

Comparative Study of S100 Marker Expression by Immunohistochemistry in Canine Melanic Tumors - Association with Macroscopic and Microscopic Aspects

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Abstract. We evaluated the expression of S100 ,compared routine immunostaining of 9 clinical specimens from melanoma cases,1 metastatic melanoma and melanocytoma using with staining for S100 as part of a diagnostic panel. All cases are positive with S100 (11/11-100%) Classification of intense of S100:9/11 (81.81%) had 51–80% positive cells, and 2/11 (18.19 %) had more than 80% positive cells. In melanoma cases the intensity of staining was high in 2/9 (33.33 %), over moderate in 7/9(77.77%), but, the staining was over moderate in the metastasis (79.84%) and in melanocytoma (54.94%). Nuclear staining was visible in 2 cases, and was staining visible in some cells and invisible in others in 5 cases and was no visible in 4 cases . All tumor cell types were reactivity for S100 .The staining in the majority of cases was heterogeneous in 8 cases, while 3 cases were homogenous. Epithelioid cells were a tendency for staining of moderate to intense between (63.83% to 98.1%). mixed epithelioid and spindle cells were a tendency for staining of moderate grade between (56.66% to 73.66%) Spindle cells were staining of moderate in (2/2) one of them is melanocytoma (54.06% - 54.94%) One balloon cells type melanoma with moderate. High percentages of marker stain accompany with medium of number of mitosis. Discussion: All cases were positive with S100.The percentages of marker stain were between (54.06% - 98.1%). All cases were between 3-4 grade that indicate to tend cells to stain .Melanoma cases distributed between moderate and intense stain while melanocytoma was moderate. Capable to stain nuclear with S100 was weak .The staining in the majority of cases was heterogeneous. Epithelioid cells were a tendency for staining of moderate to intense that indicate to capability these cells to stain with S100, Mixed epithelioid and spindle cells type,the balloon cells type and spindle cell type were a tendency for staining of moderate all cases tumoural cells were reacted diffusely in the cytoplasm.

Keywords: dogs, melanic tumors, S100

INTRODUCTION

Dermal melanomas account for 9–20% of skin tumors in dogs and generally follow a benign course. Melanomas are the most common malignant tumor of the oral cavity and digits in dogs (1, 4) at least 90% of these tumors are malignant (6) and many have metastasized by the time of diagnosis. However, few oral melanomas follow a benign course, and some dermal melanomas are malignant, making definitive prognostication for these tumors difficult. Melanocytes arise from embryonic neuroectoderm and, as such, retain the ability to differentiate into spindled or epithelioid cells, making a diagnosis of canine melanoma challenging in poorly differentiated amelanotic tumors. In these cases, determining the presence of constituent proteins whose expression is restricted to melanin-producing cells or

cells arising from neuroectodermal tissues can assist in the diagnosis. Previous studies documented the use of S100 to assist in the diagnosis of canine melanoma, but their potential prognostic significance was not examined in detail (8).

S-100 protein is a family of low molecular weight protein found in vertebrates characterized by two calcium binding sites of the helix-loop-helix ("EF-hand type") conformation. There are at least 21 different types of S100 proteins(5), S100a marker that used in my study is an isoform of a protein restricted to neuroectodermal cells. S-100 is normally present in cells derived from the neural crest (Schwann cells, melanocytes, and glial cells), chondrocytes, adipocytes, myoepithelial cells, macrophages, Langerhans cells, dendritic cells, and keratinocytes. It may be present in some breast epithelial cells.(2) S100 proteins have been implicated in a variety of intracellular and extracellular functions. Several members of the S-100 protein family are useful as markers for certain tumors and epidermal differentiation. It can be found in melanomas, (7) 50% of malignant peripheral nerve sheath tumors, schwannomas, paraganglioma stromal cells and clear cell sarcomas. S100 proteins have been used in the lab as cell markers for anatomic pathology (7).

The aim of this work is study of expression of S100 marker by immunohistochemistry in canine melanic tumors and comparative it with macroscopic and microscopic aspect these tumors.

MATERIALS AND METHODS

The database of our investigation was constituted of cadavers from the discipline of morphopathology and necropsy diagnostic, and also as samples sent from the surgery, clinic and private practitioners, for diagnostic purpose. From all cadavers and samples examined between 2001– 2010. were initially selected and reviewed to determine their suitability for the study. Cases with small samples or no tissue remaining were excluded. Those cases in which the morphologic diagnosis was not definitive were reviewed to establish consensus. This review process resulted in selection of 11 cases were diagnosed with 9 dog cutaneous melanomas and 1 melanocytomas and one metastasis melanoma in intestine for detailed study. The macroscopic study included : breed, age, sex, localization of tumor and size of tumor , and histological aspect was by formalin-fixed, paraffin-embedded tissue sections were used. Four-micrometer sections on slides and stained by Hematoxiline (Mayer's Hematoxiline : Dako) and eosine stain, for study aspect of cells, nuclei, nucleoli, type of tumor (benign, malignant) localization of the tumoural cells in tissue section, and others that were compared with Melan-A Marker by immunohistochemistry method.

For immunohistochemical method used paraffin-embedded tissue sections in positive charge slides were processed according this protocol: pretreatment with a steamer, heating the slides in antigen retrieval citrate buffer solution (target retrieval solution (10X) Dako Code S1699) at pH 6.0; then I used the primary antibody S100 (polyclonal Rabbit Anti-S100 Code No./Code/Code-Nr. Z 0311. Dako) was incubated for overnight at temperature 4 °C then secondary antibody , Streptavidin peroxidase Substrate-chromogen solution, (LSAB+System-HRP Edition 06/07 Code K0679, Code K0690) Dako With the same incubation times (30 minutes) at room temperature (RT) every one .In cases in which the amount of melanin obscured partial the immunologic reaction, tissues were counterstained with AzureB (3).

RESULTS AND DISCUSSION

In the period 2001 – 2010 in the Pathology Department, were diagnosed 9 dog cutaneous melanoma, 1 dog cutaneous melanocytoma and one metastatic melanoma in intestine. Macroscopical and microscopical aspect for these tumors and their legation with S100 immunoreaction see to (Table 1).

Tab. 1 :
Macroscopical and microscopical aspect for these tumors and their legation with S100 immunoreaction

Nr	Breed	Sex	Age	Tumoural localization	Diameter of tumors	Histologic diagnostic	Histologic type cells	Nr of mitosis	Zone of necrosis	Infiltrated limfocitar/40x >10	Activit y. junctional	Grade of clark	S100
80974	German Shorthaired Pointer	F	5	cutaneous - submandibular	2 cm	Dermal weak melanotic Melanoma	Mixed epithelioid and spindle type	53	reduced	reduced	No	4	56.66 %
81693	Doberman	M	9	Mandibular	6cm	Junctoinal dermal amelanotic melanoma	Epithelioid type	14	intense	intense	Yes	4	87.89 %
81783	tickle	F	7	Eye global	5 cm	amelanotic melanoma	Epithelioid type	7	moderate	reduced	No	4	63.83 %
81923	german sheperd	-	-	Lip	-	amelanotic melanoma	Spindle type	2	reduced	moderate	Yes	4	54.06 %
81928	-	-	7	Knee	-	Dermal amelanotic melanoma	Balloon cells	3	reduced	reduced	yes	4	69.86 %
81958	, Irish Setter	F	13	buccal cavity	3 cm	dermal weak melanotic Melanoma	Mixt epithelioid and spindle type cells	4	intense	reduced	yes	4	62.25 %
82351	tickle	F	11	Hock	2 cm	dermal weak melanotic Melanoma	Mixt epithelioid and spindle type cells	3	intense	intense	yes	4	73.66 %
81574	schnauzer	F	11	finger	¾ cm	Melanotic dermal melanoma	Epithelioid type cells	3	moderate	moderate	yes	4	66.6%
79539	common	F	10	Metastasis in intestine	13 cm	Metastasis melanic melanoma	Epithelioid type cells	2	reduced	intense	-	-	79.84 %
75688	-	-	-	knee	-	Amelanotic dermal melanoma	Epithelioid type cells	14	reduced	reduced	yes	4	98.1%
78773	<u>Metis</u>	F	6	Cutaneous-sacral	2 cm	dermal amelanotic Melanocytoma	Spindle type	3	reduced	reduced	No	4	54.94

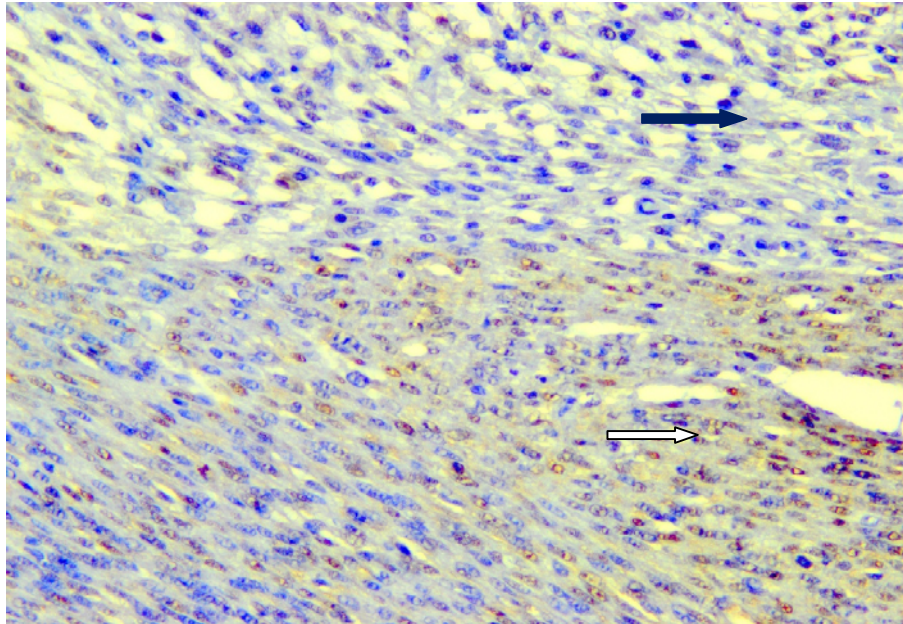


Figure 1.case Nr 78773 sarcal, cutaneous ; dog. Melanocytoma. Numerous melanoma cells are positive for S100 with a diffusion pattern of staining in zone (white arrow) but other zone is negative (black arrows) mixed visible and invisible stain nuclei Mayer's hematoxylin counterstain. 200X

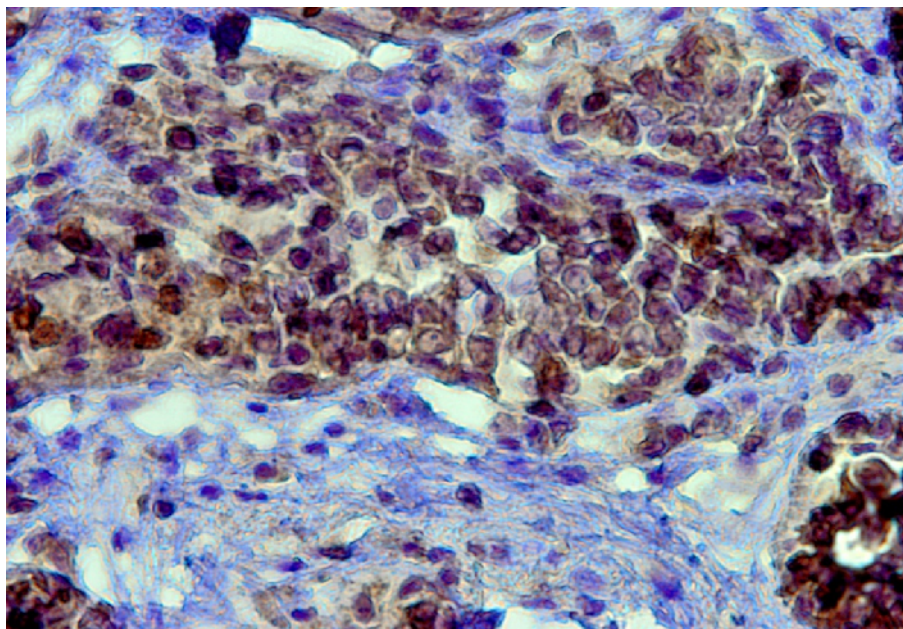


Figure.2 case Nr 76364: metastatic tumor ; dog. Melanoma. Numerous melanoma cells are positive for S100 with a diffusion pattern of staining stain and a visible stain nuclei with Mayer's hematoxylin counterstain. 400X.

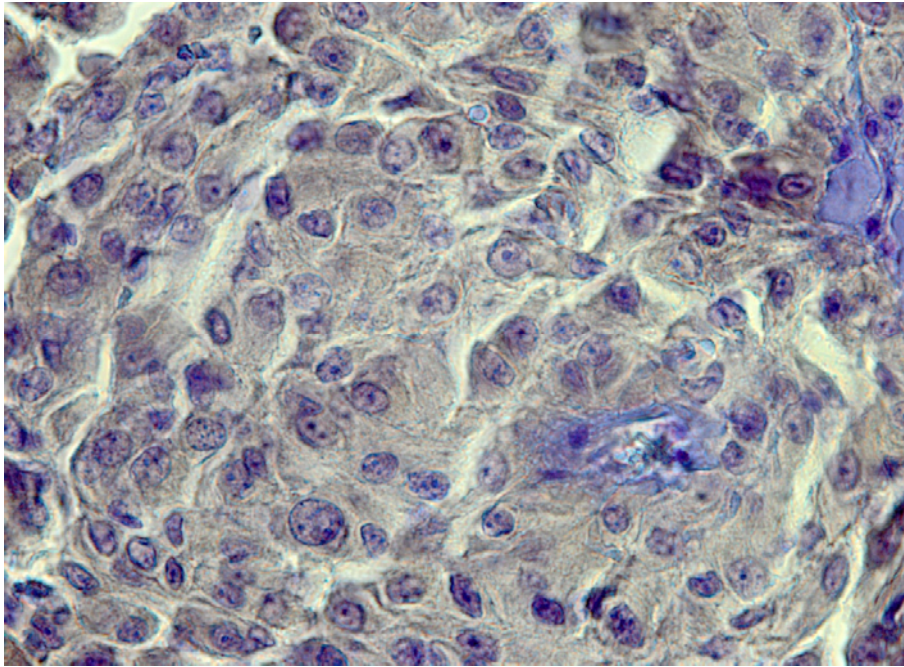


Figure 3.case Nr 81783 Eye global ; dog. Melanoma. Numerous melanoma cells are positive for Melan A with a diffusion pattern of staining, nuclear cells are not stain clearly, Mayer's hematoxylin counterstain. 400X.

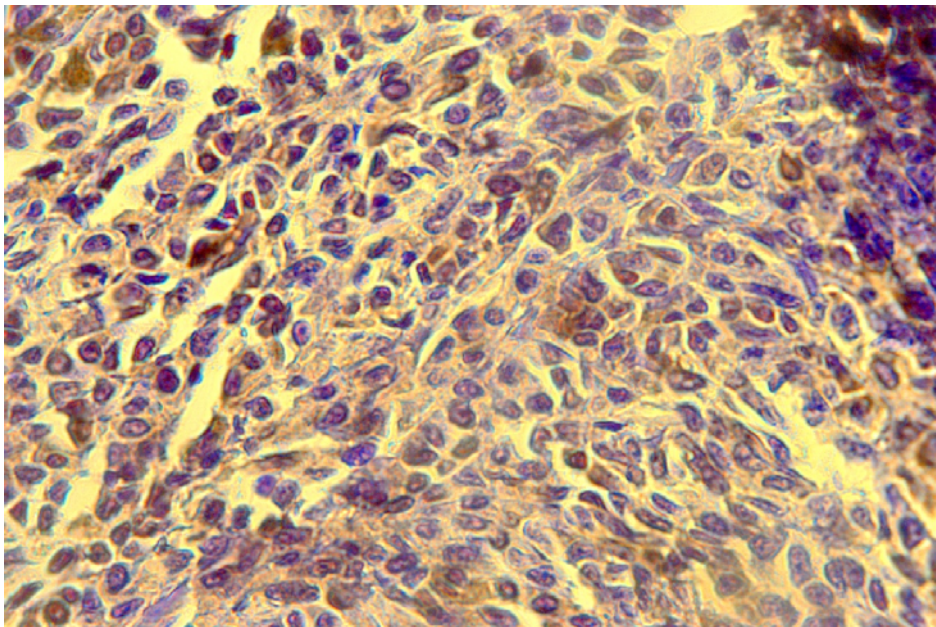


Figure 4.case Nr 80974 submandibular ; dog. Melanoma. Numerous melanoma cells are positive for Melan A with a diffusion pattern of staining in zone, mixed visible and invisible stain nuclei Mayer's hematoxylin counterstain. 400X

Immunoreactivity for S100 in melanomas.

S100 reactivity was demonstrated in 9 cases (100%) of the 9 canine melanomas examined, including. Metastases of S100 -negative primary sites was positive (1/1 cases), whereas 1/1 canine melanocytoma had positive staining for S100.

Classification of intense of S100 in these cases: zero (0%) had 5–10% positive cells, zero (0 %) had 11–50% positive cells, 9 cases (81.81%) had 51–80% positive cells, and 2 cases (18.19 %) had more than 80% positive cells. In canine cutaneous melanoma the intensity of staining was high in 2 cases (33.33 %) , over moderate in 7 cases (77.77%) , moderate in Zero (0 %) weak in 0 (0%) cases, and absent in 0 (0%) cases, but, the staining was over moderate in the metastasis (79.84%) and in melanocytoma (54.94%). Nuclear staining was visible in 0 canine amelanotic melanoma, and was staining visible in some cells and invisible in others in 2 amelanotic melanoma cases and was no visible in 3 amelanotic melanoma case but nuclear staining was visible in 1 canine melanotic melanoma, and was staining visible in some cells and invisible in others in 2 amelanotic melanoma cases and was no visible in 1 melanotic melanoma case, 1 melanocytoma case was no visible and one metastatic melanoma was staining visible in some cells and invisible in others. All tumor cell types demonstrated reactivity for S100 . The staining in the majority of cases was heterogeneous with areas not stained in 6 melanoma cases, 3 amelanotic and 3 melanotic, 1 metastatic melanoma, and 1 melanocytoma case while 3 melanoma cases were homogenous stain 1 melanotic and 2 amelanotic in the section, there was no obvious relationship between breed,sex,age, localization of tumors and diameter of tumors reactivity for S100. Epithelioid cells were a tendency for staining of moderate to intense between (63.83% to 98.1%) that indicate to capability these cells to stain with S100 .mixed epithelioid and spindle cells were a tendency for staining of moderate grade between (56.66% to 73.66%) .

Spindle cells were staining of moderate stain in (2/2) one of them is melanocytoma (54.06% - 54.94%) One balloon cells type melanoma with moderate stain with S100. In all cases tumoural cells were reacted diffusely in the cytoplasm with S100 and there aren't any cells with polar or punctuate in cytoplasm. High percentages of marker stain accompany with medium of number of mitosis, this observe in (2) cases.

CONCLUSIONS

- In the period 2001 – 2010 were diagnosed 9 dog cutaneous melanoma, 1 dog cutaneous melanocytoma and 1 metastatic melanoma in intestine.
- All cases were positive with S100 reactivity.
- The percentages of marker stain were between (54.06% - 98.1%)
- All cases were between 3-4 grade that indicate to tend cells to stain with S100 .
- Melanoma cases distributed between moderate and intense stain while melanocytoma was moderate.
- Capable to stain nuclear with S100 was weak in both amelanotic and melanotic melanoma The staining in the majority of cases was heterogeneous.
- There was no obvious relationship between breed, sex, age, localization of tumors and diameter of tumors reactivity for S100.
- All tumor cell types demonstrated reactivity for S100.
- Epithelioid cells were a tendency for staining of moderate to intense that indicate to capability these cells to stain with S100, mixed epithelioid and spindle cells type, the balloon cells type and spindle cell type were a tendency for staining of moderate stain with S100.
- All cases tumoural cells were reacted diffusely in the cytoplasm
- High percentages of marker stain accompanied with medium of number of mitosis.

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