

Clinical, Cytological, Histopathological and Immunohistochemical Features of a T-Zone Lymphoma in a Mixed-Breed Dog

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ABSTRACT

Background: Non-Hodgkin lymphoma are a group of lymphoid neoplasms originating from the proliferation of precursors or mature, T, B and/or NK lymphocytes. T-Zone lymphoma (TZL) is characterized as an indolent lymphoma due to its slow progression and poor chemotherapy's response. Dogs affected by this neoplasm may live for many years without clinical signs and are often underdiagnosed. The aim of this study was to report a TZL case in a 9-year-old male mixed breed dog, submitted to clinical follow-up and chemotherapy.

Case: A 9-year-old male mixed-breed dog was presented due to the observation of an increased left mandibular lymph node. The previous cytological examination was suggestive of reactive hyperplasia and histopathological examination, by incisional biopsy, compatible with lymphocytic low-grade lymphoma. Physical examination revealed enlarged and firm left mandibular lymph node and adequate physical condition. A cytological examination was performed on the mandibular and both popliteal lymph nodes and revealed many small lymphocytes with hyperchromatic chromatin, rarely evident nucleolus and whose cytoplasm often projected in the form of a "hand-mirror" or "comet tail", compatible with lymphocytic lymphoma (low grade) and suggestive of TZL, in the three assessed lymph nodes. The histopathological and immunohistochemical examination, of the mandibular lymph node were chosen to confirm the diagnosis. In histopathology it was observed that 40% of the sample contained a monotonous cell population, composed by small lymphocytes, with some presenting "hand-mirror" morphology. Two mitotic figures were evidenced per field of high magnification (40x), inferring a low-grade disease. Immunohistochemical analysis revealed neoplastic proliferation with immunolabeling of CD3 lymphocytes and positivity for Ki-67 in 48% of neoplastic cells, but negative for CD20, CD79a, CD45, MUM-1 and PAX-5. Although there is no consensus about requirements and treatment's efficacy, it has been chosen to treat the dog with chlorambucil, because of the elevated Ki-67 value. The patient obtained a free-progression interval higher than 820 days from the earlier investigations of lymphadenopathy, with excellent quality of life and no side effects related to the use of chlorambucil.

Discussion: Although TZL is a common subtype of lymphoproliferative disease in dog, it is still underdiagnosed. The TZL diagnosis can be suggested by cytology, from the disclosures in neoplastic cells of cytoplasmic projections, recognized as a "hand-mirror" or "comet tail", corroborated with the present case, however the histopathology is confirmatory. Immunohistochemistry in which the neoplasm cells showed a positive immunolabeling for CD3 and negative for CD79a, CD20, CD45, MUM1 and PAX5, as evidenced in this report, confirming the T-cell origin. According to the literature, lymphomas composed by small clear cells and cytoplasm projecting by cytology, immunolabeling CD3 and CD25 positive and CD45 negative are, together, findings that confirm TZL. The biological behaviour of indolent lymphoma is still largely unknown, however the prognosis of dogs, with indolent lymphoma of T or B cells, seems to be favourable. Studies showed different median survival times, such as 760 days and 4.4 years. In the present case, the dog survived more than 820 days, confirming the good prognosis and indolent behaviour. Aggressive chemotherapy protocols are not necessary for such cases and the treatment with chlorambucil, without the association of prednisolone, has been well tolerated by the patient, which showed no side effects until the moment.

Keywords: oncology, neoplasia, indolent lymphoma, cytology, immunohistochemistry, chlorambucil.

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INTRODUCTION

The current classification of non-Hodgkin lymphoma divides them into lymphomas of precursor cells (lymphoblastic) or mature cells (which include several subtypes). It also brings the concept of indolent lymphomas, represented by the T-zone lymphoma (TZL), and three B-cell lymphomas: marginal zone lymphoma (MZL), follicular lymphoma (FL) and mantle cell lymphoma (MCL) [1,10]. TZL is a rare clinical entity in humans, but apparently common in dogs, representing 5-15% of all lymphomas in this species [1,7,12]. Information about its biological behaviour and treatment are even more scarce than for other human's indolent lymphomas, and the dog may represent a possible experimental model for understanding this disease. According to Valli *et al.* [10], TZL is characterized by cellular proliferation among the follicles, being composed of a homogeneous population of small T lymphocytes (CD3+), with small nuclear indentations, unapparent nucleoli, moderate and pale cytoplasm and lack of CD45 (panleukocyte) immunolabeling. In a study performed in France, 63.8% (388/608) of canine malignant lymphoma cases were B-cell lymphoma and 36.2% (215/608) were T-cell lymphoma, and the TZL was cytologically characterized by small cells, round or slightly irregular small nuclei and extended, unipolar and pale cytoplasm ("hand mirror" morphology) [5].

The objective of this study was to report a case of TZL in a mixed breed dog, in view to increase the knowledge about the biological behaviour of this pathology, which can be extrapolated to other species, including humans.

CASE

A 9-year-old male mixed-breed dog, weighing 10.3 kg, was referred due to the observation of an increased left mandibular lymph node, with cytological examination suggestive of reactive hyperplasia and histopathological examination (incisional biopsy) compatible with lymphocytic low-grade lymphoma. The dog was under treatment with metronomic lomustine¹, at a dose of 2.8 mg/m² every 48 h, for approximately 60 days. Prednisolone² had also been used, at a dose of 2 mg/kg every 24 h, for 7 days, followed by 1 mg/kg every 24 h for over 7 days and 1 mg/kg every 48 h, for 7 more days. Physical examination revealed adequate physical condition and mental state, body

score 6 (1 - 9), a moderate increase in the volume of the left mandibular lymph node, of firm consistency, slightly fixed.

Complementary exams were performed, which included complete blood count, serum biochemistry (alanine aminotransferase, alkaline phosphatase, creatinine, urea, total protein, albumin, globulin, total cholesterol, blood glucose and triglycerides), thoracic radiography and abdominal ultrasound. Complete blood count was within normal range but biochemical tests revealed a moderate increase in the values of alanine-aminotransferase (128 UI/L; Ref: 15-58 UI/L), alkaline phosphatase (543 UI/L; Ref: 20-156 UI/L), total protein (8.45 g/dL; Ref: 5.4-7.1 g/dL), globulins (5.17 g/dL; Ref: 2.7-4.4 g/dL) and triglycerides (130 mg/dL; Ref: 20-112 mg/dL). Radiographs were unremarkable but the abdominal ultrasound showed a gallbladder with thin wall and regular contour, populated by anechoic content with amorphous echogenic material in suspension, in addition to hyperechogenic points deposited at the bottom (bile sludge), compatible with mild cholestasis and formation of biliary concretions. Spleen size, contour and echogenicity were preserved, but there was two hypoechogenic nodules, showing no distortion of the capsule of the organ, measuring 0.94 x 0.83 cm and 0.34 x 0.30 cm, compatible with lymphoid nodular hyperplasia, extramedullary hematopoiesis or neoplastic process.

Cytological examination was performed on the left mandibular and both popliteal lymph nodes, sampled by fine needle aspiration, fixed and stained with Diff-Quick method. A histopathological review was carried out from the fragment previously removed by incisional biopsy of the left mandibular lymph node, along with immunohistochemical staining for CD3³, CD79a³, CD20³, CD45³, MUM1³, PAX5³ and Ki-67³. The cytological examination allowed the identification of many small lymphocytes with hyperchromatic chromatin, rarely evident nucleolus and whose cytoplasm often projected in the form of a "hand-mirror" or "comet tail", besides the small number of erythrocytes, neutrophils and rare plasmocytes, compatible with lymphocytic lymphoma (low grade) and suggestive of TZL, in the three assessed lymph nodes (Figure 1). In the first consultation, splenic aspirates were not taken due to resistance of the person responsible for the dog, and the patient was considered in disease stage III [11].

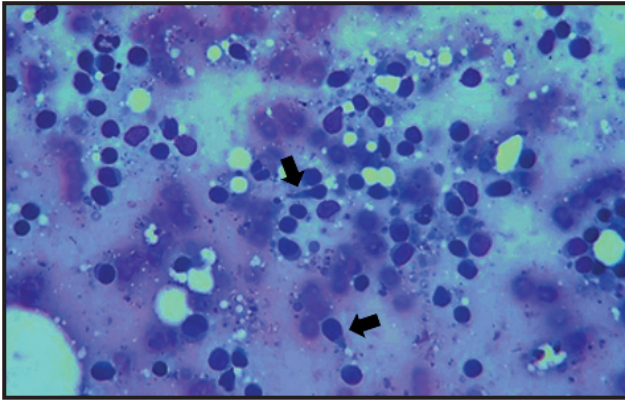


Figure 1. Cytological smear of mandibular lymph node of a 9-year-old mixed-breed dog diagnosed with T-zone lymphoma. Small lymphocytes are observed with cytoplasm often projecting in the form of a “hand-mirror” or “comet tail” (arrow). Associated with the T-zone lymphocytes there are few neutrophils and plasmacytes [Diff-Quick; 40x].

In the histopathological examination of the mandibular lymph node, approximately 40% of the sample contained a monotonous cell population, composed by small lymphocytes, sometimes with discrete nuclear indentations, with multiple nucleoli, moderate and pale cytoplasm, with some presenting projections (as a “hand-mirror” or “comet tail”). Two mitotic figures were evidenced per field of high magnification (40x), inferring a low-grade disease (Figure 2A). Immunohistochemical staining revealed neoplastic proliferation with immunolabeling of CD3³ lymphocytes (T) [Figure 2B] and a positivity for Ki-67³ in 48% of neoplastic cells (Figure 2C). There was no immunolabeling for the remaining antibodies (CD20³, CD45³, CD79a³, MUM1³, PAX5³) [Figure 2D]. It was concluded that the immunohistochemical and morphological profile favored the TZL diagnosis.

Considering the persistent abnormalities in the liver and biliary tract, oral treatment was instituted with ursodeoxycholic acid⁴ at a dose of 5 mg/kg every 12 h, along with food, omega-3 supplementation⁵ (1000 mg/day), Hepvet^{®6} (supplement based on amino acids, minerals and vitamin B complex) and silymarin⁷ (20 mg/kg) once a day for 60 days. Lomustine¹ was replaced by chlorambucil⁸ at a dose of 4 mg/m², in alternate days, with re-checks every 2 months. Ultrasonographic follow-up was also performed and the spleen nodules were still hypoechoic, measuring 0.9 x 0.8 cm and 0.3 x 0.3 cm, six months after the diagnosis. Fourteen months after the diagnosis, it was possible to identify only one nodule, this time with mixed echogenicity, measuring 0.7 x 0.6 cm. Novel cytological samples were taken from the spleen, mandibular and popliteal

lymph nodes. Spleen aspirates revealed a reactionary hyperplasia, characterized by a mixed lymphoid population with predominance of small lymphocytes and few plasma cells. No signs of malignancy were observed in these lymphoid cells. The left mandibular and both popliteal lymph nodes aspirates allowed the identification, once more, of the indolent neoplastic process. Up to the moment, 820 days after the first investigation of lymphadenopathy, and with approximately 790 days of treatment with chlorambucil⁸, the patient is otherwise healthy, without any side effects related to chemotherapy and without signs of progression of its indolent lymphoma.

DISCUSSION

In the dog 60-70% of lymphomas are derived from B cells and approximately 75% are considered high-grade lymphomas. A higher incidence is observed in Boxer, Bull Mastiff, Basset Hound, São Bernardo and Bulldogs, with an average age of 6-9 years [11]. The incidence of each histological subtype is variable according to the literature, but the diffuse B-cell lymphoma is the most frequent (50%), followed by peripheral-T-cell lymphoma not otherwise specified (PT-NOS) (15%) [12], which includes TZL [1], as evidenced in this report, which may represent 5-15% of all lymphomas in dog and 30-40% of indolent lymphomas [7,12]. Considering only the indolent lymphomas, Valli *et al.* [10], in the United States, observed greater representativeness of Golden Retriever, Labrador and Rottweiler, also commonly affected by other forms of lymphoma [12]. Mixed-breed dogs, as in the present case, represented 10% of the cases of indolent lymphomas. [10]. However, unlike the present report, which is a male dog, the females were more affected by indolent lymphomas, regardless of reproductive status, while age ranged from 1.5 - 16 years, with an average of 9 years (7.7 years for MZL, 8 years for MCL, 11 years for FL and 8.9 years for TZL) [10], coinciding with the patient's age.

The most frequent clinical presentation of indolent lymphomas is the chronic lymphadenopathy, as evidenced in this case, although patients may also be presented with inappetence, anorexia, weight loss and fever [9]. TZL are exclusive of nodal lymphomas and similar presentations in other organs may represent other forms of PT-NOS. Indolent lymphomas are also frequent in the spleen, mainly MZL and MCL, which

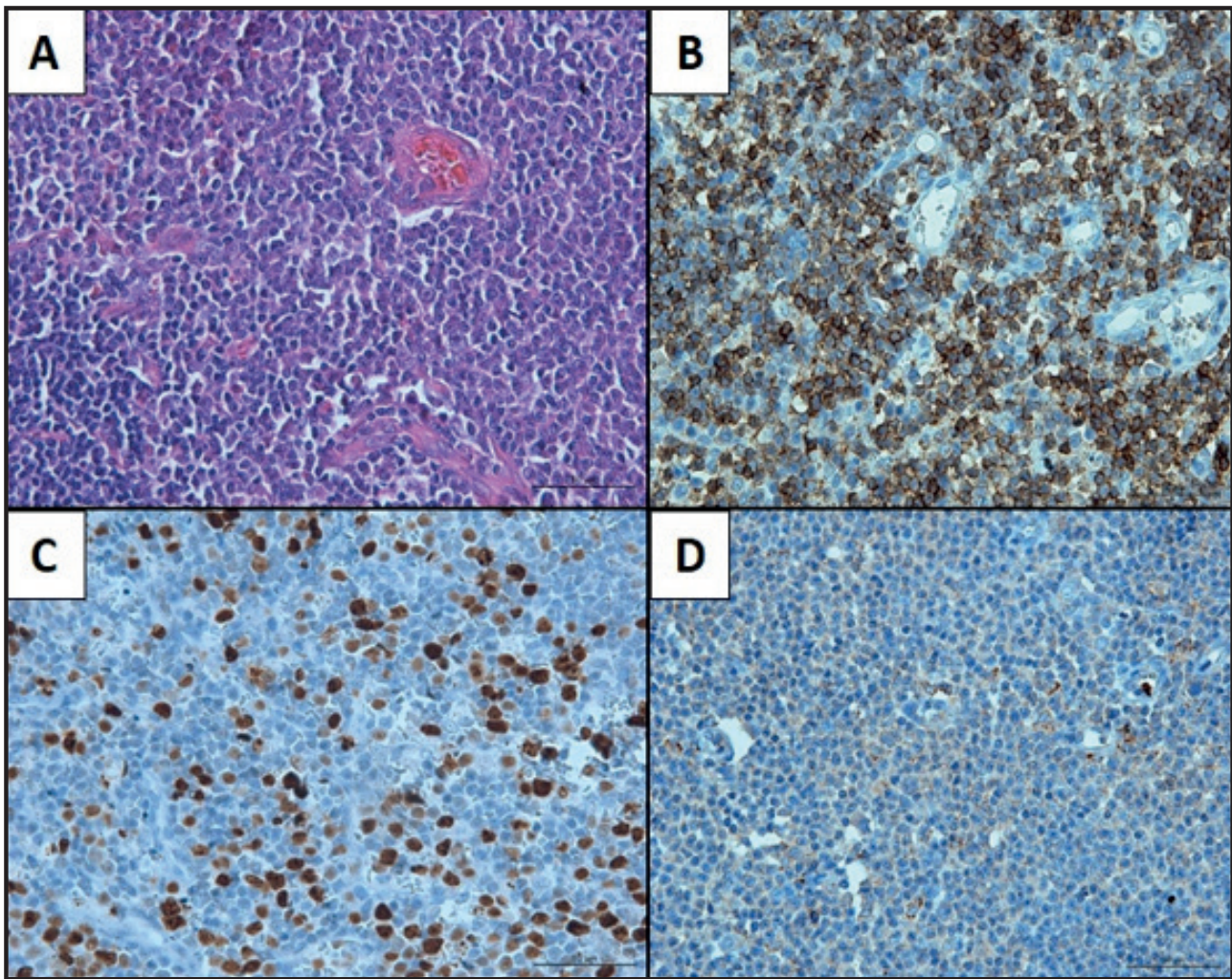


Figure 2. Histopathological and immunohistochemical photomicrographs of mandibular lymph nodes of a 9-year-old mixed-breed dog, diagnosed with T-zone lymphoma. A- Proliferation of small malignant lymphocytes, presenting the core equating to 1.0-1.5 times the diameter of the erythrocytes [HE; 40x]. B- Immunolabeling for CD3, confirming the neoplastic proliferation of T lymphocytes, marked in brown [Ac CD3; 40x]. C-Immunolabeling for Ki-67 in 48% of neoplastic cells [Ac Ki-67; 40x]. D- Negative immunolabeling for CD45 [Ac CD45; 40x].

represents the most common lymphoma subtypes in this organ (39.3% and 32.1% of cases, respectively).

Indolent lymphomas must be differentiated from lymphoid hyperplasia and other lymphomas varying from small to intermediary cells and reduced mitotic index [9, 10]. The TZL diagnosis can be suggested by cytology, from the disclosures in neoplastic cells of a cytoplasmic projection, recognized as a “hand mirror” or “comet tail “. In a retrospective study, 20 cases of TZL were cytologically characterized by small cells, round or slightly irregular small nuclei and extended, unipolar and pale cytoplasm (“hand mirror” morphology) [5]. However, as noted in this report, the retrieval of such information may be subject to the sample and/or experience of the pathologist. The histopathology is, however, confirmatory because it

highlights the neoplastic proliferation adjacent to the germinal centers (eccentric), leading to the collapse of lymphoid follicles, while the proliferation around the follicle, into a concentric pattern, refers to the MZL [12]. This might not be found in incisional biopsies. Immunophenotyping provides greater reliability to the diagnosis, which may be performed by flow cytometry, unavailable where this study was carried out, and immunohistochemistry, particularly useful in samples of incisional biopsy, as evidenced by Flood-Knapik *et al.* [1] and also in this report, in which the neoplasm showed a positive immunolabeling for CD3, confirming the T-cell origin, and negative for CD79a and CD20 (expressed in B lymphocytes and inconstant in plasmocytes), CD45 (expressed in all leukocytes but absent in TZL), MUM1 (expressed in

plasmacytes and inconstant in B lymphocytes) and PAX5 (expressed in B lymphocytes) [7,12]. According to recent studies, lymphomas with a small clear cell appearance and “hand mirror” morphology by cytology [2], immunolabeling CD3 and CD25 positive and CD45 negative [2,3] are, together, findings that confirm TZL.

In one study with 66 dogs diagnosed with indolent lymphoma, it was observed that 72.7% (48/66) were represented by MZL (35 nodal and 13 splenic), 15.2% (10/66) by TZL (all in lymph nodes), 7.6% (5/66) by nodal FL and 4.6% (3/66) by splenic MCL [10]. However, in one retrospective study, including 75 dogs with lymphoma whose histopathological descriptions presented small or intermediate cells and/or reduced mitotic index, it was observed that the TZL was the most frequent, representing 61.7% of the cases (37/75), followed by MZL (25%), FL (6.7%) and MCL (1.7%), while the remaining diagnoses were T-cell rich B-cell lymphoma (3.3%) and lymphocytic lymphoma (1.7%) [1]. In the same study, immunophenotyping proved to be useful by allowing change in the diagnosis in 20.4% of cases (particularly in MZL for TZL), and allowed the final diagnosis of nine TZLs, five MZL and one FL. Despite the reduced mitotic index, compatible with low-grade lymphoma, evidenced in the cytology and histopathology, in this case, the Ki-67 value was higher than the mean observed for indolent lymphomas in the study of Flood-Knapik *et al.* [1], who obtained an average of 21% of stained neoplastic cells (17.6% for the TZL, 20% for MZL and 59% for FL).

The biological behaviour of indolent lymphoma is still largely unknown, however the prognosis of dogs with indolent lymphoma of T or B cells, seems to be favourable [1,2,9,10]. A study performed with 51 dogs diagnosed with TZL, showed 760 days of median overall survival [2]. In another study [1], it was observed a median of 4.4 years for indolent lymphomas, and no statistical difference was observed in the survival of dogs with TZL (33.5 months) or MZL (21.2 months) [$P = 0.5$]. The stage of the disease, including bone marrow infiltration, mitotic index, cell size, immunophenotype, value of Ki-67 and treatment did not influence the survival, however, lymphocytosis above 9212 cells/ μ L had an impact on prognosis, resulting in a median of 15.4 months, while the median of the remaining dogs was not achieved ($P = 0.05$).

Treatment does not seem to influence the survival and quality of life of dogs affected by indolent lymphomas [10]. In the study of Flood-Knapik *et al.* [1], no difference was observed in the survival of dogs with indolent lymphoma which received no treatment (52.4 months), prednisone (33.5 months) or chemotherapy ($P = 0.07$), with chlorambucil and prednisolone (median not reached) or combination of prednisolone, cyclophosphamide, vincristine, doxorubicin and L-asparaginase (21.6 months) [$P = 0.06$]. It is a slow-progressive disease and the effect of systemic treatment should be determined by future studies [1]. There is no consensus and treatment may be indicated, at the discretion of the clinician, in the presence of lymphocytosis, advanced stage, high mitotic index or percentage of expression of Ki-67, choosing the monotherapy with prednisolone, its association with alkylating agents, or even protocols with combination of multiple drugs [10]. Although bone marrow aspirates were not taken, the patient of the reported case was considered in stage III. It was chosen to initiate the treatment due to the disease advanced stage (involvement of at least three lymph nodes) and high growth fraction evidenced by Ki-67, using the chlorambucil as a single agent due to hepatic abnormalities showed in the serum biochemistry and ultrasound, probably related to the vacuolar hepatitis, by obesity and use of glucocorticoids. The patient showed no side effects and remains in follow-up every 2 months, with an excellent quality of life.

TZL is a common subtype of lymphoproliferative diseases in dogs, however it is still underdiagnosed. TZL must be suggested by cytology, although the definitive diagnosis is conditioned to histopathology, and may receive support from immunohistochemistry or flow cytometry. TZL presents a good prognosis and an indolent behaviour, as shown in the described case, despite the high Ki-67 value, resulting in a high period free of progression and survival. Aggressive chemotherapy protocols are not necessary for such cases and the treatment with chlorambucil, without the association of prednisolone, has been well tolerated by the patient, which showed no side effects until the moment.

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