CLINICAL, ULTRASONOGRAPHIC AND IMMUNOHISTOCHEMICAL STUDY OF DIFFUSE LARGE B-CELL LYMPHOMAS IN DOGS

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INTRODUCTION

Diffuse large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma in people and dogs, with differing survival times and variable aggressiveness. The incidence of lymphoma in dogs has increased significantly in recent decades, similarly to what has happened in humans, with the so-called Non-Hodgkin lymphoma. Although multicentric lymphoma is one of the most responsive neoplasms to polychemotherapy treatment, about 65 to 90% of cases will have complete remission with treatment, the rate of disease recurrence in the middle or after completion of the protocol is also very high, about 75% of treated lymphoma cases will experience a relapse of the disease. ARFI elastography is a non-invasive medical imaging technique that helps determine the stiffness of organs and other structures , qualitatively through the color map called an elastogram, in which shades of blue indicate that the tissue is softer, and quantitatively through the measurement of shear-wave velocity, the higher the velocity, the more rigidity the tissue has. This technique has been used for monitoring and diagnosis of neoplasms (1).

OBJECTIVE

This study aimed to establish better prognostic characterization and therapeutic monitoring of the DLBCL in dogs, using immunohistochemistry, histopathology, and ultrasonography techniques.

MATERIALS AND METHODS

The ultrasonographic features of peripheral lymph nodes were evaluated in 16 dogs with DLBCL (GL), diagnosed through histopathology and immunohistochemistry (IHC), and in 12 healthy dogs (GC). B mode ultrasonography, quantitative and qualitative elastography and cytology were performed serially during CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy treatment, at T0, T5, T10, T15 and T19 weeks. The expression of alpha-SMA, the biomarker of activated fibroblasts, (Table.1) was evaluated by IHC, along with the expression of type I and III collagen in Picrosirius red stained sections, in the stroma of lymph node biopsies. These evaluations aimed to establish a possible correlation with the therapeutic response and survival time.

Table 1: Alpha-SMA antibody, IHC methodology

Antibody	Clone	Diluition	antigenic recovery	Incubation	Detection system	Positive Control	Score -Image J
Alfa-SMA.	1A4	1:2000	Pascal Citrate Solution, pH 6 at 95º, water bath for 30 min.	4 hours	Novolink polymer detection system (Leica)	Dog dermis sample	0- negative, 1- low < 25%, 2- moderate- 25%-50%, 3- High expression-> 70% of positive cells.

RESULTS

A-SMA expression

No statistical differences were found between groups, GC and GL regarding alpha-SMA (P> 0,05)

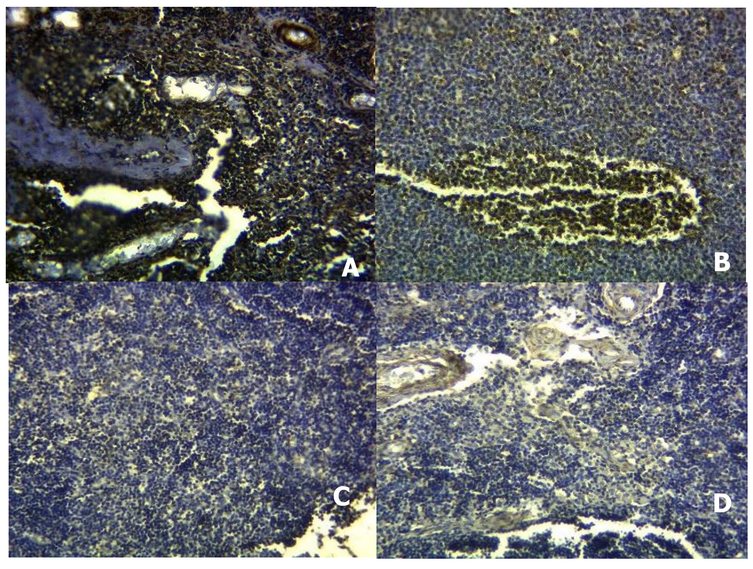

Figure 1: a. Strong immunostaining for α-SMA, more than 70% of positive cells, score 3, at sample of Lymphoma Group (DAB,400x); **b.** Moderate immunostaining for α-SMA, 25%-50% of positive cells, score 2, at sample of Lymphoma Group (DAB,400x); c. & d. negative and weak immunostaining for α-SMA, score 0 and 1, at sample of Control Group (DAB,400), respectively.

Colagen expression.

No statistical differences were found between groups, GC and GL regarding Colagen expression (P> 0,05). However, there was a difference in the structure of the capsule of the lymph nodes in the GL compared to the CG; with a predominance of type III collagen fibers, immature and less thick in diseased animals (Figure 2C and 2D).

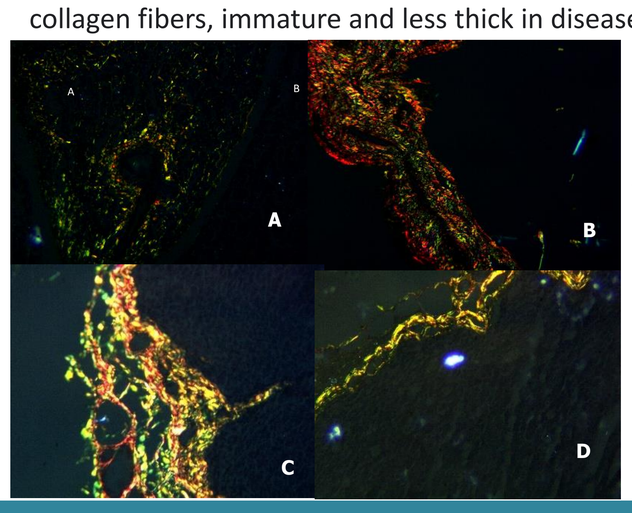

Figure 2: A. & B. Photomicrographs of dog lymph node with DLBCL, stromal portion. Polarized light, histochemical staining of picrosirius red. **A)** Region with high expression of type III collagen fibers (green), immature collagen. **B)** Photomicrographs of healthy dog lymph node (GC), stromal portion, polarized light, picrosirius red histochemical staining Region with mixed expression of collagen fibers type I (red) and type III (green). 20x magnification**. C)**: Photomicrographs of lymph node (CG), capsular portion. Region with high expression of type I and III collagen fibers, with greater density and organization. **D)**: Lymphoma Group (LG) , capsular portion. Predominance of immature collagen, type III. Thinner and faulty fibers.

Elastography

There was a statistically significant (P<0.05) between the qualitative and quantitative ARFI elastography and the minor axis/major axis ratio measured using the B mode in the lymph nodes, among the different lengths of therapy and the therapeutic response identified by cytology. More rigid structures, evaluated by qualitative elastography, were correlated significantly with shorter patient survival (P=0.026).

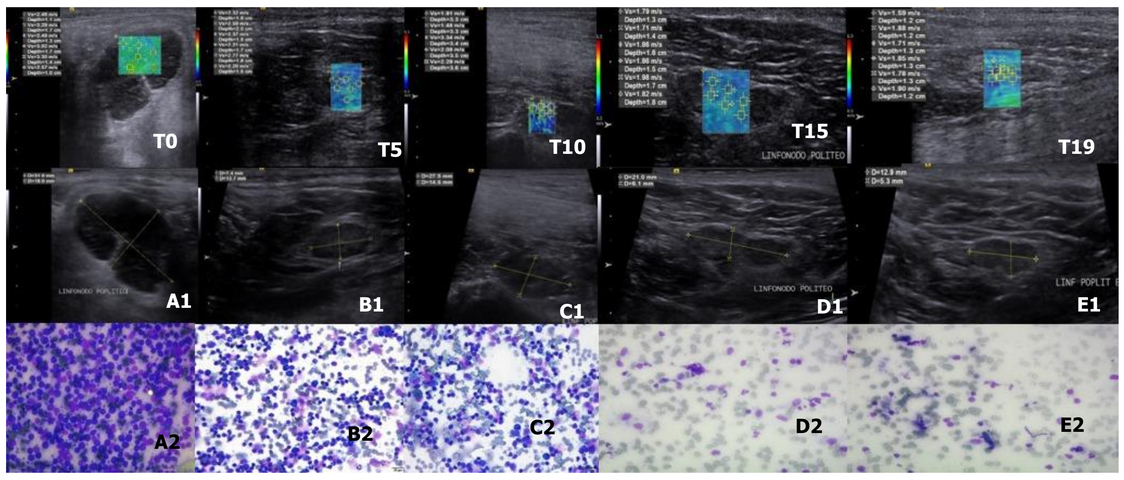

Figure 3: Evaluation moments T0, T5, T10, T19 - elastography, major / minor axis ratio and cytology of the popliteal lymph node of a dog diagnosed with LDGCB.

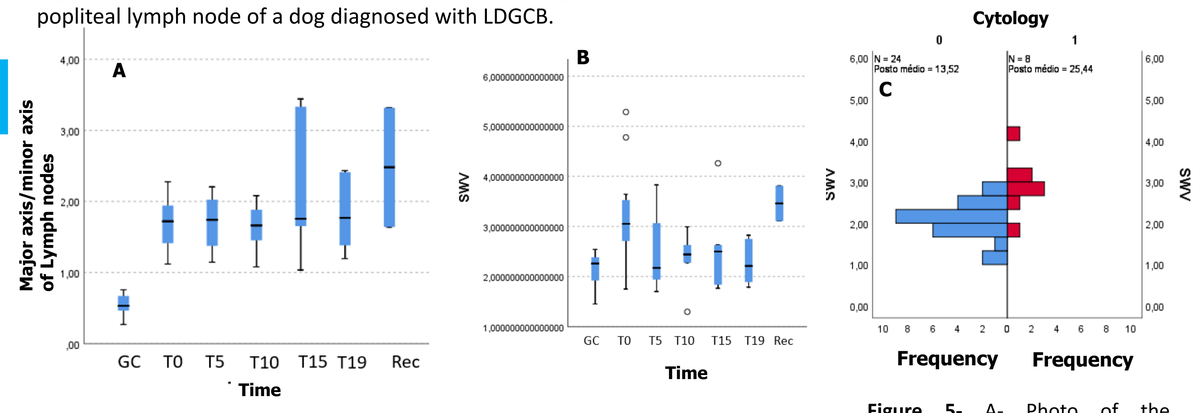

Figure 4-A: Boxplot Graphic Major axis/minor axis **B:** Shear-wave Velocity**,** relationship of the GC and LG lymph nodes during the CHOP chemotherapy treatment. **C:** Correlation between cytological evaluation and shear velocity determined by ARFI elastography

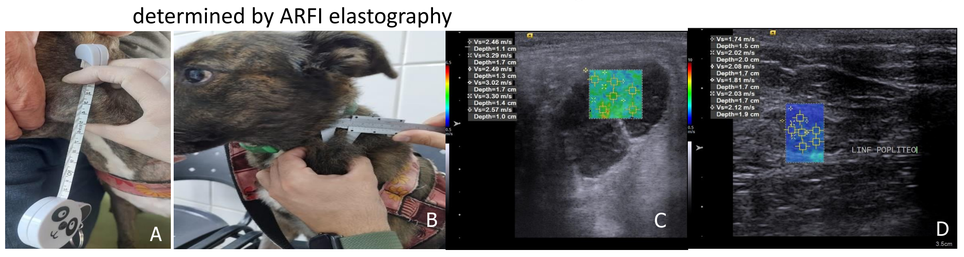

Figure 5- A- Photo of the measurement of the affected ventral cervical lymph node in a patient with DLBCL at the time of diagnosis. B- Same patient in complete remission, photo of measurement of the same lymph node evaluated in the tenth week of CHOP chemotherapy treatment, C- Evaluation by ARFI elastography, of the same patient, at time T0, diagnosis and D- Evaluation by ARFI elastography, at time T10.

DISCUSSION AND CONCLUSION

In veterinary oncology, elastography has already demonstrated significant value for the prognostic evaluation of mammary tumors in female dogs, as well as for detecting the presence of metastasis in regional lymph nodes, in mammary tumors (3). Other applications of ARFI elastography involve the standardization of reference values for evaluating splenic lesions in adult canines (1) and distinguishing malignant from benign cutaneous lesions (2). In this study, there was also a correlation between the type of therapeutic response and the survival time with the elastographic findings. Statistical results indicate that a more rigid elastogram, as well as high shear-wave velocity values, are associated with the presence of the disease detected by cytology and with shorter survival times.

The findings and results obtained suggested that elastography is an auxiliary, non-invasive diagnostic tool in veterinary oncology to monitor the therapeutic response during chemotherapy in canine DLBCL.

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