Title
Pancytopenia in a 13-year-old dog

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Specimen
Bone marrow aspiration
Liver fine-needle aspiration
Spleen fine-needle aspiration

Signalment
13-year-old neutered female, Cairn Terrier dog

History
The dog was referred to the ICU of the veterinary teaching hospital of Lyon, France, for the medical management of pancytopenia in a context of weight loss and fatigue that had been evolving for 4 months. A biochemistry panel and urine dipstick also revealed slightly increase of alkaline phosphatase (354 U/L; reference interval 23-212 U/L) and moderate bilirubinuria respectively. Total bilirubin was within reference intervals and SNAP 4Dx Plus (detection of *Dirofilaria immitis* antigen, antibodies to *Anaplasma phagocytophilum*, *Anaplasma platys*, *Borrelia burgdorferi*, *Ehrlichia canis*, *Ehrlichia ewingii*, IDEXX Laboratories, Westbrook, USA) was negative. No other prior results were available.

Clinical findings
Clinical examination revealed pale mucous membranes, prolonged capillary refill time, tachycardia and heart murmur at the apex (4/6). Blood group was determined as DEA1- and a blood transfusion was performed.

Hematology
A CBC performed on Sysmex XT-2000iV analyzer (Sysmex, Kobe, Japan) revealed moderate macrocytic normochromic non regenerative anemia, panleukopenia and thrombocytopenia (confirmed on the smear)(Table 1). Blood smear revealed frequent ovalocytes, a left shift with presence of toxic neutrophils (not depicted), granular lymphocytes, and activated monocytes, and confirmed thrombocytopenia (Figure 1).

Figure 1: Blood smear, Romanowsky stain, 50x objective.

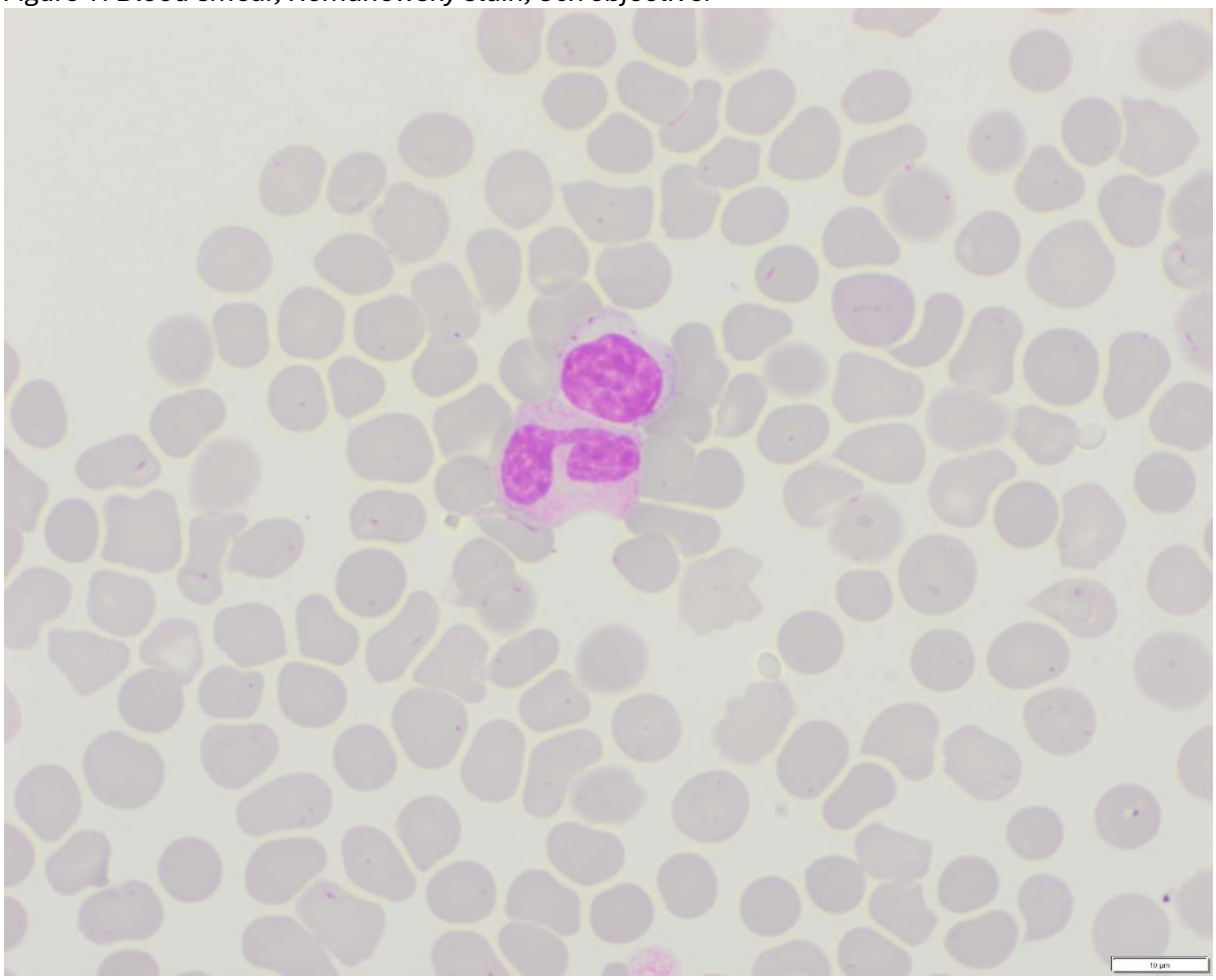


Table 1: CBC results Sysmex XT-2000iV

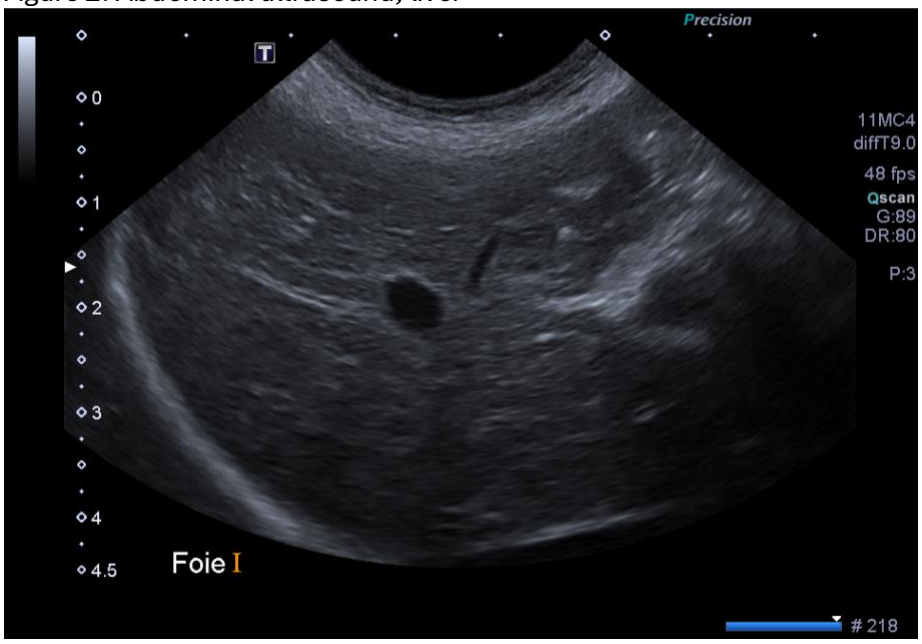
Measurand	Units	Result	LRL of RI	URL of RI
RBC	10^{12}/L	**1.7**	5.5	8.5
HGB	g/dL	**5.3**	12	18
HCT	%	**15.3**	37	54
MCV	fL	**90**	60	71
MCH	pg	**31.2**	17	23
MCHC	g/dL	34.6	31	36
RET	%	3.54		
Corrected RET	%	**1.2**	0	1
RET	10^9/L	60.2	0	80
WBC	10^9/L	**1.6**	6	17
Neutrophils	10^9/L	**0.7**	2.9	13.6
Lymphocytes	10^9/L	**0.7**	1.1	5.3
Monocytes	10^9/L	**0.2**	0.3	1.6
Eosinophils	10^9/L	**0**	0	1.4
Basophils	10^9/L	**0**	0	0.1
PLT-O	10^9/L	**92**	140	600

RBC: red blood cell; HGB: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RET: reticulocyte; WBC: white blood cell; PLT-O: platelet count by optic method; RI: reference interval; LRL: lower reference limit; URL: upper reference limit.

Abdominal Ultrasound examination (Figures 2, 3 and 4)

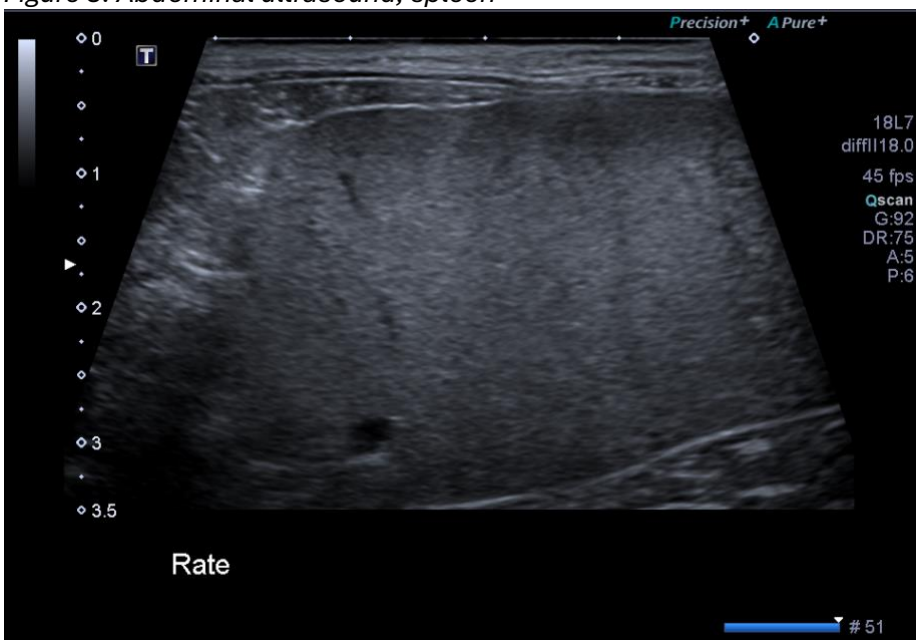
Abdominal Ultrasound examination revealed marked splenomegaly and hepatomegaly with diffuse hyperechoic structure. Splenic and ileocolic lymph nodes were also slightly increased.

Figure 2: Abdominal ultrasound, liver



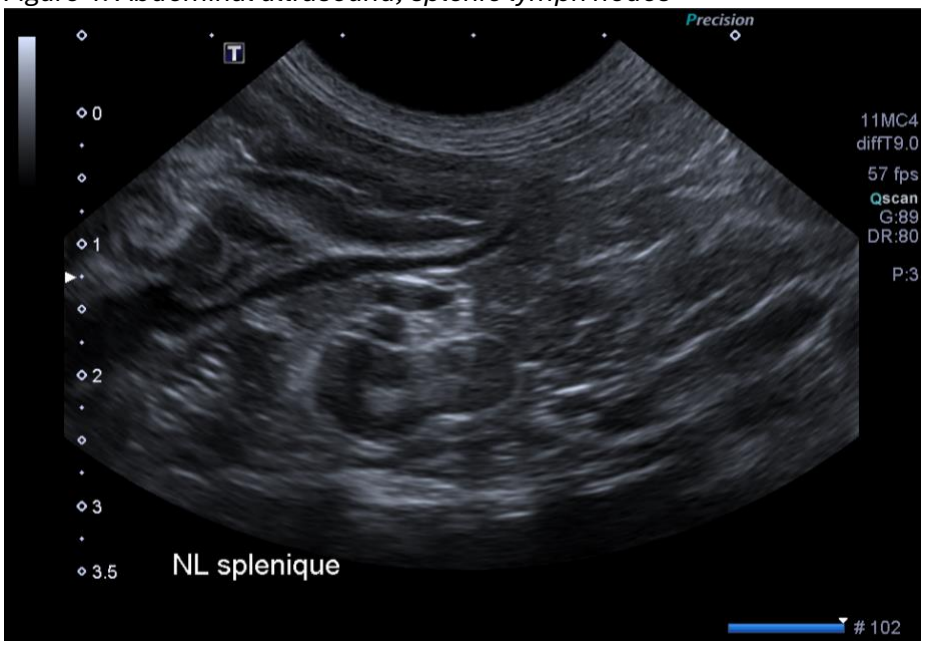
Credit: VetAgroSup, Diagnostic Imaging Department

Figure 3: Abdominal ultrasound, spleen



Credit: VetAgroSup, Diagnostic Imaging Department

Figure 4: Abdominal ultrasound, splenic lymph nodes



Credit: VetAgroSup, Diagnostic Imaging Department

Considering these initial results, bone marrow, liver, spleen, and lymph nodes fine-needle aspirations were performed.

Figure 5: Fine needle aspirate biopsy of the liver, Romanowsky stain, 50x (A) 100x (B) objectives.

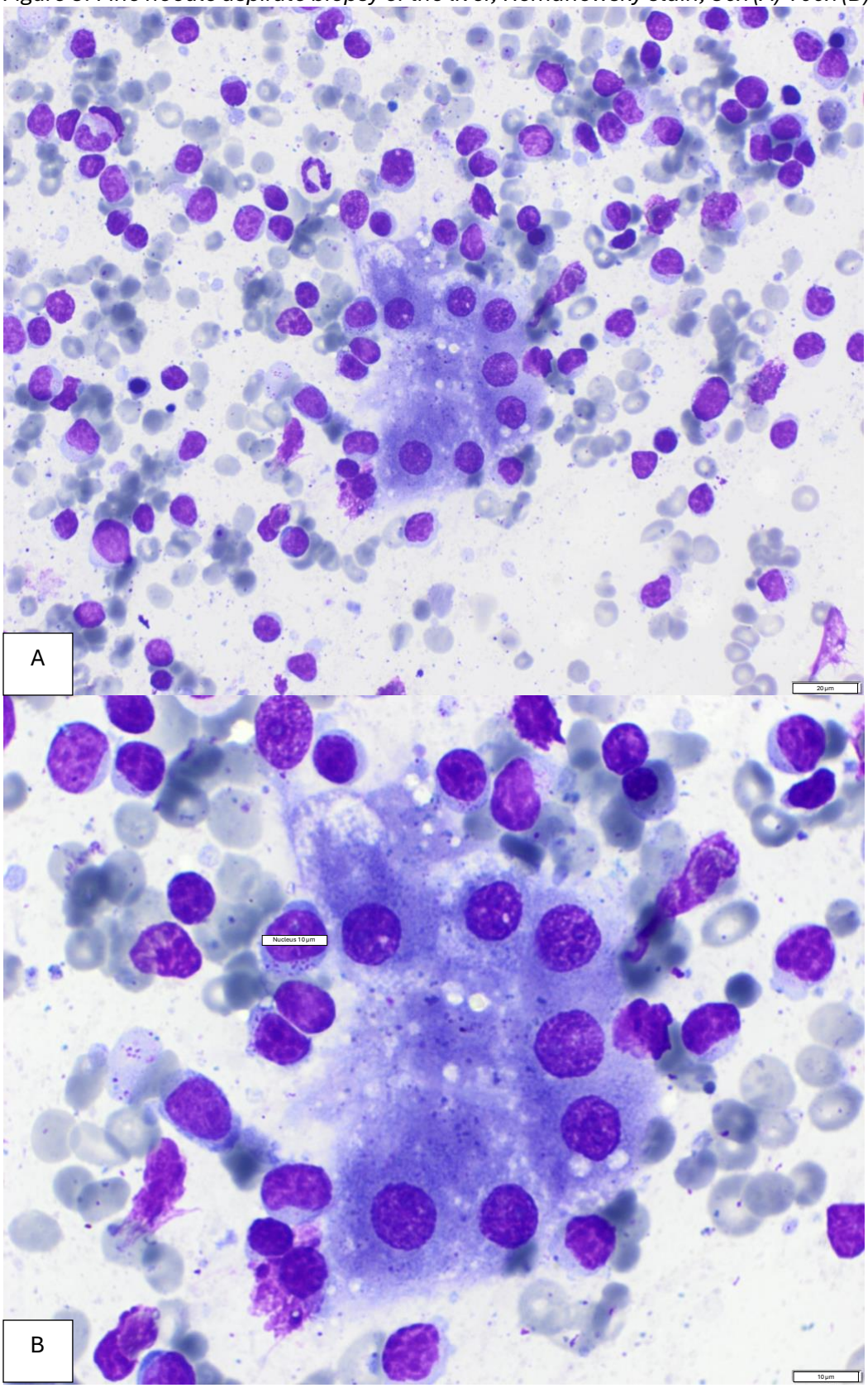


Figure 6: Fine needle aspirate biopsy of the spleen, Romanowsky stain, 50x (A) 100x (B) objectives.

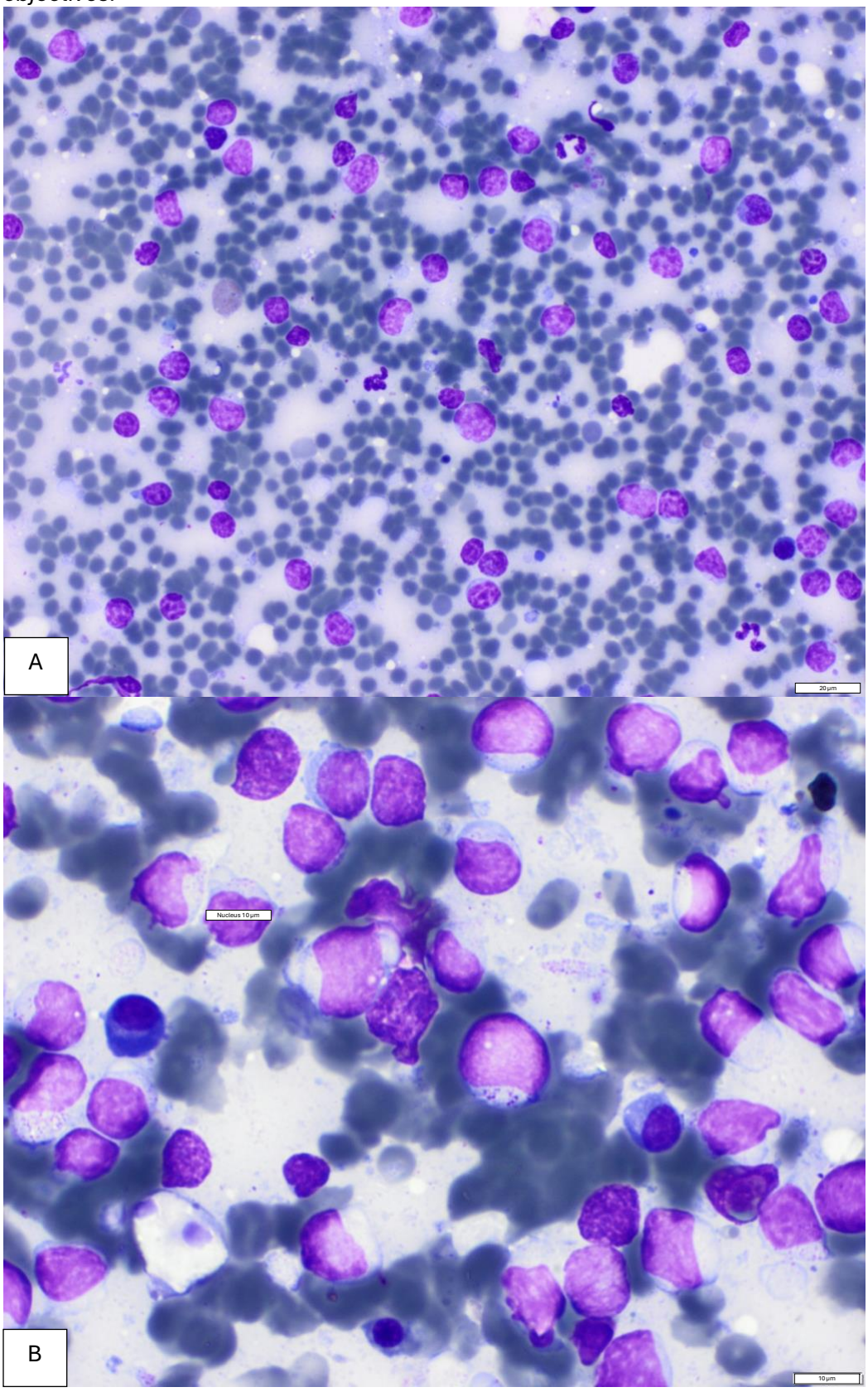
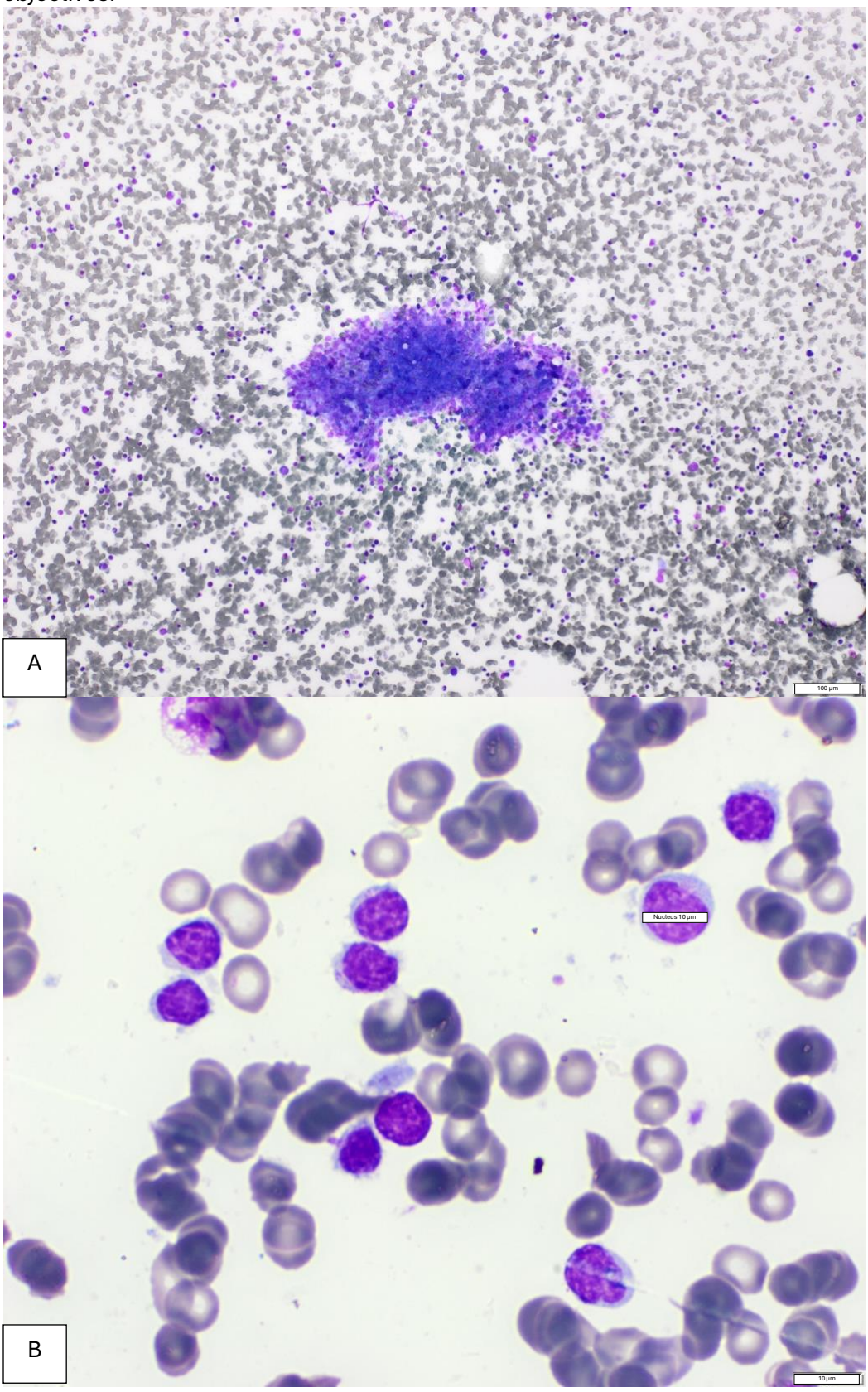


Figure 7: Fine needle aspirate biopsy of the bone marrow, Romanowsky stain, 10x (A) 100x (B) objectives.



Unfortunately, fine-needle aspirate biopsy of lymph nodes were not representative (aspiration of adipose tissue only).

Questions
What do the 3 aspirations have in common?
What could be your first hypothesis for the origin of pancytopenia?
Which additional data could confirm your hypothesis?

Interpretation/Diagnosis
Liver fine-needle aspiration (Figure 5):
The slides were of excellent quality and cellularity. The background was hemorrhagic. Clusters of well-differentiated hepatocytes were noticed. A monomorphic lymphoid population was observed, composed of small granular lymphocytes, with round to ovoid nuclei (10 µm), clumped chromatin, no prominent nucleoli, slightly basophilic cytoplasm, and small granules.
Conclusion: We concluded on an infiltration of the liver by a lymphoid population, most likely malignant.

Spleen fine-needle aspiration (Figure 6):
The slides were of excellent quality and cellularity. The background was hemorrhagic. Clusters of reticulated cells associated with platelets clumps were noticed (not depicted), suggestive of the red pulp and validating the splenic origin of the sample. A large lymphoid population was observed, composed of small mature lymphocytes, plasma cells and 40% monomorphic small to large granular lymphocytes (same morphology as granular lymphocytes in the liver); some hematopoietic progenitor cells of the 3 lineages and rare mast cells, isolated, well-differentiated were also present (not depicted).
Conclusion: An infiltration of the spleen by the same malignant lymphoid population as in the liver was highly suspected. An extramedullary hematopoiesis was also reported.

Bone marrow aspiration (Figure 7):
The slides were of poor to moderate quality with only few spicules overall. Based on these spicules, increased cellularity was suspected. The differential count revealed a predominance of lymphoid cells with 77% of mostly small monomorphic granular lymphocytes, similar to those observed in liver and spleen, with round to ovoid nuclei (10 µm), clumped chromatin, no prominent nucleoli, slightly basophilic cytoplasm, and small granules. Erythroid, granulocytic, megakaryocytic lineage were represented, with complete and ordered differentiation and moderate signs of dysplasia (not depicted): dyserythropoiesis (nuclear and cytoplasmic asynchrony, abnormal nuclear shapes, micronuclei) and dysmegakariocytopoiesis (dwarf megakaryocytes). Rare images of erythrophagocytosis were also noticed (not depicted).
Conclusion: Hypercellularity of bone marrow aspirates due to lymphoid infiltration, with myelophtisis and secondary hypoplasia of the erythroid, myeloid, and megakaryocytic series. A hematologic malignancy of lymphoid origin with small granular lymphocytes (NK or T-cells more likely) was suspected. Dyserythropoiesis and dysmegakariocytopoiesis were secondary. An infectious complication could not be excluded (left shift, activated monocytes).

Conclusion of the bone marrow, spleen and liver aspirations: A hematologic malignancy of lymphoid origin with small granular lymphocytes (NK or T-cells more likely) was highly suspected. The differential includes low-grade lymphoma stage V, chronic lymphocytic leukemia, hepatosplenic T-cell lymphoma, hepatocytotropic T-cell lymphoma, T-acute lymphoblastic leukemia, lymphoblastic T-cell lymphoma…

What do the 3 aspirations have in common? Marked infiltrations by a monomorphic lymphoid population, most likely malignant, composed of mostly small granular lymphocytes, with round to ovoid nuclei (10 µm), clumped chromatin, no prominent nucleoli, slightly basophilic cytoplasm, and small granules.

What could be your first hypothesis for the origin of pancytopenia? Pancytopenia could be secondary to myelophtisis (replacement of the erythroid, myeloid, and megakaryocytic series by malignant lymphoid infiltration).

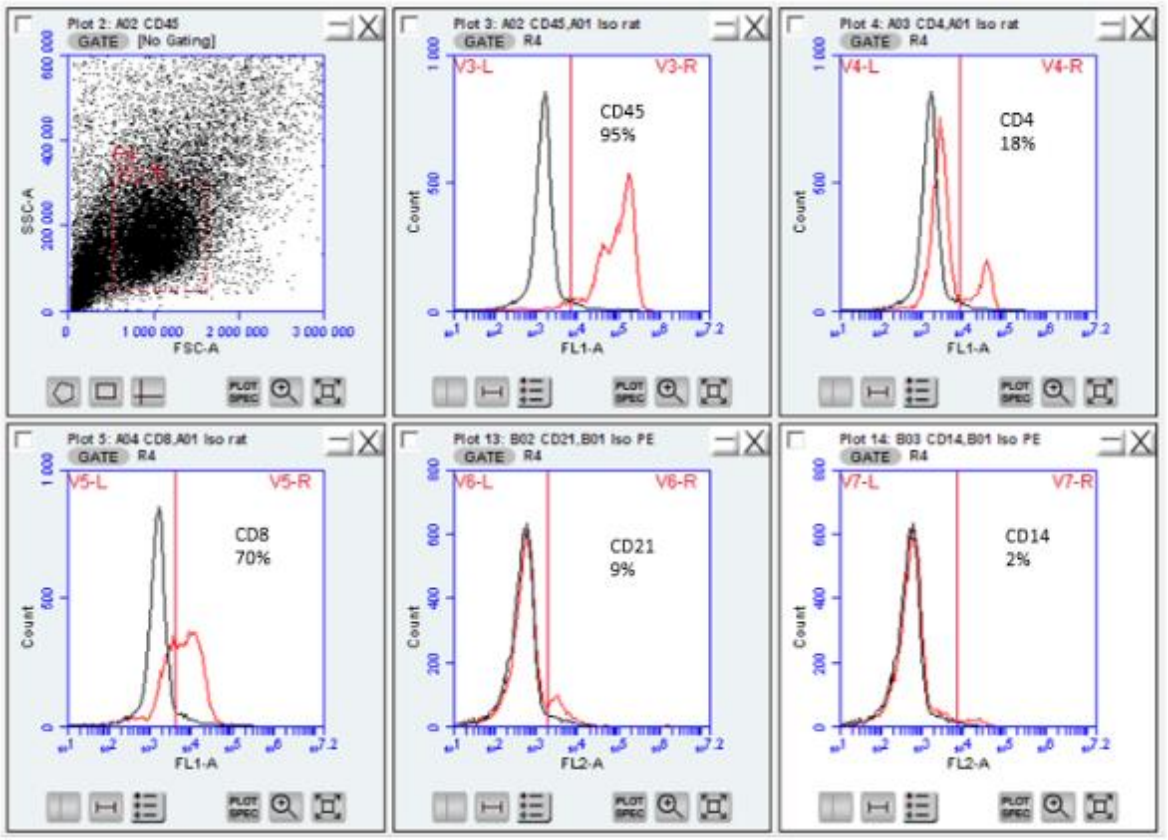
Which additional data could confirm your hypothesis? PARR to assess clonality and flow cytometry to characterize the lymphoid population.

Additional information

Flow cytometry was performed on spleen sample (Figure 8). 95% of the total cells were CD45+ (leucocyte common antigen). The immunophenotype of the lymphoid population was : 70% suspected neoplastic cells T-cells CD8+ ; less than 20% T-cells CD4+ and less than 9% B-cells CD21+.

Conclusion: A hematologic malignancy of lymphoid origin involving small granular cytotoxic T-cell lymphocytes (CD8+) was highly suspected.

Figure 8: Flow cytometry results on spleen sample.



Follow up and clinical outcome

A modified L-COP protocol was induced, followed by metronomic chemotherapy (chlorambucil, prednisolone) with partial clinical response to therapy and biological improvement: anemia became regenerative, hematocrit/hemoglobinemia/RBC and platelet counts increased but persistent leukopenia/neutropenia, spleen/liver and bone marrow involvement were noticed. 7 months after treatment initiation, pancytopenia recurred. The dog died 11 months after initial presentation.

Discussion

A hematologic malignancy of lymphoid origin involving small granular lymphocytes CD8+ was diagnosed. PARR is mandatory in human medicine to assess clonality and, therefore, establish an accurate diagnosis. Unfortunately, PARR was not available in our case. However, expansion of lymphoid population secondary to infectious disease such as vector-borne diseases (*e.g.* ehrlichiosis) seemed unlikely as serology testing was performed twice and was negative. Several hypothesis were thus considered including aleukemic chronic lymphocytic leukemia, hepatosplenic T-cell lymphoma with bone marrow involvement, hepatocytotropic T-cell lymphoma or precursor cell neoplasms like T-acute lymphoblastic leukemia, and lymphoblastic T-cell lymphoma. But all criteria were not met for any of these hypothesis and, even if we found some atypical case reports in literature, some items of our case were very unusual. Is it possible to exclude some of these tentative diagnosis? Let's summarize pros and cons for each hypothesis (Table 2).

Table 2: hypothesis, pros and cons (adapted from Schalm's, 2022)

	Pros	Cons
T-Acute Lymphoblastic Leukemia	Severe pancytopenia Small lymphocytes with scant amount of cytoplasm Myelophtisis Extensive bone marrow involvement (77% infiltrate) Alkaline phosphatase increased Some granular lymphocytes on blood smear	No lymphocytosis No prominent nucleoli, mostly mature chromatin CD8+ = mature post-thymic phenotype Uncommon diagnosis for dogs
Lymphoblastic T-cell lymphoma		No hypercalcemia Bone marrow involvement ++ > lymph nodes No mitoses CD8+ Survival > 5 months with chemotherapy
Chronic Lymphocytic Leukemia	Small lymphocytes No prominent nucleoli Cytoplasmic granules Splenomegaly Indolent disease with non-specific clinical signs, medium age patient No hypercalcemia, no hyperphosphatemia CD8+ Most common malignant hemopathy in dogs	No lymphocytosis Severe pancytopenia Bone marrow involvement ++ Survival < 930-1098 days
Hepatosplenic T-Cell Lymphoma	Pancytopenia No lymphocytosis Spleen++/liver+/bone marrow involvement Weight loss/fatigue for 4 months at initial presentation Cytoplasmic granules, medium lymphocytes CD4-CD8+ Relapses in most cases	No fever Small lymphocytes No/rare erythrophagocytosis/thrombophagocytosis Survival > 1 month
Hepatocytotropic T-Cell Lymphoma	Alkaline phosphatase and alanine aminotransferase increased Hepatocytes/neoplastic lymphocytes close association CD8+	Splenomegaly No icterus/cholestasis Anemia

As a mature post-thymic T-cell phenotype was determined by flow cytometry and no or too few arguments were listed for these hypothesis, T-acute lymphoblastic leukemia, lymphoblastic T-cell lymphoma, hepatocytotropic T-cell lymphoma were considered unlikely and were excluded from the differential diagnosis. Two hypothesis remained to be explored.

On the one hand, there were some arguments for the chronic lymphocytic leukemia (CLL) hypothesis: in particular, CD8+ T-cell is the most frequent phenotype of CLL with a high prevalence of Large Granular Lymphocyte (LGL) subtype (Tasca 2009); but, also, some cons. Indeed, in our case, there was no lymphocytosis, whilst in veterinary clinical pathology, lymphocytosis was reported as a hallmark of CLL and even participated to its definition (Deravi, Schalm's veterinary hematology 2022, p610). However, some human similar cases of CLL without lymphocytosis were reported (Bagacean 2017). Moreover, in our case, the main clinicopathological finding was severe pancytopenia but in the literature, even if mild anemia was frequently reported (75-86% of the cases), thrombocytopenia was rare (15-45% of the cases) and neutropenia unusual in dogs (Tasca 2009). Still, several cases also reported significant neutropenia and thrombocytopenia in dogs (Elliott 2018, Museux 2019, Capasso 2023) and neutropenia was commonly reported in human LGL leukemia (WHO 2017). Finally, the large predominance of lymphoid neoplastic cells in our patient's bone marrow did not perfectly fit with common description of CLL: *"splenic involvement is extensive in most cases and may result in splenomegaly, while bone marrow infiltration is sparse and likely occurs late in disease"* (Schalm's veterinary hematology 2022, p610). But major bone marrow involvement was occasionally observed at the very end of the disease progression in dogs (Capasso 2023) and a bone marrow infiltrate higher than 10% was reported in LGL leukemia in humans.

On the other hand, spleen, liver, and bone marrow involvements were typical features of hepatosplenic T-cell lymphoma (Keller 2012, WHO 2017). Additionally, lymphoproliferative diseases involving granular lymphocytes in dogs frequently originated from spleen (McDonough 2000). However, the clinical course of our case did not fit with the typical presentation of the entity. Indeed, this condition was typically described as an aggressive subtype of extranodal lymphoma with mostly short survival time (23 days without treatment) and, in some cases, 196 days with chemotherapy (Keller 2012).

Unfortunately, it was not possible conclude on the exact type of lymphoid malignancy in our case. This was due not only to the lack of data available from the case but also to the state of art in this topic in veterinary medicine.

It would have been interesting to add β_2 integrin antigens (CD11d) immunophenotyping to confirm splenic origin (CD11d+)(Keller 2012). A more comprehensive antigen panel could have been helpful to better characterize the lymphoid population, to identify some aberrant immunophenotype and to exclude some differential diagnosis (CD3 for T-cell lineage, CD56 to exclude NK cells, CD34 to exclude stem cells, TCR γδ to study hepatosplenic T-cell lymphoma hypothesis in details, granzyme B to help differentiate T-Cell LGL Leukemia granzyme B positive and hepatosplenic T-cell lymphoma granzyme B negative in humans…)(WHO 2017). Unfortunately, we had some limited choice for this patient due to financial and technical limitations.

Conclusion

In summary, atypical T-cell chronic lymphocytic leukemia and hepatosplenic T-cell lymphoma hypothesis best fit this clinical case presentation.

This case illustrates the atypical clinical presentations that some hematologic malignancies can express and the importance of ancillary tests such as PARR and flow cytometry in the diagnostic process. It also emphasizes the need to keep differential diagnoses open for a better management of patient health.

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