

## 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<sup>®</sup> 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).

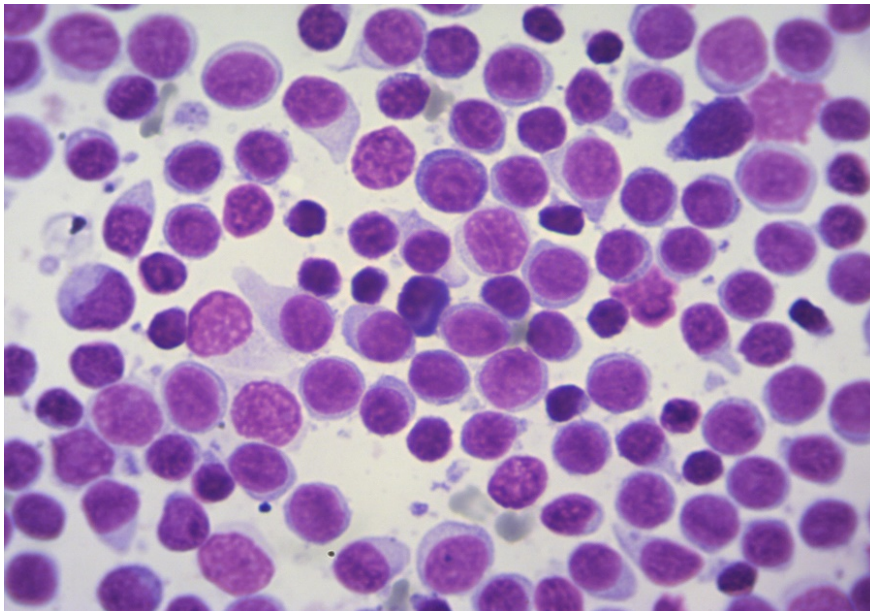
A comprehensive serum biochemistry was unremarkable.

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

HEMATOLOGY	15/May/2018	RIIs
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)

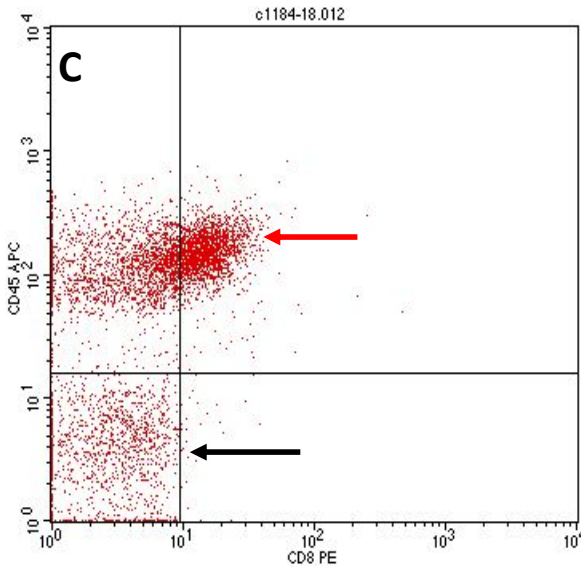
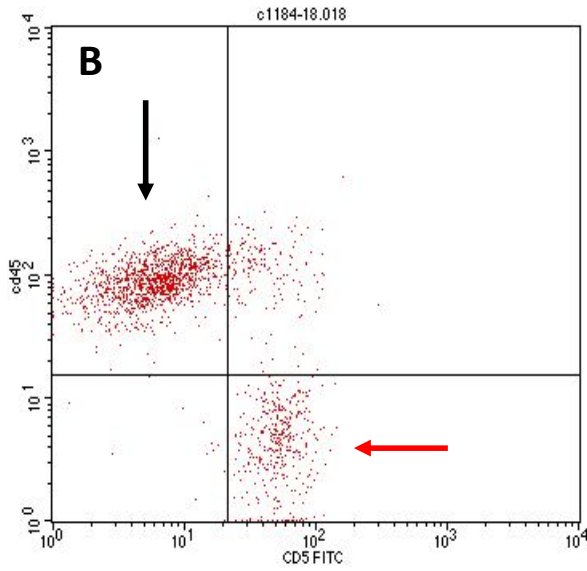
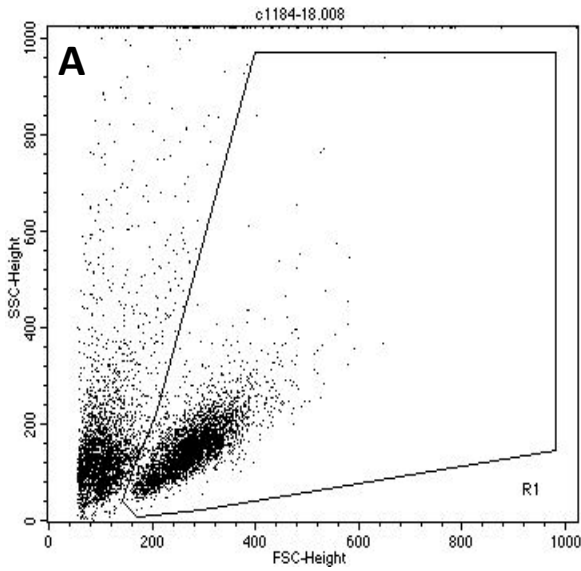
Anti – CD117 (mast cells, precursor cells)

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> (red arrow) and CD45<sup>+</sup> CD5<sup>-</sup> (black 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 such an immunophenotypic pattern?

What else might be done?