



## RESEARCH PAPER

## OPEN ACCESS

## Immunohistochemical markers of mammary tumors in female dogs from Northeastern Algeria

Zahra Gabli<sup>1\*</sup>, Leila Beddar<sup>2</sup>, Zouhir Djerrou<sup>3</sup>

<sup>1</sup>*Institut des Sciences Vétérinaires, Université des Frères Mentouri-Constantine 1, Constantine, Algérie*

<sup>2</sup>*Laboratoire de Recherche de Biologie et de Génétique Moléculaire, Université des Frères Mentouri-Constantine 3, Constantine, Algérie*

<sup>3</sup>*Département des Sciences de la Nature de la Vie, Faculté des Sciences, Université 20 Août 1955 Skikda, Algérie*

**Key words:** Mammary Gland Tumors, Female dogs, Immunohistochemical markers.

<http://dx.doi.org/10.12692/ijb/12.4.330-335>

Article published on April 28, 2018

### Abstract

The present study was undertaken to investigate the importance of immunohistochemical markers (cytokeratins AE1/AE2, CK20, CK5, 6, RE, vimentin and P63) in tumor type diagnosis in the case of canine mammary tumors. Thirteen (13) tumors tissue specimens were obtained from 42 female dogs in different ages and breeds. They had been classified according to WHO method after histopathological examination. The tumors were diagnosed as Squamous cell carcinoma (3 cases), Spindle cell carcinomas (2 cases), Sarcomas (3 cases), Carcinoma simple cribriform (2 cases) and the epithelial component is malignant, and the myoepithelium is benign (3 cases). Cytokeratins AE1/AE2 were seen in all cases except in the spindle cell carcinoma. Vimentin can be used as a myoepithelial and mesenchymal cell marker in all cases except in the malin myoepithelioma, P63 is a sensitive and specific myoepithelial marker in canine mammary tumors. Whereas CK 5, 6 and RE were specifically detected in simple cribriform Carcinoma, cytokeratins CK20 were expressed in all cases except in the Squamous cell carcinoma. These results emphasize the interest of immunohistochemical markers to identify the humoral cell origin in canine mammary tumors.

\* **Corresponding Author:** Zahra Gabli ✉ [zahrascom@gmail.com](mailto:zahrascom@gmail.com)

## Introduction

Research in the field of canine tumors has evolved and become more relevant in recent years; not only because the frequency of canine tumors is constantly increasing in veterinary medicine, but also because the mammary tumors of the canine species is a good model for breast cancer in women (Dias *et al.*, 2016). Mammary gland tumors are the most common tumors in the female dog (Karayannopoulou *et al.*, 2005). The risk in male dogs is 1% or less than that in female dogs. The risk of developing mammary tumors is closely linked to exposure to the female sex hormones estrogen and progesterone in early developmental years. However, conflicting reports have been obtained from *in vivo* and *in vitro* studies regarding the role of progesterone, particularly in mammary tumorigenesis (Rao *et al.*, 2009). The most common types are tumors from glandular tissue and include adenoma, carcinoma and adenocarcinoma. Benign mammary tumors appear earlier in life than malignant tumors, and younger animals usually have dysplasia or hyperplasia (Perez *et al.*, 2000). The incidence of malignant forms varies from 26 to 73% (Perez *et al.*, 2000). Morrison (1998) reported that carcinoma is being the most common malignant type. Surgical treatment remains the treatment of choice, except for inflammatory carcinoma or presence of distant metastases (Karayannopoulou and Lafioniatis, 2016).

Canine mammary tumors may originate from different cell types including luminal epithelial, myoepithelial and stroma cells. For accurate diagnosis and prognosis of canine mammary tumors, differentiation of these cell types is very important. Therefore, some specific markers such as cytokeratins, vimentin and RE were used by immunohistochemical techniques in the canine mammary tumors (Griffey *et al.*, 1993; Toniti *et al.*, 2010; Vos *et al.*, 1993; Mallofré, 2003).

The aim of the present study was to investigate the use of the immunohistochemical markers in the diagnosis of mammary gland tumors in bitches in the northeastern of Algeria.

## Material and methods

### *Animals and tumors tissues collection*

The present study was carried out on female dogs, with mammary gland tumors suspicion, from for provinces located in the North-east of Algeria (Annaba, Skikda, Constantine, Oum El Bouaghi). All animals were examined clinically before specimen collections. After general anesthesia, mastectomy of all tumoral glands was carried out following routine surgical techniques. The tissue specimens from tumors were obtained from 13 female dogs and fixed in formalin 10% as described elsewhere (Aydogan and Metin, 2013). All experimental procedures were approved by Institute of Veterinary Sciences of Constantine 1 University, Algeria.

### *Immunohistochemical study*

For the immunohistochemical staining, tumor sections were routinely processed according to standard protocols and described elsewhere (Aydogan and Metin, 2013). Avidin-biotin peroxidase complex method was used on tumor sections (avidin-biotin peroxidase complex, Invitrogen Histostainplus Detection Kit, USA).

Tissue sections were deparaffinised, rehydrated and then antigen retrieval was applied by microwave heat for 10 minutes at medium voltage in a 10 mM citrate buffer, pH 6.0. After cooling at room temperature, the sections were incubated in 3% hydrogen peroxide ( $H_2O_2$ ) for 30 minutes and then washed by phosphate buffer saline (PBS), pH 7.2, 3 times. Nonspecific staining was eliminated by 10 minutes incubation with normal goat serum at the room temperature. Excess normal serum was removed and slides were then incubated with primary antibody (Cytokeratin AE1/AE2, Vimentin, P63, CK20, CK5, 6& RE) at 4°C overnight. After washing the slides, the sections were incubated with biotinylated secondary antibody for 15 minutes and replaced in the streptavidin horseradish peroxidase (HRP) conjugate for 15 minutes at the room temperature. The color was developed with 3, 3'-diaminobenzidine tetrahydrochloride (DAB, DAKO)- $H_2O_2$  in PBS for 5 minutes. Slides were counterstained with Harris Haematoxylin,

dehydrated and mounted with Entellan (Merck). In all slides, non-tumoral areas were used as internal positive control and specificity of primary markers was confirmed.

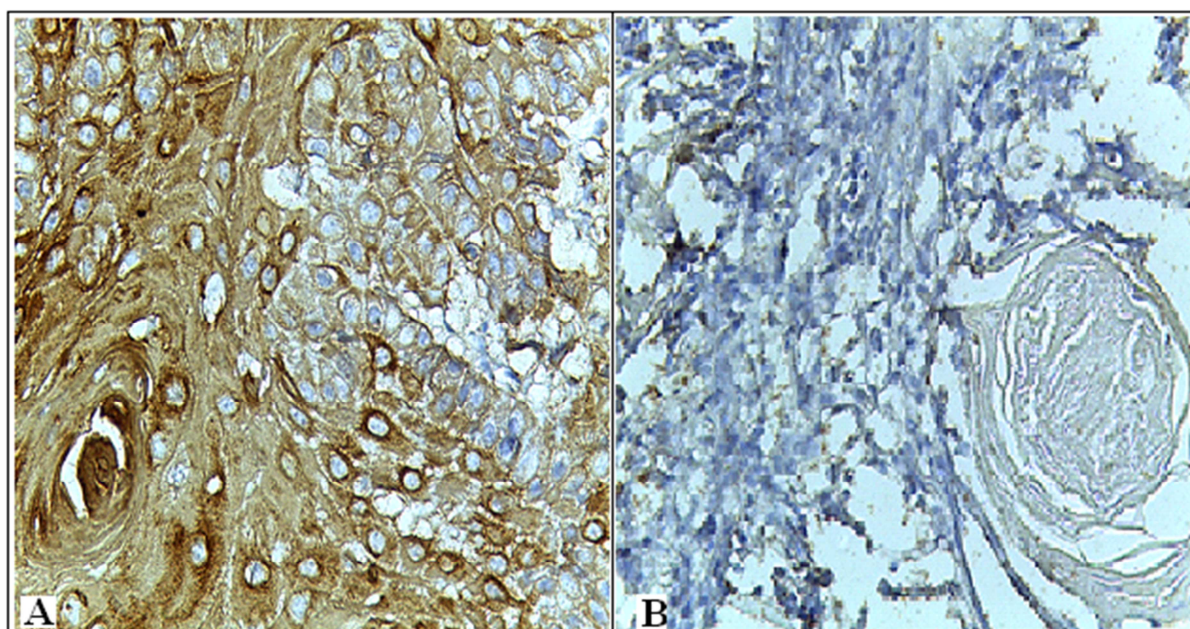
On each slide, different fields were observed and immunopositive reactions were demonstrated by the presence of brown cytoplasmic staining. The results were evaluated semi-quantitatively.

The semi-quantitative scoring (SQS) was performed as follows: +, weak expression; ++, moderate expression; +++, strong expression; -, negative. The tumors were classified according to WHO-AFIP

(World Health Organization–Armed Forces Institute of Pathology) classification.

### Results

All of the cases used in this study were examined histopathologically, and then underwent an immunohistochemical examination. The next step was the expression intensity of the different immunohistochemically markers investigated in the 13 canine malignant mammary tumors. Evaluation of immunohistochemical data positivity was indicated by the presence of distinct dark brown nuclear or cytoplasmic staining and all markers are summarized in the following figures (Fig. 1-3).



**Fig. 1.** Immunohistochemical Staining of Luminal Epithelial Tumors (spindle cell carcinoma). Note dark brown color of positive staining of AE1/AE2 (A) in cytoplasm but negative for CK20 (B). Counterstaining : Harris Hematoxylin.

The tumors were diagnosed as Squamous cell carcinoma (3 cases), Sarcomas (3 cases), Spindle cell carcinomas (2 cases), cribriform simple Carcinoma (2 cases) and the epithelial component is malignant, and the myoepithelium is benign in 3 cases. Cytokeratins AE1/AE2 was seen in all cases except in the spindle cell carcinoma. Vimentin can be used as a myoepithelial and mesenchymal cell marker in all cases except in the malinmyoepithelioma, P63 is a recently characterized p53 homologue, necessary to maintain an epithelial stem cell population. P63 is a

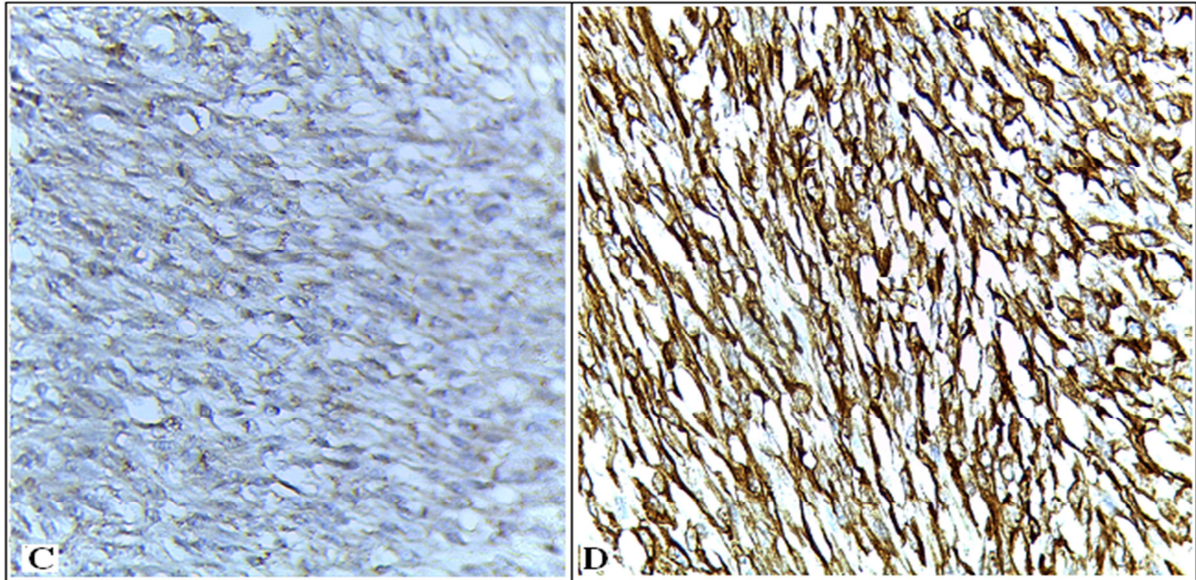
sensitive and specific myoepithelial marker in canine mammary tumors. Whereas CK 5,6 and RE were specifically detected in simple cribriform Carcinoma, cytokeratins CK20 were expressed in all cases except in the Squamous cell carcinoma.

### Discussion

In this study cytokeratins (cytokeratins AE1/AE2, vimentin, P63, CK20, CK5, 6 and RE) were used as immunohistochemical markers; they allowed the identification of the lumino-epithelial and

myoepithelial origin of tumor cells in canine mammary tumors (female dogs). Normal and tumoral myoepithelia have a complex immunophenotype (epithelial and smooth muscle

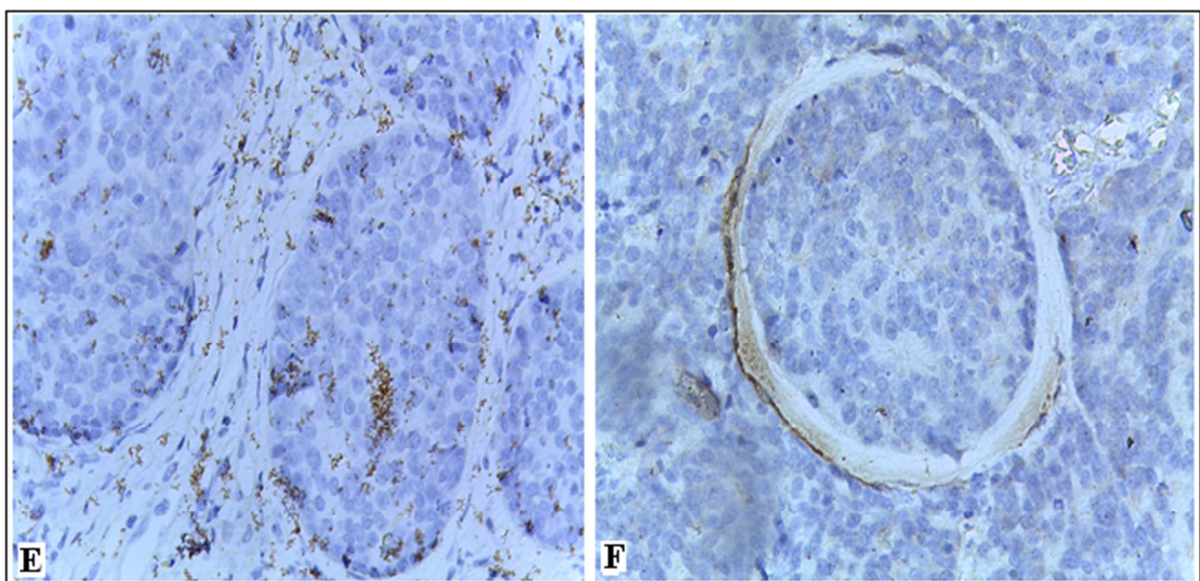
characteristics) and because of this, high-molecular-weight cytokeratins and RE have been used as markers of myoepithelial origin in tumors (Destexhe *et al.*, 1993; Griffey *et al.*, 1993; Yaziji *et al.*, 2000).



**Fig.2.**Immunohistochemical Staining of Myoepithelial Tumors (Mammary Sarcomas).Note dark brown positive staining of vimentin in myoepithelial cells and mesenchymal cells (C) but negative for p63 (D).Counter staining: Harris's Hematoxylin.

The cytokeratin AE1/AE2 monoclonal antibody is a combination of 2 monoclones (AE1 and AE2) and these antigens are expressed during epithelial cell differentiation in tumors. Cytokeratins are specific epithelial markers and the detection of their

expression in tumors has been widely used for specification of the epithelial origin of malignant cells (Bonnie, 2002; Bussolati *et al.*, 1986; Mallofré *et al.*, 2003).



**Fig.3.** Immunohistochemical Staining of cribriform simple Carcinoma: Note negative for CK5, 6 and RE (E and F). Counter staining: Harris's Hematoxylin.

In the present study, cytokeratins AE-1/AE-2 were detected in luminal epithelial and myoepithelial cells in all cases except in the spindle cell carcinoma case. This finding revealed that the spindle shaped cells have not a myoepithelial origin.

Vimentin is a 57 kDa intermediated filament protein, it is stated as an important diagnostic marker in the histogenesis of tumors cells and mesenchymal components (Van Houdt *et Hellmén*, 2005). In this study, vimentin was observed in mesenchymal cells and myoepithelial cells.

In previous study, p63 gene is expressed in the basal cells of several organs, including myoepithelial origin of mammary gland tumor. It is highly specific because neither stromal fibroblast nor vascular smooth muscle cells are not colored (Gama *et al.*, 2003). P63 antigen displays a nuclear staining pattern. While vimentin and AE1/AE3, CK20, CK5, 6, RE antigens display a cytoplasmic staining pattern. The positive results would show brown-stained as reported elsewhere (Gama *et al.*, 2003; Gärtner *et al.*, 1999).

### Conclusion

In the current study, the use of immunohistochemical markers (AE1/AE2, CK20, CK5, 6, RE, vimentin& P63) allowed distinguishing between canine mammary tumors of myoepithelial and lumino-epithelial cells lineage in female dogs. Based on these results, histopathological findings should be sustained by immunohistochemical markers before a definite diagnosis for canine mammary tumors.

### References

**Aydogan A, Metin N.** 2013. Detection of cell origin by immunohistochemistry in canine mammary tumours. *Revue de Médecine Vétérinaire* **164**(7), 395-399.

**Bonnie LK.** 2002. Immunohistochemical analysis of breast cells obtained by ductal lavage. *Cancer Cytopathology* **96**, 244-249.

<http://dx.doi.org/10.1002/cncr.10719>

**Bussolati G, Gugliotta P, Morra I, Pietribiasi F, Berardengo E.** 1986. The immunohistochemical detection of lymph node metastases from infiltrating lobular carcinoma of the breast. *British Journal of Cancer* **54**, 631-63.

**Destexhe E, Lespagnard L, Degeyter M, Heymann R, Coignoul F.** 1993. Immunohistochemical identification of myoepithelial, epithelial and connective tissue cells in canine mammary tumors. *Veterinary Pathology* **30**, 146-154.  
<http://dx.doi.org/10.1177/030098589303000207>

**Gama A, Alves A, Gartner F, Schmitt F.** 2003. p63: A novel myoepithelial cell markers in canine mammary tissues. *Veterinary Pathology* **40**, 412-420.  
<http://dx.doi.org/10.1354/vp.40-4-412>

**Gärtner F, Geraldés M, Cassali G.** 1999. DNA measurement and immunohistochemical characterization of epithelial and mesenchymal cells in canine mixed mammary tumors: Putative evidence for a common histogenesis. *Veterinary Journal* **158**, 39-47.  
<http://dx.doi.org/10.1053/tvj.1998.0333>

**Griffey SM, Madewell BR, Dairkee SH, Hunt JE, Naydan DK, Higgins RJ.** 1993. Immunohistochemical reactivity of basal and luminal epithelium-specific cytokeratin antibodies within normal and neoplastic canine mammary glands. *Veterinary Pathology* **30**, 155-161.  
<http://dx.doi.org/10.1177/030098589303000208>

**Karayannopoulou M, Kaldrymidou E, Constantinidis TC, Dessiris A.** 2005. Histological grading and prognosis in dogs with mammary carcinomas: Application of a human grading method. *Journal of Comparative Pathology*, **133**, 246-252.  
<http://dx.doi.org/10.1016/j.jcpa.2005.05.003>

**Karayannopoulou M, Lafioniatis S.** 2016. Recent advances on canine mammary cancer chemotherapy: A review of studies from 2000 to date. *Revue de Médecine Vétérinaire* **167**(7-8), 192-200.

**Mallofré C, Castillo M, Morente V, Solé M.** 2003. Immunohistochemical expression of CK20, p53, and Ki-67 as objective markers of urothelial dysplasia. *Modern Pathology* **16(3)**, 187–191.

<http://dx.doi.org/01.MP.0000056628.38714>

**Dias ML de M, Joanlise M, Leon Andrade, Márcio B. de Castro, Paula D. Galera.** 2016. Survival analysis of female dogs with mammary tumors after mastectomy: epidemiological, clinical and morphological aspects. *Pesquisa Veterinária Brasileira* **36(3)**, 181–186.

<http://dx.doi.org/10.1590/S0100736X2016000300006>

**Morrison WB.** 1998. Canine and feline mammary tumors. In: *Cancer in Dogs and Cats: Medical and Surgical Management*, WB. Morrison, Ed., Williams and Wilkins, Baltimore, Maryland, pp. 591–598.

**Perez A, Pena L, Del Castillo N, Nieto AI.** 2000. Factors influencing the incidence and prognosis of canine mammary tumors. *Journal of Small Animal Practice* **41**, 287–291.

<http://dx.doi.org/10.1111/j.17485827.2000.tb03203.x>

**Rao NAS, Van Wolferen ME, Gracanin A, Bhatti SFM, Krol M, Holstege FC, Mol JA.** 2009. Gene expression profiles of progestin-induced canine mammary hyperplasia and spontaneous mammary tumors. *Journal of Physiology and Pharmacology Suppl* **1**, 73–84.

**Toniti P, Sirivisoot S, Jandee P, Srimontri P, Puchadapirom P, Doungchawee G, Kasorndorkbua C.** 2010. AE1/AE3, Vimentin and p63 Immunolocalization in canine mammary gland tumours: roles in differentiation between luminal epithelial and myoepithelial lineages. *Asian Pacific Journal of Cancer Prevention* **11**, 227–230.

**Van Houdt L, Hellmén E.** 2005.

Immunohistochemical study of canine mammary gland tumors. Uppsala University Rudbeck Laboratory Department of Genetics and Pathology, Research Project, 29–55 P.

**Vos J.H, Van DenIngh TS, Misdorp W, Molenbeek RF, Van Mil FN, Rutterman GR, Ivanyi D, Ramaekers FCS.** 1993. Immunohistochemistry with keratin, vimentin, desmin, and  $\alpha$ -smooth muscle actin monoclonal antibodies in canine mammary gland: malignant mammary tumors. *The Veterinary Quarterly* **14**, 96–102.

<http://dx.doi.org/01652176.1993.9694382>

**Yaziji H, Gown AM, Sneige N.** 2000. Detection of stromal invasion in breast cancer: the myoepithelial markers. *Advances in Anatomic Pathology* **7**, 100–109.

<http://dx.doi.org/10.1097/00125480-200007020-00000>