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FOUR IS BETTER THAN ONE?

Evaluation and validation of an immunohistochemical panel for feline melanomas

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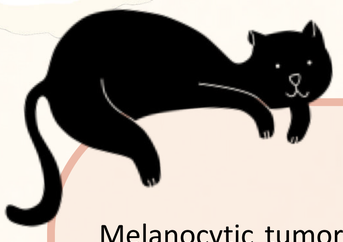
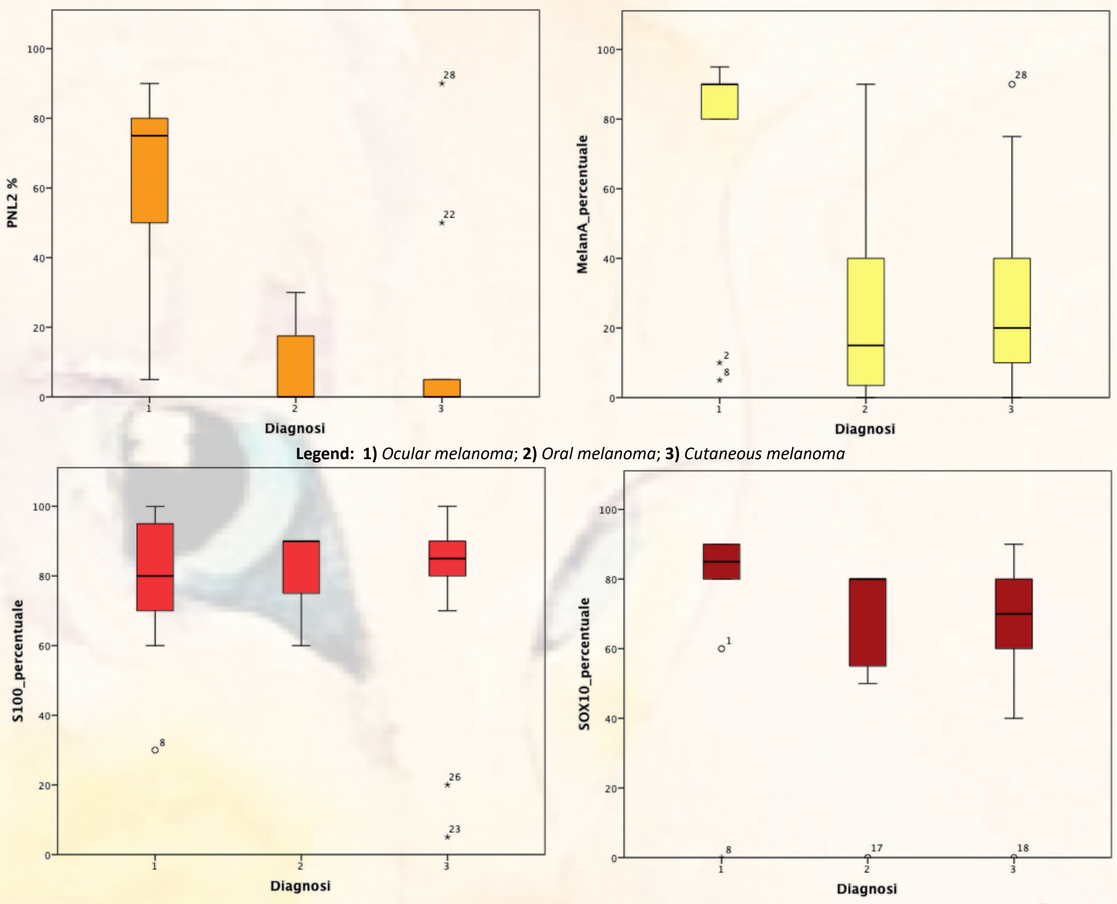


INTRODUCTION

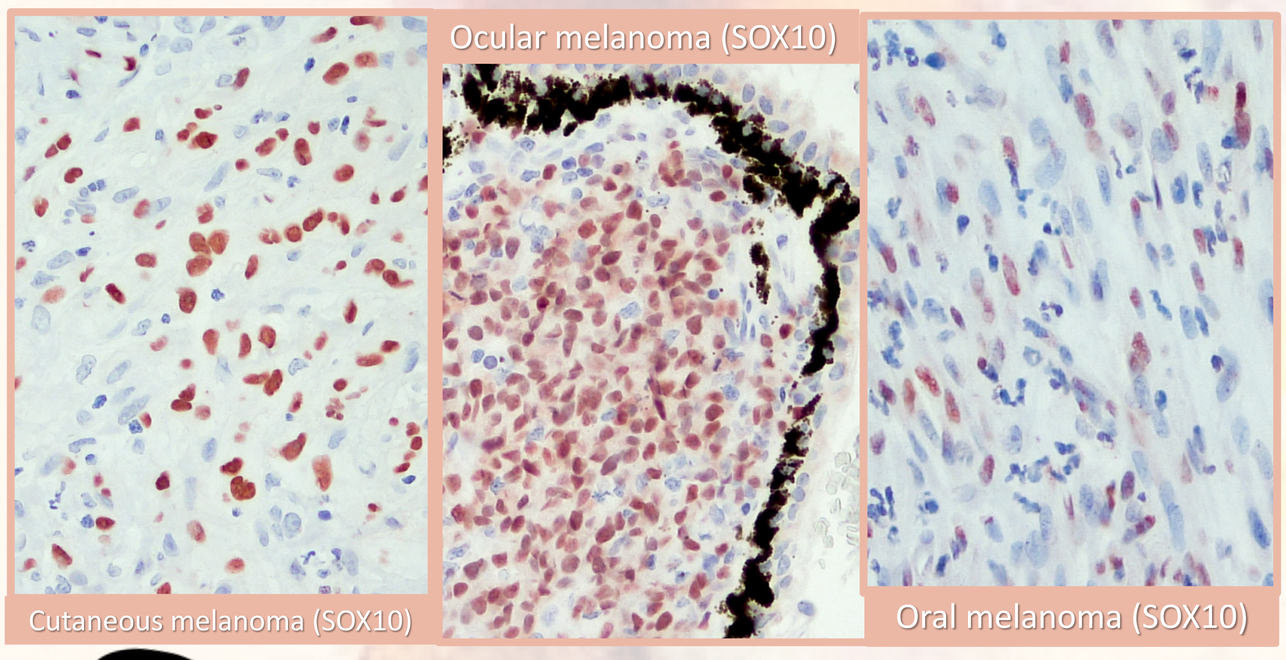
Melanocytic tumors are uncommon in cats, with a lower prevalence of melanocytomas than their malignant counterpart. Diffuse iris melanomas are the most common neoplasms, followed by those originating in the oral cavity and the skin [1].
Histologically, melanocytic neoplasia may have a variable melanin content and amelanotic tumors often represent a diagnostic challenge for the pathologist.
Currently, the definitive diagnosis of amelanotic melanomas relies on the use of antibodies such as **Melan-A, S100 and PNL2** [2], [3]. S-100 has been shown to have high sensitivity but low specificity, since other neoplasms may be immunoreactive to this marker.
Tyrosinase-related protein 2 (TRP-2), an enzyme involved in melanocyte differentiation, has been tested in dogs and found to be a promising marker for melanomas [4].
Moreover, in human medicine, **Sry-related HMg-Box gene 10 (SOX-10)**, a nuclear transcription factor involved in the differentiation of neural crest progenitor cells to melanocytes, has been validated for both primary amelanotic melanomas and their metastases [5].

AIM

The aim of this study is to **evaluate SOX10 and TRP-2 immunolabeling** in feline melanocytic tumors in comparison to other validated markers (Melan-A, PNL-2 and S100) to establish a valid IHC panel for diagnostic purpose. Moreover, for every case **histological features** were evaluated (percentage of necrosis, Breslow thickness, Clark level, lymphovascular invasion, mitotic index, nuclear atypia, cellular pleomorphism, histotype and pigmentation degree) to compared them with immunohistochemistal expression.



Legend: 1) *Ocular melanoma*; **2)** *Oral melanoma*; **3)** *Cutaneous melanoma*



DISCUSSION

In this study **S100** and **SOX-10** proved to be the two most performing marker for feline melanomas. However, S100 is expressed in nonneoplastic tissue derived from the neural crest, namely Schwann cells, glial cells and melanocytes, but also chondrocytes, adipocytes, myoepithelial cells, macrophages, dendritic cells and their relative tumors (for example, peripheral nerve sheet tumors, paraganglioma, schwannomas, meningioma, clear cell carcinoma). Thus, S100 is considered to have a low specificity for melanocytic neoplasms.
In humans SOX-10 is considered a *specific and sensitive marker for pigmented and amelanotic melanoma* [5], however also for this marker positivity for other types of neoplasia (alveolar rhabdomyosarcoma, myoepithelial/basal cell epithelial neoplasms, malignant peripheral nerve sheath tumors) have been documented, questioning its specificity. In veterinary medicine, **SOX-10** has been used recently in a few cases, but there are no articles that thoroughly investigates its performance in melanocytic neoplasms.
In this study, the specificity of this marker in feline tissues has been documented by the positivity in *nonneoplastic intraepidermal and follicular melanocytes*, being negative in other skin cells ; nevertheless, its specificity for melanocytic tumors still need to be proved by testing other kinds of neoplasia. We encourage the validation of this marker since it appeared to be more sensitive then PNL2 and, although the sensitivity was equal to the Melan A, the nuclear pattern made the assessment by the pathologist much easier.
Regarding histological features investigate, although ***Breslow thickness and Clark levels*** are not yet been investigated in feline melanoma as prognostic factors as in dogs, they may deserve further investigation.
In conclusion, even working with a limited number of melanocytic neoplasms, and given the lack of specificity assessment for this new marker, the results suggest that **SOX-10 should be included with PNL-2, MelanA and S100 as part of an immunohistochemical panel** in suspected cases of melanocytic neoplasm in cats to increase diagnostic power.

RESULTS

Thirty cases of feline melanoma were included in this study. Breeds were represented by DSH and Persian cats. The mean age at presentation was 8 years (±3,5 years), females (47%) and males (40%) were similarly represented. Diagnosis of 13 **cutaneous**, 7 **oral**, and 10 **ocular melanomas** was achieved by histological features (presence of pigmentation and cellular atypia) or, in case of amelanotic melanomas, positivity to at least one of specific melanocyte markers.
Analysis of the histological features evaluated showed a significant positive correlation between cellular pleomorphism and nuclear atypia, Clark level and percentage of necrosis were inversely correlated.
At immunohistochemistry, all the investigated melanomas demonstrated nuclear and cytoplasmic **S100** expression (100% positivity).
PNL-2 was found to be mostly expressed in ocular tumors, compared to cutaneous and oral neoplasms (with positivity of 90, 42, and 41%, respectively) and *negatively correlated* with the percentage of necrosis.
Melan A results as positive as S100 only in ocular melanomas (100%), while showed less positivity than S100, but more than PNL-2, for cutaneous (92%) and oral (85%) tumors. Furthermore, these two markers resulted *positively correlated* with the degree of tumor pigmentation and inversely related to Breslow thickness.
SOX-10 exhibited nuclear expression either in neoplastic cells and in the epidermal and follicular normal-appearing melanocytes. It showed 100% positivity in ocular melanomas and was less positivity in oral (85%) and cutaneous (92%) neoplasms; in all examined tumors the *expression was diffuse*. This marker showed a *significant correlation* with both PNL-2 and MelanA expression.
Epithelioid, mostly seen in ocular neoplasms, and spindle histotype were *significantly correlated* with PNL-2 and MelanA expression, respectively.
Any positivity was seen in neoplastic tissues by using **TRP-2 marker**, although in some samples nuclear positivity was occasionally detected in nonneoplastic intraepithelial melanocytes.

REFERENCES
[1] Smith et al. A Comparative Review of Melanocytic Neoplasms, Veterinary Pathololgy, 39:651–678, 2002. [2] Chamel G et al. Non-ocular melanomas in cats: a retrospective study of 30 cases, Journal of Feline Medicine & Surgery, 19:351–357, 2017. [3] Sabattini S. Evaluation of Ki-67 expression in feline non-ocular melanocytic tumours, BMC Veterinary Research, 14:309, 2018. [4] Smedley et al. Immunohistochemical Diagnosis of Canine Oral Amelanotic Melanocytic Neoplasms, Veterinary Pathology, 48:32-40, 2011. [5] Mohamed A et al. SOX10 Expression in Malignant Melanoma, Carcinoma, and Normal Tissues, Appl Immunohistochem Mol Morphol, 21:506-10, 2013.