Clinical and pathological prognostic factors
in canine mammary tumors

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Summary. - In the present study, clinical and reproductive variables and pathological features were evaluated as prognostic factors in canine mammary tumor disease. Seventy-four female dogs free of distant metastases at diagnosis were followed-up during a period of 18 months after the surgical excision of mammary nodules. Age at presentation, tumor size, lymph node involvement, histological malignant grade and nuclear grade were proven to act as variables able to predict the overall survival time in dogs with mammary neoplasms. Disease free survival time was associated to the age, the presence of multiple malignant tumors, tumor size and ulceration of the skin.
Key words: canine mammary tumors, prognosis.

Riassunto *(Fattori prognostici clinici e patologici nei tumori mammari del cane)*. - In questo studio sono state valutate le variabili cliniche e riproduttive e le caratteristiche patologiche quali fattori prognostici della malattia tumorale mammaria nel cane. Il follow-up è stato realizzato su settantaquattro cagne senza metastasi a distanza al momento della diagnosi per un periodo di 18 mesi dopo la rescissione chirurgica dei noduli mammari. L'età alla presentazione, la dimensione del tumore, il coinvolgimento linfonodale, il grado istologico di malignità e il grado nucleare rappresentano variabili in grado di predire il tempo di sopravvivenza in cani con neoplasie mammarie. Il tempo di sopravvivenza libero da malattia è invece associato all'età, alla presenza di tumori maligni multipli, al volume tumorale e all'ulcerazione cutanea.
Parole chiave: tumori mammari, cane, prognosi.

Introduction

Mammary tumors represent the most common neoplasm of the bitch [1, 2]. Malignant tumors range from 41 to 53% of total mammary neoplasms [3]. The clinical and histopathological features are extremely variable ranging from nodules of benign appearance to large masses with clinical characteristics of malignancy. For clinicians, it is interesting to predict the evolution of mammary tumor disease. Nowadays, new prognostic factors in canine mammary tumors (CMT) are being studied and nevertheless, an important part of the prognostic information has to be based on clinical and histopathological data.

The present study is focused on clinical features, including reproductive history, and pathological characterictics as basic prognostic factors in CMT.

Materials and methods

Animals and clinical evaluation

Seventy-four female dogs presented in the Small Animal Clinics of the Veterinary School of Madrid with spontaneous primary mammary tumors were included in the study.

Clinical examination was performed at the first presentation and included the collection of data such as age, breed, weight and height. Data on the reproductive history were also obtained, including intact or neutered state, date of the eventual ovariectomy, number of full term pregnancies, and age at the first pregnancy and the presence of pseudopregnancies in the past. The age at first estrous, duration and regularity of estrous, number per year and hormonal treatments to prevent estrous used in the past were recorded. With regard to the nodules, the date of first presentation and the rate of growth were also obtained.

After the complete physical examination, mammary glands and the regional lymph nodes (axilar, inguinal) were evaluated. Several features were obtained from each tumor. Size in three dimensions (largest was used for the statistical comparisons) was recorded. According to the WHO's system, the tumors were classified as T1 < 3 cm, T2 3-5 cm, or T3 > 5 cm. Adherence to underlying tissues and skin, and the occurrence of ulceration were also registered.

In all dogs included in the study, a radiological evaluation of the thorax (2 lateral projections) was performed in order to evaluate the presence of distant metastases.

The animals were clinically staged using the TNM system [4]. The lymph nodes enlarged were histopathologically or cytologically examined to define their involvement. Using this system, four clinical stages were established: local (without lymph node involvement), advanced local (severe and advanced local invasion), regional (lymph node affectation), distant (presence of distant metastases). None of the animals included in the study presented distant metastases at first presentation.

Histopathology

After the surgical removal of mammary nodules, a total of 115 tumors were obtained. Samples were fixed in buffered formalin, paraffin-embedded, cut in 4 mm sections and haematoxylin-eosin stained. Histopathological examination was performed according to the World Health Organization's classification system for tumors in domestic animals [5]. Using the Scarff-Bloom-Richardson grading system [6, 7] for human breast cancer and malignancy criteria for canine mammary tumors [8], three degrees of histological malignant grade (HMG) were assigned to malignant tumors. This grading was based on the degree of differentiation, nuclear pleomorfism, mitotic index and invasiveness of lymphatics and/or blood vessels. Likewise, three degrees of nuclear grade of malignancy (NG) were recognized and categorized as I (well differenciated), II (moderately differenciated) and III (poorly differenciated).

Follow-up study

After the surgical excision of the tumors, dogs were followed-up for a period of 18 months. Clinical examinations were done every 3-4 months including radiological study of the thorax. The presentation of tumor recurrences and distant metastases were recorded along with the time and cause of the death. Disease free survival (time from surgical excision to the occurrence of metastases or recurrences, DFS) and overall survival (time from surgery to death, OS) times were obtained for each dog and both categorized as low (0-6 months), medium (7-17 months) and high (18 months).

Statistical analyses

Several variables of the animals and tumors were examined with Biomedical Processing Software [9], using the BMDP7D program (F test, Tukey and Duncan tests) for continuous variables. For categprical variables, the BMDP4F program (χ^2 test) was used. In cases with more than one malignant neoplasm, that with the worst clinical and pathological prognosis was chosen for the statistical analysis. A p value < 0.05 was considered significant.

Results

Clinical results

Seventy-four female dogs were included in the study. The age ranged from 5 to 13 years (mean = 9.77; SD = 2.25). Most of them were mixed breeds (no. 27). German shepherd (no. 14) was the most common among the pure breeds. Body weight ranged between 3.60 to 59.40 kg (mean = 18.42 kg; SD 11.85) and height between 18 to 63 cm (mean = 41.41; SD = 13.48).

Sixty-six dogs were intact and 8 ovariohysterectomized. Regarding the estrous cycle, in most of the dogs it was regular (no. 46). The mean number of estrous per year was 1.73 (range = 0 to 2; SD = 0.64) and the mean estrous duration ranged from 0 to 30 days (mean = 15.94; SD = 6.33). The age at first estrous ranged from 6 to 24 months (mean = 8.41, SD = 3.48) (no. 28). Fifty-seven dogs had never received hormonal treatments to prevent estrous and 17 had been treated at least once.

The occurrence of pseudopregnancy was not frequent among of the dogs included in the study (no. 25). With regard to full term pregnancies, 33 dogs had at least one before the presentation of mammary tumors and the mean age at first pregnancy was 2.96 years (range = 0 to 8; SD = 1.99).

At first examination, 35 animals presented only dysplasias and/or benign tumors and 39 had at least one malignant tumor. The animals with malignant tumors were clinically staged. Nine presented a regional stage (with histologically confirmed lymph node involvement) and 30 a local stage.

Tumor data

Among the 115 mammary nodules included in the study, 15 were dysplasias, 60 benign tumors and 40 malignant. In the group of dysplasias the most frequent was the lobular hyperplasias (no. 12). Two epitheliosis were found and 1 intraductal papillomatosis.

The benign tumors group consisted of complex adenomas (no. 32), benign mixed tumors (no. 23), fibroadenomas (no. 2) and condromas (no. 1). One angioma and 1 hemangiopericytoma were included in this category.

Simple adenocarcinoma was the most frequent malignant tumor (no. 11). Seven complex carcinomas, 6 malignant mixed tumors, 5 squamous cell carcinomas, 4 solid carcinomas, 3 in situ carcinomas, 2 spindel cell carcinomas, 1 osteosarcoma and 1 sarcoma were other malignant tumors included. For the statistical comparisons, malignant tumors were separated in two groups. Malignant group 1 consisted of simple and complex adenocarcinomas and in situ carcinomas (no. 21). Malignant group 2 included solid carcinomas, squamous cell carcinomas, spindel cell carcinomas, mixed malignant tumors and sarcomas (no. 19).

Clinical and histopathological variables of dysplasias and benign tumors and of malignant neoplasms are detailed in Table 1.

Table 1. - Clinical and histological variables in 115 dysplasias and mammary tumors

	Tumoral group				
	Benign (no. 75)			Malignant (no. 40)	
Clinical variables					
Tumor size	T1	51		T1	14
	T2	15		T2	11
	T3	9		T3	15
Lymph node affect				N0	31
				N1	9
Rate of growth [a]	Slow	36		Slow	14
	Medium	25		Medium	12
	Fast	13		Fast	14
Ulceration	Yes	2		Yes	13
	No	73		No	27
Fixation to tissues	Yes	29		Yes	25
	No	46		No	15
Histopathological variables					
Tumor type	Dysplasias	15		Malignant 1	21
	Benign tumors	60		Malignant 2	19
HMG [b]				I	7
				II	14
				III	14
NG [b]				I	2
				II	16
				III	17

(a) One unknown value; *(b)* Five unknown values. HMG: histological malignant grade; NG: nuclear grade of malignancy.

Follow-up study

During the follow-up period, 19 neoplasms recurred after the surgical excision. Twelve malignant tumors metastatized and six of them also recurred. Mixed malignant tumor was the tumor type which most frequent recurred, but not at a statistical level of significance. With regard to metastases, solid carcinoma and spindel cell carcinoma were the most frequent tumor which metastatized.

Disease free survival was less than seven months in 17 cases; in 14 dogs ranged from 7 to 17 months. Regarding the overall survival, 49 dogs were alive at the end of the study, 18 died due to the tumoral disease and 4 because of other reasons.

Univariate statistical analyses were performed to know which tumor-related and/or animal-related variables were significantly associated to prognostic variables: recurrences, metastasis, DFS and OS.

With regard to the recurrence, there were no tumor or dog-related variable significantly associated to this variable.

Metastasis displayed a significant correlation with involvement of regional lymph nodes ($p = 0.05$). The rate of tumor growth and the fixation to underlying tissues were not significantly associated to metastasis, however a significative association was found between ulceration and this variable ($p = 0.02$).

There were not significant relations between the histological type of tumor (malignant 1 or 2 group), the HMG, nor the NG and the occurrence of metastasis.

Disease free survival. - The DFS was significantly lower in old dogs than in youngs ($p = 0.02$). Regarding reproductive variables, an early age at first estrous was related with higher DFS ($p = 0.03$). The presence of multiple malignant tumors ($p = 0.01$), and a low OS were also related with low DFS ($p = 0.01$).

Large tumor size and ulceration of the skin were significantly related with a low DFS ($p = 0.04, 0.02$). Rate of growth and fixation to underlying tissues were not significantly associated with this survival time.

Disease free survival was not significantly different between the 2 groups of malignant tumors, nor between tumors of different HMG and NG.

Overall survival. - Advanced age of the animal (p = 0.04), short estrous duration (p = 0.02), short DFS (p = 0.01) were related with a low OS; likewise, tumor size (p = 0.04), regional lymph node involvement (p = 0.05). Histological malignant grade (p = 0.04) and NG (p = 0.02) were significantly associated with a low OS.

Results of univariate analysis of DFS and OS and tumor variables are summarized in Table 2.

Discussion

In this study several clinical and pathological variables are evaluated as basic prognostic factors in CMT.

None of the variables studied were significantly related with the presence of recurrences. Local recurrences sometimes are due to the type of surgery, specially in those cases not very agressively treated.

The occurrence of distant metastases during the follow-up period was significantly related to the cause of death by the tumor, the lymph node involvement and the ulceration of the skin. The role of lymph node affectation on prognosis of CMT is confuse; Misdorp and Hart did not found a relation between the lymph node involvement and survival in dogs with mammary tumors [19].

Ulceration of the skin has been found one of the clinical variable most frequently related to a poor prognosis, and thus associated to the presence of distant metastases. In our opinion, this clinical variable has not been enough evaluated in other studies on CMT.

The histological malignant types included in the malignant group 2 (solid carcinomas, squamous cell carcinomas and spindle cell carcinomas, mixed malignant tumors and sarcomas) metastatized more frequently than tumors included in malignant type 1; however, the

Table 2. - Univariate analysis of clinical and pathological variables and disease free survival and overall survival

	DFS	OS
Clinical variables		
Tumor size	p = 0.04	p = 0.04
Lymph node involvement	NS	p = 0.05
Rate of growth	NS	NS
Ulceration	p = 0.02	NS
Fixation to tissues	NS	NS
Histopathological variables		
Tumor type	NS	NS
HMG	NS	p = 0.04
NG	NS	p = 0.02

HMG: histological malignant grade; NG: nuclear grade of malignancy, DFS: disease free survival; OS: overall survival; NS: non significant.

difference was not statistically significant. Several authors describe the presence of metastases as more frequent in sarcomas than in carcinomas [10, 8, 11].

Age at presentation and age at first estrous were clinical variables significantly associated to disease free survival. The mean age of CMT incidence is 9 and 10 years [3, 8]. Age has been associated to the presence of anaplastic carcinomas [12]; however, other authors describe that age does not affect tumor behavior once it has clinically developed [8]. In another study of our group, it has been found that with advanced age, rate of tumor growth increases [13]. An advanced age at tumor diagnosis has been related with low DFS and OS [12, 14]. The age at first estrous was known in a low number of dogs included in the study, thus more studies are necessary to confirm this result.

Breed and reproductive history were not significantly related to DFS nor OS. Only one author describe the German shepherd as the breed with poorer prognosis compared with other breeds [15]. Regarding reproductive history, ovariectomy does not affect the prognosis of dogs with malignant mammary tumors [16]. Furthermore, the role of ovariectomy as adjuvant therapy in CMT has not been cleared yet [17]. None of several reproductive variables such as hormonal treatments were related to prognosis according to others in CMT [14].

Tumor size and ulceration of the skin were significantly related to DFS. Tumor size has been associated with the presence of metastases not taking into account the moment of its occurrence [10, 8]. Large sized and ulcerated tumors are difficult to be agressively resected, thus the development of recurrences seems to be more expected than in smaller nodules.

All dogs with multiple malignant tumors presented a low DFS. However, other authors did not find such association [8] or even reported the opposite one [14].

The associations between the HMG, NG or the different histological type of tumor with the DFS did not reach the statistical significance. Mammary neoplasms with NG II has been correlated with low DFS [8].

An advanced age has been related with low OS, and it has been described as a very good predictive factor of OS [14]. Several aspects of reproductive history has not been related with OS in previous studies [14]. Likewise, rate of growth, ulceration and adherence to underlying tissues, have not been associated to OS in other studies. Only Hellmén *et al.* [14] found a rapid rate of growth and ulceration related to a low OS. According to others, lymph node affectation is related to a low OS [18, 14]. Tumor size [12, 19], HMG [19, 20] and NG were also significantly associated to a lower OS.

Significant differences in the OS of dogs with different histological tumor types has been described by others [17, 11], especially between sarcomas and the rest of malignant types [10, 8, 14].

In conclusion, the age at presentation, tumor size, lymph node involvement and HMG and NG are clinical and histopathological variables that could predict overall survival of dogs with CMT. Disease free survival is related to the age, the presence of multiple malignant tumors, tumor size and ulceration of the skin. In our opinion, these variables are necessary to establish the basic prognosis on canine mammary tumor disease by clinicians.

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