

## Use of Markers Ki- 67, CD3 and CD79a as Immunohistochemistry's Technique for Classification of Canine Lymphoma - Case Report

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**Abstract:** *Lymphomas, cancers originating in components lymphopoietic, are very common in dogs. The most used diagnostic methods for canine lymphoma are histopathology and immunohistochemistry. Immunohistochemistry is a technique employing labeled antibodies able to identify antigens "in situ"; which score in this type of neoplasm and the degree of cell proliferation. The most commonly used markers for canine lymphoma are B lymphocytes (CD79a), T (CD3) lymphocytes and cell proliferation marker Ki -67. This paper reports a case of a dog, the English Bulldog breed, which was diagnosed lymphoma, and by immunophenotyping showed positive to negative for B (CD79a) T lymphocytes (CD3) and lymphocytes, and demonstrated a high degree of malignancy based the pattern of cell proliferation by Ki -67. Histological classification type of cell pattern was seen centroblastic representing high-grade malignancy.*

**Keywords:** *Canis familiaris, dog, neoplasia, immunophenotyping.*

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### I. Introduction

Lymphomas are cancers more frequent in the canine specie. Actually it is necessary to have an evaluation, in addition to the morphological and immunophenotypic classification, as well as the index of cell proliferation [1-4]. Its etiology is multifactorial and, therefore, well questioned by you may not be able to isolate the etiologic agent as in cats, usually infected with feline leukaemia virus [2,5]. The canine lymphomas are classified in a multicenter, alimentary, mediastinal or thymic, cutaneous and extranodal or lonely, being the way the multi more common [2,6,7]. The clinical finding more important in dogs with lymphoma multicenter is the generalized lymphadenopathy of superficial lymph nodes and cutaneous masses [7]. The cell proliferation related cancers, has been widely studied by show good correlation with the biological behavior of these tumors in addition to contributing to the orientation of the treatment and prognosis of the disease [4].

In the cutaneous form nodules occur solitary or multiple in the skin with higher incidence in animals of advanced age, and may also occur in the oral mucosa, in the form of nodules or erythematous plaques, and cause metastases in other organs such as the lymph nodes, spleen, liver and bone marrow [6]. The cutaneous form is classified into epitheliotropic lymphoma or not epitheliotropic lymphoma [8]. The radiographic examination and ultrasound, dogs with lymphoma present with clarity, increased volume of lymph nodes dissection, sub-injury to the spleen and liver. Studies highlight that dogs with lymphoma in multicentric have enlarged lymph nodes chest and diffuse pulmonary infiltrate that are clearly visible on the radiograph [9,10].

The diagnosis of lymphoma can be done from the assessment of samples taken by needle aspiration (FNA) or by puncture with fine needle (PAF) of lymph nodes or other tissues affected [11]. According to the literature, most cases of lymphoma multicentric study were diagnosed correctly by means of cytology, whose advantages are numerous as quickly and efficiently diagnosis, do not have a collection technique, be of low cost, becoming an extension of the physical examination [11-14]. In man and in dogs there are increasingly more studies that employ the PAAF as a technique for the diagnosis of lymphoma [14]. Two systems of histopathological classifications of human lymphoma were adapted to better characterize the canine lymphoma: the "Working Formulation" (National Cancer Institute) and the Sistema de Kiel-Lennert [12]. These two systems identify whether the tumor is of low or high malignancy and the "Working Formulation" adds an intermediate degree of malignancy [13]. When the methods cytological and histopathological examination are not sufficient to identify the tumor cell, the examination of immunohistochemistry is used [13], which helps in determining the origin of metastasis [14].

Because it is a recent technique, the immunophenotyping still presents great difficulty in availability and standardization of markers for cells of lymphoid origin of dogs. For this reason, we use markers of T cells (CD3) and B cells (CD79a) that have proven to be very effective in marking of T cells and B of the dog [12]. Some researchers have described the application of methods immunohistochemical for immunophenotyping classification of canine lymphoma in histological sections of tissue embedded in paraffin using polyclonal anti-

CD3 to mark T-cell lymphomas and the monoclonal anti-MIB1(CD79a) to mark lymphomas of B-cell lymphoma. With the development of the technique of immunohistochemistry, we found that the diagnosis and the survival of the animal can increase by the fact of knowing, with greater speed, what type of cell is involved [12]. Immunohistochemical methods have been used for the detection of proteins associated with cell proliferation in human neoplasms such as in Veterinary Medicine. The main marker of proliferating cells is the Ki-67 (MIB-1) [4]. It is a protein that recognizes all active phases of the cell cycle, except the rest phase (G<sub>0</sub>), thus becoming an excellent marker to determine the growth phase of a cell population [3]. Studies indicate that quantify the antigen Ki- 67 results in an increase in the accuracy of the classification of lymphomas canines [12,13]. Some authors have observed a positive correlation between the proportion of cells positively stained for Ki-67, cell morphology, immunophenotyping and the degree of malignancy of the neoplasms [5]. In humans, its determination has prognostic value in non-Hodgkin lymphomas [5].

The anti-CD3 is a human polyclonal antibody. In recent years, it has been shown that markers of T and B cells of humans are effective, also in the marking of T and B cells of some animals, including the canine species [13]. The anti-CD3 recognizes a protein associated with receptors of mature T cells, while the immature T cells are negative for CD3 with expression in membrane, but often positive for cytoplasmic expression using polyclonal antibodies, being quite specific for blastic lymphomas of T cells [14,16]. The CD3 works specifically with the antigen C3 that is accompanied by T cells present in both normal cells and neoplastic diseases and there is no indication of this antigen expression in other cell. The CD3 is expressed in approximately 80% of T-cell lymphomas [14]. The anti-CD79a is a human monoclonal antibody that reacts with a polypeptide which identifies a protein portion of the receiver of B cells associated with the expression of an immunoglobulin of surfasse [16]. The dial pattern is intracytoplasmic, being that this antibody recognizes virtually all neoplasms of B cells, except plasmocytomas where up to 50% may be negative [13].

The prognosis of canine lymphoma involves several factors that influence the evolution of the disease making it highly variable and difficult to be established. The classification of the "Working Formulation" diseases of high degree of malignancy showed a lower survival. The phenotype is extremely important, because lymphomas of origin of T cells are associated with a shorter duration of remission and survival of the animal. Evaluate the proliferation of neoplastic cells by means of a marker Ki- 67 has been shown to be very effective for the prognosis. However Couto [5] does not recommend to wait for the outcome of immunophenotyping for starting therapy because this will interfere with the prognosis only. The objective of this work was to use the technique of staining with the use of markers Ki- 67, CD3 and CD79a for the classification of lymphoma in waxed material collected from a dog breed English Bulldog. This research was approved by the Ethics Committee on Animal Use of Unifeso.

## **II. Case Report and Discussion**

A male dog of 4 years, breed English Bulldog was submitted to a clinical evaluation because it had, according to the owner, tumors of the limbs that appeared relatively quick. After history taking, physical examination revealed an increase of right lymph nodes popliteal and inguinal (Figure 1) generating the suspicion of neoplasia in organs lymphoid [1,2]. Was performed fine needle aspiration (FNA) of the right popliteal lymph node where there was scarce material, making a better evaluation of the sample. This finding may be due to inappropriate collection of material [15].

There was, then, by infiltration of local anesthesia lidocaine around the lymph node, excisional biopsy of the inguinal lymph node [13] and fragments were fixed in 10% formalin were sent for histopathological analysis. The samples were submitted to histological processing of routine, stained by the method of Hematoxilin and eosin (HE) and classified according to Kiel [12]. For the determination of the immunophenotype, were employed lymphoid markers for T lymphocyte, the polyclonal anti-CD3, and for B lymphocytes, the monoclonal anti-CD79a. It was used to determine the rate of cell proliferation antibody Ki-67 clone MIB-1. The cuts were distillate to 60 °C for 20 minutes, then were hydrated in a series of descending ethyl alcohol until the water, and washed in phosphate buffer saline (PBS) for 5 minutes. Subsequently, it was done the blocking of endogenous peroxidase in two baths of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) at 3% in methanol, 10 minutes each and washing with water and PBS (saline solution buffered with phosphate). After this stage, were washed in sequential on PBS (5 minutes each) and incubated with primary antibody (specific to each marker) in a moist chamber at 4°C overnight. As a result, must be hatched secondary antibody, in a humid chamber at 37°C for 30 minutes, more rinses in PBS. Then one more incubation with streptavidin complex-biotin-peroxidase in a moist chamber at 37°C, for 30 minutes, more rinses in PBS. The revelation will be made by immersion in a solution of diaminobenzidine (DAB) [15]. The histopathology in HE (hematoxylin and eosin) revealed a neoplastic process, with lymphoid cells showing hyperchromatism, with discreet pleomorphism and moderate mitotic atypia (Figure 2) with intense infiltration of these cells in the dermis and layer of hypodermis. The finding was consistent with lymphoma [4,16,18].

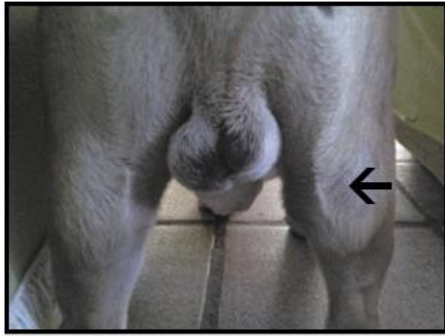


Figure 1 - Dog of the present study showing lymphadenopathy. Increase in the right popliteal lymph node (arrow)

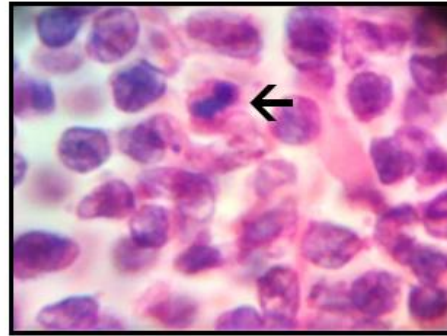


Figura 2 - Dog. Lymphoma. Presence of figure of atypical mitosis (arrow). Large increase, HE

After a month of the first consultation, the animal presented generalized lymphadenopathy and the enlarged lymph nodes showed ulceration (Figure 3), in addition to the presence of nodules disseminated in the region of the dorsum (Figure 4) [7]. This finding is in agreement with the literature [7,17,19], that describes the cutaneous form presenting nodules with alopecia, ulcerations exudative or hemorrhagic, single or multiple in the body of the animal.



Figure 3 - Dog of the present study showing an increase in the axillary lymph node with ulceration (arrow)

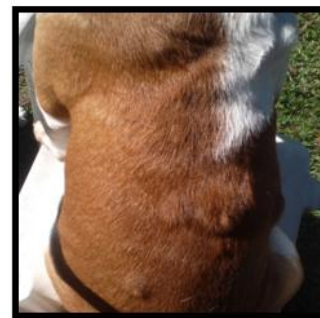
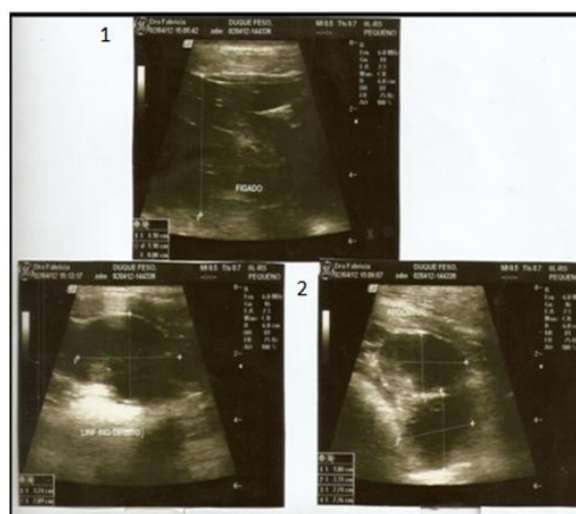


Figure 4 - Dog of the present study showing widespread nodules in the region of the back

In addition, in the same day the animal was subjected to abdominal ultrasound which showed enlarged lymph nodes in the hepatic chain, spleen and inguinal lymph nodes (Figure 5) [11].



**Figure 5-** Dog. Abdominal ultrasound of the animal in the present study. Increase in liver (1) and inguinal lymph node right (2)

Was collected, also, by venous puncture blood for laboratory tests. Was observed normocytic normochromic anemia arregenerativa, besides leukocytosis with neutrophilia and monocytosis and eosinopenia [17]. With the purpose of classification of tumor were performed the techniques of immunohistochemistry and cell proliferation. The immunohistochemical examination showed positivity for T lymphocytes (CD3) (Figure 6) and negativity for B lymphocyte (CD79a) (Figure 7), confirming the higher occurrence in Brazil, of T-cell lymphoma [6]. The examination of cellular proliferation Ki- 67 (Figure 8) showed a high degree of malignancy. The histological classification in HE (hematoxylin and eosin) of this report was based on "Working Formulation" shown large cells do not ruptures with a high degree of malignancy (Figure 9) [11,18-20].

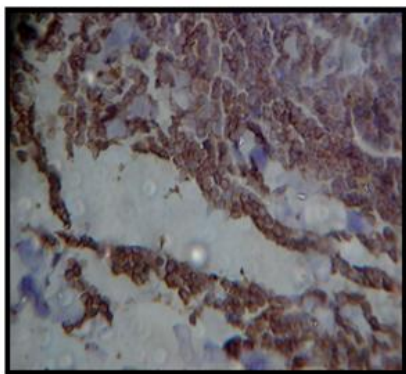


Figure 6 - Dog. Positive result for CD3 because the brown-brownish of tumor cells. Immunohistochemistry, 400X

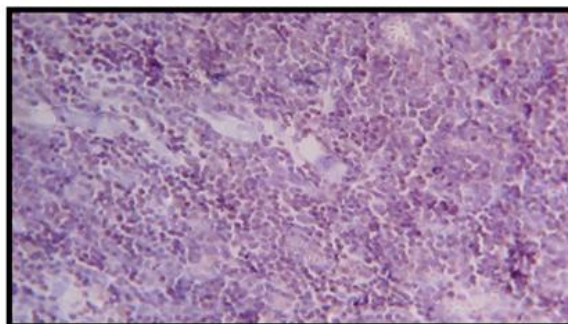


Figure 7 - Dog. A negative result for CD79a. Absence of brown-brownish. Immunohistochemistry. 400X

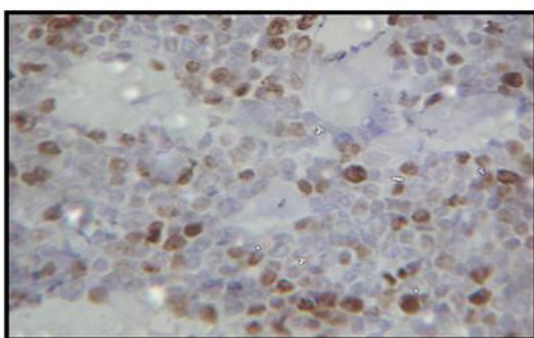


Figure 8 - Dog. Immunohistochemistry. A positive result for K i-67. We observed tumor cells with a brown brownish. 1000X

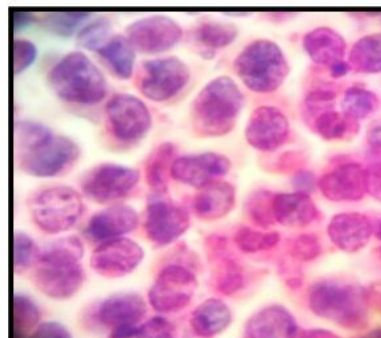


Figure 9 - Dog. Centroblástico lymphoma. The presence of large cells, not clivadas, vesicular chromatin, evident nucleoli. HE

Started the chemotherapy treatment, the dog showed no positive response, which resulted in a poor prognosis [6,11,17,19]. Due to the worsening of the animal, the owner decided to eutanásia [7].

### III. Conclusions

Although the histopathology has been inconclusive for diagnosis of lymphoma, the immunohistochemistry has proved to be indispensable for the classification of the tumor in relation to the type and degree of malignancy.

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