

## MANDIBULAR LYMPH NODE ENLARGEMENT AND LYMPHOCYTOSIS IN A SHIH TZU

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### Signalment

“Cookie”, dog, Shih Tzu, 11 years old, castrated male

### History and clinical findings

The dog was presented to the Veterinary Teaching Hospital, Thessaloniki, Greece, for a submandibular lymphadenomegaly during the previous 6 months.

The dog lived in the urban area of Thessaloniki (GR), was updated with vaccinations, and received regularly deworming and ectoparasite treatments.

The clinical examination confirmed the enlargement of right submandibular lymph node and a milder enlargement of the left lymph node. Historically and on physical examination, the dog was bright, alert and responsive.

### Diagnostic procedure

Complete blood count (CBC) (ADVIA 120, Siemens, Tarrytown, NY, USA), serum biochemistry and lymph node cytology were performed.

Serological testing was also performed: 4DX Idexx SNAP® was negative for *Dirofilaria immitis*, *Borrelia burgdorferi*, *Anaplasma* spp. and *Ehrlichia canis*. Immunofluorescence for *Leishmania infantum* gave a positive result with a 1:200 antibody titer (cut-off: 1:100).

Complete blood count revealed a mild leucocytosis with lymphocytosis. No cytopenia or other abnormalities were found (Table 1).

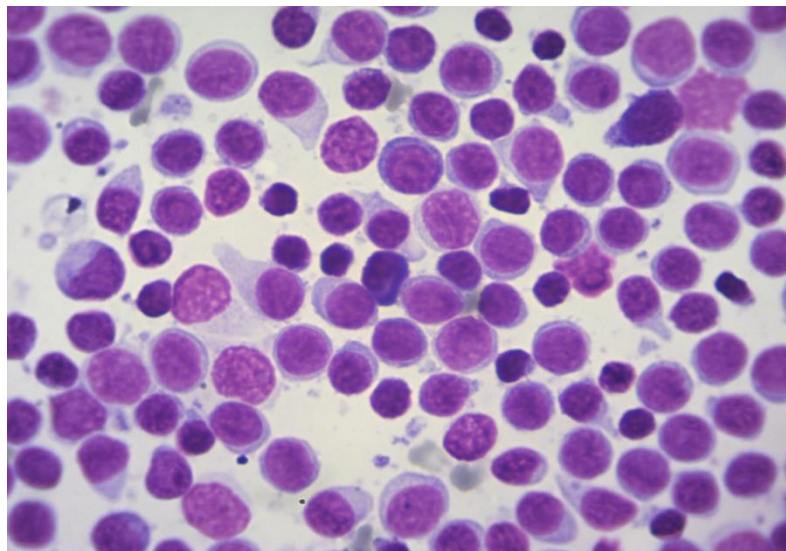
A comprehensive serum biochemistry was unremarkable.

Diagnostic imaging (thoracic/abdominal radiography and abdominal ultrasonography) was unremarkable.

HEMATOLOGY	15/May/2018	RI
HCT	52.3	37-55%
WBC	24,500	6,000-17,000 / $\mu$ L
Segmented neutrophils	7,400	3,000-11,000 / $\mu$ L
Monocytes	1150	150-1350 / $\mu$ L
Lymphocytes	15500	1,000-4,800 / $\mu$ L
Eosinophils	450	100-1100/ $\mu$ L
Platelets	309,000	200,000-500,000 / $\mu$ L

**Table 1.** Cookie's CBC analysed with ADVIA 120.

Lymph node fine needle biopsy cytology showed a predominance of medium-sized to large-sized lymphocytes, with a few cells showing a "hand mirror" appearance. No evidence of *Leishmania infantum* amastigotes was found (Figure 1).



**Figure 1.** Cytology of right mandibular lymph node. Medium-sized lymphocytes and some "hand mirror" shape and small lymphocytes.

Following cytological examination and laboratory work up, flow cytometric analysis was performed on lymph node cytology specimens and peripheral blood. Samples were shipped to the University of Milan Flow Cytometry Service, and the following antibodies were tested:

Anti - CD45, CD44, CD18 (pan-leukocyte)

Anti – CD5, CD3, CD4, CD8 (T-lymphocytes subsets)

Anti – CD21 (B-lymphocytes)

Anti – CD34 (precursor cells)

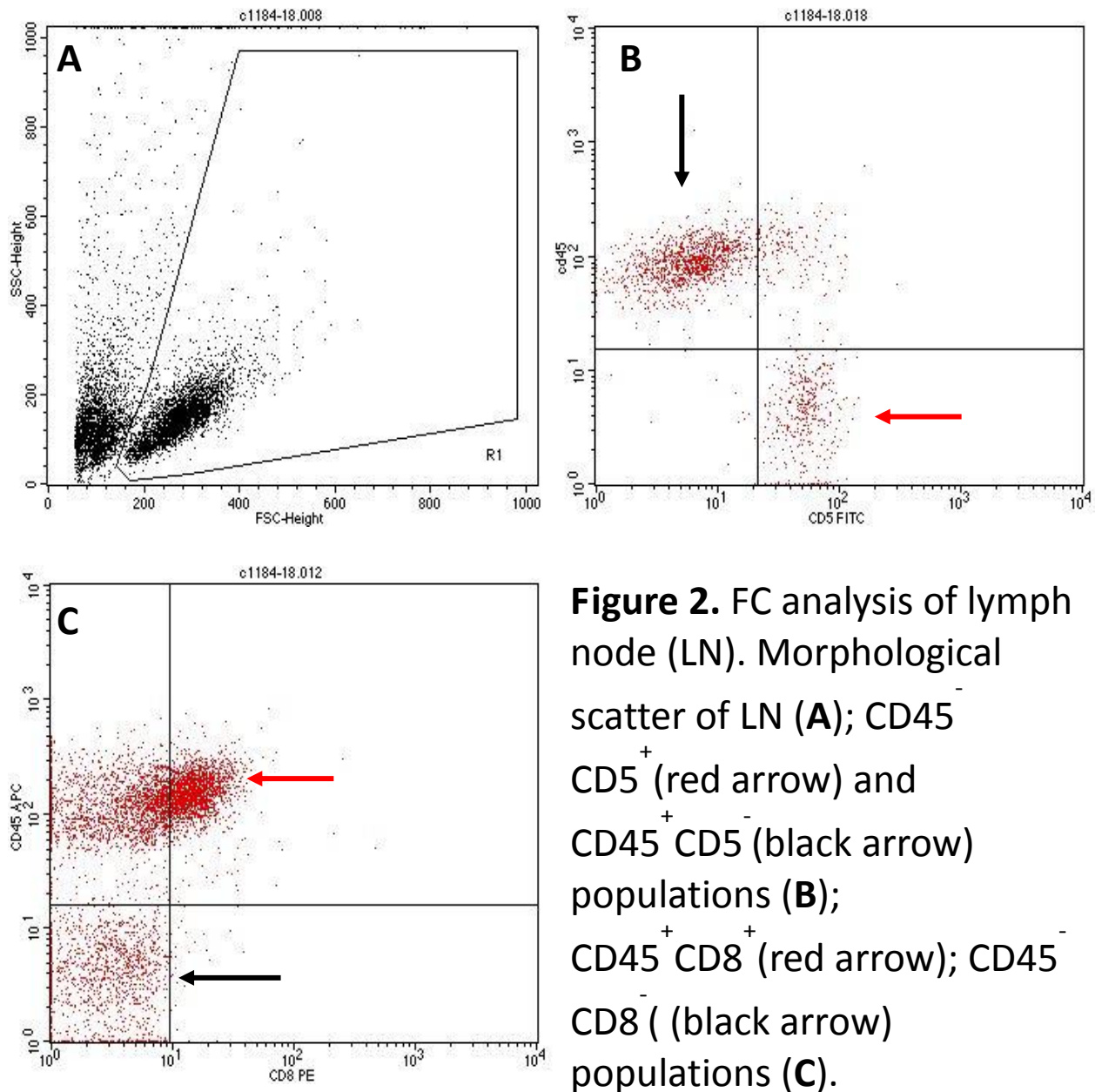
Anti – CD117 (mast cells, precursor cells)

Anti – MHCII (leukocytes, monocytes).

The entire panel was performed on the lymph node sample.

Lymph node aspirate showed two different lymphoid populations (Figure 2): the prevalent population accounted for 40% and the cells were medium-sized expressing CD45 and CD8, staining negative for CD5; the other population accounted for 30% and the cells were small-sized, staining positive for CD5 and CD21 and staining negative for CD45, CD4 or CD8.

However, peripheral blood was composed mainly by disrupted cells but a population of small-sized lymphocytes staining positive for CD5 and negative for CD45 and CD8 was detected, accounting for the 54% of nucleated cells; a small population of CD45<sup>+</sup>CD5<sup>+</sup>CD8<sup>+</sup> cells, accounting for the 5,6% was also present.



**Figure 2.** FC analysis of lymph node (LN). Morphological scatter of LN (**A**); CD45<sup>+</sup> CD5<sup>-</sup> (black arrow) and CD45<sup>+</sup> CD5<sup>+</sup> (red arrow) populations (**B**); CD45<sup>+</sup> CD8<sup>+</sup> (red arrow); CD45<sup>+</sup> CD8<sup>-</sup> (black arrow) populations (**C**).

**What is your diagnosis?**

**Are the lymph node subpopulations representative for a lymphoid hyperplasia?**

**Is one or more neoplasia possible with a such an immunophenotypic pattern?**

**What else might be done?**

The immunophenotypic pattern is highly suggestive of lymphoma; since the same CD45 negative population has been detected in the lymph node and peripheral blood, being thus the main population detected, T zone lymphoma (TZL) is the most probable neoplasm. The presence of a second clone of CD8+T cells showing aberrantly negative to CD5 is suggestive of a second neoplasm or a transformation of TZL. PARR was performed to understand if clonal rearrangement of T-cell receptor with two different clones was present, suggesting a double neoplasm, instead of a single rearrangement supporting the hypothesis of transformation. Two clonal rearrangements of the TCR gene were detected on lymph node aspirate. However, no clonal rearrangement was detected in peripheral blood, but this was supposed to be due to the poor quality of blood sample.

The dog hasn't been treated since no symptoms were evident and the diagnosed lymphoma was low grade.

## Discussion

T zone lymphoma is an indolent lymphoma of canine species<sup>1-4</sup>. Cytologically, "hand mirror" shape of small clear lymphocytes is a suggestive sign, although non conclusive<sup>5</sup>. The diagnostic challenge is to differentiate a reactive hyperplasia of lymphoid tissues from low grade lymphomas and until a few years ago, histopathology together with immunohistochemistry were considered mandatory to establish a reliable diagnosis. However, recently, some scientific data have been published, concerning the diagnosis of TZL, providing a strong evidence of a typical aberrancy of TZL immunophenotype, which includes the lack of expression of CD45 antigen<sup>1-2</sup>. This feature is apparently due to the absence of gene transcription, since CD45 gene was detected in neoplastic cells<sup>4</sup>.

Thus, currently, small clear cell appearance, T-zone pattern and CD45 negativity together (cytologically, histologically and immunophenotypically, respectively) are considered diagnostic of TZL. Importantly, the clinical presentation seems to play a distinguishing role, too<sup>2,3</sup>. In particular, TZL is a disease of adult dogs, with no sex predilection; in one study<sup>2</sup>, a large proportion of dogs were Golden Retrievers, whereas this wasn't confirmed in another study<sup>3</sup> and a different genetic background among countries (American vs European blood lines) has been recently suggested<sup>7</sup>. In the first study, Shih Tzu was the second most represented breed<sup>2</sup>. A remarkable peripheral blood infiltration (stage V) is commonly described similar the case presented here, but this isn't considered a negative prognostic factor for this disease and it is often concomitant with a low infiltration of bone marrow and typically a very low prevalence of cytopenias. Cases with a poorer prognosis were overtly ill (substage b)<sup>3</sup>.

Overall, stage V, no blood cytopenias and a good prognosis with long survival times can be considered as hallmarks of TZL.

There is no consensus treatment to date for TZL and chemotherapy should be decided on a case-by-case basis, according to the health state of the patient.

Thus, according to the general presentation of Cookie, TZL is strongly suspected, even if a second homogeneous T cell population was detected both by flow cytometry and PARR and consequently, the coexistence of two different neoplasms is the first hypothesis. Definitely, histological evaluation of the analysed lymph node might be interesting. A transformation was also suspected at the beginning and can't be ruled out at this point of the disease, but it is less likely, since in the transformation cases reported in literature, clones detected by PARR were alike<sup>8</sup>. Follow up of this patient and re-evaluation of the lymph node subpopulations could possibly clarify the situation and eventually lead to a therapeutic decision.

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