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Review Article

Consensus on the diagnosis, prognosis, and treatment of canine and feline mammary tumors: solid arrangement – 2023

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Abstract

The purpose of this paper is to discuss and update the criteria that guide the diagnosis, prognosis, and treatment of canine and feline neoplasms. This work was developed during the 5th Meeting of Mammary Pathology and the 1st Latin American Congress of Mammary Pathology, held on October 5th and 6th, 2023, in Belo Horizonte, MG, Brazil. The event was organized by the Laboratory of Comparative Pathology, Department of General Pathology, ICB/UFMG, with the support of the Brazilian Association of Veterinary Pathology (ABPV), Brazilian Veterinary Oncology Association (ABROVET) and the Latin American Society of Veterinary Oncology (SLOVET). The primary goal of the meeting was to unite academics and professionals in veterinary mammary oncology to discuss the morphology, diagnostics criteria, and prognosis of solid mammary tumors in dogs and cats, and to provide updates on treatment guidelines for mammary tumors.

Keywords: mammary neoplasms, cats, dogs, mammary gland, veterinary oncology.

Introduction

Canine and feline mammary tumors are morphologically and biologically heterogeneous, which has led to several attempts to classify them based on their histopathological characteristics, given their importance in predicting tumor biological behavior (2, 3, 30). This classification is performed based on the evaluation of several tumor characteristics such as cellular differentiation, components and arrangement, as well as invasion of the basement membrane (4, 5).

Among the histological types described (Table 1), solid carcinoma is a common pattern of mammary tumor

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in dogs. In an epidemiological survey, a prevalence of 8.06% of solid carcinoma cases was found among 1,310 malignant neoplasms of the female mammary gland of dogs. In this study, only carcinomas in mixed tumors presented a greater number of cases, representing 44.18% of the total sample (21).

Table 1. Histological classification of canine and feline mammary tumors

Histological classification of canine and feline mammary tumors
1. Non-neoplastic epithelial lesion
1.1 Ductal hyperplasia
1.2 Lobular Hyperplasia
1.3 Adenosis
1.4 Duct ectasia
1.5 Columnar cell lesions
1.5.1 Columnar cell alteration
1.5.2 Columnar cell hyperplasia
1.5.3 Atypical columnar cell lesions
2. Benign tumors
2.1 Adenoma
2.2 Adenomyoepithelioma
2.3 Myopithelioma
2.4 Basaloid adenoma
2.5 Fibroadenoma
2.6 Benign mixed tumor
2.7 Ductal papiloma
2.8 Phyllodes tumor
3. Malignant tumors
3.1 Carcinomas
3.1.1 *In situ* carcinoma
3.1.2 Ductal in situ carcinoma
3.1.3 Lobular in situ carcinoma
3.1.4 Carcinoma in a mixed tumor
3.1.5 Papillar carcinoma (invasive and noninvasive)
3.1.6 Solid papillar carcinoma
3.1.7 Tubular carcinoma
3.1.8 Basaloid carcinoma
3.1.9 Cribriform carcinoma
3.1.10 Invasive carcinoma with solid pattern
3.1.11 Micropapillary carcinoma
3.1.12 Pleomorphic lobular carcinoma
3.1.13 Secretory carcinoma
3.1.14 Mucinous carcinoma
3.1.15 Lipid-rich carcinoma
3.1.16 Glycogen-rich carcinoma
3.1.17 Squamous cell carcinoma
3.1.18 Spindle-cell carcinoma
3.1.19 Carcinoma with sebaceous differentiation
3.1.20 Carcinoma with neuroendocrine differentiation
3.1.21 Neuroendocrine carcinoma
3.1.22 Carcinosarcoma
3.2 Myoepithelial neoplasias
3.2.1 Malignant adenomyoepithelioma
3.2.2 Malignant myoepithelioma
3.3 Sarcomas
3.3.1 Fibrosarcoma
3.3.2 Osteosarcoma
3.3.4 Sarcoma in a mixed tumor
3.3.5 Condrosarcoma
3.3.6 Liposarcoma
3.3.7 Hemangiosarcoma
3.3.8 Phyllodes sarcoma

What morphologically characterizes this histological type is the dense arrangement of epithelial cells, anchored in a scarce or inapparent stroma, with nests of invasive cells forming solid masses and with rare tubular formations. Cells are polygonal to oval and often have poorly delineated cell borders and scarce cytoplasm, which may be slightly eosinophilic to basophilic. The nuclei are round to oval and often hyperchromatic with coarsely stippled chromatin and evident, sometimes multiple, nucleoli. Anisokaryosis and anisocytosis are moderate to marked and the number of mitoses is variable. Infiltration of neoplastic cells can be found, as well as metastases to regional lymph nodes (4, 11).

In some cases, solid carcinoma probably represents a more advanced stage of other histological types, as is frequently observed when tumors develop over long periods without surgical intervention (4).

According to survival studies in female dogs, this histological type has a poor prognosis (21). Rasotto et al. (2017) demonstrated in their work that female dogs with solid carcinoma had a median survival of 8 months; only anaplastic carcinomas and carcinosarcomas had shorter survival, with 3 months. Nunes et al. (2018) observed a similar result, with a median survival of 268 days for solid carcinoma, only longer than micropapillary carcinoma (120 days) and carcinosarcoma (113 days).

It is now known that tumors previously classified as solid carcinomas are, in fact, an arrangement of cells that can occur in several histological types (19). The correct histological classification of solid-array tumors is extremely important, since the great heterogeneity of histological types, which present a solid arrangement, can determine differences in behavior, prognosis, and treatment.

Given the new questions and the search for understanding the prognostic and predictive factors of solid-array tumors, the 5th Meeting of Mammary Pathology and the 1st Latin American Congress of MammaryPathology was held on October 5th and 6th, 2023 in Belo Horizonte, Minas Gerais, Brazil. This meeting was organized by the Laboratory of Comparative Pathology – Department of General Pathology – ICB/UFMG, with the support of the Brazilian Association of Veterinary Pathology (ABPV), Brazilian Veterinary Oncology Association (ABROVET), and the Latin American Society of Veterinary Oncology (SLOVET). The objective of this event was to bring together university professors, researchers, professionals, and postgraduate students who work in clinical, surgical, and pathological practice focused on mammary oncology to discuss the morphological aspects, as well as the prognostic and predictive factors of tumors with a solid arrangement in the canine and feline mammary glands.

Tumor with solid pattern

The term ‘tumor with solid pattern” was determined in consensus, since this group of neoplasms includes

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proliferations of epithelial and/or myoepithelial origin, and the term carcinoma is used only for epithelial origin.

Tumors with solid pattern are a common type of mammary tumor in dogs, representing approximately 8% of 1,310 malignant neoplasms of the mammary gland of female dogs (21). However, unlike an architectural characteristic of a specific histological type of neoplasm, it can characterize a more advanced stage of the disease, particularly when tumors develop over long periods, without surgical intervention. What morphologically characterizes a tumor with a solid pattern is the dense disposition of epithelial and/or myoepithelial cells, anchored by a scarce or inapparent stroma, with nests of invasive cells forming solid masses, with rare or absent tubular formations.

This group of tumors includes the following histological types: malignant adenomyoepithelioma, malignant myoepithelioma, invasive carcinoma with solid pattern, neuroendocrine carcinoma, basaloid carcinoma, and solid papillary carcinoma (Table 2).

Malignant adenomyoepithelioma

Adenomyoepitheliomas (AMEs) are biphasic tumors that can affect the skin, salivary glands, and mammary gland. Histologically, AMEs show a predominantly solid pattern, but they may exhibit tubular or papillary areas. Regardless of the growth pattern, two types of cells are observed (8, 10, 14).

Malignant AMEs with a solid pattern are composed of solid cell nests, some small and others large coalescent nests (Fig. 1A-B), often delimited by a delicate fibrous stroma. Cells are round, with an epithelioid pattern, scant to moderately clear cytoplasm, and most cells exhibit vacuolization. The nuclei are round and present varied, but generally moderate to marked pleomorphism, with evident nucleoli. In this pattern, epithelial and myoepithelial cells are difficult to differentiate from each other in routine staining, except when there is still tubule formation by luminal epithelial cells. Otherwise, the distinction between invasive carcinoma, solid pattern, and malignant myoepithelioma should be made by immunohistochemistry, following the definitions in Table 2, and AME is considered malignant if there is p63 staining in 10 to 90% of neoplastic cells (Fig. 1C-D). In this pattern, both types are most frequently malignant, but sometimes tubular formations can be observed, containing a layer of well-differentiated luminal epithelial cells, with low pleomorphism, without invasion of the adjacent stroma, in which case it is considered benign epithelial proliferation. The mitotic count in these cases of solid pattern is variable, but generally presents a high mitotic index (5, 19). Additionally, necrotic areas are frequent, and fibrosis and inflammation (mainly lymphoplasmacytic) can occur with variable intensity. The formation of a myxoid matrix by myoepithelial cells is not frequent but may occasionally occur.

Table 2. Solid tumor subtypes, immunohistochemistry and morphology

Subtype	Immunohistochemistry	Morphology
Malignant adenomyoepithelioma	p63: positive in 10 to 90% of neoplastic cells	Myoepithelial cells with an epithelioid appearance, usually round, with moderately sized, clear, and vacuolized cytoplasm. It is difficult to distinguish between epithelial and myoepithelial cells in HE.
Malignant myoepithelioma	p63: positive in more than 90% of neoplastic cells	Cells with a moderately sized cytoplasm with cytoplasmic vacuolization. Predominantly epithelioid pattern and, in some cases, may present a slightly elongated cytoplasm and a scant extracellular myxoid matrix.
Invasive carcinoma with solid pattern	p63: positive in less than 10% of neoplastic cells	Cells with scant cytoplasm, high nucleus-to-cytoplasm ratio, rounded nuclei with evident nucleoli.
Neuroendocrine carcinoma	Significant expression of chromogranin A and another neuroendocrine marker in neoplastic cells	Cells with moderately sized, slightly eosinophilic cytoplasm, with varying degrees of fine eosinophilic granulation. Clear nucleoli and stippled chromatin, with a "salt and pepper" appearance. Some cases present cells with eosinophilic cytoplasm, with no granulation, hyperchromatic nuclei, or pseudorosette formation.
Basaloid carcinoma	Positive immunolabeling for cytokeratin-14	Solid nests delimited by a layer of palisade cells at the periphery, with hyperchromatic nuclei and scant cytoplasm. Cells in the central regions of solid nests have moderately sized cytoplasm and nuclei with less dense chromatin than peripheral cells. Areas of squamous metaplasia are frequently observed.
Solid papillary carcinoma	Without a specific labeling	Coalescent papillary projections due to the overgrowth of numerous layers of cells, supported by a thin and delicate fibrovascular stroma.

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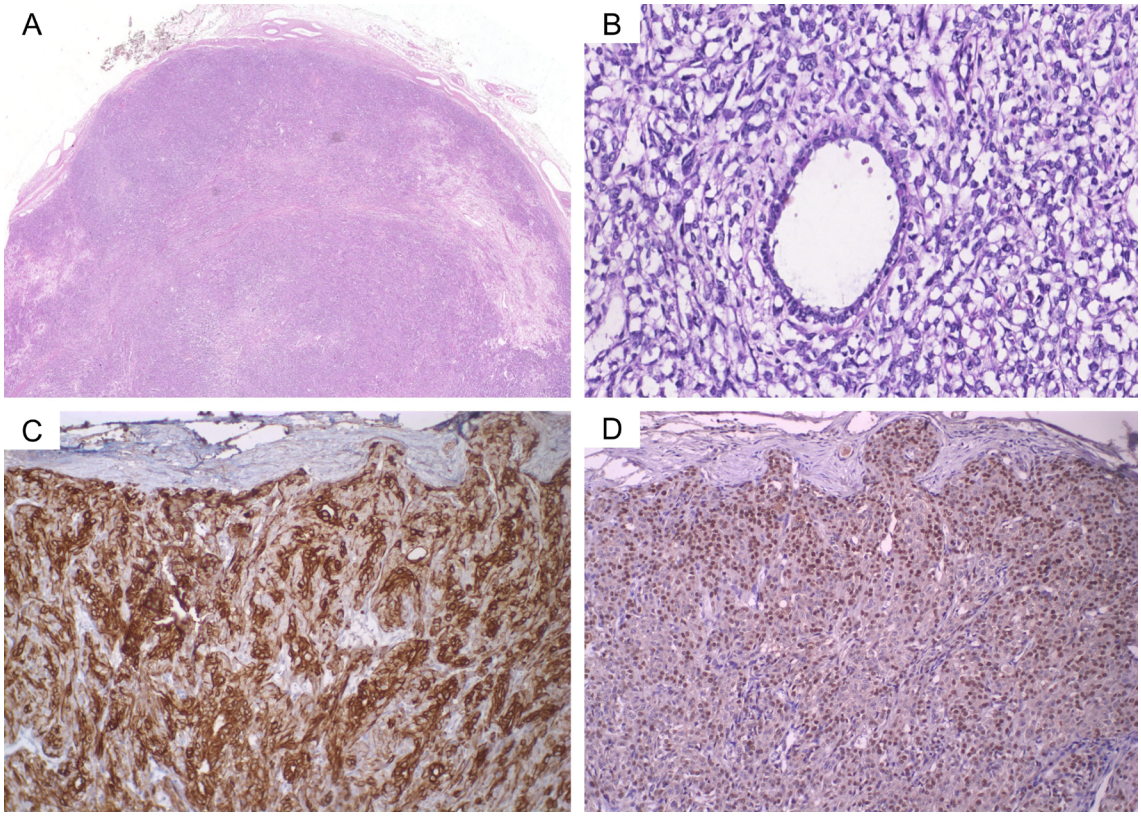

Figure 1. Malignant adenomyoepithelioma. A- Solid and well-defined proliferation, consisting of more densely and less densely cellular areas. At this magnification, it is not yet possible to differentiate the epithelial and myoepithelial populations. HE, 1.9x. B- Tubular formation outlined by epithelial cells and surrounded by a solid proliferation of spindle-shaped myoepithelial cells with vacuolated cytoplasm. HE, 37.2x. C and D- Cytoplasmic staining of pancytokeratin (C) of epithelial neoplastic cells interspersed with nuclear staining of p63 in neoplastic myoepithelial cells (D).

Malignant myoepithelioma

Pure malignant myoepithelioma is a tumor composed almost exclusively of malignant myoepithelial cells (p63 positive in more than 90% of neoplastic cells) (Table 2) (Fig. 2D). The histology of these cases can be heterogeneous. It appears that tumors with myoepithelial differentiation may have well or poorly differentiated features paralleling those seen in common invasive carcinomas. Tumors that demonstrate myoepithelial differentiation may be the result of neoplastic transformation of a stem cell capable of dual differentiation (8).

In dogs, malignant myoepitheliomas are tumors that originate in myoepithelial cells of the mammary gland and are quite rare. In many cases, immunohistochemical differentiation is necessary to distinguish between mesenchymal neoplasms when the cells are spindle-shaped, and epithelial neoplasms when the cells present an epithelioid pattern. Histologically, in most cases, they present as large solid nests of round to spindle-shaped cells, with moderate to abundant cytoplasm, exhibiting moderate to intense vacuolization, and round to oval nuclei, usually with single and small nucleoli (Fig. 2C). The mitotic index is usually moderate (5, 19).

Invasive carcinoma with solid pattern

Invasive carcinoma with solid pattern is a type of carcinoma with scarce fibrovascular stroma, without any special type. Its closest counterpart in humans is grade III invasive carcinoma (5).

In dogs, this histological type is uncommon, representing less than 1% of all histological types and approximately 16% of carcinoma with a solid pattern. Among the histological types that present a solid arrangement, invasive carcinoma with solid pattern is the one with the highest regional metastasis rate, with 62.5% metastases in 16 lymph nodes analyzed (19). Cells are positive for cytokeratin AE1AE3 and negative or exhibit less than 10% positivity for p63.

Microscopically, cells are arranged in large solid nests, sometimes coalescing, with scant supporting fibrovascular stroma. Cells have indistinct borders, slightly eosinophilic cytoplasm, round nuclei, and large, evident nucleoli, generally marked anisokaryosis (Fig. 2E). Mitotic activity is moderate to high and histological grade is usually between II and III. Vascular invasion can often be found at the time of diagnosis (19).

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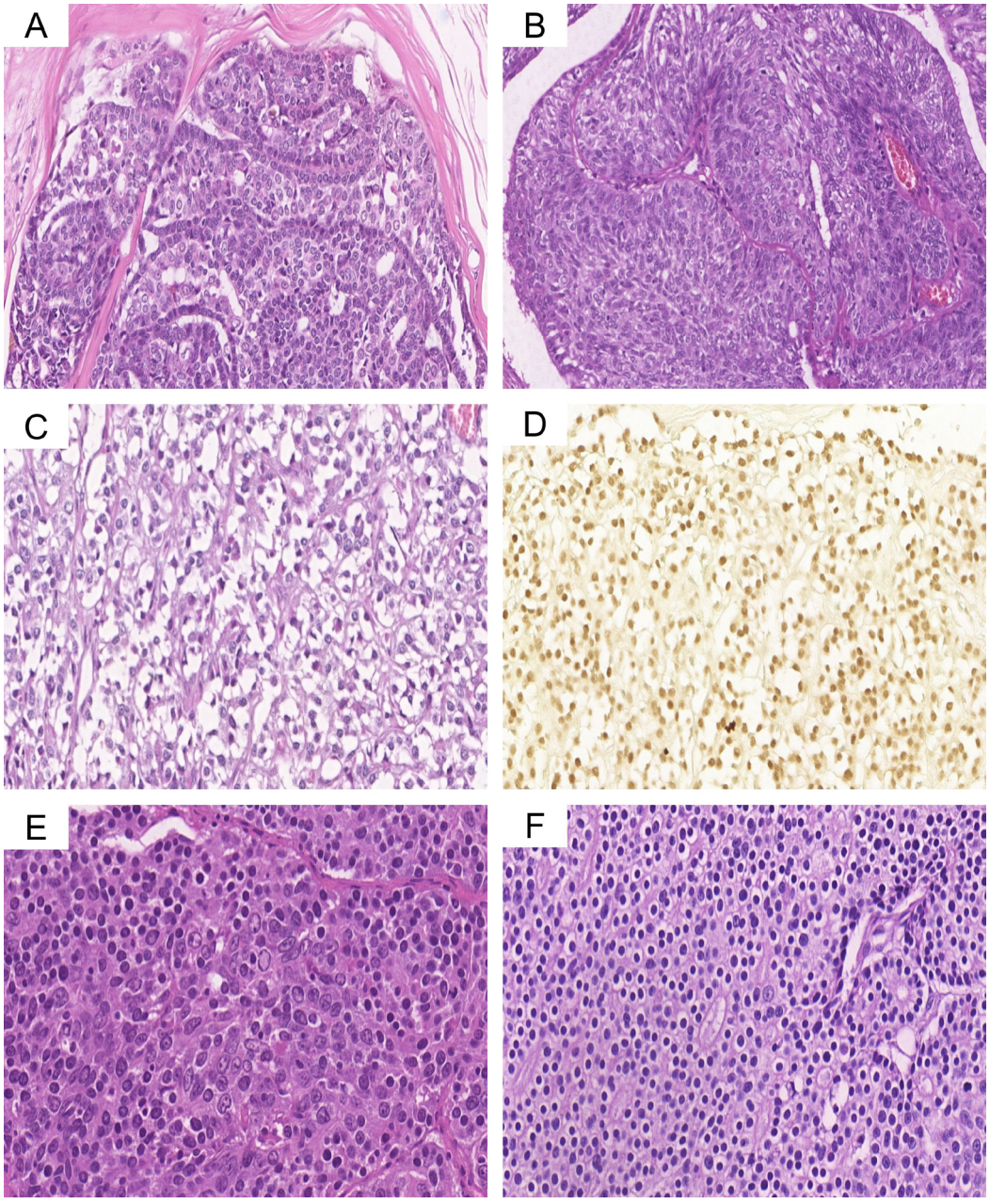

Figure 2. Tumors of solid pattern. A- Basaloid carcinoma. Solid nests delineated by cells arranged in a palisade pattern. The peripheral cells exhibit hyperchromatic nuclei and scant cytoplasm, whereas the central cells exhibit nuclei with stippled chromatin and moderate cytoplasm. HE, 43.1x. B- Solid papillary carcinoma. Densely cellular coalescent papillary projections supported by thin fibrovascular shafts. HE, 33x. C- Malignant myoepithelioma. Mantle of myoepithelial cells with vacuolated cytoplasm, in epithelioid pattern, interspersed with discrete fibrous stroma. HE, 45.2x. D- Malignant myoepithelioma, p63. Positive nuclear staining for p63 in more than 90% of neoplastic cells. 40x. E- Invasive carcinoma with solid pattern. Solid proliferation with scarce interspersed fibrous stroma, cells with scant cytoplasm, high nuclear-to-cytoplasmic ratio, and evident nucleoli. HE, 63.8x. F- Neuroendocrine carcinoma. Solid proliferation interspersed with pseudorosettes, cells with moderate and slightly granular cytoplasm, and round and hyperchromatic nuclei. HE, 65.7x.

Neuroendocrine carcinoma

Neuroendocrine tumors are a group of biologically and clinically heterogeneous neoplasms that most commonly originate in the lungs, gastrointestinal tract, and pancreas. Although their occurrence is rare, several cases of neuroendocrine neoplasms have been reported in the mammary gland in women and a few cases in dogs (5, 17, 20)

The histogenesis of these tumors is debated mainly due to the difficulty in finding neuroendocrine cells in normal

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mammary glands. The absence of neuroendocrine cells during the development of the mammary gland indicates that the neuroendocrine part of a mammary cancer does not originate from a normal component but is the result of neuroendocrine differentiation during neoplastic progression (32).

In women, the overall incidence is less than 1% of all breast carcinomas. However, the true incidence of mammary neuroendocrine neoplasms is difficult to assess, because neuroendocrine markers are not routinely used in the immunohistochemical panel for breast cancer diagnosis (33). In dogs, a prevalence of 7.4% was found among solid carcinomas of the mammary gland (19). Mammary cancers with neuroendocrine differentiation are categorized into three groups, namely invasive carcinoma with neuroendocrine differentiation, neuroendocrine tumor (NET), and neuroendocrine carcinoma (NEC) (15). When neuroendocrine morphological characteristics and expression of neuroendocrine markers are focal or not distinctly enough to classify a neoplasm as NET or NEC, an invasive carcinoma with neuroendocrine differentiation should be considered (24).

In human medicine, from a clinical point of view, the importance of neuroendocrine differentiation in invasive human breast carcinoma is unclear, as some studies have stated that there is no prognostic value for its identification, while others have shown that it is associated with a better or worse prognosis (34). In the literature, a median survival of approximately 60.5 days is reported for dogs diagnosed with neuroendocrine carcinoma (19).

Microscopically, these tumors show an infiltrative growth pattern, with cells arranged in small solid nests of cells delimited by a delicate fibrovascular stroma. Cells mostly have moderate-sized, slightly eosinophilic cytoplasm with varying degrees of fine granulation. Nuclei are usually large, round to oval, and moderate to high anisokaryosis (Fig. 2F). In most cases, granular "salt and pepper" nuclear chromatin is observed; in others, prominent nucleoli are evident. Neuroendocrine tumors are easier to identify on routine staining because they present a carcinoid-like pattern. Cells are arranged in some areas in a palisade pattern, often forming pseudorosettes. The cytoplasm in these cases is scarce and slightly eosinophilic, with small, hyperchromatic oval nuclei and single, occasionally prominent nucleoli (5, 19). Care must be taken to differentiate pseudorosette formation from pseudolumen formation, which is frequently observed in cribriform carcinomas.

Among neuroendocrine cell markers, chromogranin A and synaptophysin are used most frequently and reliably. However, other neuroendocrine markers such as NSE, PGP 9.5, CD56, and INSM1 can be used in the diagnosis. It is recommended to use at least two neuroendocrine markers to confirm the diagnosis (5).

Basaloid carcinoma

Basaloid carcinoma of the mammary gland in dogs is very similar to basal cell adenocarcinomas of the human salivary gland. The immunophenotype of this tumor simulates the embryonic development of salivary gland tissues (22).

In dogs, this histological type represents less than 1% of mammary neoplasms. Morphologically, proliferation in this histological type presents a solid arrangement, sometimes multinodular (Fig. 2A) separated by moderate to abundant fibrous stroma. There are solid nest formations and trabeculae of cells with a larger cytoplasm in the internal region, delimited by a layer of palisade cells on the periphery, with hyperchromatic nuclei and scarce cytoplasm (Figure 2A). Anisokaryosis is moderate but can be marked in some cases. The mitotic index is moderate to high. Areas of squamous metaplasia are frequently observed, which helps in the diagnosis of this histological type. Immunohistochemical staining by CK14 is observed mainly in palisade cells at the periphery of the nodules but, can be found in a large part of the neoplasm (Fig. 9.34A) (18, 19)

The differentiation of basaloid adenoma from basaloid carcinoma consists mainly of the mitotic index, which is low in adenoma and moderate to high in carcinomas, and the presence of areas of stromal invasion in carcinomas (18).

There are still few studies on the prognosis of basaloid carcinomas, but the first two cases reported in the literature presented regional lymph node metastasis and one of them lung metastases (18).

Solid papillary carcinomas

Mammary papillary carcinomas in dogs and cats are tumors histologically characterized by arborescent epithelial proliferation with central fibrovascular stroma. Microscopically, papillary lesions can be classified as papillomas, carcinomas *in situ* in papillomas, papillary carcinomas *in situ*, noninvasive and invasive papillary carcinomas, and more rarely, solid papillary carcinomas (5).

Some papillary carcinomas exhibit epithelial overgrowth, with their projections converging into solid cellular masses, and are often misclassified as solid-pattern invasive carcinomas, due to the failure to visualize the delicate fibrovascular stroma (5)

In women, solid papillary carcinomas are low-grade tumors frequently located in the central/subareolar area of the breast. According to the WHO classification of breast neoplasms in humans, solid papillary carcinomas can be *in situ* or invasive, often show neuroendocrine differentiation, and are biologically indolent (15).

In women, this histological type accounts for less than 1% of breast carcinomas and is considered a rare entity that occurs preferentially in elderly women. In cases associated with invasive carcinoma, the prognosis will depend on the invasive component of the tumor, morphology, and grading. In these cases, metastases may occur (28). In a survey carried out in Brazil on solid pattern in female dogs, 3.7% of the cases were classified as solid papillary carcinoma (19).

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Under microscopy, a neoplastic proliferation is observed, composed of a growth of papillary projections and contains numerous layers of cells supported by a thin and delicate fibrovascular stroma (Figure 2B). As a result of the large number of cell layers, the projections coalesce, generating a solid appearance. The cells have a moderate eosinophilic cytoplasm, with indistinct boundaries, large nuclei, and evident nucleoli. They can be *in situ* (when they do not exceed the limits of the duct) or invasive. Special stains, mainly Masson's trichrome and Gomori's trichrome, help to highlight the connective stroma supporting the epithelial cells (5, 19).

Anatomopathology of the primary tumor and lymph nodes

Surgical specimen

A complete evaluation of the tumor mass is recommended, as mammary neoplasms in female dogs present great architectural heterogeneity (tubular, papillary, solid, micropapillary, etc.), cellular heterogeneity (epithelial, myoepithelial and mesenchymal), and behavioral heterogeneity (benign, malignant, *in situ*, invasive). Therefore, an incisional biopsy is not recommended, as it may not be representative of the entire lesion. In case of multiple tumors, the surgical specimen must be sent as a block, indicating the surgical margins and identifying all lesions.

Intraoperative pathological evaluation

Intraoperative evaluation consists of analyzing samples during the surgical procedure, which can be performed by cytology and frozen sections. For tumors of solid pattern, as well as for other mammary neoplasms, this technique has benefits and limitations. Just as the incisional biopsy may not be representative of the histological type of the neoplasm, in the frozen section biopsy, the sampling may also be insufficient for a conclusive diagnosis. In some cases, the technique can help differentiate between processes (inflammatory versus proliferative).

Intraoperative evaluation of the lymph node is not recommended, since the result will not interfere with the surgical decision, as excision of regional lymph nodes is recommended in all mammary neoplasms.

Finally, as applied to other neoplasms, intraoperative evaluation can provide information on the margins (preserved, narrow or dirty), allowing the surgeon to widen the margins if necessary (25). However, most mammary neoplasms can be completely excised without major difficulties and the cutting and freezing technique should be reserved for larger and infiltrated tumors, with rational use of this resource.

Prognostic and predictive factors

Although specific prognostic and predictive factors are not yet well established for these types of tumors, factors that are already well established for other histological types can be applied and it is recommended that they are described in the results of the histopathological examination. These include: histological grading, evaluation of regional lymph nodes and immunohistochemistry (Ki-67, ER, PR, Cox-2). Additionally, it is important to describe the presence of vascular invasion, invasion of adjacent tissues, and evaluation of surgical margins.

Histological grading

Histological grading is a well-established prognostic factor that is only applied to invasive carcinomas and considers the following parameters: tubular formation, nuclear pleomorphism and mitotic count (Table 3).

In solid tumors, two histological types (malignant adenomyoepithelioma and malignant myoepithelioma) cannot be graded histologically because they present myoepithelial proliferation without tubular formation, also making mitotic counting impossible, which should be considered in grading only in invasive epithelial cells. In these cases, it is recommended that nuclear pleomorphism (mild, moderate, and marked) and the overall mitotic count be described in the histopathological report, since in many cases it is not possible to differentiate between epithelial and myoepithelial cells.

Table 3. Summary of the semiquantitative method to assess histological grade in canine and feline mammary carcinomas*

Feature	Score
Tubule formation	
Most of the tumor (>75%)	1
Moderate degree (10-75%)	2
Little or none (<10%)	3
Nuclear pleomorphism	
Small, regular uniform cells	1
Moderate increase in size and variability	2
Marked variation	3
Mitotic count**	
0-8	1
9-16	2
>17	3
In 10 high-power fields (40x)	
Field diameter: 0.55 mm	
Field area: 0.23 mm²	

*According to Elston and Ellis (30, 31)
**Assessed as number of mitoses per 10 fields at the tumor periphery.

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Assessment of regional lymph nodes

Evaluation of regional lymph nodes is a very well-established prognostic factor for mammary neoplasms in dogs and cats (3) and should also be applied to solid tumors.

All lymph nodes should be analyzed, since there is a direct correlation between the number of affected lymph nodes and shorter survival (1). It is recommended to classify the types of metastases into macrometastasis (>2.0 mm), micrometastasis (0.2-2.0 mm) and isolated tumor cells (<0.2 mm), since the presence of macrometastasis indicates a worse prognosis compared to micrometastasis and isolated tumor cells (Fig. 3A-D) (1, 3).

Regarding the prognosis, a higher risk of death and a shorter survival time were identified, when the presence of extracapsular extension of the neoplasia and tumor implants in adjacent adipose tissue was observed in the affected lymph node with metastasis (1).

Immunohistochemistry

Considering that tumors with solid pattern have been recently described (19), there is insufficient data in the literature on the clinical impact of establishing the immunophenotype of these neoplasms. However, as with other mammary neoplasms, it is recommended to perform the routine prognostic and predictive panel: Ki-67, ER, PR and Cox-2 (5). Taking into account the difficulty of morphological differentiation, the inclusion of p63, pancytokeratin, cytokeratin 14 and chromogranin in this panel should also be considered.

Treatment

Locoregional therapies

The treatment of tumors with solid pattern in dogs should continue to be based on the surgical approach, as the first choice and main therapeutic modality capable of interfering with the natural progression of the disease. The cure rates for mammary cancer in dogs are mainly attributed to surgical treatment (13).

Despite advances in the histopathological diagnosis of mammary neoplasms, it is important to emphasize that, to date and supported by the current literature, surgical treatment is still generally indicated, based on clinical aspects, before

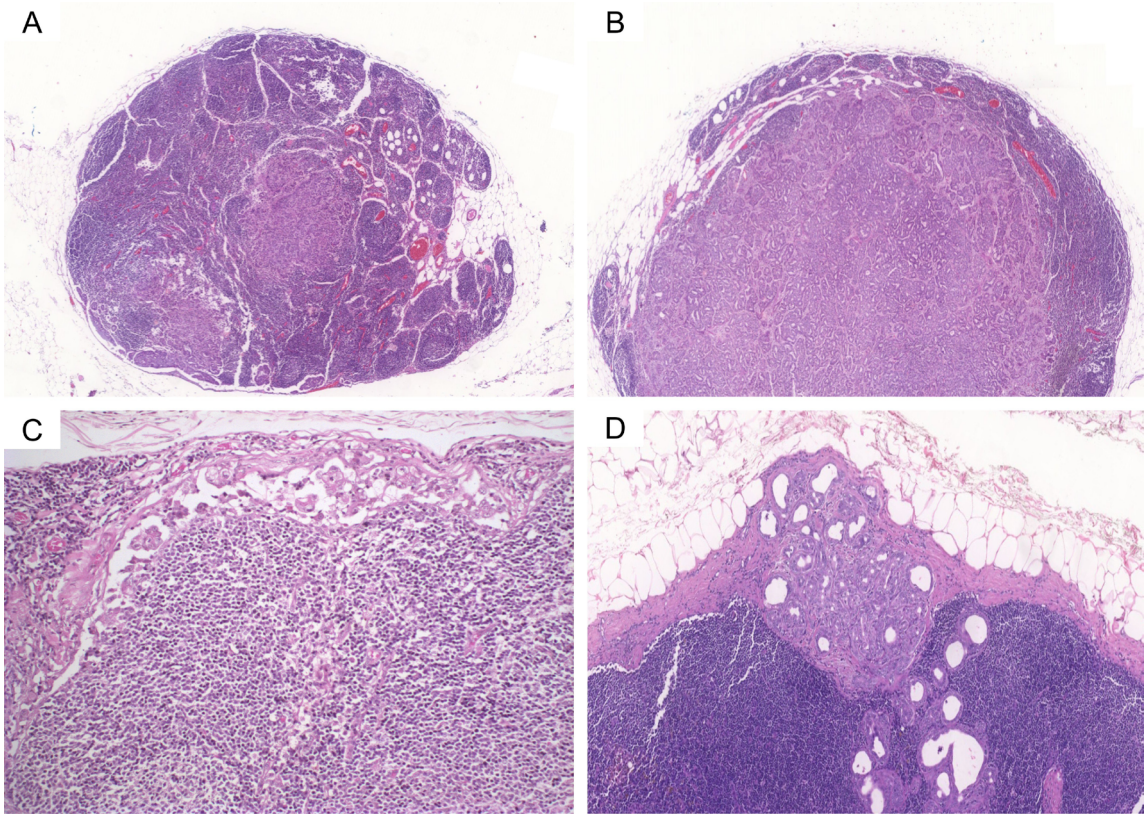

Figure 3. Lymph node metastasis. A- Micrometastasis. Metastatic focus measuring between 0.2 and 2.0 mm in diameter. HE, 3.1x. B- Macrometastasis. Metastatic focus measuring more than 2.0 mm in diameter, replacing most of the tissue architecture. HE, 3.7x. C- Isolated cells. Isolated cells and in small nests, in foci less than 0.2 mm, in the subcapsular sinus. HE, 20x. D- Subcapsular extension. Metastatic focus that extends beyond the nodal capsule and infiltrates adjacent adipose tissue. HE, 11.4x.

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histopathological diagnosis or even confirmation of malignancy, which can be attributed to limitations of cytology and incisional biopsies given the heterogeneity of mammary neoplasms in dogs. However, given the risk of at least 50% malignancy and considering the realization of definitive surgery with curative intent, it is essential to obtain maximum clinical information to direct surgical therapy without excesses or failures. In this context, before surgery, it is essential to perform a clinical staging, based on the TNM system (Table 4), including at least a clinical evaluation of lymph nodes, three-view thoracic radiography, and abdominal ultrasound (5).

The surgical approach in female dogs with mammary neoplasms remains a controversial subject with no consensus yet, and although new studies have not yet been published, the recommendation proposed by Cassali et al. (2020) persists, as an attempt to unify procedures and generate data that can be discussed among specialists (Table 5).

For potentially curative surgery, one should invest in the search for the sentinel lymph node, either through direct or indirect lymphography techniques and/or with the use of intraoperative facilitators such as 2.5% patent blue (29). In female dogs with mammary neoplasms, the sentinel lymph node, defined as the first lymph node to receive lymphatic flow from a solid neoplasm, usually corresponds to the regional lymph node(s) of the inguinofemoral and/or axillary lymph center. However, anatomical changes may occur in the number and location of lymph nodes, as well as lymphatic drainage, as a result of tumor lymphangiogenesis. The oncologist must be aware of this possibility and the limitations of preoperative and intraoperative lymph node identification techniques (23). In general, it is recommended that inguinal lymph node resection be performed when there is resection of the abdominal and inguinal mammary gland; and axillary lymph nodes, when there is resection of the thoracic and cranial abdominal mammary glands (5, 29).

In turn, the extent of mastectomy is based on clinical staging, tumor size, number and location of lesions, and lymphatic drainage (Table 5). The decision-making process for the extent of mastectomy in female dogs, proposed by Cassali et al. (2020), provides that animals with tumors larger than 3 cm, or when located in the cranial abdominal mammary gland, regardless of size, undergo unilateral radical mastectomy or, in the presence of lesions in both chains, bilateral, preferably in two stages. On the other hand, it is assumed that in bloc or regional mastectomy, an approach with effective curative intent can also be represented, particularly in dogs with tumors smaller than 3 cm, without involvement of the cranial abdominal mammary gland; or complete mastectomy, including resection of the muscular fascia and abdominal musculature. It is important to emphasize that the advantages and disadvantages of less aggressive procedures can be

Table 4. Clinical staging in dogs with mammary neoplasms according to the TNM system.

Primary tumor– T
T_1: <3cm
T_2: 3–5cm
T_3: >5cm

Regional lymph node – N
N_0: Non metastatic
N_1: Metastatic

Distant metastasis – M
M_0: Absent
M_1: Present

	T	N	M
Stage I	T_1	N_0	M_0
Stage II	T_2	N_0	M_0
Stage III	T_3	N_0	M_0
Stage IV	Either T	N_1	M_0
Stage V	Either T	Either N	M_1

Table 5. Guidelines to determine the surgical technique and extension of single canine mammary tumors, depending on location, as defined in Consensus 2019.

Single tumor localization	Tumor Size	Surgery Type
M1*	< 3 cm (T1)	Regional mastectomy (M1-M2 + axillary lymph node)
	>3cm (T2 or T3)	Unilateral mastectomy
M2*	< 3 cm (T1)	Regional mastectomy (M1, M2, M3 + axillary lymph node)
	>3cm (T2 or T3)	Unilateral mastectomy
M3*	Any size (T1, T2 or T3)	Unilateral mastectomy
M4*	< 3 cm (T1)	Regional mastectomy (M3, M4, M5 + inguinal lymph node)
	>3cm (T2 or T3)	Unilateral mastectomy
M5*	< 3 cm (T1)	Regional mastectomy (M4-M5 + inguinal lymph node)
	>3cm (T2 or T3)	Unilateral mastectomy

*Tumors associated with other negative prognostic factors should be subjected to unilateral mastectomy.

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discussed with more informed owners and that, until there is new evidence on the best surgical approach, shared decisions can offer more personalized actions aimed at better disease control and better quality of life for patients.

The surgeon's definition of safety margins is based on elusive criteria, as there is a lack of studies with a careful evaluation of surgical margins (12, 26). Radical surgeries do not necessarily provide microscopic margins free of neoplastic cells (16). Fossum (2014) suggests, in the treatment of mammary gland neoplasms, lateral margins of at least 1 cm, with divulsion along the abdominal fascia; however, it is admitted that larger tumors and more aggressive histopathological types may require more extensive resections for complete excision.

In the study by Rasotto et al. (2017), infiltrated margins were observed in 24% (42/169) of female dogs undergoing mastectomy; however, a recurrence rate of only 29% (12/42) was observed, statistically higher than the recurrence rate of 15% (19/127) observed in dogs with descriptions of cell-free neoplastic margins ($p = 0.03$). In cats, in which tumors tend to assume more infiltrative behavior, Chocteau et al. (2019) observed "dirty" margins in 45.6% of cases (180/395).

Although radiation therapy is widely used as an adjuvant local therapy for women with breast cancer, studies in dogs are limited to those with inflammatory mammary carcinoma (27). Electrochemotherapy has become widely available for local treatment of several cancers in various countries (31). However, to date, there have been no studies on the use of electrochemotherapy in dogs with mammary gland tumors, the results are not warranted by previous research, and its use cannot be supported at this point, although it can be used in selected cases according to the discretion of the oncologist, especially considering that "dirty" surgical margins are related to an increased risk of local recurrence (26).

Chemotherapy

The decision to administer chemotherapy to patients with tumors with a solid pattern still requires studies, both in terms of choosing the candidates who will benefit from the therapy and the ideal protocol to be implemented.

In general, the decision about which patients should undergo systemic therapy is based on histological subtype, grade, clinical staging, and immunohistochemical prognostic panel. In solid tumors, it can be challenging to make a decision based on morphological criteria of subtype and grade, since there are few articles that validate the individual behavior of each of the histological subtypes that comprise this group and not all solid tumors are graded, since this classification is exclusive to neoplasms with a strictly epithelial component and is not applicable to neoplasms such as malignant adenomyoepithelioma and malignant myoepithelioma. Although these two subtypes are not graded, it is important to consider their morphological factors that can predict the behavior of the neoplasia, such as the high proliferation index through the mitotic count and nuclear pleomorphism.

Due to these limitations, other criteria prevail in the systemic therapeutic decision, such as advanced staging (IV-V), immunohistochemical panels with characteristics suggesting aggressiveness, such as high Ki-67 (although individual cutoff values are still lacking for this subgroup of tumors) and high expression of COX-2. In cases where grading is applied, it should be noted that grade III tumors exhibit aggressive behavior and are candidates for systemic therapy. As challenging as the selection of animals to benefit from chemotherapy is the decision on the best protocol to be adopted, since studies on the subject are not specific to this subgroup of tumors with solid pattern. Therefore, conventional protocols used for other aggressive mammary neoplasms have been suggested, such as those based on carboplatin, or combinations such as carboplatin and doxorubicin, doxorubicin and cyclophosphamide, carboplatin and gemcitabine, or cyclophosphamide and 5-fluorouracil.

New studies must be carried out to outline the morphological criteria for solid arrangement tumors to select candidates for chemotherapy, as well as the best protocol to be instituted in each case.

Conclusion

The need to establish criteria for diagnosing solid mammary tumors in dogs and to advance discussions about treatment options and surgical approaches led to the 5th Meeting of Mammary Pathology and the 1st Latin American Congress of Mammary Pathology. The histopathological classification and therapeutic protocol recommendations for mammary gland lesions in dogs and cats present significant challenges due to their morphological complexity and varying progression. Standardizing diagnostic criteria and treatment approaches is essential for developing more individualized therapeutic protocols, always aiming to improve the patient's quality of life. Through multidisciplinary meetings in the field of veterinary mammary cancer research, we have made considerable progress. The possibility of multicentric collaboration in Latin America, sharing the expertise of various institutions, is now a reality in mammary oncology, particularly in Brazil.

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Declaration of conflict of interest

The authors declare no competing interests.

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