The Clinical and Pathological Characteristics of Mammary Neoplasms with Malignant Mesenchymal Components in Female Dogs

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Abstract

Mammary neoplasms with malignant mesenchymal components are not common in female dogs, and they are poorly understood. As such, this study aimed to describe the clinical presentation, histological findings, and the COX-2 immunohistochemical expression of mammary neoplasms in female dogs with malignant mesenchymal components, as well as verify the relationships between the different neoplasm types and these aspects. We selected 41 female mammary neoplasms (23 carcinosarcomas, 16 sarcomas, and 2 sarcomas in mixed tumors). Medical records were reviewed to obtain clinical data. Subsequently, histological slides were analysed to establish histological parameters, and immunohistochemistry was used to assess the expression of COX-2 receptors. Carcinosarcomas and sarcomas developed as large tumours, mainly in the abdominal and inguinal mammary glands, with frequent intratumoral necrosis and a low frequency of nodal metastasis. Fifty-eight percent of the cases of malignant mesenchymal proliferation were identified as osteosarcomatous, and 24.5% chondrosarcomatous and fibrosarcomatous each. The osteosarcomatous pattern was the most predominant type in sarcomas and carcinosarcomas, and was the only one that resulted in vascular invasion, regional lymph node metastases, and higher histologic grades. High COX-2 expression was detected in 10% of the carcinosarcomas and 25% of the sarcomas. In conclusion, sarcomas and carcinosarcomas showed similar results regarding the clinical and pathological aspects. Discovering carcinosarcomas and sarcomas with high COX-2 expression suggests that, in some cases, these neoplasms may respond to therapy with COX-2 inhibitors.

Introduction

Neoplasms with malignant mesenchymal components in the mammary glands of female dogs are called sarcomas, carcinosarcomas, and sarcomas in mixed tumors. They are characterized by neoplastic cells with poorly differentiated connective tissue (Cassali et al. 2011; Goldchmidt et al. 2017). Sarcomas comprise approximately 1% of mammary neoplasms in female dogs, while carcinosarcomas may account for 4% (Nunes et al. 2018) and their presence generally points toward a poor prognosis and a high occurrence of metastases (Hellmen et al. 1993). Malignant mesenchymal proliferation can have different histologic presentations, namely osteosarcomatous, fibrosarcomatous, chondrosarcomatous, liposarcomatous, or hemangiosarcomatous forms (Dolka et al. 2013; Nunes et al. 2018).

Clinical and pathological characteristics, such as size, lymph node status, distant metastasis, vascular invasion, and the expression of immunohistochemical markers like cyclooxygenase-2 (COX-2), have been used to assess and issue the prognoses of female dogs with malignant mammary neoplasms (Queiroga et al. 2007; Nunes et al. 2018).

COX-2 is an inducible enzyme that is involved in inflammatory processes, neoplastic transformation, and tumour progression (Heller et al. 2005). COX-2 overexpression has been recognized as a negative prognostic marker in canine mammary malignant tumours (Millanta et al. 2006; Lavallo et al. 2009; Queiroga et al. 2010; Carvalho et al. 2017; Pastor et al. 2020). There have been suggestions that COX-2

expression can be used to guide therapeutic planning involving selective COX-2 inhibitors for female dogs with mammary carcinomas (Millanta et al. 2006; Souza et al. 2009; Lavallo et al. 2012).

And enhance cell motility and adhesion (6). Overexpression of COX-2 causes tumorigenesis in animal models, and its inhibition has significant effect on reducing the incidence and progression of tumors in animal models and in the treatment of some cancer patients (7, 8). Evaluation of COX-2 expression and its roles in treatment and prevention has been performed mostly on tumors of epithelial origin; little is known about the roles of COX-2 in tumors of mesenchymal origin, especially GIST and enhance cell motility and adhesion (6). Overexpression of COX-2 causes tumorigenesis in animal models, and its inhibition has significant effect on reducing the incidence and progression of tumors in animal models and in the treatment of some cancer patients (7, 8). Evaluation of COX-2 expression and its roles in treatment and prevention has been performed mostly on tumors of epithelial origin; little is known about the roles of COX-2 in tumors of mesenchymal origin, especially GIST. However, due to the considerable histologic variety of malignant mesenchymal proliferation, and the low frequency of occurrence of malignant mesenchymal mammary neoplasms, very little is known about COX-2 expression and the clinical and pathological profiles of these tumours.

As such, this study aimed to describe the clinical presentation, histological findings, and the COX-2 immunohistochemical expression of malignant mesenchymal mammary neoplasms in female dogs, as well as verify the relationships between different types of neoplasms and these aspects.

Materials And Methods

Case selection

Formalin fixed paraffin embedded canine neoplasms with malignant mesenchymal components sampled between 2014 and 2018 were selected from pathological archives of the Setor de Patologia Veterinária da Universidade Federal do Rio Grande do Sul, SPV-UFRGS. The criteria of inclusion were diagnoses of carcinosarcoma, sarcoma, or sarcoma in a mixed tumour. We excluded cases with diagnoses of other types of malignant mammary neoplasms; cases for which the slides could not be accessed; and cases with histologic sections that were not representative of the lesions (Araújo et al. 2017).

Exam request records were examined to collect the following information: age, breed, affected mammary glands, lesion size, and regional lymph node, and distant metastases.

Histological assessment

The selected cases were histologically reviewed on Hematoxylin and Eosin stained sections. Standard criteria were followed for the classification of neoplasms such as sarcomas and (Misdorp et al. 1971; Goldschmidt et al. 2011) and carcinosarcomas (Misdorp et al. 1999; Cassali et al. 2020). The mammary origin of the neoplasms was determined based on the presence of mammary acini in the histological section.

The histological parameters evaluated were: type of sarcomatous proliferation, intratumoral necrosis (present or absent), vascular invasion (lymphatic and hematogenic), lymph node invasion (present or absent), and grade. Lymphatic invasion was defined as the presence of neoplastic cells in spaces with an endothelium lining. Vascular invasion was defined as the presence of neoplastic cells in spaces with an endothelium lining, muscle and elastic fibres, and intraluminal erythrocytes.

We followed the established criteria for grading soft tissue sarcomas in humans as a grading scheme for our study (Trojani et al. 1984). This has been previously done to grade mammary sarcomas in female dogs (Dolka et al. 2013). The most representative area of the sarcoma was used to examine the predominant tumour type. This assessment took into consideration: the degree of differentiation in the malignant mesenchymal component, the mitotic rate (that was calculated by counting of mitotic figures in ten high-power fields (400x, 0.239 mm² area), and intramural necrosis. The mitotic rate was established by counting at the periphery of the neoplastic proliferation. Areas affected by necrosis, haemorrhage, inflammation or artifacts were excluded.

We used the Nottingham system, modified by Elston and Ellis (1998), to grade the carcinoma components of carcinosarcomas.

Histological sections of the lymph nodes were reviewed to assess for neoplastic cells and the type of metastatic proliferation (epithelial, mesenchymal, or both). Lymph nodes were considered positive for metastasis when isolated cells or groups of non-lymphoid neoplastic cells were detected in the subcapsular sinus or lymph node parenchyma.

Immunohistochemistry (IHC)

Histological sections 4 µm thick were sliced from each paraffin block and put on gelatinized slides that were dewaxed in xylol and rehydrated in a series of progressively diluted alcohols. For antigenic recovery, the sections were incubated in a water bath containing citrate buffer (Recovery solution, Dako Cytomation, Carpinteria, CA, USA) pH 6.0 for 20 minutes. Endogenous peroxidase was blocked by incubating the slides in 0.3% hydrogen peroxide with absolute methanol for 15 minutes at room temperature. The sections were then incubated with the following antibodies: NovocastraTM Liquid Mouse Monoclonal Anti-Multi-Cytokeratin, Product code NCL-L-AE1/AE3, Leica Biosystems; Newcastle, United Kingdom (1: 150); NovocastraTM Liquid Mouse Monoclonal Antibody Vimentin, Product code NCL-L - VIM-V9; Leica Biosystems, Newcastle, United Kingdom (1: 800) and rabbit anti- COX2 Monoclonal Antibody, Clone SP21, Product code MA5-14568, Thermo Scientific, Invitrogen, Waltham, Massachusetts, USA (1:80) for one hour in a humid chamber. Subsequently, a NovolinkTM Polymer Detection Systems (Leica Biosystems, Newcastle, United Kingdom) was used for incubation, according to the manufacturer's instructions. Immunoreactivity was visualized by using chromogen 3,3'-diaminobenzidine 4 HCL (DAB) (DAB Substrate System, Dako, Carpinteria, CA, USA) and counterstaining with Harris hematoxylin.

Sections of canine breast tumours that had previously tested positive for vimentin and cytokeratin AE1/AE3 were used as positive controls. For COX-2, sections of canine kidney that had previously tested

positive were used as a positive control. For negative control, PBS was used to omit the primary antibody in the three markers.

Assessing immunomarkers in tumoral cells

The intracytoplasmic staining patterns of antibodies were used to examine vimentin, cytokeratin AE1/AE3, and COX-2 expression.

The expression of COX-2 in the tumour cells was evaluated in a semi-quantitative way, taking into account the distribution parameters and intensity of positive markings in the five high-power fields [19]. To assess distribution, markings were evaluated at: 0 (no immunostaining); 1 (less than 10% immunostaining); 2 (between 10% to 30% immunostaining); 3 (between 31% to 60% immunostaining); 4 (more than 61% immunostaining). The intensity of the immunostaining was assessed as follows: 0 (absent); 1 (+); 2 (++); 3 (+++). Additionally, for each field, the scores were multiplied, and the result was the average of all fields. The final scores were divided into two extracts: from 0 to 5 and from 6 to 12 with scores of 0 to 5 considered low and scores from 6 to 12 considered high (Lavalle et al. 2009).

Statistical analysis

The data were recorded in an electronic Excel ®2010 spreadsheet. Subsequently, we analysed the absolute frequencies and percentages to relate variables. We evaluated the possible association between histological types (sarcomas and carcinosarcomas) and clinical and pathological variables (size, intratumoral necrosis, intratumoral vascular invasion, the histologic grade of the mesenchymal component, and lymph node mesenchymal metastasis). We also examined the possible association between mesenchymal subtypes (osteosarcomatous versus non-osteosarcomatous) and the morphological variables (necrosis, intratumoral vascular invasion, mesenchymal lymph node metastasis, and the histologic grade of the mesenchymal component). All associations were analysed using Fisher's exact test ($p < 0.05$) and STATA 14.0 software (Stata Corp LP).

Results

There were 1621 diagnosed cases of mammary neoplasms in female dogs diagnosed in the study period and 12.2% (198) presented a malignant mesenchymal component. Carcinosarcomas accounted for 7.8% (126/1621) of the total number of neoplasms, while sarcomas made up 3.2% (52/1621) and sarcomas in mixed tumours 1.2% (20/1621). We selected 41 of these cases for analysis, of which 16 were sarcomas (Fig. 1a), 23 were carcinosarcomas (Fig. 1b), and 2 were sarcomas in mixed tumors.

In total, 29.2% of the female dogs (12/41) were mixed breed dogs (MBD) and 70.7% were purebred (29/41). The mean age was 11.3 years (ranging from 5 to 17 years). Non-spayed female dogs made up 60.9% (25/41) of the cases. Tumour sizes varied between 2.5 cm and 20 cm, with an average of 10.5 cm as the largest dimension. We had access to the radiologic exam results for distant metastasis for 26 cases; of these, 7.6% (2/26) presented images that suggested pulmonary metastasis.

The most frequent type of mesenchymal proliferation was osteosarcomatous; it made up 58.5% (24/41) of the sample. Pure sarcomas presented malignant mesenchymal differentiation of the following types: osteosarcomatous 62.5% (10/16), fibrosarcomatous 31.2% (5/16), and liposarcomatous 6.2% (1/16). Among the carcinosarcomas, 56.5% (13/23) of the cases showed a malignant mesenchymal component of the osteosarcomatous type, 21.7% (5/23) of the liposarcomatous type, 17.4% (4/23) of the chondrosarcomatous type, and 4.3% (1/23) of the fibrosarcomatous type. Half (1/2) of the SMT exhibited malignant osteosarcomatous differentiation and the other half (1/2) fibrosarcomatous differentiation. Concerning the carcinomatous component, we observed a simple papillary pattern in 60.8% (14/23) of the cases, 26.1% (6/23) tubular patterns, 8.7% (2/23) solid patterns, and 4.3% (1/23) comedocarcinoma patterns.

The osteosarcomatous component was characterized by the proliferation of highly pleomorphic and poorly differentiated osteoblasts of an ovoid and fusiform shape, intermixed with varying quantities of cartilaginous, collagenous, or osteoid matrix (sometimes mineralized), frequent mitotic figures, and occasional giant multinucleated cells. Chondrosarcomas were characterized by a proliferation of pleomorphic and poorly differentiated ovoid cells, intermixed with islands of a disorganized and moderately to intense cellularized hyaline matrix. The fibrosarcomatous component was characterized by the proliferation of fusiform cells, with moderate pleomorphism, arranged in multidirectional bundles, with elongated or ovoid nuclei and infrequent mitoses. In turn, the liposarcoma component presented polygonal, elongated, or fusiform cells, interspersed with lipid vacuoles of varying sizes and peripheral nuclei and few mitotic figures.

In our study, the osteosarcomatous subtypes presented patterns: osteoblastic (12/24) (Fig. 1c), 4.2% (1/24) chondroblastic (Figure 1D), 20.8% (5/24) fibroblastic, 8.3% (2/24) telangiectatic (Fig.1f), and 16.6% (4/24) were rich with giant cells (Fig. 1e). The osteoblastic subtype showed an abundant atypical proliferation of ovoid to spindle-shaped osteoblasts interspersed with a variable amount of osteoid matrix. In the chondroblastic subtype, we observed chondroid matrix formation associated with neoplastic osteoblasts. In the subtype rich with giant cells, there was an intense proliferation of multinucleated giant cells. In the telangiectatic subtype, spaces of varying sizes formed and were filled with a large amount of blood. In the fibroblastic subtype, we noted the proliferation of spindle-shaped osteoblasts, similar to fibroblasts, interspersed with an osteoid matrix.

There was no statistical association between the osteosarcomatous and non-osteosarcomatous mesenchymal subtypes (chondrosarcomatous, fibrosarcomatous, and liposarcomatous) or the histologic grade of the mesenchymal component ($p = 0.274$). The association between the other variables (intratumoral necrosis, mesenchymal vascular invasion, and lymph node metastasis of the mesenchymal component) and the mesenchymal subtype was not tested due to the insufficient number of samples for statistical analysis. The distribution of variables according to the type of mesenchymal proliferation is shown in Table 1.

Adjacent lymph node samples were available for 58.5% (24/41) of the cases. Whenever there was a metastasis of the mesenchymal component, the proliferation was of the osteosarcomatous type. The frequency of lymph node metastases for sarcomas and sarcomas in mixed tumours was calculated at 30% (3/10), while 21,5% (3/13) of carcinosarcomas presented mesenchymal metastasis. There was lymph node metastasis of carcinomatous components in 7.1% (1/14) of the carcinosarcomas. This was of the anaplastic subtype, but the primary tumour exhibited a simple papillary pattern. None of the lymph nodes we assessed presented metastasis with both components. There was intratumoral vascular invasion by the mesenchymal component in 19.5% (8/41) of the cases; of these four cases of invasion occurred in blood capillaries and two cases in lymphatic vessels. In two cases, lymphatic and blood invasion occurred (4.7%).

Low-grade mesenchymal components were well-differentiated, with less than 50% of the parenchyma affected by necrosis, and with up to nine atypical mitoses in 10 high-power fields. The intermediate grade components presented poorly differentiated parenchyma, with variable degrees of intratumoral necrosis and ten to nineteen atypical mitoses. The high histologic grade components were undifferentiated, with necrosis in more than 50% of the parenchyma, and more than 20 atypical mitoses in 10 high-power fields. A high degree of the mesenchymal component was observed in 78% (32/41) of the sarcomas and carcinosarcomas, an intermediate degree in 9.7% (4/41), and a low degree in 12.2% (5/41) of the cases. Carcinomatous components were classified as grade I in 62.5% (15/24) of the cases, and grade II in 37.5% (9/24).

Results revealed high COX-2 expression in 10% (2/10) of the carcinosarcomas and in 25% (4/16) of the sarcomas and sarcomas in mixed tumors. (Table 2). One carcinosarcoma presented high COX-2 expression in the carcinomatous component that was cytoplasmatic and multifocal. Another carcinosarcoma showed high expression in the sarcomatous component. In the sarcomas, this expression was cytoplasmatic and multifocal and was detected in osteoblasts, fibroblasts, chondroblasts, and occasionally in multinucleated giant cells. COX-2 expression was also observed in areas affected by inflammation and necrosis. However, these were not considered in the analyses.

There was no statistically significant association between histological subtypes (sarcoma and carcinosarcoma) and the clinical and morphological characteristics we evaluated (Table 2).

In immunohistochemical tests for cytokeratin AE1/AE3, intracytoplasmic staining was observed in carcinomatous components (Fig. 2a) and lymph node metastasis of the anaplastic type. Intracytoplasmic staining for vimentin was observed in the sarcoma component of the four histologic types: fibrosarcoma (Fig. 2b), chondrosarcoma (Fig. 2c), osteosarcoma (Fig. 2d), and liposarcoma. The same pattern of vimentin marking was noted in cases of lymph node mesenchymal metastasis of the osteosarcomatous component.

Discussion

This study allowed us to comprehensively explore the histological variations of sarcomas and carcinosarcomas, as well as possible relations between these variations, clinical presentations, and morphological criteria of aggressiveness.

Few reports have addressed the frequency of mammary sarcomas in female dogs. In the female dogs we assessed, the frequency of mammary neoplasms with malignant mesenchymal components was similar to data from a previous report (13%) (Misdorp et al. 1971). However, in a recent survey with a fuller description, other authors reported that neoplasms with malignant mesenchymal components accounted for 5% of the total number of tumours (Nunes et al. 2018).

As previously published (Nunes et al. 2018), carcinosarcomas were the most frequent type of malignant mesenchymal neoplasm. The low frequency of pure sarcomas and sarcomas in mixed tumors corroborated the understanding that these histologic types are extremely rare in the mammary glands of female dogs (Nunes et al. 2018).

Among the assessed cases, the prevalence of carcinosarcomas and sarcomas larger than 5.0 cm is consistent with the unfavourable prognoses that are usually attributed to these types of tumours. Since survival rates are lower among female dogs with larger-diameter tumours, tumour size is an important prognostic factor for female dogs with mammary neoplasms (Ferreira et al. 2009). Besides this, large tumours have been associated with other prognostic factors, such as the histologic subtype, losses in hormone receptor expression, and the proliferative index (Langenbach et al. 1998; Nieto et al. 2000; Sarli et al. 2002; Ferreira et al. 2009).

The first challenge in performing a histologic diagnosis of mammary neoplasms with mesenchymal components is determining the benign or malignant character of the mesenchymal proliferation. Carcinosarcomas must be histologically distinguished from carcinomas in mixed tumors that are characterized by carcinomatous and benign mesenchymal components. To do so, the key is to identify malignant characteristics in the mesenchymal cells, such as the level of differentiation in the matrix, high cellularity, elevated nuclear and cellular pleomorphism, karyomegaly, irregular nuclear membrane, prominent nucleoli, and multinucleation (Cassali et al. 2017; Goldschmidt et al. 2017). The same aspects should be considered when distinguishing between sarcomas and benign mesenchymal tumours.

For all the cases in our study, we performed an immunohistochemical analysis of the expression of intermediate vimentin and cytokeratin AE1/AE3 filaments. This made it possible to discriminate between epithelial and mesenchymal proliferation. All of the mesenchymal components of the sarcomas and carcinosarcomas presented vimentin markers (cell markers of mesenchymal origin), and all the epithelial components of the carcinosarcomas presented cytokeratin AE1/AE3 markers (cellular markers of epithelial origin) (Boos et al. 2011; Dolka et al. 2013). Besides this, in some cases, the use of immunohistochemistry made it possible to detect small foci of sarcomatous and carcinomatous proliferation in lymph nodes. These were not detected with H&E staining.

The correct classification of sarcomas by the type of proliferation is another challenge in morphological diagnosis. This is due to the varying nature of the matrix in the same tumour. Different combinations of mesenchymal proliferation (osteosarcomatous, chondrosarcomatous, fibrosarcomatous, and liposarcomatous) and epithelial proliferation were observed. Similarly, this has previously been described in human (Yakan et al. 2014) and canine mammary glands (Cassali et al. 2017). The histologic variety of the mesenchymal components of neoplasms has been attributed to myoepithelial cell transformations (Cassali et al. 2012) epithelial-mesenchymal transition (EMT) (Kokkinos et al. 2007), or totipotent cells with a high differentiation capacity (Hellmen and Lindgren 1989).

We classified all tumours that presented a malignant osteoid matrix as osteosarcomas because, among the sarcomas found in different anatomical locations, this type exhibits the most aggressive type of proliferation (Goldschmidt et al. 2017).

Although the most frequent mesenchymal subtype in our study was osteosarcoma, another report on sarcomas in the mammary glands of female dogs demonstrated a higher frequency of fibrosarcomas (Dolka et al. 2013). On the other hand, another investigation revealed that, among extraskelatal osteosarcomas in dogs, the mammary gland is the most frequent location (Kokkinos et al. 2007). There were no pure chondrosarcomas in our findings. However, this histologic type has already been found in the mammary glands of female dogs (Serin and Aydogan 2009; Tavasoly et al. 2013).

Whether they are located in the mammary glands or other anatomical locations, osteosarcomas can be classified into different types, due to the heterogeneity of their cellular populations and diverse extracellular matrix formation. At times, the same neoplasm can simultaneously present different types and this can make them difficult to classify. Moreover, it corroborates the highly pleomorphic behaviour of this component. Any correlations between histologic types and prognoses are still uncertain, but skeletal fibroblastic osteosarcomas have been associated with better prognoses while the opposite has been reported for the telangiectatic type (Thompson and Dittmer 2017). Other researchers have found no correlation between the histologic subtypes of skeletal osteosarcomas and histologic grades (Nagamine et al. 2015). Similarly, we found no correlation between histologic subtypes and other clinical or pathological variables. However, other studies with larger numbers can give answers about the importance of this correlation.

Our findings concerning the highest frequency of the mesenchymal lymph node metastasis corroborate those of previous studies that related mammary gland sarcomas in female dogs with greater metastatic potential and a worse prognosis (Hellmen et al. 1993). However, the similarity between the frequency of lymph node metastases in sarcomas and carcinosarcomas (30% and 21,5% respectively), substantiated the malignancy of both histologic types (Goldschmidt et al. 2017). The prevalence of mesenchymal lymph node metastases with osteosarcoma components confirmed its malignancy and capacity to disseminate along the lymphatic pathway (Hellmen 2014) and reinforces the importance of assessing regional lymph nodes.

Our finding of a low frequency of cases with radiographic evidence of distant metastasis was different from other results in the literature. These studies examining the mammary glands of humans and female dogs related mammary gland sarcomas to the frequent occurrence of metastasis to other organs (Benjamin and Lee 1999; Yakan et al. 2014). The lack of radiographic exam results weakens knowledge regarding the behaviour of the tumours, from the time of the first diagnosis onwards, and this divergence was probably due to issues of access to the results of pre-surgical radiographic exams, as well as the ones carried out during the clinical history.

Determining the histologic grade is important for issuing a prognosis and deciding on a therapeutic plan for invasive mammary carcinomas (Karayannoupoulou et al. 2005). However, there is no established system for grading the mesenchymal components of mammary sarcomas in female dogs.

A previous study used a grading system to assess mammary sarcomas in female dogs, and most of the cases demonstrated a high histologic grade (Dolka et al. 2013). By using a grading system, the authors also observed that sarcomas with a higher histologic grade were associated with more proliferative activity (Ki67 index). This suggests there may be implications regarding the grade and prognosis of these neoplasms. We used the same grading system and also observed a prevalence of high-grade sarcoma components among the sarcomas and carcinosarcomas we assessed. However, further prognostic studies with larger sample sizes are needed to validate this grading system.

The interest in evaluating COX-2 expression in canine malignant mesenchymal tumours stems from research findings regarding the association between COX-2 overexpression (6-12) and lymph node metastasis by the time of surgery, the development of distant metastasis, and the decline in both overall survival and disease-free survival times (Queiroga et al. 2010; Lavallo et al. 2012; Carvalho et al. 2016). Besides this, the enzyme is a therapeutic target for non-steroidal anti-inflammatory drugs that have already been included in therapy protocols for the treatment of other histological types of mammary neoplasms in female dogs (Souza et al. 2009; Lavallo et al. 2012).

There are few published reports regarding COX-2 expression in the mesenchymal components of the mammary glands in female dogs. One study found intense expression in mammary fibrosarcoma (Arenas et al. 2016). Another investigation discovered significant COX-2 expression in appendicular osteosarcomas in dogs (Millanta et al. 2012).

The percentage of carcinosarcomas with a high COX-2 score was lower than the number previously described by Queiroga et al. (2010) and Carvalho et al. (2016). However, it was greater than the percentage reported by Lavallo et al. (2012). Probable justifications for these differences in results include the use of dissimilar antibodies, inherent distinctions in components and antibodies, or inherent variations in immunohistochemical exams.

Recently, COX-2 overexpression was associated with more aggressive histological types, such as carcinosarcomas (Pastor et al. 2020). This overexpression in carcinosarcomas and other mammary carcinomas was also linked to immunohistochemical markers for angiogenesis, proliferation and

inflammation, shorter disease-free survival, and shorter global survival (Queiroga et al. 2010; Carvalho et al. 2016).

In conclusion, sarcomas and carcinosarcomas were found to be clinically similar and developed as large tumours, mainly in the abdominal and inguinal mammary glands, with frequent intratumoral necrosis but a low frequency of nodal metastasis. Sarcomatous histological patterns varied and, when compared with the other types of mesenchymal proliferation, the osteosarcomatous subtype was the only one that resulted in vascular invasion, metastasis in regional lymph nodes, and a higher histologic grade. Besides this, the findings of carcinosarcomas and sarcomas with high COX-2 expression suggest that, in some cases, these types of neoplasms may respond to therapy with COX-2 inhibitors. However, the usefulness of COX-2 inhibitors in treating these neoplasms remains to be further investigated.

Declarations

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Conflicts of interest: The authors declare that *there is no conflict of interest*.

Availability of data and material: The datasets generated during the current study are available from the corresponding author on request.

Code availability: Not applicable.

Author Contributions: Conceptualization: [Angélica Cavalheiro Bertagnolli Rodrigues, Saulo Petinatti Pavarini, Luciana Sonne, David:Driemeier], Methodology: [Angélica Cavalheiro Bertagnolli Rodrigues, Saulo Petinatti Pavarini], Formal analysis and investigation: [Klaus Scherer Prates, Priscilla Lucas de Oliveira, Thaís Silveira Bueno, Karine Araújo Damasceno], Writing - original draft preparation: [Klaus Scherer Prates]; Writing - review and editing: [Angélica Cavalheiro Bertagnolli Rodrigues, Saulo Petinatti Pavarini, Karine Araújo Damasceno], Funding acquisition: [Angélica Cavalheiro Bertagnolli Rodrigues], Resources: [Angélica Cavalheiro Bertagnolli Rodrigues, Saulo Petinatti Pavarini, Luciana Sonne, David:Driemeier], Supervision: [Angélica Cavalheiro Bertagnolli Rodrigues, Saulo Petinatti Pavarini].

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Tables

Table 1: Clinical and histological aspects according to the predominant subtypes of mesenchymal proliferation (n= 41).

	Osteosarcoma		Non-osteosarcoma	
	N	%	N	%
Intratumoral necrosis	24	100	17	100
Absent	0	0	2	11.1
Present	24	100	16	88.8
Mesenchymal vascular invasion	24	100	17	100
Absent	16	66.6	17	100
Present	8	33.3	0	0
Mesenchymal lymph node metastasis	11	100	13	100
Absent	7	63.6	13	100
Present	4	36.4	0	0
Histologic grade	23	100	17	100
Low and intermediate	3	12,5	6	35.3
High	21	87,5	11	64.7
Total	24	100	18	100

Table 2: Clinical and pathological characteristics of neoplasms with malignant mesenchymal components.

	Carcinosarcomas		Sarcomas and SMT		P
	N	%	N	%	
Size	23	100	18	100	0.498
Up to 5.0 cm	5	21.7	3	16.6	
>5.0 cm	18	78.2	15	83.3	
Location	17	100	14	100	0.613
Thoracic	2	11.7	1	7.1	
Abdominal or inguinal	15	88.2	13	92.8	
Mesenchymal vascular invasion	23	100	18	100	0.256
Absent	20	89.9	13	72.2	
Present	3	13.1	5	27.7	
Necrosis	23	100	18	100	a
Absent	1	4.3	0	0	
Present	22	95.6	18	100	
Lymph node metastasis	13	100	10	100	1.000
Absent	10	78.5	7	70	
Present	3	21.5	3	30	
Sarcomatous component grade	23	100	18	100	0,254
Low	4	17.4	1	5.5	
Intermediate	3	13.0	1	5.5	
High	16	69.5	16	88.9	
COX-2 ^b	20	100	16	100	0.350
Negative	6	30	5	31.2	

0-5	12	60	7	43.7
6-12	2	10	4	25

^a Statistical analysis was not possible due to insufficient number of samples.

^b Evaluation of COX-2 staining was not possible in all 41 sarcomas, carcinosarcomas and SMT.

Figures

Figure 1

Malignant mesenchymal histologic subtypes in the mammary glands of the female dogs. Mammary fibrosarcoma. One can observe the malignant neoplastic proliferation of fusiform cells, arranged in multidirectional bundles with accentuated anisocytosis and anisokaryosis, and a large quantity of mitotic figures. H&E, 40X (a) Mammary carcinosarcoma. The malignant proliferation of epithelial cells, arranged in multifocal islands with accentuated pleomorphism and frequent mitotic figures, surrounded by the proliferation of malignant fusiform to polygonal cells that are highly pleomorphic and intermixed with accentuated amorphous eosinophilic material (osteoid matrix). One can also see a focal area of haemorrhage. H&E, 40X (b) Mammary osteosarcoma. The proliferation of osteocytes intermixed with a large quantity of amorphous eosinophilic material (osteoid matrix). H&E, 40X (c) Mammary osteosarcoma. The malignant proliferation of osteocytes intermixed with an osteoid matrix, as well as multifocal pre-chondroid forms and focal areas of necrosis. H&E, 40X (d) Mammary osteosarcoma. The malignant proliferation of osteocytes intermixed with a large quantity of osteoid matrix, in addition to an

accentuated proliferation of giant multinucleated cells and an extended focal area of necrosis. H&E, 40X (e) Mammary osteosarcoma. The malignant proliferation of osteocytes surrounded by an osteoid matrix and intermixed with great vascular spaces containing large quantities of red blood cells. H&E, 40X (f)

Figure 2

Intermediate filament expression. Mammary carcinosarcoma. Cytokeratin AE1/AE3 markers in malignant epithelial cells arranged in tubules and surrounded by a malignant proliferation of poorly differentiated osteocytes. DAB, 10X (a) Mammary fibrosarcoma. Vimentin markers in fusiform cells. Note the absence of markers in the adjacent epithelial cells. DAB, 40X (b). Mammary osteosarcoma. Vimentin markers in a malignant proliferation of osteocytes, with pre-chondroid areas. DAB, 40X (c) Mammary osteosarcoma. Vimentin markers in an accentuated proliferation of osteocytes. Note the absence of markers in the epithelial cells. DAB, 40X (d)