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Feline breast carcinoma microstroma profile

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Abstract: The incidence and complexity in the clinical outcome of mammary tumors in domestic cats have aroused special interest in the study of prognostic factors. One of the complementary forms of prognosis assessment of malignant feline mammary tumors, by classical histopathology, corresponds to the stroma tissue analysis. This study aimed to characterize the mammary carcinomas microscopic stroma in female cats. Following the animals' mastectomy, samples (n = 24) were collected, being fixed thereafter. After that, the material was submitted to gross exam and subsequently subjected to classic histological processing. The stroma component of the mammary carcinomas corresponded to necrosis areas (92%), vascular invasion by tumor cells (62.5%), desmoplasia (21%) and microcalcifications (4%). For domestic cat mammary gland carcinomas, beyond the description of histomorphological type and degree of tumor differentiation, a detailed microscopic evaluation of the stroma tissue becomes essential, as it provides useful and relevant prognostic information in feline oncology.

Key words: cat, neoplasia, mammary gland, prognosis

Perfil microestromal dos carcinomas mamários felinos

Resumo: A incidência e complexidade na evolução clínica dos tumores de mamas de gatas domésticas têm despertado interesse especial no estudo de fatores de prognóstico. Uma

das formas complementares de avaliação do prognóstico das neoplasias mamárias malignas felinas, pelo exame histopatológico clássico, corresponde à análise do estroma tecidual. O presente trabalho objetivou a caracterização microscópica estromal dos carcinomas mamários de gatas. As amostras (n=24) foram coletadas após a execução de mastectomia e em seguidas fixadas. Posteriormente, o material foi avaliado macroscopicamente e posteriormente submetido ao processamento histológico clássico. Os componentes estromais dos carcinomas mamários corresponderam a áreas de necrose (92%), invasão vascular (62,5%), desmoplasia (21%) e microcalcificações (4%). Para os carcinomas da glândula mamária de gatas domésticas, além da descrição do tipo histomorfológico e grau de diferenciação tumoral, torna-se fundamental a avaliação microscópica detalhada do tecido estromal, uma vez que fornece informações prognósticas úteis e aplicáveis na oncologia felina.

Palavras-chave: gato, neoplasia, glândula mamária, prognóstico

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Introduction

The annual incidence of feline mammary tumors is estimated at 12.8 to 25.4 per 100,000 cats (BURRAI et al., 2010). The majority (80% to 96%) of these malignant growths shows potential malignancy, in divergence to canine species, which reveal malignant characteristics in 41 to 53% of the

mammary gland neoplasms. The vast majority (over 80%) of feline malignant mammary tumors is equivalent to carcinomas (FILGUEIRA, 2011). Various clinical parameters have attracted interest, with potential prognostic usefulness in feline mammary oncology. Tumor size, stage of disease, lymph node involvement, metastases and extent of surgery are some of those parameters. However, as the prognosis for cat breast neoplasms is often from reserved to unfavorable, the association with other factor s of pathologic nature is essential, being histopathology cited, with applicability in both the diagnostic process and in providing relevant prognostic information (HUGHES & DOBSON, 2012; ZAPPULLI et al., 2014). The morphologic diagnosis is a key initial step and tends to follow a standardized system to ensure proper evaluation. Currently, we use the classification according to the World Health Organization guidelines, with some modifications recently suggested (ZAPPULLI et al., 2014). The histological type and degree of tumor differentiation provide a useful prognostic knowledge (FILGUEIRA, 2011). However, another prognosis complementary form of the malignant mammary tumor, by the classic histopathological examination, is equivalent to the tissue stroma analysis (FILGUEIRA et al., 2007).

One tumor, regardless of its biological behavior, consists of its own cells and clonal tumor cells, corresponding to the parenchyma and a non-neoplastic portion, called stroma. It contains connective tissue, blood vessels, and a variety of cells (MCGAVIN & ZACHARY, 2009). It is equivalent to a fibro vascular component that provides the supporting structure for growth as well as the necessary nutrients for the neoplastic cell maintenance (FILGUEIRA et al., 2007). Several lines of evidence indicate that the s tumoral stroma can originate from the proliferation of preexisting stroma cells by *in situ* stem cells differentiation in

normal adjacent tissues or through progenitor cells recruitment from the bone marrow (HANAHAN & WEINBERG, 2011). The parenchyma and stroma contain different types and subtypes of cells that collectively allow the neoplasm progression. It is increasingly evident that cancer cells respond complexly, with all abundance, histological organization and phenotypic characteristics of their stroma tissues. This interaction modulates the rate of development, differentiation state and behavior of both stromal and parenchymal cells, thereby reflecting on the acquired capacity related to tumor invasive growth and metastasis (MCGAVIN & ZACHARY, 2009; HANAHAN & WEINBERG, 2011). In bitches, the microscopic description of the stroma elements of malignant mammary proliferations has already been held (FILGUEIRA et al., 2007). In order to contribute information to the feline oncology, the present study aimed to characterize the stroma components of the mammary gland carcinomas in domestic cats, emphasizing their respective prognostic correlations.

Materials and Methods

The studied material was the result of surgical resections of domestic cat mammary gland proliferations. These procedures were performed during routine of Veterinary Hospital of the Rural Federal University of the Semi-Arid, Mossoró, RN, Brazil). The collected samples were fixed in 10% formalin for more than 24 hours. After that, the material was sent to gross exam and, at the end, sections were performed, yielding fragments to be used for the classical histological processing. Then, they were subjected to dehydration process in increasing ethanol concentrations, diaphanized in xylene, embedded in paraffin and sectioned in 5µm micrometer. The sections were stained with hematoxylin-eosin (HE). The histological preparations were examined in optical microscopy, in 75, 150 and 600 × objectives. A total of 24 mammary tumors,

all classified as carcinomas with distinct subtypes and degree of morphological differentiation, were analyzed.

Results and Discussion

Among the most frequent microstroma characteristics, the areas of necrosis were highlighted (Figure 1a), with a percentage of 92% of all feline female mammary carcinomas (Figure 2). These data were similar to another study in which domestic cat mammary tumors showed mostly (86.87%) necrosis, and such signal covered a large part (over 30%) of the neoplastic tissue (SCHIRATO et al., 2012). In bitches, the necrotic fields of mammary gland malignant tumors showed a value of 59.2% and also corresponded to the most frequent stroma changes (FILGUEIRA et al., 2007). However, in those animals, the value presented was inferior to the ones verified in the tumors of the cats in the research in question. This higher value for the cats could be associated with the larger dimensions usually found in mammary carcinomas of the species, once the tumor tissue necrosis indicates a rapid cells' growth rate and also relates to more anaplastic neoplasms and higher capacity for embolization and metastasis. Thus, the prognosis is worse in the presence of tumor necrosis (HATAKA, 2004). It has been shown in a multivariate analysis that the extent of necrosis in feline mammary carcinomas maintains a prognostic value (ZAPPULLI et al., 2014). In the research discussed, there was a higher incidence of necrotic regions in the microscopic field when compared to the gross exam this could be explained by the fact that tumor necrotic process occurred earlier and more intensely from the histopathological point of view. Thus, breast carcinomas that have not yet clinically exhibited points of necrosis may have already presented such changes microscopically.

The mechanism for the formation of necrotic areas can be justified by the fact that, as the rapid growth and infiltration of malignant tumors happen,

there is a compression of adjacent cells and blood vessels. Being so, a hypoxic condition is installed, resulting from ischemia and consequent atrophy or necrosis (JONES et al., 2000). Additionally, necrosis correlates well with tumor immunity. Specific tumor antigens (detected only in tumor cells) induce both a humoral immune response and a cellular one, being the latter more effective. Thus, macrophages acting together with T lymphocytes can destroy tumor cells by oxygen free radical generation and cytokine secretion such as tumor necrosis factor-α (Jones et al., 2000). In general, the necrotic lesions presence cause the recruitment of immune system inflammatory cells (consisting mainly of lymphocytes, plasma cells and neutrophils) in order to remove tissue debris. However, in the context of cancer, several lines indicate that inflammatory cells have the ability to promote tumor cell proliferation, invasion and angiogenesis. Consequently, the occurrence of necrosis regions, while apparently useful for counteracting neoplasm tissue hyperproliferation, may trigger more harm than good (HANAHAN & WEINBERG, 2011; SHAFIEE et al., 2013).

Vascular invasion by tumor cells (Figure 1b) was observed in 62.5% of the cases (Figure 2). In a research, when examining the microscopic characteristics of mammary carcinomas in cats, the presence of vascular invasion by neoplastic cells was found in a frequency from 70.8 to 75%, varying according to the histological pattern (TRAVASSOS, 2006). However, in dogs, vascular invasion was observed in 29.70% of malignant breast neoplasms (FILGUEIRA et al., 2007). The higher infiltration into the vascular tissue (by neoplasm cells), in the feline species in relation to canines, came to corroborate the high potential of malignancy (and consequent poor prognosis) of breast carcinomas in cats compared with other species. The lymphovascular invasion is an extremely important prognosis indicator.

Lymphatic emboli were strongly associated with disease-free survival, accounting for five months when present, over 14 months when absent (ZAPPULLI et al., 2014). One study found that female feline mammary carcinoma carriers with vascular infiltration by tumor cells showed average survival of seven months (TRAVASSOS, 2006). Contrary to the canine species, all cats' malignant mammary neoplasms with vascular invasion by neoplastic cells had metastases to lymph nodes (COSTA, 2010). Such an observation led to the hypothesis that, in cats, there were favorable local conditions for the metastasis phenomenon. In feline mammary carcinoma, the lymphatic system is an important pathway for metastasis. Carcinomas may also diffuse directly into the blood vessels. Considering that most of the lymph ends up returning to the bloodstream, it is not uncommon to secondary haematogenous spread of carcinomas (JONES et al., 2000; MCGAVIN & ZACHARY, 2009). Thus, infiltration and metastasis are malignant tumor hallmarks.

A series of steps becomes necessary for the tumor cells to lose their cohesion from a primary mass, to penetrate the blood or lymphatic vessels, and to produce a secondary growth at a distant site. Intercellular junction rupture and extracellular matrix invasion, due to the adhesion between tumor cells and the basal membrane of laminin receptors, initially occur. Some proteolytic enzymes are secreted (as collagenase type I, and urokinase plasminogen activator). Thus, there is a degradation of the basement membrane and subsequent migration of cancer cells to the extracellular matrix, mediated by cytokines such as autocrine motility factor. Then, the cells eventually reach the vascular system (MCGAVIN & ZACHARY, 2009; KUMAR et al., 2010). Vascular invasion and metastasis comprise one of the biological capabilities acquired during various stages of tumor development (internationally named as one

of the Cancer Hallmarks). It is equal to one of the organizing principles to streamline the neoplastic disease complexity (HANAHAN & WEINBERG, 2011).

Desmoplasia (Figure 1c) was present in 21% of the analyzed mammary carcinomas (Figure 2). Some mammary cancers are characterized by formation of a collagenous tissue stroma. This phenomenon is known as desmoplastic reaction. The connective tissue proliferation represents a host reaction to neoplastic cells invasion. This response seems to be an attempt to isolate the invading cells (JONES et al., 2000). For the data under discussion, fewer than a quarter of the malignant mammary tumors presented desmoplasia. Thus, the reduced frequency of this feature can have explained the vascular invasion in more than a half of the carcinomas examined, since there was not the constant presence of a barrier to limit the tumor tissue spread. However, even if desmoplasia were present on a regular basis, neoplastic cells could have used some of the mechanisms mentioned above, for lysis and vascular tissue penetration. In cats' malignant mammary tumors, desmoplasia ranges from mild to moderate (FERREIRA & AMORIM, 2003). In well-developed neoplasms, there was extensive desmoplasia which afforded greater adhesion to the skin and abdominal wall, hindering the *in situ* mobility of the mammary tumor. Desmoplasia is increased in breast carcinomas probably as a result of the factors secretion by the tumor cells. These are known as activity biosynthetic modulators of mesenchymal cells such as tissue growth factor type α, tissue growth factor type β and fibroblast growth factor (MARTINS et al., 2002).

The microcalcification areas (Figure 1d) occurred in only 4% of the diagnosed cancers (Figure 2). In humans, mammary microcalcifications, which may represent a mammary cancer, are structures of size equal to or smaller than 0.5 mm, and are frequently detected in

mammograms. It was found that, among the malignant mammary tumors in women, 62% had evidence of calcification (MARTINS et al., 2010). The focal calcification is occasionally observed in the feline mammary carcinoma (FERREIRA & AMORIM, 2003). Therefore, the presence of such a stroma characteristic, in the mammary carcinomas of the cats studied, proved to be unusual when compared to the human species. The pathologic calcification or mineralization is characterized when calcium salts as phosphates, carbonates, citrates and others are deposited in non-osteoid tissues. Such a change can be seen in dystrophic and metastatic forms. However, dystrophic calcification is the most frequent and occurs locally. This is detected in some tumors (such as mammary carcinomas), old necrosis areas which were not reabsorbed, tendons, sclerotic vascular tunics, and cardiac valves (BRASILEIRO FILHO, 2009). The dystrophic calcification significance may correspond to an alert signal to a prior cell injury (KUMAR et al., 2010). Although uncommon (as demonstrated in the work in question), areas of calcification in the feline breast tissue can serve as an indicator for the presence of a malignant neoplastic process.

The feline mammary carcinoma reveals similarities with some aspects of human breast cancer, such as age group affected, risk factors, pathologic features, biologic behavior, metastasis pattern, and response to therapy. A large number of studies have investigated the feline mammary tumors in an attempt to identify prognostic indicators and generate comparative analyzes with mammary malignancies in women (SEIXAS et al., 2007; ZAPPULLI et al., 2005).

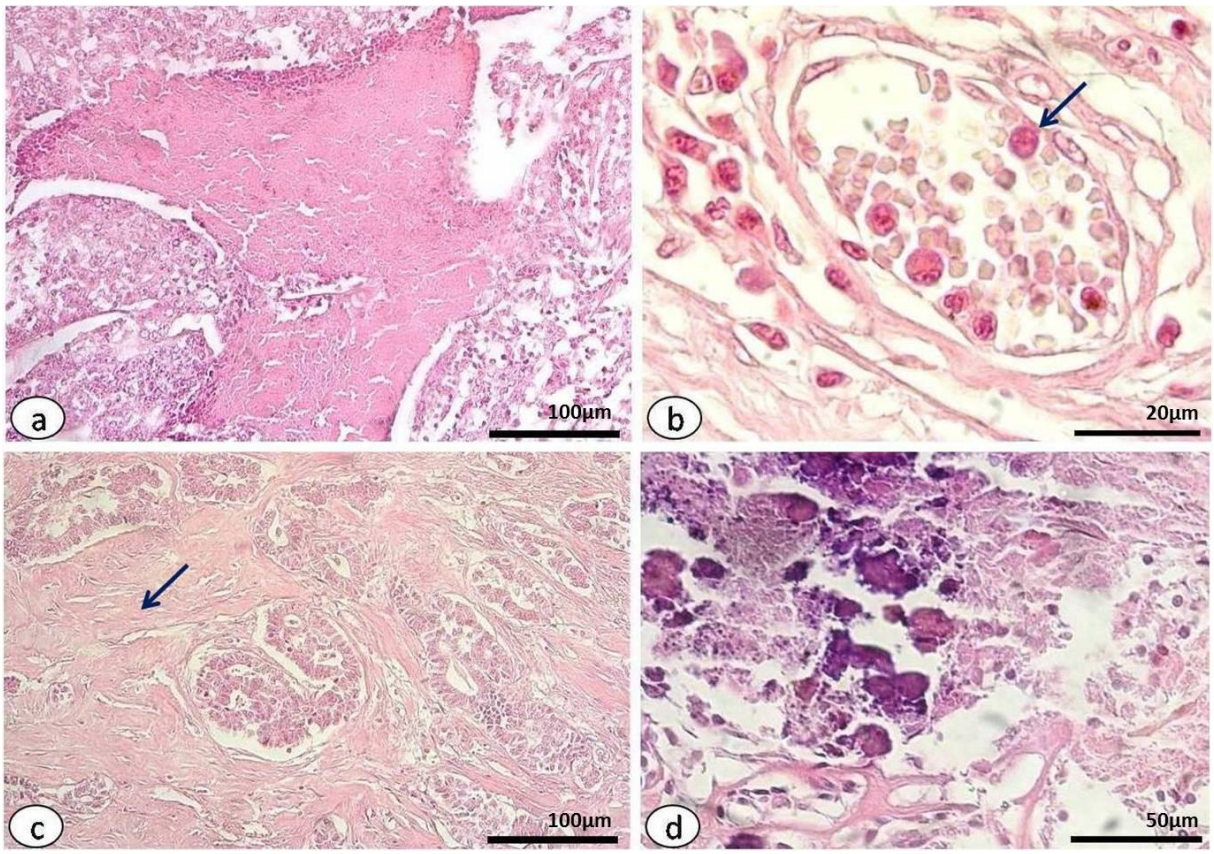

Figure 1. Histopathologic photomicrograph of feline mammary carcinoma stroma components. a: necrosis areas (center of figure); b: vascular invasion (arrow); c: desmoplasia (arrow); d: microcalcifications, characterized in the figure as basophilic aggregates (HE staining; a and c: 20x objective. Bar: 100 µm; b: objective 100x. Bar: 20µm, d: objective 40x Bar: 50 µm).

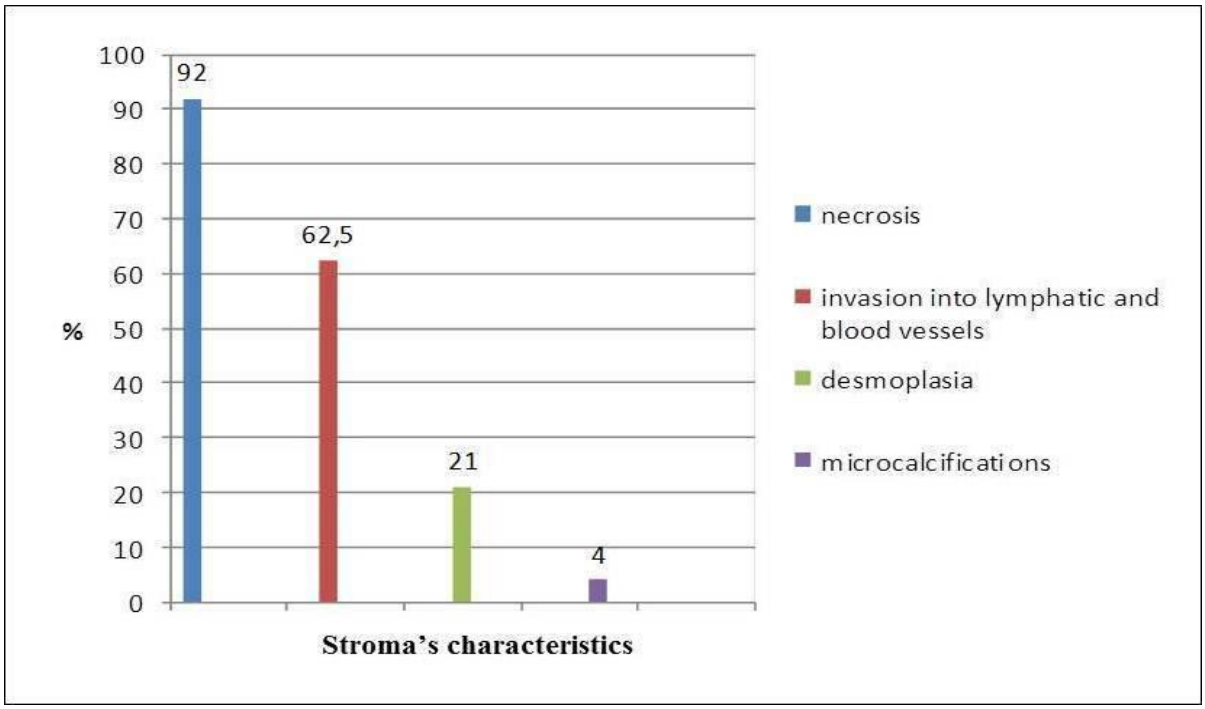

Figure 2. Percentage distribution of stroma characteristics of the feline mammary carcinomas.

Conclusion

The stroma microscopic appearance of the domestic cat malignant tumors, besides demonstrating prognosis applicability in feline oncology, can be extrapolated to the study of the human malignant breast disorder.

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