prospective studies correlating surgical techniques with tumor location represent an area for further research.

In accordance with other studies[18-19, 21], size of the tumor is another important clinical parameter with prognostic value, with malignant tumors being generally larger than benign ones. Our results show that among malignant tumors, larger size corresponds to higher malignancy grade and lower DFS.

We conclude that spaying at the time of mastectomy should always be considered in intact bitches with mammary tumors, possibly followed by the additional assessment of hormone receptors presence on the removed tumors. Intact bitches around 9 years old, have higher probability to develop mammary tumors and older age of bitches and tumors size larger than 2 cm are more commonly related to malignant neoplasms. Location should be carefully considered when designing the surgical plan, because bitches with nodules located in the cranial thoracic mammary glands have a shorter time free of mammary tumors. This will help the clinician to make a more precise prognosis to the patient.

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6. TABLES

Table 1. Frequencies of some parameters of bitches with CMTs (n = 225) and tumors (n = 489).

	Spaying status			Breed		Number of tumors†		Location of tumors				
	Intact	Spayed	N/A	Purebred	Mixed breed	Single tumor	Multiple tumors	I	II	III	IV	V
n	141	31	53	145	80	78	147	22	56	104	143	164
Percentage (%)	62.7	13.8	23.5	64.4	35.5	34.7	65.3	4.4	11.5	21.3	29.2	33.6

†Bitches with single or multiple neoplasms.

Table 2. Mean and standard deviation (SD) of age of the bitches included in the study (n = 225) and size of the tumors.

	Mean	±SD
Age (years)	9.8	2.8
Size of tumors (cm)	2.1	4.8

Table 3. Frequency of benign and malignant tumors with degree of malignancy

	Benign tumors	Malignant tumors		
		I degree	II degree	III degree
n	88	134	43	20.4
Percentage (%)	29.5	63.9	20.4	15.7

Table 4. Histological diagnosis (number: n and percentage: %)

	n	*Percentage (%)*
Simple benign tumors		
Adenoma, simple	32	10.7
Ductal-associated benign tumors		
Intraductal papillary adenoma	26	8.9
Nonsimple benign tumors		
Complex adenoma	10	3.4

Benign mixed tumor	12	4
Fibroadenoma	8	2.7
Simple carcinoma		
Carcinoma, simple	27	9.1
Tubopapillary carcinoma	51	17.1
Solid carcinoma	2	0.6
Nonsimple carcinoma		
Carcinoma in a benign mixed tumor	21	7
Complex carcinoma	99	33.2
Others		
Adenosquamous carcinoma	4	1.3
Carcinosarcoma	3	1
Myoepithelioma	2	0.6
Osteosarcoma	1	0.4

Table 5. Frequencies of surgical techniques for mastectomy in 116 bitches.

	n	*Percentage (%)*
Lumpectomy	14	12
Simple mastectomy	15	13
Regional mastectomy	39	33.5
Unilateral mastectomy	18	15.5
Combination of techniques	30	26%

Table 6. Differences (mean and standard deviation: SD) in age and tumor size in bitches with benign or malignant tumors.

	Age (years)			Size (cm)		
	Mean	SD	P-value	Mean	SD	P-value
Benign tumors	9.1	2.8	0.007*	1.34	1.82	0.004*
Malignant tumors	10	2.3		2.17	2.31	

*Significance for P < 0.05