

Spontaneous skin canine tumors: toluidine blue stain detection of mast cells in tissue section

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Abstract

Dog mast cell tumor (MCT) is common in dog. The etiology of canine MCTs is unknown, but it is probably multi-factorial. Its incidence is higher than it has found in human. There are demonstrated several common biological and clinical characteristics in both species. Cutaneous mast cells are located in the dermis and hypodermis. The objective of this study is to detect of MC on Toluidine Blue stained slides. There were examined 74 dogs of difference breeds and aged, from Tirana city. Six of them demonstrated the skin canine tumors. Skin samples were obtained from these animals. Macroscopic examination of the tumor revealed nodular ulcerated lesion with areas of necrosis and hemorrhage, accompanied with normal superjacent epidermis and annexes. Serial sections obtained from biopsy specimens were processed with toluidine blue staining pH 4.5, specific for MC identification. This study suggests that Toluidine blue, pH 4.5 stain may give a good information about skin tumors in dog, histologically with benign behavior.

Key words: cutaneous, mast cell, dog, neoplasm, toluidine blue

1. Introduction

Skin is a very extensive organ that covers the external surface of the animal body; therefore it represents a barrier between the internal and external environments [14]. Mast cells originate from the bone marrow and are normally found throughout the connective tissue and around blood vessels as normal components of the immune system. MCs are present in highest number in the skin, the lung and the gastrointestinal tract, etc, [2, 6]. There's a single nucleus, and numerous large and obvious granules in the cytoplasm. Mast cells can release several biologically active amines such as histamine and heparin, and lysosomal enzymes, which can be much damaged to the tissues [5, 16]. MC may act as a reservoir of pro-angiogenic factors, suggesting that they are correlated with MVD in normal and tumour cutaneous tissues [8]. The word mastocytoma (MCT) means stuffed nodular mass of mast cells (MC) in connective tissue of skin. Spontaneous mast cell tumors (MCT) are one of the most common cutaneous tumors in the dog, accounting for 7–21% of all canine skin tumors and with an incidence much higher than human ones [10, 12]. The breeds considered to be most susceptible are Boxer, Boston and Bull terriers,

and Labrador retriever, and the mean age of affected dogs is 8.5 years [7, 15]. Based on grade of the differentiation the dog cutaneous MCT are classified in MCT G1 (well differentiated), MCT G2 (intermediate), MCT G3 (poor differentiated or anaplastic) [9, 11]. The molecular events and etiology of MCT development and progression are not well clarified, but it is thought to be multifactorial [13, 12].

The specificity of several staining methods for mast cells provides the pathologist with a useful means for the differential diagnosis of mast cell tumors [4]. One of them is the toluidine blue stain. This staining method, as the basic color, showing that content of these granules is associated with the red color (metachromatic characteristic).

Although special stains have been used to diagnose canine mast cell tumors, a systematic comparison has not been done yet [1, 3].

The purposes of this study were to evaluate the presence of MC using the toluidine blue stain, in formalin-fixed tissues diagnosing mast cell tumors, and to determine if this technique would be helped in differentiation of their grade on spontaneous cases of skin tumors in dogs.

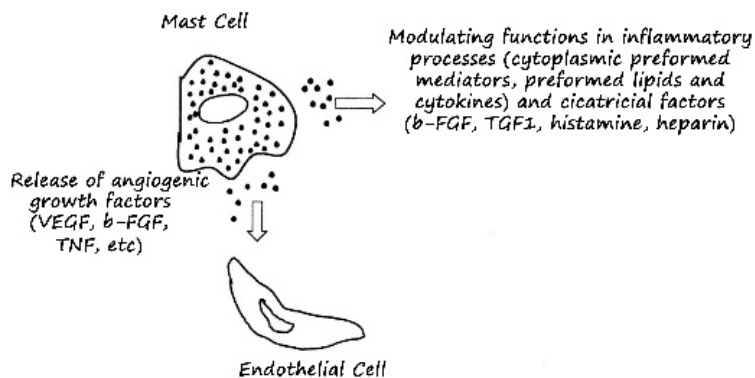


Figure 1. Schematic representation of mast cell multiple functions

2. Material and method

Specimens of cutaneous MCTs from dogs of different ages, breeds, and sexes, submitted to Small Animal Clinic at Faculty of Veterinary Medicine, Agricultural University of Tirana, Albania, were included in this study. The materials were collected also from some private veterinary hospitals and clinics of Tirana metropolis.

From seventy-four dogs under control, nineteen of them resulted with different skin tumors. Six of nineteen dogs with these pathologies resulted with mastocytomas. Samples of dog skin with mastocytomas were fixed in formalin 10 % and paraffin embedded. Five μm thick serial sections, for each sample, were cut. They were stained with toluidine blue, cytochemical method specific for MC identification, to evaluate the presence of them in tissue and around blood vessels. A dilute aqueous solution (<0.1%) of toluidine blue is made at pH 4.5 with dilute acetic acid. Slides are stained for 1 hour, rinsed quickly in water, differentiated in 70% alcohol, then dehydrated, cleared and cover slipped.

We made the observation under the MOTIC, BA 210 microscope, and the estimate of MC in these samples was carried out.

3. Results and Discussion

Spontaneous mast cell tumors (MCT) are one of the most common cutaneous tumors in the dog. There were examined seventy-four dogs of different breeds and aged, from Tirana city.

Six of them have demonstrated the MCT. Macroscopic examination of them revealed to nodular ulcerated lesions, with difference size dimensions and white – reddish on section, with areas of necrosis and

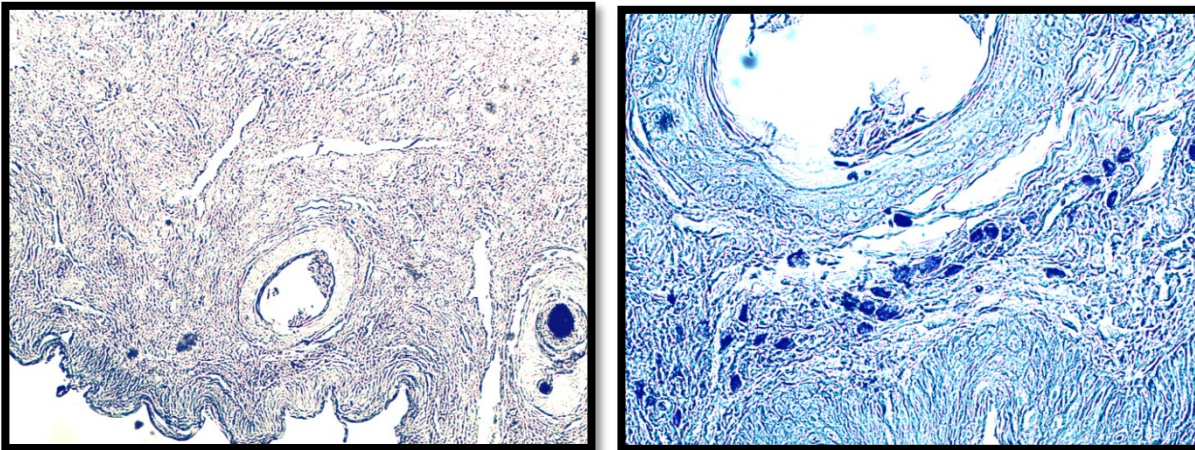
hemorrhagic. Each tumor was classified histologically according Patnaik with MCT grade G1 (well differentiation), grade G2 (intermediate differentiation) and MCT G3 (poor differentiated or anaplastic) [7]. Staining properties of mast cell tumors varied greatly and tended to be associated with the number and size of cytoplasmic granules and the degree of cell differentiation. The metachromatic reaction of the granules with toluidine blue dye allowed us to identify the MC in the skin tissue sections. The study of the distribution of MC in the skin becomes necessary to understand the pathogenesis of the diverse diseases that affect this tissue [3].

Well differentiated mast cell tumors were present in three dogs. The affected dogs were of different breeds (Husky, Pastor and Rottwieler), the animals aged between 8-15, and all of them were male. MCT were found in different locations and their size were between 3-7 mm. Macroscopically they were presented like skin masses of solitary hairless lesions, strong consistence, red color and round shape. The masses were surrounded to a connective tissue capsule. The mast cells from well differentiated cutaneous tumors were regularly round with low cellularity and a lot of cytoplasmic granules (figs. 3 and 4). They were found especially out around blood vessels (Fig. 4). In grade I tumor (well differentiated) there is a lower number of MC full of cytoplasmic granules. Those cutaneous tumors are benign and they are the best type of the mast cell tumors.

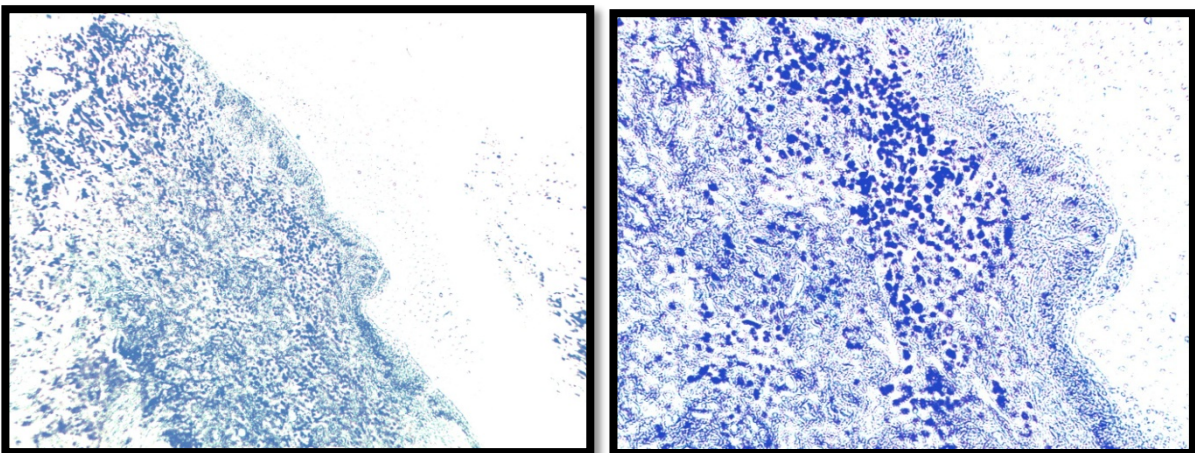
Three others samples were found out in dogs of different breeds (Rottwieler, Doberman Pince and Spits). The animals aged between 9-13, and of different sexes. These tumors were located on the trunk and the limbs, their size were between 4.7–6 cm

in dimensions. Macroscopically, the masses had appearance of a classic button-shaped, and hairless nodule.

Skin mast cell tumors of these animals were presented by highly cellular of MC and poorly differentiated (G3) grade (fig. 4 and 5).



Figures 2 and 3: Mast cell tumor (well differentiated). A) Magnification X100 stained with Toluidine Blue. Mast cells demonstrated cytoplasmic methachromasia. B) X400.



Figures 4 and 5: Mast cell tumor (poorly differentiated) in papilla of the dermis. A lot of mast cells are presented on connective tissue of dermis, A) x100 and B) x400

In poorly differentiated tumors, the cytoplasm of mast cells stained the same color as other cells. In this case, they have a few or no cytoplasmic granules (fig. 5). This stain is particularly helpful when we need to evaluate animals with pathological conditions that involve mast cell tumors.

This study found that classification of skin tumors as well differentiated and poorly differentiated provided the good prognosis information for MCTs.

4. Conclusion

This study represents a first research on the identification of canine mast cell tumors, which was committed with the specific method of toluidine blue. Our results, suggest a value information about macroscopic and microscopic characteristics of mast

cell tumors in dogs based on the grade of differentiation and the different color technics.

This study suggests that Toluidine blue stain may give a good information about skin tumors in dog, histologically with benign behavior.

5. References

1. Becker AL, Chung KF, MacDonald DM: **Mast cell heterogeneity in dog skin.** *Anat Rec* 1985, 3(12):477-480.
2. Caughey GH: **The structure and airway biology of mast cell proteinases.** *Am J Respir Cell Mol Biol* 1991, 4:387-394.
3. Kiupel M, Webster J.D, Kaneene J.B, Miller R.A, Yuzbasiyan-Gurkan V: **The use of kit and tryptase expression patterns as prognostic tools for canine cutaneous mast cell**

- tumors.** *Veterinary Pathology* 2004, **41**(4): 371–377.
4. Lillie RD, Fullmer HM: **Histopathology technic and practical histochemistry**, 4th ed, 1976, 657, 671-678. McGraw-Hill, New York, NY.
 5. Michel G. M, Knapp D. W, Denicola D. B, Glickman N. and Bonney P: **Prognosis Following Surgical Excision of Canine Cutaneous Mast Cell Tumors with histopathologically Tumor-Free versus Nontumor-Free Margins: A Retrospective Study of 31 Cases.** *Journal of the American Animal Hospital Association* 2002, **38**(5): 458-466.
 6. Page C, Foreman JC: **Introduction to mast cells and basophils, in JC PCF (ed): Immunopharmacology of mast cells and basophils.** *Academic press*, 1993, 1-4
 7. Patnaik AK, Ehler WJ, Macewem E G: **Canine cutaneous mast cell tumor: morphologic grading and survival time in 83 dogs.** *Vet Pathol* 1984, **21**(5):469–474.
 8. Ranieri G, Passantino L, Patrulo R, Passantino G, Jirillo F, Catino A, Mattioli V, Gadaleta C, Ribatti D: **The dog mast cell tumor as a model to study the relationship between angiogenesis, mast cell density and tumor malignancy.** *Oncol. Rep.* 2003, **10**(5), 1189-11
 9. Scase T.J, Edwards D, Miller J, Henley W, Smith K, Blunden A, Murphy S: **Canine mast cell tumors: Correlation of apoptosis and proliferation markers with prognosis.** *Journal of Veterinary Internal Medicine* 2006, **20**(1): 151–158.
 10. Thamm D.H, Vail D.M: **Mast cell tumors.** In: Withrow and MacEwen's *Small Animal Clinical Oncology*, Fourth Ed. Saunders Elsevier, St. Louis 2007:402–424.
 11. Turin L, Acocella F, Stefanello D, Oseliero A, Fondrini D, Brizzola S, Riva F: **Expression of c-KIT proto-oncogene in canine mastocytoma: A kinetic study using real time polymerase chain reaction.** *Journal of Veterinary Diagnostic Investigation* 2006, **18**:343–349.
 12. Vail D.M: **Mast cell tumors.** In: *Small animal clinical oncology*, ed. Withrow SJ, MacEwen EG, 2nd (ed). 1996, 192–210. WB Saunders Co, Philadelphia, PA.
 13. Walder EJ, Gross TL: **Canine mast cell tumors.** In: *Veterinary dermatopathology-a macroscopic and microscopic evaluation of canine and feline skin disease*, (ed). Gross TL, Ihrke PJ, Walder EJ, 1992: 470–473.
 14. Walsh L J, Lavker R M, and Murphy G F: **Biology of disease: determinants of immune cell trafficking in the skin.** *Lab Invest* 1990, **63**:592-600.
 15. Webster J, Yuzbasiyan Gurkan V, Miller R, Kaneene J, Kiupel M: **Cellular proliferation in canine cutaneous mast cell tumors: Associations with c-KIT and its role in prognostication.** *Veterinary Pathology* 2007, **44**(3):298–308.
 16. Weisse V. M. D. C. and Sorenmo C. M. V. K: **Recurrence Rates and Sites for Grade II Canine Cutaneous Mast Cell Tumors Following Complete Surgical Excision.** *Journal of the American Animal Hospital Association* 2002, **38**(1):71-73.