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CASE REPORT
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Cutaneous Epitheliotropic Lymphoma in a Lhasa Apso

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ABSTRACT

***Background*:** Lymphoma neoplasms originate from the lymphocytes. Anatomically, these tumors can be classified into multicentric, digestive, mediastinal, and cutaneous forms. The etiology of cutaneous lymphoma remains unclear; however, it has been associated with chronic skin inflammation. The definitive diagnosis is based on histological analysis and immunohistochemistry, although fine-needle aspiration cytology has shown good results. The aim of this paper is to describe the clinicopathological aspects of a case of cutaneous epitheliotropic T cell lymphoma, classified as mycosis fungoides, in a Lhasa Apso dog.

***Case*:** A 8-year-old bitch Lhasa Apso with multiple non-pruritic skin nodules and history of 10-day evolution was referred to the Veterinary Hospital of the Centro Universitário do Espírito Santo (UNESC), Colatina, ES, Brazil. The nodules were erythematous, exophytic, firm, circumscribed, and measured 0.2-4 cm in diameter in locations throughout the animal's body. An incisional biopsy was performed with an 8-mm punch and sent for histopathological examination. An infiltrative, poorly demarcated, non-encapsulated, densely cellular neoplasm, which was replacing the dermal collagen and displacing the adnexa, was observed in the dermis. The tumor was composed of a population of round cells, with generally distinct cell borders and a small-to-moderate amount of eosinophilic cytoplasm. The nuclei were irregularly rounded and occasionally edentulous, with vesicular chromatin, a visible nucleus, and 11 mitotic figures in an area of 2.37 mm^2. The immunohistochemical test, which was positive for the CD3 marker, confirmed the diagnosis of T cell lymphoma. On an ultrasound to identify metastasis, the liver showed heterogeneous parenchyma, heterogeneous expansive formation, areas of cavitary appearance, and cytology compatible with lymphoma. Antineoplastic chemotherapy was administered using the CHOP regimen (cyclophosphamide, doxorubicin, vincristine, and prednisone). However, the animal died after 45 days.

***Discussion*:** A diagnosis of the mycosis fungoides type of cutaneous epitheliotropic T cell lymphoma was established based on clinical, laboratory, anatomopathological, and immunohistochemical findings. Pruritus is a common clinical condition in animals with mycosis fungoides, particularly in those with the erythrodermic form of the disease. Epitheliotropic lymphomas have no sexual or racial predilections and usually affect dogs over 9 years of age. The Cocker Spaniel, English Bulldog, Boxer, Golden Retriever, Scottish Terrier, Briard, English Springer Spaniel, Beagle, German Shepherd, and English Cocker Spaniel breeds are frequently affected by these lymphomas. These neoplasms can have a primary skin origin, or they can be secondary and associated with lymphoma found elsewhere in the body. Chemotherapy is the treatment of choice, especially in cases with multifocal distribution. Protocol preference varies with disease stage, patient clinical and laboratory conditions, and the degree of toxicity. Commonly used chemotherapy regimens include L-CHOP (vincristine, cyclophosphamide, doxorubicin, L-asparaginase, and prednisolone), CHOP, COP (cyclophosphamide, vincristine, and prednisone), LAP (lomustine, L-asparaginase, and prednisolone), LOPP (lomustine, vincristine, procarbazine, prednisolone), chlorambucil, and prednisolone. The prognosis of canine epitheliotropic cutaneous lymphoma is unfavorable, with a survival time ranging from a few months to 2 years. The animal in this study survived for 105 days. In addition, epitheliotropic cutaneous T cell lymphoma is aggressive, which may result in a shorter survival time in animals affected by this type of tumor.

Keywords: epitheliotropic lymphoma, tumor, oncopathology, immunohistochemistry, mycosis fungoides.

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INTRODUCTION

Lymphomas are neoplasms that usually originate in lymphoid organs from lymphocytes but can manifest in other tissues of the body because the cells continuously migrate [5]. Anatomically, these tumors can be classified into multicentric, digestive, mediastinal, and cutaneous forms. Approximately 80% of cases occur in the multicentric form [15].

The etiology of cutaneous lymphoma remains unclear; however, these tumors have been associated with chronic skin inflammation, since the proliferation of activated lymphocytes is stimulated by the constant presentation of environmental antigens [6].

The cutaneous form of lymphoma is rare in dogs. In addition, it has no sexual predisposition and is more commonly observed in older animals [14]. The prognosis is poor because the cancer has a short remission time, ranging from a few months to two years [2,3].

The definitive diagnosis is based on histological analysis and immunohistochemistry; however, fine needle aspiration cytology (FNAC) has shown excellent results for screening [7,14].

The aim of this report is to describe the clinicopathological aspects of a case of cutaneous epitheliotropic T cell lymphoma, classified as mycosis fungoides, in a Lhasa Apso dog.

CASE

A 8-year-old bitch Lhasa Apso with a history of multiple cutaneous nodules with a 10-day evolution was admitted to the Joaquim Rossi Veterinary Hospital. The dog's owner reported no pruritus. Upon clinical examination, erythematous, exophytic, firm, circumscribed tumors measuring 0.2-4 cm in diameter were observed throughout the animal's body (Figure 1A). Erythematous plaques were also observed on the face, neck, back, and limbs (Figure 1B). Therefore, we opted for an incisional biopsy with an 8-mm punch and local anesthesia (lidocaine 1 mL/kg)[1]. The fragments were fixed in 10% formalin, sent for histopathological examination, processed in the usual way, and embedded in paraffin. Histological sections were prepared with thicknesses of 3-5 µm and stained with hematoxylin and eosin[2].

Histological sections of the skin were subjected to immunohistochemistry techniques (IHC). The sections were 4 µm in size. They were deparaffinized in xylene, hydrated in decreasing concentrations of ethanol, and washed in distilled water. They were then subjected to antigenic recovery and were heated in a 10 mM citrate solution (pH 6.0) in a Dako Pascal pan[3]. The slides were subsequently cooled to room temperature for 20 min and washed with deionized

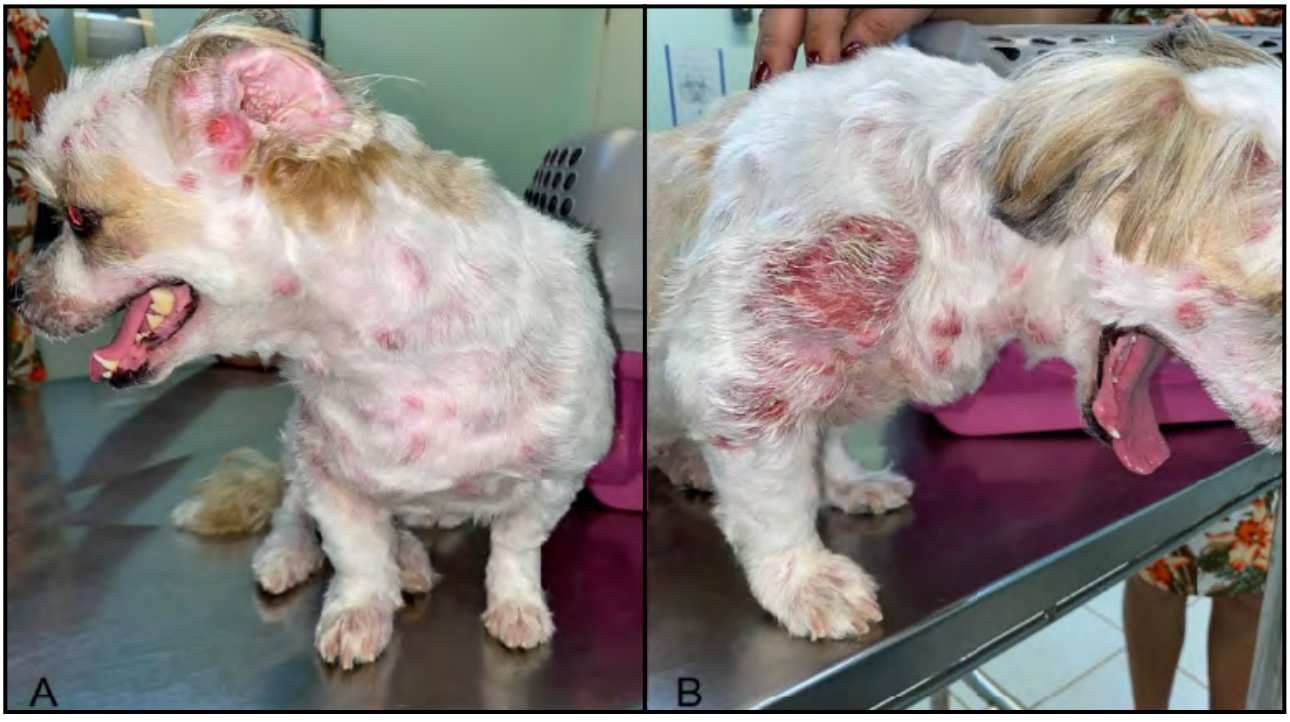

Figure 1. Epitheliotopic T lymphocyte lymphoma (mycosis fungoides). A- Erythematous, exophytic, firm and circumscribed nodules throughout the animal's body. B- Erythematous plaques on the face, neck, and limbs.

water. After antigen retrieval, endogenous peroxidase was blocked by immersing the slides in ready-to-use Peroxide Block[4]. The sections were then washed in Tris solution (pH 7.4), and the nonspecific sites were blocked with Protein Block[3] (nonspecific reaction). The cells were incubated with the primary antibodies (anti-CD20[3] and anti-CD3[3]) for 18 h at 4°C. Dako Envision[3] and DAB chromogen[3] were used as the amplification and detection systems, respectively. Slides were counterstained with Harris' hematoxylin[5].

Microscopically, an infiltrative, poorly demarcated, non-encapsulated, densely cellular neoplasm was observed in the dermis; it was replacing dermal collagen and displacing attachments. The tumor was composed of a population of round cells, with generally distinct cell borders and a small-to-moderate amount of eosinophilic cytoplasm. The visible nuclei were centrally irregularly rounded and occasionally edentulous, with vesicular chromatin. Eleven mitotic figures were observed in an area of 2.37 mm^2. Multifocal ulcerations and mild diffuse parakerotic hyperkeratosis were present. Based on these observations, the lesion was classified as epitheliotropic cutaneous lymphoma (Figure 2A). Immunohistochemical staining revealed that the neoplastic cells were strongly positive for anti-CD3 and negative for CD20; thus, the neoplasm was characterized as T cell lymphoma (Figure 2 B and C).

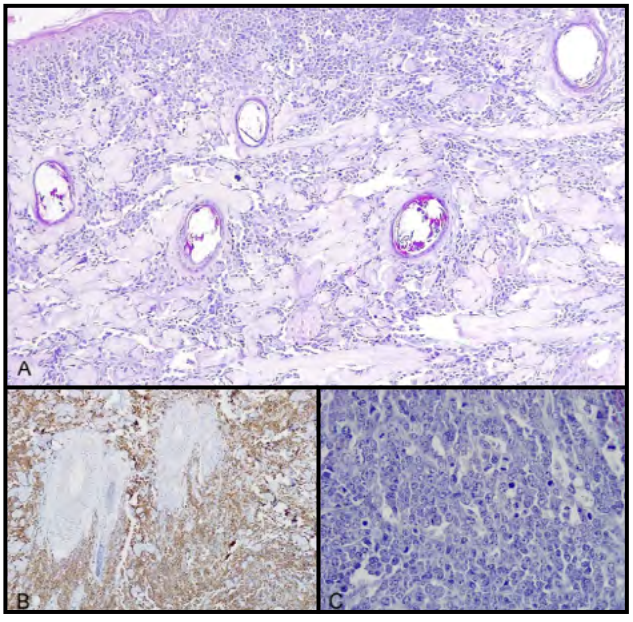

Figure 2. Epitheliotopic T lymphocyte lymphoma (Mycosis fungoides). A- Infiltration of neoplastic lymphocytes replacing dermal collagen and displacing attachments [HE; Obj.40x]. B- CD3 positive immunostaining of neoplastic T cells infiltrated in dermis [IHC; Obj.40x]. C- CD20 negative immunostaining of neoplastic cells in the skin [IHC; Obj.40x].

Chest radiography and abdominal ultrasonography were performed to detect metastases. No radiographic changes were observed. Ultrasound showed that the liver had heterogeneous parenchyma, and there was a heterogeneous expansive formation, along with areas that appeared cavitary. After FNAC was performed on the liver nodule, the cytology was found to be compatible with lymphoma (Figure 3 A and B). Antineoplastic chemotherapy was performed with the CHOP regimen. The patient achieved complete remission after the first chemotherapy session. The disease-free period was 60 days, but there was subsequent recurrence of the condition. Treatment with lomustine[4] 70 mg/m^2 and prednisolone was initiated, but the patient did not respond and died 45 days later.

DISCUSSION

The diagnosis of the mycosis fungoides type of cutaneous epitheliotropic T cell lymphoma was established based on clinical, laboratory, anatomopathological, and immunohistochemical findings. The proliferation of mononuclear cells and the observations

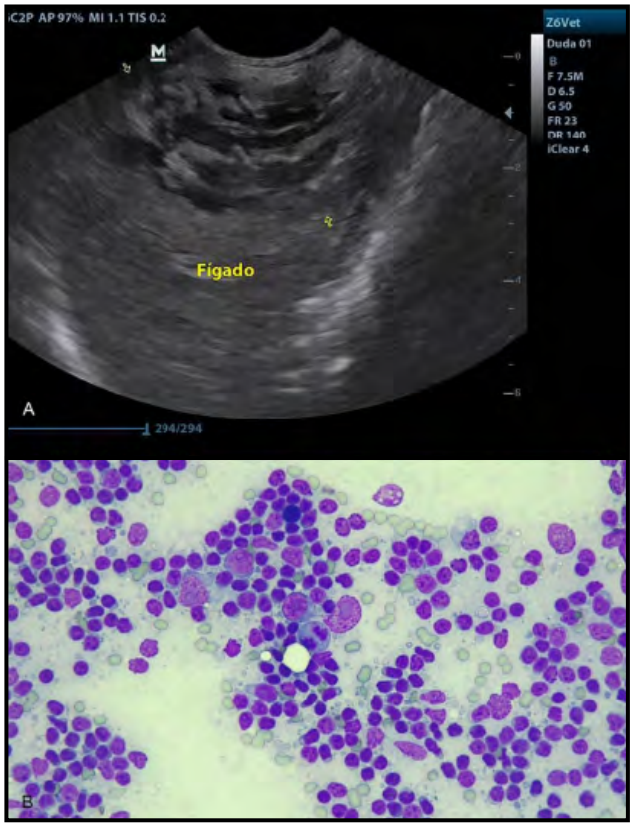

Figure 3. Epitheliotopic T lymphocyte lymphoma (Mycoses fungoides). A- Ultrasound showing liver with heterogeneous parenchyma and a heterogeneous expansive formation with areas of cavitary appearance. B- Neoplastic process with cytomorphology suggestive of hepatic lymphoma [CIT; Obj.40x].

of epidermotropism, such as invasion into the hair follicles and adnexal glands, indicated that histological examination might show epitheliotropic lymphoma [5]. Immunohistochemistry was used to determine the tumor histogenesis. The cells showed positive immunostaining for CD3 antibody, which indicated a T lymphocytic lineage, [14] and negative immunostaining for CD20 antibody. The immunohistochemical findings, along with the clinical-dermatological presentation, were determinants for the diagnosis of mycosis fungoides.

Mycosis fungoides is the most common form of epitheliotropic cutaneous lymphoma, and it usually originates from T cells and causes the infiltration of neoplastic lymphocytes in the epidermis and adjacent structures [5]. In Sézary syndrome, there is an accentuated presence of neoplastic cells in peripheral blood. These cells are rare and aggressive, constituting the leukemic form of mycosis fungoides in addition to pagetoid reticulosis, which is characterized by the presence of malignant cells exclusively in the epidermis [5].

Pruritus is a common clinical condition in animals with mycosis fungoides, particularly in those with the erythrodermic form of the disease [4,11,12]. However, in this study, the patient had a mixed form of the disease (nodule-tumor and erythrodermic) and did not experience itching. Other dermatological clinical alterations were similar to those described in the literature.

Epitheliotropic lymphomas have no sexual or racial predilections and usually affect dogs over 9 years of age. The Cocker Spaniel, English Bulldog, Boxer, Golden Retriever, Scottish Terrier, Briard, English Springer Spaniel, Beagle, German Shepherd, and English Cocker Spaniel breeds are frequently affected by these lymphomas [6].

Cutaneous lymphoma can have either a primary origin in the skin or a secondary origin, in which it is associated with lymphoma found elsewhere in the body [15]. In the present case, the animal had hepatic lymphoma in addition to cutaneous lymphoma. Thus, it was not possible to determine whether the epitheliotropic lymphoma was of primary or secondary origin. In epitheliotropic cutaneous lymphomas, tumor evolution and dissemination of neoplastic lymphocytes to other organs, lymph nodes, and blood may occur [9].

The clinical forms of mycosis fungoides must be differentiated from other skin diseases, especially from proliferations of other round cells, such as histiocytic and Merkel cells, which are difficult to differentiate histologically from lymphocyte neoplasms [8].

Chemotherapy is the treatment of choice for epitheliotropic cutaneous lymphoma, especially in cases with multifocal distribution. Protocol preference varies with disease stage, patient clinical and laboratory conditions, and degree of toxicity [4]. The use of lomustine for the treatment of high-grade lymphomas has shown good results and acceptable levels of toxicity [13]. The use of L-asparaginase has shown a better response to lymphoma treatment when implemented in the therapeutic protocol [10].

Commonly used chemotherapy regimens include L-CHOP, CHOP, COP, LAP, LOPP, chlorambucil, and prednisolone [3,13]. However, the use of therapeutic protocols highlighted in the literature does not guarantee long-term survival, but these treatments do increase the quality of life of patients [9]. Protocols based on COP present a less aggressive approach, while CHOP and L-CHOP are classified as more aggressive chemotherapies and are commonly used for the treatment of lymphomas [2]. The treatment of the patient in this report was based on the CHOP chemotherapy protocol with initial remission and subsequent relapse. The chemotherapy protocol was changed to lomustine and prednisolone; however, the patient did not respond, and later died.

The prognosis of dogs with epitheliotropic cutaneous lymphoma is unfavorable, with a survival time ranging from a few months to 2 years. The animal in this study survived for 105 days. In addition, epitheliotropic cutaneous T cell lymphoma is considered aggressive, which may result in a shorter survival time in animals affected by this type of tumor.

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