Chronic lymphocytic leukemia/Small cell lymphoma in a horse

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Signalment:
Horse, Irish Draft mare, 16 years old

History:
The horse was admitted to the Chiltern Equine Clinic for an annual health check.

Clinical findings:
Clinical examination revealed normal vital parameters, excellent body condition with normal appetite and no history of recent weight loss. Mild/moderate generalized lymphoadenomegaly was identified.

Diagnostic procedures:
On ultrasound, palpable lymph nodes appeared enlarged, with a hyperechoic, homogeneous texture. Rectal palpation and trans-rectal ultrasonography revealed prominent mesenteric lymph nodes. Transcutaneous abdominal ultrasonographic evaluation was unremarkable.

Haematology and biochemistry, fine needle aspirations of regional lymph nodes and a bone marrow aspirate were performed. Results are shown below.

Hematology:

	Value	Reference interval
WBC	63.06 x 10^9 / L	5.40 - 14.30
Neutrophils	2.6 10^9 / L	2.3 – 8.9
Lymphocytes	58.2 10^9 / L	1.5 – 7.7
Monocytes	2.0 10^9 / L	0.0 – 1.5
Eosinophils	0.2 x 10^9 / L	0.7 - 4.9
Basophils	0.1 x 10^9 / L	0.0 - 0.3
RBC	6.04 x 10^12 / L	5.50 - 9.50
Hb	10.30 g / dl	8.00 - 14.00
HCT	0.298 L / L	0.275 - 0.450
MCV	49 fl	37 – 59
MCH	17.1 pg	12.3 - 19.7
MCHC	35 g / dl	40 - 46.7
Platelets	253 x 10^9 / L	100-350
PCV (spun)	28 %	24 – 44
Plasma protein	65 g / L	60 – 80
Fibrinogen	4 g / L	1 – 4

Biochemistry:

	Value	Reference interval
Sodium	139 mmol / L	135 – 155
Potassium	6.3 mmol / L	2.7 -5.5
Chloride	96 mmol / L	98 – 106
Total CO2 (bicarbonate)	36 mmol / L	14 – 36
Na : K ratio	22	> 27
Anion Gap	13	11 – 25
Urea	5.6 mmol / L	3.3 – 6.7
Creatinine	122 mmol / L	77 – 145
Glucose	<0.6 mmol / L	3.4 – 6.4
Total protein	75 g / L	55 – 75
Albumin	36 g / L	25 – 50
Globulin	39 g / L	17 – 35
Albumin : Globulin ratio	0.9	0.8 – 2.0
Calcium	3.43 mmol / L	2.50 – 3.50
Phosphate	1.18 mmol / L	0.90 – 1.60
AST	343 IU / L	183 – 497
CK	200 IU / L	113 – 333
ALP	294 IU / L	153 – 311
GGT	35.2 IU / L	< 50.0
GLDH	1.4 U / L	2.7 – 14.1
Bile acids	3 µm / L	2 – 53
Total bilirubin	19.8 µm / L	0.0 – 34.0
Direct bilirubin	6.6 µm / L	< 7.0

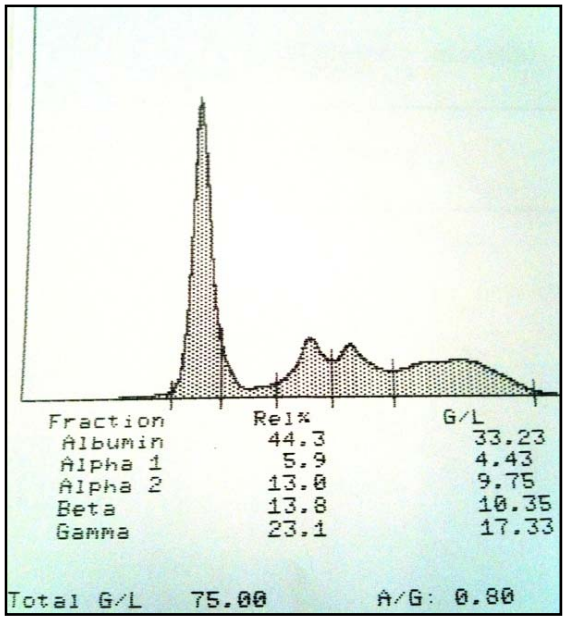

Fig. 1 Serum protein electrophoresis

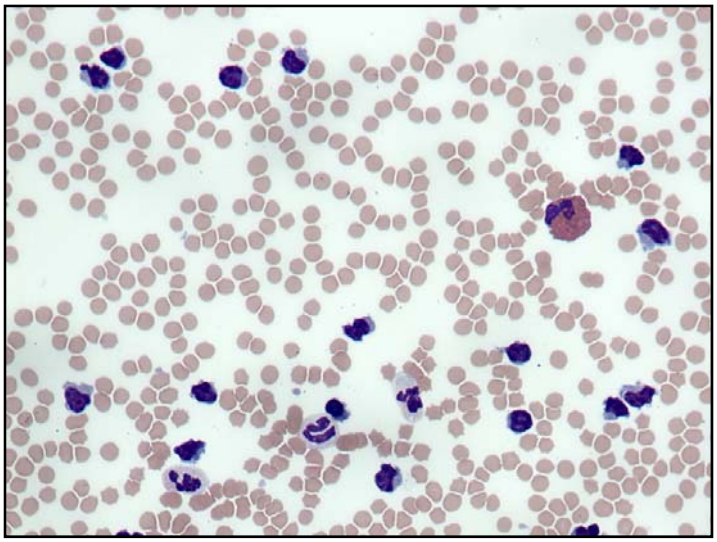

Fig.2 Peripheral blood smear, May-Grunwald Giemsa stained, x20

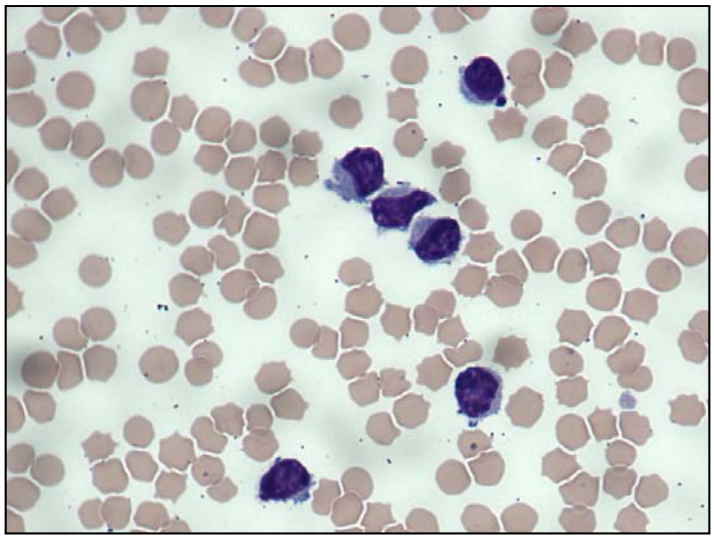

Fig.3 Peripheral blood smear, May-Grunwald Giemsa stained, x50

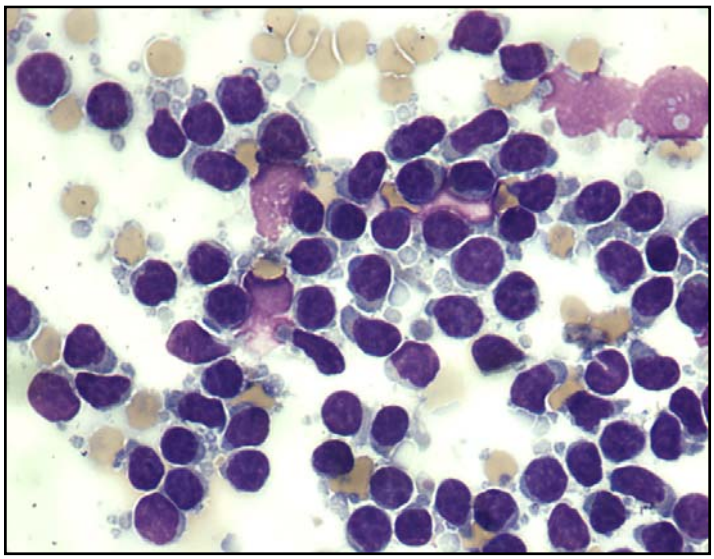

Fig. 4 Lymph node (FNA), May-Grunwald Giemsa stained, x50

Questions:

1. What are your main differential diagnoses?
2. Which further tests would you suggest to confirm your diagnosis?

Further investigations and discussion:

Hematology revealed a marked leukocytosis (63.06 x10^9/l) with lymphocytosis (58.2 x10^9/l) with the majority being small lymphocytes. No evidence of anemia or thrombocytopenia was found.
The serum biochemistry profile showed mild hyperglobulinemia (39g/L). Serum protein electrophoresis performed to investigate this was unremarkable. Severe hypoglycemia (0.6 mmol/l) and mild elevation in potassium (6.3 mmol/L) were likely to be due to delayed analysis (delayed separation of cells from serum).
Fine needle aspirates of multiple lymph nodes and a bone marrow aspirate were performed. Lymph node aspirates were characterized by a monomorphic population of small lymphocytes, similar to those identified in the peripheral blood. The bone marrow had low-normal cellularity with few spicules and megakaryocytes observed but all hematopoietic cell lines were present and there was a prevalence of small lymphocytes, accounting for >60% of the total bone marrow cells.
These results indicated the presence of either a leukemic small cell lymphoma or a chronic lymphocytic leukemia (CLL). Considering that the lymphadenomegaly was identified at the same time as the lymphocytosis, the distinction between these two conditions could not be made.

Flow cytometry and immunocytochemistry:

Other tests were carried out in order to further investigate the condition. Flow cytometry and immunocytochemistry on peripheral blood were performed.
Flow cytometry on peripheral blood was performed using an antibody panel for equine surface antigens. The antibodies used were as follows: mouse anti-equine CD5 (HT23A, VMRD, Inc., Pullman, U.S.A.), mouse anti-equine CD8 (HT14A, VMRD, Inc., Pullman, U.S.A.), mouse anti-equine CD4 (HB61A, VMRD, INC., Pullman, U.S.A.), bovine anti-equine B-cells (B29A, VMRD, INC., Pullman, U.S.A.).
Briefly, for these non-conjugated monoclonal antibodies an indirect labeling using fluorescein isothiocyanate-conjugated rabbit anti-mouse IgG was used. Data acquisition was performed with a flow cytometer FACSCalibur (Becton Dickinson, San Jose, California) operating with Cell Quest software (Becton Dickinson). Typically, 10000 events were collected from the gated region. Lymphocytes were negative to CD4, CD8, and B lymphoid antigen and expressed CD5. Variable intensity to CD5 was noted with two distinct populations clearly identified.

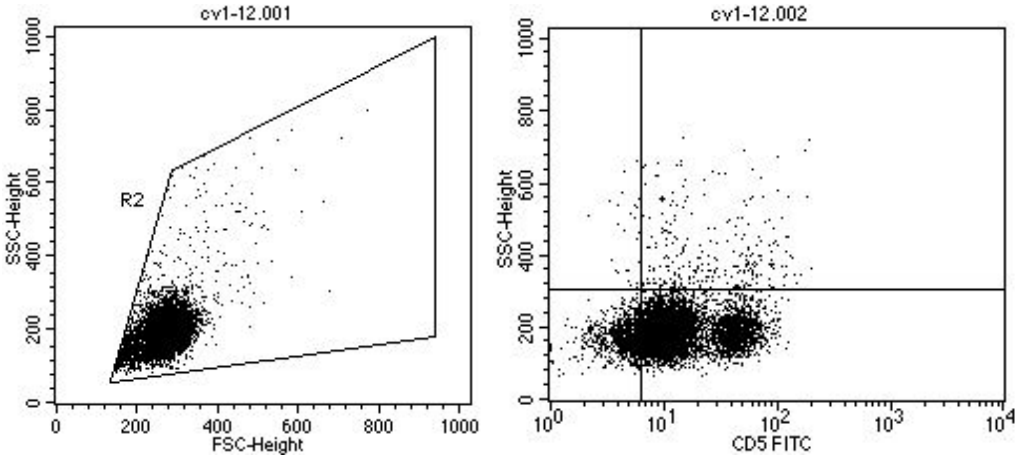

Fig. 5 Results of the flow cytometric analysis of lymphocytes stained with a monoclonal antibody recognizing equine leukocyte antigen CD5

Immunocytochemical staining for CD3 (monoclonal mouse anti-human antibody, clone F7.2.38, DAKO, Denmark) and CD79αcy (monoclonal mouse anti-human antibody, clone HM57, DAKO, Denmark) was performed using a Dako autostainer. All lymphocytes showed a moderate membrane and cytoplasmic positivity to CD3 and were all negative to CD79, indicating a T-cell phenotype.

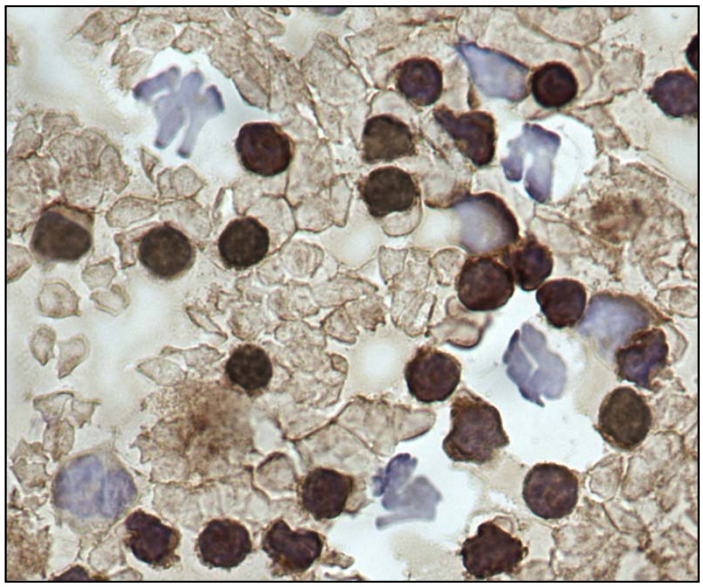

Fig. 6 Peripheral blood smear, Immunocytochemistry, CD5

Overall, the results were consistent with a leukemic small T-cell lymphoma/chronic T-cell lymphocytic leukemia (CLL).

Treatment:

As the horse remained clinically normal no treatment was given though regular re-examinations were undertaken.

Discussion:

Lymphoproliferative diseases are uncommon in horses (Scheneider et al., 2003). They include lymphoma, lymphoid leukemia, and multiple myeloma. Lymphoma typically involves lymph nodes and thoracic and abdominal organs although solitary tumours at extranodal sites have been reported (Meyer et al., 2006, Kelley et al., 1998). Peripheral blood involvement has also been described in advanced stages (Meyer et al., 2006). Conversely, in primary leukemia, neoplastic cells arise in the bone marrow and secondarily spread into the peripheral blood. In advanced cases, neoplastic lymphocytes may infiltrate lymphoid and other tissues (Rendle et al., 2007, Dascanio et al., 1992). This may prevent accurate differentiation of primary and secondary leukemia and only a thorough clinical history may help to distinguish between them.
However, in most species this distinction is considered of secondary importance. According to the WHO classification of hematopoietic tumours of domestic animals, both leukemias and lymphomas derived from the same neoplastic clone are classified in the same category (e.g. B/T cell lymphoblastic leukemia/lymphoma, B/T-cell chronic lymphocytic leukemia/lymphoma) (Valli et al., 2002).
In this case the marked peripheral lymphocytosis and the prevalence of small lymphocytes in all the enlarged palpable lymph nodes were considered sufficient to emit a diagnosis of

leukemic small cell lymphoma / chronic lymphocytic leukemia. The distinction between these conditions was not possible because lymphocytosis was observed concurrently with the generalized peripheral lymphadenomegaly.

A mild diffuse enlargement of palpable peripheral and mesenteric lymph nodes was identified on physical examination and ultrasound but no other clinical signs were identified. Lethargy, inappetence, and weight loss are frequently observed in horses with lymphoma and lymphoid leukemia although absence of clinical signs has previously been described (Rendle et al., 2007). Considering the advanced stage of the disease with both peripheral blood and lymph nodes affected, the absence of clinical signs is considered unusual.

No cytopenias were identified on hematology. This is unusual considering the bone marrow infiltration. Possible explanations for this are the presence of foci of extramedullary hematopoiesis in spleen and liver. However, considering the hemodilution and the low cellularity of the sample, part of the lymphocytes observed in the bone marrow aspirate might be blood derived and the bone marrow involvement might have been over-interpreted.

The mild elevation in globulin with a normal electrophoretic trace was possibly due to mild chronic inflammation. In horses with lymphoma and lymphoid leukemia moderate to marked hyperglobulinemia with increases in the beta and gamma globulin fractions have been frequently described (McClure et al., 2001,). Immunoglobulin deficiencies (mainly IgM) have also been associated with lymphoproliferative diseases in several horses (Dascanio et al., 1992, Furr et al., 1992). Quantitation of immunoglobulins by single-radial immunodiffusion was not performed therefore evaluation of the individual classes of immunoglobulins could not be assessed.

Further tests were performed in order to immunophenotype the cells. Flow cytometry and immunocytochemistry showed the cell population had a T cell phenotype. According to the literature, in horses lymphoma and lymphoid leukemia are more commonly of T cell phenotype although a large retrospective study of equine lymphoma identified a prevalence of B-cell forms (Rendle et al., 2007, Meyer et al., 2006, Kelley et al.,1998). Chronic leukemias in horses seem to show analogies with chronic leukemia in the dog and humans with slow progression and long survival times often exceeding one year. Lower survival times have been associated with advanced stages (Rendle et al., 2007). The persistent absence of clinical signs in this case is unexpected considering the advanced stage of the disease.

Flow cytometric analysis showed the lymphoid cells had a variable expression of CD5 with a majority of these having a mild expression of this surface marker and a lower proportion showing a marked positivity. This is likely due to a down regulation of the expression of surface markers, condition previously described in lymphoproliferative diseases in domestic animals (Gelain et al., 2007). The lack of expression of CD4 and CD8 would not be expected in a population of normal lymphocytes and is considered another indicator of neoplasia.

Immunocytochemical investigations showed positivity of all lymphoid cells to CD3, a surface antigen expressed by T-lymphoid cells during all stages of development (Wilkerson et al., 2005) The monoclonal mouse anti-human CD3 used in the present study has been reported to cross react with equine lymphocytes (Morrison et al., 2008). CD3 has not been tested on flow cytometry although CD5 is reported to have a similar expression (Wilkerson et al., 2005). These results show that immunocytochemistry may be an alternative to flow cytometry in order to immunophenotype lymphoproliferative diseases, when flow cytometry is not available. Flow cytometry requires a fresh blood sample and the availability of specific monoclonal antibodies. On the other hand, immunocytochemistry is characterized by a limited panel of antibodies available compared with flow cytometry.

In summary, this study reports an unusual case of leukemic small T-cell lymphoma / chronic T-cell lymphoid leukemia characterized by the absence of clinical signs in spite of the advanced stage of the disease. Moreover, the population of neoplastic lymphocytes showed an unusual phenotype with expression of CD3 and CD5 and not CD4 and CD8. Finally, to the author knowledge this is the first report of the use of immunocytochemistry for immunophenotyping a lymphoid neoplasm in the horse.

References:

1. Dascanio JJ, Zhang CH, Antczak DF, et al. Differentiation of chronic lymphocytic leukemia in the horse. J Vet Intern Med 1992; 6(4): 225-229.

2. Furr MO, Cristman MV, Robertson J, et al., Immunodeficiency associated with lymphosarcoma in a horse. J Am Vet Med Assoc 1992; 201: 307-309.

3. Gelain ME, Mazzilli M, Riondato F, et al., Aberrant phenotypes and quantitative antigen expression in different subtypes of canine lymphoma by flow cytometry. Vet Immunol Immunopathol 2008; 15 (121): 179-188.

4. Kelley LC, Mahaffey EA. Equine malignant lymphomas: morphological and immunoistochemical classification. Vet Pathol 1998; 35: 241-252.

5. McClure JT, Young KM, Fiste M, et al. Immunophenotypic classification of leukemia in three horses. J Vet Intern Med 2001; 15: 144-152.

6. Meyer J, DeLay J, Bienzle D. Clinical, Laboratory, and Histopathological Features of Equine Lymphoma. Vet Pathol 2006; 43: 914-924.

7. Morrison LR, Freel K, Henderson I, et al., Lymphoproliferative Disease with Features of Lymphoma in the Central Nervous System of a Horse. J Comp Pathol 2008; 139(4): 256-261.

8. Rendle DI, Durham AE, Thompson JC, et al. Clinical immunophenotypic and functional characterization of T-cell leukemia in six horses. Equine Vet J 2007; 39(6): 522-528.

9. Schneider DA. Lymphoproliferative and myeloproliferative disorders. In: Current therapy in equine medicine, ed. Robinson NE, 5th ed, 359-361. WB Saunders, Philadelphia, PA, 2003

10. Wilkerson MJ, Dolce K, Koopman T, et al., Lineage differentiation of canine lymphoma/leukemias and aberrant expression of CD molecules. Vet Immunol Immopathol 2005; 15(106): 179-196.

11. Withrow SJ, Vail DM, Withrow and MacEwen's small animal oncology (4th edition). Philadelphia, PA: WB Saunders; 2007.