RELATO DE CASO

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IMMUNOHISTOCHEMICAL CHARACTERIZATION AND CLINICAL MANAGEMENT OF SOLID ARRANGEMENT CARCINOMA IN A CANINE MAMMARY TUMOR

ABSTRACT

Canine mammary tumors (CMTs) are a prevalent condition in female dogs, particularly in those that are unspayed, while being rare in males (<1%). These tumors are predominantly malignant, exhibiting diverse histological classifications and a high mortality rate. Solid carcinomas within CMTs represent a crucial area of research due to their prevalence and varied prognostic implications. A comprehensive understanding of their biological behavior, as obtained through detailed histological and immunohistochemical analyses, is essential for the development of tailored treatment strategies. The identification of specific subtypes of solid carcinomas allows for improved prognostication and the implementation of more efficacious therapeutic interventions, thereby potentially enhancing both the quality of life and survival rates of affected canines. This study presents a case of an 8-year-old Pinscher diagnosed with solid arrangement carcinoma, characterized by positive CK14 staining indicative of poorly differentiated basaloid carcinoma. The dog was treated with chemotherapy comprising doxorubicin and carboplatin. This case illustrates the importance of precise immunohistochemical and histological classification of CMTs in facilitating accurate prognosis and treatment planning, even for small nodules. It is noteworthy that the dog survived for a duration exceeding the typical median survival for solid carcinomas, which serves to illustrate the potential for enhanced outcomes with appropriate clinical management. In conclusion, this case study highlights the necessity for a comprehensive approach to the clinical, histopathological, and molecular evaluation of canine cutaneous mast cell tumors (CMTs), with the aim of optimizing treatment strategies and improving clinical outcomes.

Keywords: basaloid; chemotherapy; histopathology; prognosis; staging.

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1 INTRODUCTION

Solid arrangement in canine mammary tumors (CMTs), account for approximately 8% of CMTs, are notable for their aggressive nature and unfavorable prognosis, with a median survival of 8 months (Nakagaki *et al.*, 2022). Morphologically, they are characterized by a dense arrangement of epithelial cells supported by a sparse or inapparent stroma, with nests of invading cells forming solid masses with rare tubular formations (Rasotto *et al.*, 2017; Nunes *et al.*, 2018).

These tumors exhibit significant immunophenotypic heterogeneity, rendering them difficult to differentiate cell of origin using hematoxylin and eosin (H&E) staining to accurately predict their progression. The combination of immunohistochemistry (IHQ) with histopathological evaluation is essential for accurately classifying and subtyping CMTs. This comprehensive approach facilitates a more nuanced understanding of tumor behavior and aids in the selection of appropriate therapeutic strategies (Cassali; Nakagaki, 2023). In accordance with this approach, the objective of this study was to present a case of solid mammary carcinoma subclassified as basaloid carcinoma through IHQ and to illustrate how this classification enhanced the clinical management of the patient.

2 CASE REPORT

An eight-year-old, 3.4 kg, intact, non-pregnant Pinscher was presented at the Veterinary Hospital with the presence of nodules in the right cranial abdominal mammary gland (M3D), left caudal abdominal mammary gland (M4E), and left inguinal mammary gland (M5E). The dimensions of these nodules were all under 1 cm and the consistency was firm. After hematological testing, radiographic imaging, ultrasonographic examination, and cytological analysis, the animal was recommended for mastectomy and ovariohysterectomy. The surgical specimens were subjected to comprehensive routine histopathological (H&E) and immunohistochemical (IHC) analyses. The panel included HER-2 (polyclonal rabbit/1: The following immunohistochemical stains were performed: estrogen receptors (1D5)/1), progesterone receptors (hPRa2/1:50), COX-2 (SP21/1:50), Ki67 (Mib-1/1), pancytokeratin (AE-1-AE3/1:500), p63 (4A 4/1:100), and CK14 (LL002/1:1000).

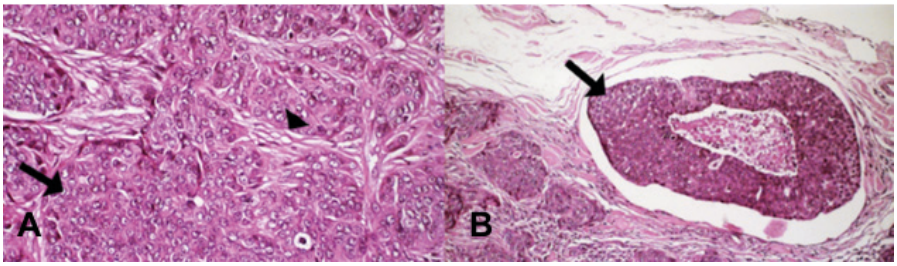
For immunohistochemical analysis, sections of 3 µm in thickness were prepared from primary tumors and mounted on common slides. The antigen was immunodetected using the detection system anti-mouse/anti-rabbit (Novolink Polymer Detection System, Leica Biosystems, Newcastle Upon Tyne, United Kingdom) in accordance with the manufacturer's instructions. Endogenous peroxidase activity was inactivated by the addition of a 10% hydrogen peroxide (H2O2) solution in methanol. The reagents were manually applied, and immunoreactivity was visualized by incubating the slides with the chromogen diaminobenzidine (DAB Substrate System, Dako, Carpinteria, CA, USA) for three minutes.

3 DISCUSSION

The histopathological findings revealed the presence of a mixed tumor carcinoma in M3D and a solid carcinoma with neoplastic embolism in the lymphatic vasculature in M4E. No evidence of metastasis was identified in the regional lymph nodes or in distant sites by imaging studies. Given the size of the nodule and the absence of metastasis, the patient was classified as grade I according to the TNM clinical staging system established by the World Health Organization (Yamagami *et al.*, 1996).

Despite the absence of regional or distant metastases during the follow-up period, the presence of embolism suggests the potential for aggressive behavior (Figure 1). Tumoral lymphatic vessel invasion in dogs has been associated with reduced survival rates following surgical intervention and an increased propensity for more aggressive neoplasms (Rasotto *et al.*, 2017).

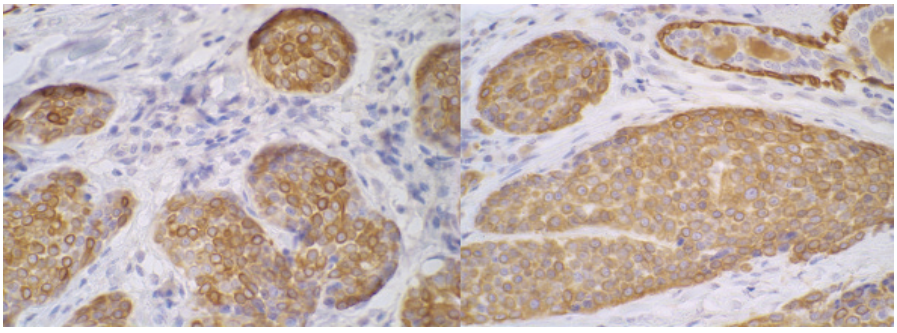
Figure 1 - Canis familiaris, or the domestic dog. The mammary gland. A diagnosis of solid carcinoma is indicated. A) The cells are organized in nests (arrow), interspersed by dense connective tissue stroma (arrowhead). Histological examination. Magnification: 40x. B) Tumor embolization exhibiting a cribriform arrangement within the luminal region of a lymphatic vessel (arrow) (HE). Magnification: 40x



Source: prepared by the authors.

The immunohistochemical analysis indicated a basaloid profile for the solid carcinoma, with positive staining for CK14 (Figure 2). It is therefore essential to classify these neoplasms on the basis of their immunophenotypic profile in order to determine their biological behaviour and prognosis, and to provide appropriate guidance on treatment (Nakagaki *et al.*, 2022).

Figure 2 - Positive cytoplasmic immunostaining for cytokeratin 14 in neoplastic cells at the periphery of the nests and in the central cells



Source: prepared by the authors.

The Ki-67 cellular proliferation index is subject to variation; however, the 2019 consensus suggests a cutoff point of 20%, with higher values associated with an unfavorable prognosis and a greater risk of metastasis (Cassali *et al.*, 2020). The patient exhibited a Ki-67 index value of approximately 20%, indicating the presence of approximately 20% neoplastic cells that were positively marked.

In light of the evidence indicating infiltrative tumor behavior, the IHC panel nevertheless suggested a favorable prognosis for the patient, who exhibited a luminal B HER-2 negative profile with weak HER-2 expression, positive PR and ER, and absence of COX-2 expression. This indicates hormonal dependency and a favorable prognosis. This profile, though less common and with a slightly less favorable prognosis than luminal A, is still associated with superior outcomes compared to other subclassifications (Nunes *et al.*, 2018). The final decision regarding treatment should be made on the basis of a comprehensive evaluation of the patient clinical condition.

In light of the histopathological and IHC essay findings, the patient was referred for oncological follow-up with chemotherapy. The treatment regimen consisted of alternating cycles of doxorubicin and carboplatin, administered every 21 days for a total of six cycles. At the conclusion of the sixth chemotherapy session, hematological and biochemical examinations were conducted, along with thoracic radiography and abdominal ultrasound, for the purpose of monitoring the patient condition.

Prior research has demonstrated that basaloid tumors exhibit heightened sensitivity to cytotoxic agents, including carboplatin (Wu *et al.*, 2013). The case in question exemplifies the intricacies of CMT management, necessitating a meticulous assessment of clinical, histopathological, and molecular characteristics to ascertain prognosis and identify optimal therapeutic strategies. The patient remains clinically stable at the time of this report, with a survival time of 420 days, which exceeds the median survival reported in the literature for solid carcinomas.

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