

## Features of the clinical manifestation of the cutaneous form of mastocytoma in dogs

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### Abstract

According to foreign researchers, mastocytoma is one of the most common (7–12 %) skin tumors in dogs. The study aimed to study the features of the clinical manifestation of cutaneous mast cells according to the criteria of the clinical TNM classification. We analyzed 24 cases of cutaneous mastocytoma in dogs during 2016–2020, of diagnostic and therapeutic measures in the Department of Surgery and Clinic of the Small Pets Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv. According to the criteria of the international clinical TNM classification at the time of admission to the clinic in 7 (29.2 %) dogs established stage I of the tumor, 10 (41.7 %) – II, 6 (25 %) – III, and 1 (4.1 %) animals – stage IV. Based on cytological evaluation of biopsy material selected from tumors and regional lymph nodes of low-grade mastocytes, 17 (70.8 %) animals were found, and in 7 (29.2 %) cases, neoplasia was characterized as high-grade.

**Keywords:** dog, mast cell tumor, clinical signs, cytological examination, mast cell classification.



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## 1. Introduction

Mast cell tumors (MCTs) of skin neoplasms, the proportion of mast cells can be 7 to 21 % MCTs are characterized by excessive proliferation of atypical mast cells. Also, by their activation and degranulation (mast cell activation syndrome), active substances, mediators, and specific IgE antibodies. Under such conditions, the growth and development of mast cells are usually masked by inflammation, diffuse edema, allergic reactions, and other pathological processes that cause a variety of appearances and symptoms of these neoplasms and cause periodic fluctuations in tumor size. It is also noted that the clinical course of mastocytoma is usually accompanied by periods of exacerbation and remission, so it is difficult to assess biological behavior and predict the prognosis. According to the generalized results of studies for identifying MCTs in dogs, cytological examination of the biopsy obtained by fine-needle aspiration tumor biopsy (FNA) is sufficient. However, the final diagnosis of mastocytoma and its classification according to the degree of malig-

nancy and the appointment of adequate treatment, prediction of biological behavior, and survival of animals are based on the results of histological examination (Thompson et al., 2010; Blackwood et al., 2012; Camus et al., 2016; Hergt et al., 2017).

A meta-analysis of current scientific sources shows that due to advanced technologies to study MCTs' molecular genetic and immunobiological properties, many researchers have identified many changes and atypicalities in subcellular structures that may indicate malignant neoplasia. However, as the present shows, approaches to the pathomorphological criteria for mast cell verification and their evaluation systems are constantly being refined and improved and are often the subject of debate. Thus, for a long time in veterinary oncology, the three-stage histological classification of MCTs by Patnaik et al. (1984). According to this classification, mastocytomas are divided into high- (G1), moderate- (G2), and low-differentiated (G3). The most characteristic morphological features of grade I mast cells are considered, as a rule, their limited skin location the presence of well-

differentiated with noticeable metachromatic cytoplasmic granules. At the same time, low degree of cell differentiation, aggressive growth, moderate or high mitotic activity, mitotic atypia, the presence of single or no cytoplasmic granules are the main signs of grade III neoplasia. Grade II mastocytomas are an intermediate variable between the two previous groups in this context. Accordingly, when assessing the potential biological behavior of MCTs, the highest probability of survival is inherent in animals with the established category G1 and the worst prognosis (survival up to one year) for G3. According to numerous researchers, neoplasia with moderate differentiation (G2) is the most problematic in predicting their biological behavior, as pathomorphologists may include neoplasms of both G1 and G3 in doubtful or vague histological signs. Therefore, it is difficult to predict the aggressiveness of the further development of such tumors.

In 2011, a team of scientists from different countries proposed a two-stage classification of MCTs, dividing mastocytomas into high-grade and low-grade. According to researchers, the two-stage classification (Kiupel et al., 2010) eliminates the prognostic uncertainties of the Patnaik system, has high predictive value, minimal variability, and high rate (96.8 %) consistency in pathomorphological assessment. However, it does not provide 100 % accuracy in predicting the biological behavior of MCTs. In recent years, many papers have been published indicating that the histological method of cell phenotyping should be considered the basis for the final diagnosis and classification of neoplasms. However, regardless of the evaluation system used, the prognosis should be based on a combined approach. According to the literature, prognostic information should take into account clinical signs and anatomical location of the tumor (Thompson et al., 2010; Garrett, 2014), growth rate, size, general appearance (Mullins et al., 2006; Pierini et al., 2019), clinical-stage (Stefanello et al., 2015; Sabattini et al., 2015; Warland et al., 2015), recurrences, metastases (Ferrari et al., 2018; Horta et al., 2018; Cruz et al., 2020), immunohistochemical and molecular markers (Sabattini et al., 2015; Sledge et al., 2016; Kiupel & Camus, 2019).

Based on this, **our research aimed** to establish the features of the clinical manifestation of the cutaneous form of mastocytoma in dogs.

## 2. Materials and methods

The study included 24 dogs with mastocytoma of the skin. During 2016–2020 underwent an entire course of diagnostic and therapeutic measures in the Department of Surgery and Clinic of the Small Pets Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv. The animals were of different breeds and genders, aged 4 to 16 years. Upon admission to the clinic, cancer patients were examined according to clinical (TNM) classification of tumors (Owen, 1980), using clinical, instrumental, and laboratory research methods.

The cytological examination was performed to verify neoplasia at the stage of preoperative examination. Selection of material from tumor-like skin formations and regional lymph nodes was performed by fine-needle biopsy (FNA) in two variants. When the puncture was performed by “blind” method and by targeted biopsy under ultrasound control “ESAOTE MyLab 40” 5.0–7.5 and 15 MHz, respectively).

The 2-3 smears were made from the obtained aspirates, dried in the air, fixed with methyl alcohol for 5 minutes, stained according to Romanovsky-Gimza, and studied under a microscope using an immersion system. The criterion for verifying mastocytoma was detecting homologous round tumor cells with azurophilic granules in the cytoplasm in cytological preparations.

All dogs, after cytological confirmation of MCTs, underwent surgical treatment. Removal of tumors was performed under general anesthesia following the rules of ablastics and antiblastics.

## 3. Results and discussion

During 2016–2020, diagnostic tests and treatment of 24 dogs (13 dogs and 11 bitches, different breeds, aged 4 to 16 years) with skin mastocytoma were performed. In 22 (91.7 %) studied dogs, neoplasia was single and only in 2 (8.3 %) cases – multiple nodular lesions. The most common sites of MCTs were: torso (45.8 %), limbs (37.5 %), less common head and neck, axillary, inguinal, and perineal-perianal areas (16.7 %). At the initial examination, at the time of admission to the clinic, among these animals found stage I of the tumor process (T1aN0M0, T1aN1aM0) in 7 (29.2 %) dogs, 10 (41.7 %) animals – II (T2aN0M0, T2aN1aM0, T2aN1bM0, T2bN1bM0), in 6 (25 %) – III (T3aN0M0, T3aN1bM0, T3bN1aM0) and in 1 (4.1 %) animals – stage IV (T3b, in N1bM1). A detailed analysis of the epidemiological situation, the results of clinical trials of dogs, and the dynamics of development of MCTs in them was presented by us in a previous study (Ivashkiv et al., 2020). However, due to the importance of establishing a pathomorphological diagnosis of clinical data: symptoms of neoplasia, its location, growth rate and doubling of tumor size, germination in subcutaneous tissue, lesions and spread (metastasis) to regional lymph nodes, internal organs, etc., we in this the article focuses only on clinical trial data. In particular, in the studied dogs, stage I neoplasia had the appearance of a small, dense, well-demarcated, mobile tumor node located in the thickness of the skin. Stage II tumors were 3–5 cm in size, and stage III tumors were 6–10 cm or more. In some dogs, eczema-like skin lesions similar to nodular panniculitis occurred. In 10 (41.7 %) of the studied animals, the growth rate of neoplasms was extremely rapid because, in  $56.5 \pm 1.91$  days, the tumors doubled in size. In 7 (29.2 %) dogs, the doubling time of the primary tumor reached, on average, four months ( $122.1 \pm 10.6$  days), and in 5 (20.8 %) dogs, this period lasted for two years. Information on tumor development in 2 (8.3 %) dogs was unknown. For most mast cells, in contrast to other solid tumors, the increase in their size was provoked, as a rule, by episodic inflammation and edema in the tumor and surrounding tissues, which is associated with mast cell activation and degranulation syndrome. And to a lesser extent, the growth of tumor mass. It was noted that the rapidly progressing course of the disease was characteristic of mast cells localized in the pelvic limbs, groin, perineum, neck, namely areas freely available for permanent injury and, above all, self-injury (combing, licking, gnawing). Many researchers also report the aggressive development of MCTs with rapid metastasis to regional lymph nodes with the localization of lesions in the muzzle, including the lips, perineum, foreskin, scrotum

The results of the study of the local process showed that in more than 87 % of cases, the characteristic signs of mastocytoma were: swelling and inflammation in the tumor and surrounding tissues, alopecia, erythema and skin ulcers, periodic fluctuations in tumor size, pain in palpation, itchy skin in the tumor area. Examination of regional lymph nodes in 11 (45.8 %) dogs with cancer did not reveal any changes in the latter. In 8 (33.3 %) animals, the regional lymph nodes are slightly enlarged but not painful, mobile, not compacted, with a smooth surface. At the same time, in 5 (20.8 %) dogs with tumors of considerable size (more than 10 cm in diameter), lymph nodes were 2–3 cm in size, passive, dense consistency. In one of these animals, the regional lymph node palpation was complicated by its growth and fixation to the surrounding tissues.

Following the generally accepted recommendations for verifying neoplasms at the stage of preoperative examination, fine-needle biopsy (FNA) of tumors and regional lymphatic and cytological examination of aspirates was performed. It was noted that the result of such a study largely depends on the quality of the biopsy. Unlike other skin tumors, the clinical manifestation of mastocytoma is often accompanied by inflammation, diffuse edema, and other pathological processes, which usually cause structural changes in the tumor site and surrounding tissues and create

difficulties in the biopsy. According to the results of comparative studies of biopsy material obtained by the "blind" method of fine-needle biopsy and target biopsy performed under ultrasound control (Fig. 1), we have substantiated the significant advantages of the latter. It was found that more than 37 % of biopsies obtained by "blind" biopsy were unsuitable or uninformative for cytological studies due to insufficient or poor (unpresentable) cellular content of the obtained material, and this required a repeat procedure.

Furthermore, such a biopsy poses a serious risk of dissemination of tumor cells in cases of penetrating the tumor node or getting a needle into a vessel. The use of sonography allowed to establish the boundaries, shape, and size of the tumor, its echotexture, tissue perfusion (Doppler). Accordingly, it provides visual control of the targeted selection of material from specific foci of neoplasia. In addition, sonography also provided additional clinical information on the state of neoplasia in real-time. For example, sonographic visualization of solid hypervascular foci with a central type of vascularization, against the background of diffuse infiltration of neoplasia into the subcutaneous tissue, with a pronounced perinodular inflammatory response of surrounding tissues is evidence of mastocytoma malignancy. Undoubtedly, such data are essential to the cytological evaluation of the biopsy and interpretation of the diagnosis.



**Fig. 1.** Performing a targeted fine-needle biopsy under ultrasound control

Thus, it can be noted that despite the more painstaking preparation and time spent on fine-needle biopsy under ultrasound control, this manipulation allowed us to obtain quality material for cytological verification of mastocytoma.

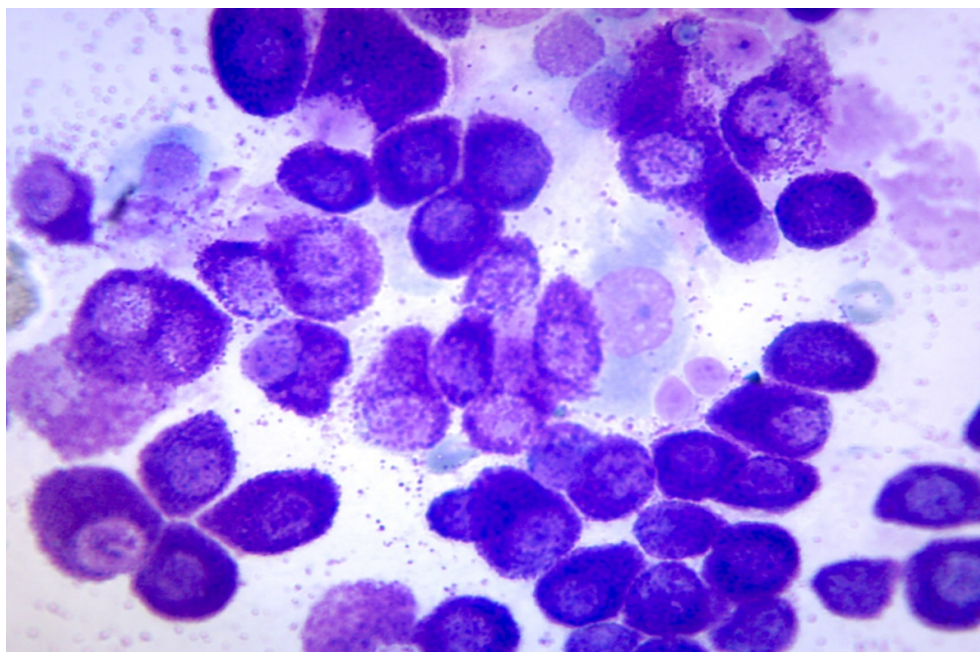
According to numerous literature sources, screening for verification of mastocytoma is the detection of atypical mast cells by cytological examination of tumor biopsies. Thus, our study's establishment of the primary diagnosis was based on the cytological identification of mast cells of large round cells with purple granules in the basophilic cytoplasm. It should be noted that the cytological study also focused on the morphological structure of mast cells. In particular, they were evaluated for intracytoplasmic granules, nuclear pleomorphism, mitotic figures, bi- or multinucleated, and anisocariasis. The two-level classification criteria according to the Kiupel system, according to which MCTs are divided

into low- and high-malignant tumors. The granularity of the cytoplasm was assessed as intensely granular and lightly granular, sometimes mixed (with the simultaneous presence of the first and second). Nuclear pleomorphism was assessed as present if no rounded nuclear forms were present and absent if only rounded or ovoid forms were noted. Collagen fibrils, mitotic figures, and binuclear or multinucleated cells were assessed as present or absent. Anisocariasis was defined as >50 % of nucleus size fluctuations. According to the research results, the above morphological parameters are easily identifiable by cytological evaluation of aspirates. They have high specificity (94–97.3 %) for histological evaluation of mastocytes and are essential for developing treatment strategies and tactics.

For MCTs of low malignancy, the most typical cytological feature was detecting round or oval mast cells, 1.5–3

times larger than neutrophils, which were located in groups with different cell numbers (Fig. 2). The nuclei of such mast cells are round or oval, clearly delineated. Visualization of the nucleus is problematic because it is poorly stained and covered with granules that absorb large amounts of dye and shade it. The cytoplasm of most cells is densely filled with dark purple granules, as a result, such cells had an intense blue-violet color. At the same time, cells with a lower content of granules in the cytoplasm acquired a light purple hue. Single binuclear and giant mast cells were rarely found in the smears, and single cells with high magnification (400 times) were found in the cytograms of individual tumors in a state of mitosis.

For cytograms of MCTs, the high degree of malignancy (Fig. 2) was characterized by visualization in the studied smears of atypical mast cells located in dense clusters or layers. The cytoplasm of most cells with fuzzy contours and low content of light purple granules. In some cells, intracytoplasmic granules are absent altogether. Cells with irregularly shaped nuclei and binuclear and, less frequently, multinucleated mast cells were often found in the smears studied. At the high magnification of the microscope in some cytological smears, the number of detected mitoses could reach five figures.



**Fig. 2.** Cytogram of mastocytoma with a low degree of malignancy. Clusters of cells of homogeneous structure. Mixed-grained mast cells (most cells are highly granular and fewer less granular) with minimal anisocariasis; According to Romanovsky-Gimza. EP. 10, lens 100



**Fig. 3.** Cytogram of mastocytoma of high malignancy. Mast cells with a small number of intracytoplasmic granules and slight anisocariasis; nuclear pleomorphism - the presence of non-rounded nuclear forms (1); the presence of dinuclear (2) or multinucleated cells. According to Romanovsky-Gimza. EP. 10, lens 100

It is now known that an important prognostic indicator for the diagnosis and evaluation of the biological behavior of MCTs is the detection of metastases in regional lymph nodes and internal organs. Based on the research results, cytological criteria for diagnosing lymphogenic metastases have been developed. Many researchers have found that metastasis to regional lymph nodes in dogs with cutaneous MCTs correlates with reduced survival time and a high risk of spreading to remote areas. Reports at the stage of preoperative examination for neoadjuvant therapy and lymphadenectomy, or not. According to the results of our studies, a correlation was established between the clinical manifestation of MCTs and cytology data was found. Cytological evaluation of aspirates of regional lymph nodes from seven dogs and lymphoid cells and neutrophils revealed an accumulation of atypical polymorphic mast cells in the form of nests of 3–5 cells, which is evidence of tumor metastasis. According to the clinical (TNM) classification in all these dogs, stage III and IV tumors were large, and regional lymph nodes were enlarged. Cytological smears of eight dogs with clinical stage I and II neoplasia showed many neutrophils and lymphoid elements: large lymphocytes, prolymphocytes, lymphoblasts and, at the same time, the presence of single mast cells. Considering the physical examination data, ulcers on the skin surface in the tumor area, lymphadenomegaly, it was possible to predict reactive lymphadenitis. Cytological examination of aspirates from lymph nodes that were not enlarged pathomorphological changes was not found.

#### 4. Conclusions

According to the results of preoperative clinical examination of 24 studied dogs and cytological evaluation of biopsy material selected from tumors and regional lymph nodes of low-grade mastitis, 17 (70.8 %) animals and 7 (29.2 %) cases of neoplasia were characterized as high-grade.

#### Conflict of interest

The authors declare that there is no conflict of interest.

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