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DIAGNOSIS AND TREATMENT OF CHRONIC T-LYMPHOCYTIC LEUKEMIA IN A SPOTTED HYENA (*CROCUTA CROCUTA*)

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Abstract: Physical examination of an asymptomatic 20-yr-old intact female spotted hyena (*Crocuta crocuta*) revealed a midabdominal mass. A complete blood count (CBC) revealed peripheral lymphocytosis. Abdominal ultrasonography and laparoscopy confirmed severe splenomegaly. Cytologic examination of a bone-marrow core and histologic examination of spleen and liver biopsy samples revealed neoplastic small lymphocytes. Immunohistochemical staining of liver and spleen samples with the use of leukocyte-specific monoclonal antibodies showed that the neoplastic lymphocytes were immunoreactive to T-lymphocyte CD3 receptor and immunonegative to B-lymphocyte CD79a receptor. The morphology and distribution of neoplastic T-lymphocytes within the spleen, liver, peripheral blood, and bone marrow was most consistent with chronic T-lymphocytic leukemia. Treatment with chlorambucil and prednisone effectively decreased the lymphocyte count, but was associated with thrombocytopenia, which resolved after chlorambucil treatment was temporarily discontinued. Chemotherapy was resumed with a single dose of L-asparaginase, followed by a lower dosage of chlorambucil and continued prednisone. Two years after initial diagnosis, the hyena developed a hemoabdomen and was euthanized. Neoplastic T-lymphocytes were present in spleen, liver, visceral and peripheral lymph nodes, lungs, heart, kidney, adrenal glands, mesentery, intestines, pancreas, brain, and bone marrow.

Key words: Chemotherapy, *Crocuta crocuta*, immunohistochemistry, leukemia, spotted hyena.

BRIEF COMMUNICATION

A 20-yr-old, 66.5-kg female spotted hyena (*Crocuta crocuta*) was anesthetized for routine examination (week 0). Anesthesia was induced with ketamine (KetaVed™, Phoenix Scientific, Inc., St. Joseph, Missouri 64503 USA; 7 mg/kg, i.m.), xylazine (Xyla-Ject®, Phoenix Scientific; 0.8 mg/kg, i.m.), and atropine sulfate (Phoenix Scientific; 0.02 mg/kg, i.m.). Anesthesia was maintained with the use of isoflurane (USP, Phoenix Pharmaceutical, Inc., St. Joseph, Missouri 64503, USA) in oxygen delivered through a face mask. Physical examination revealed an approximately 15 × 15 × 10-cm midabdominal mass. A CBC showed moderate leukocytosis (48,000 cells/μl; reference range[2] = 5,800–26,900 cells/μl) with 87% lymphocytes (41,760 cells/μl; reference range[2] = 1,080–7,520 cells/μl) and moderate microcytic, normochromic anemia (26%; reference range[2] = 32.9–51%). The lymphocytes were small to intermediate in size, with a high nuclear-to-cytoplasm ratio, a round to slightly indented nucleus with coarse chromatin, and no visible nucleolus (Fig. 1). No pulmonary nodules or infiltrates were visible on thoracic radiographs. Abdominal radiographs revealed a soft-tissue mass effect in the midabdomen, which was confirmed by ultrasound exam to be a severely enlarged spleen. A fine-needle aspirate of the spleen yielded mostly small lymphocytes, similar in morphology to those in the peripheral blood smear. The tentative diagnosis was lymphocytic leukemia.

The geriatric hyena remained asymptomatic; therefore, minimally invasive diagnostic tests and treatments were elected. During weeks 4 and 8, the hyena was anesthetized as previously described to obtain additional diagnostic samples. Physical exam findings and thoracic and abdominal radiographs were unchanged; however, the lymphocyte count had risen to 224,300 cells/μl during week 4 and to 302,800 cells/μl during week 8. Bone marrow samples from the right tibia and left femur were nondiagnostic.

Abdominal laparoscopy was performed to evaluate the abdominal organs. The spleen was markedly enlarged and had regions of capsular fibrosis and mottling. The liver appeared normal. Multiple biopsies were obtained from the spleen and liver.

Histologic examination of the spleen showed dense sheets of neoplastic small lymphocytes (Fig. 2). The liver contained similar-appearing neoplastic lymphocytes that expanded and disrupted portal regions and dissected along sinusoids.

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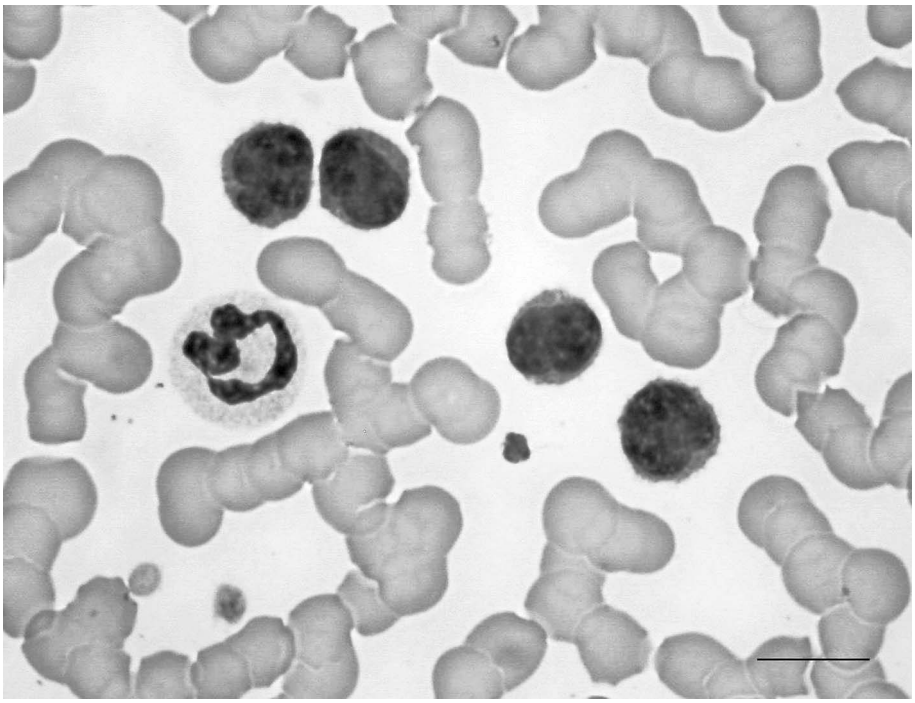

Figure 1. Photomicrograph of a peripheral blood smear from a spotted hyena with chronic T-lymphocytic leukemia. Neoplastic small lymphocytes are characterized by a high nuclear to cytoplasm ratio, a round to slightly indented or lobulated nucleus with coarsely clumped chromatin, and no apparent nucleolus. Wright-Giemsa stain; bar = 10 μm.

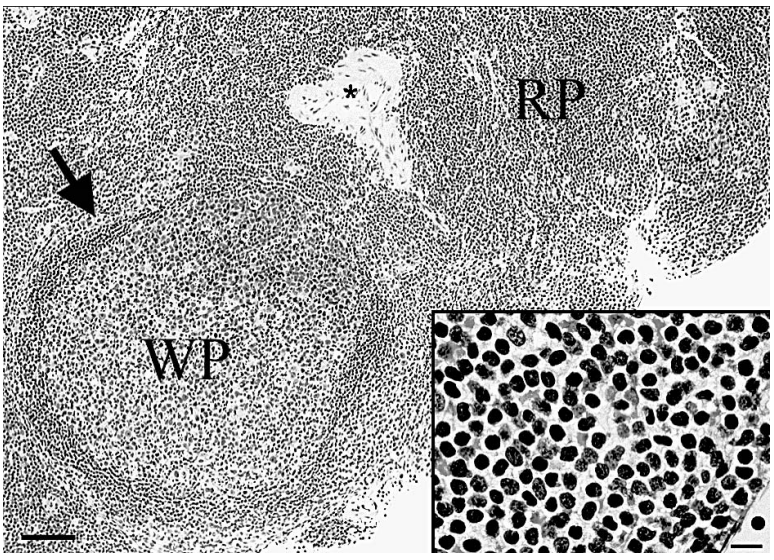

Figure 2. Photomicrograph of a section of the splenic biopsy from a spotted hyena with chronic T-lymphocytic leukemia. A dense sheet of neoplastic small lymphocytes effaces the red pulp (RP) and most of the white pulp (WP). The marginal zone (arrow) and periarteriolar lymphoid sheaths of nodular arterioles (asterisk) are obscured, and neoplastic lymphocytes infiltrate the remnant intact corpuscle. H&E stain; bar = 100 μm. Inset: Neoplastic lymphocytes are small to occasionally medium in size with a scant amount of amphophilic cytoplasm and a round nucleus with occasional shallow indentations, coarsely clumped chromatin, and 1–3 infrequently apparent pinpoint nucleoli. Mitotic figures are rare. Smooth-muscle trabecula (circle). H&E stain; bar = 10 μm.

Immunohistochemical staining was performed on liver and spleen samples. Neoplastic lymphocytes were immunoreactive for CD3, a T-lymphocyte receptor epitope, using canine CD3-12 antibody (Leukocyte Antigen Biology Laboratory, Davis, California 95616, USA) and immunonegative for CD79a, a B-lymphocyte receptor epitope, using human HM57 antibody (Dako Corporation, Carpinteria, California 93013, USA).

The lack of clinical signs, severe lymphocytosis, and morphology, distribution, and immunophenotyping of lymphocytes in this geriatric hyena were most consistent with chronic T-lymphocytic leukemia (CLL).[6,9,10] Oral chemotherapy was initiated during week 8 with administration of prednisone (Mutual Pharmaceutical Co., Inc., Philadelphia, Pennsylvania 19124 USA; 60 mg, 1 mg/kg, p.o., s.i.d.) and chlorambucil (Leukeran®, Glaxo-Wellcome Oxford House, Oxford Road, Aylesbury, Bucks HP21 8SZ, England; 10 mg, 0.16 mg/kg, p.o., e.o.d.).

Three weeks after the start of chemotherapy (week 11), the lymphocyte count decreased from 302,800 cells/μl to 146,600 cells/μl, and the platelet count decreased from 135,000 cells/μl to 88,000 cells/μl. Five weeks after starting chemotherapy (week 13), the hyena developed intermittent unilateral epistaxis. A CBC showed thrombocytopenia (10,000 cells/μl) and a further reduced lymphocyte count (86,390 cells/μl). Treatment with chlorambucil was temporarily discontinued because of suspected bone marrow toxicity. For the next 4 wk, the hyena was treated with prednisone alone, and the epistaxis resolved.

Three weeks after chlorambucil was discontinued (week 16), the lymphocyte count had risen to 150,600 cells/μl, the platelet count had risen to 230,000 cells/μl, and the hyena had lost 4 kg of body weight. A bone marrow aspirate from the right humerus showed a predominance of small to intermediate mature lymphocytes. After 9 wk of treatment with prednisone alone (week 22), the hyena was given a single dose of L-asparaginase (Elspar®, Merck, Whitehouse Station, New Jersey 08889 USA; 10,000 IU, 167 IU/kg, i.m.), and chlorambucil was restarted at a lower dosage (6 mg, 0.1 mg/kg, p.o., e.o.d.). A single dose of L-asparaginase was administered because of concerns that the lower dose of chlorambucil would be ineffective at reducing the lymphocyte count before other clinical signs developed. The prednisone was continued at the same dosage.

The hyena was anesthetized nine more times during the next 14 months (through week 82) for continued evaluation. During that time, the lymphocyte count fluctuated between 176,130 and 81,450 cells/μl (weeks 32 and 70, respectively), and the hyena showed no clinical signs of disease or drug toxicity.

Two years after initial diagnosis of leukemia, the hyena developed hemoabdomen and extreme lymphocytosis (518,300 cells/μl). Because of deterioration in quality of life, the hyena was euthanized. At necropsy, a severely enlarged and ruptured spleen was discovered. Neoplastic T-lymphocytes were present in spleen, liver, visceral and peripheral lymph nodes, lungs, heart, kidney, adrenals glands, mesentery, intestines, pancreas, brain, and bone marrow.

Chronic lymphocytic leukemia (CLL) is a neoplastic proliferation of T- or B-lymphocytes that manifests as a persistent, marked peripheral lymphocytosis.[6,10] In dogs, CLL occurs most commonly in middle-aged to older animals, but it is rarely reported in cats.[6,7,10] Leukemia has not been reported in hyenas, but other forms of lymphoid neoplasia have been documented (J. Parker, pers. comm.).[3,4]

Most animals with CLL are asymptomatic, and the disease is discovered on routine examination, as was the case with this hyena. In dogs and cats, diagnosis of CLL is based on physical examination, CBC, cytology of peripheral blood smears, and cytology or histology of biopsy specimens.[6,10] Clinical signs develop as the degree of lymphocytosis, bone marrow involvement, or other organ involvement progresses.[6,10] Dogs and cats with CLL may be presented for lethargy, reduced appetite, weight loss, pale oral mucous membranes, lymphadenopathy, or abdominal enlargement. Physical examination may reveal pallor, lymphadenopathy, or abdominal organomegaly.

In dogs and cats, the primary hematologic abnormality present with CLL is persistent, mild to marked, lymphocytic leukocytosis (>30,000 cells/μl).[5,6,10] Differentiating benign from malignant causes of lymphocytosis can be difficult.[9] A diagnosis of CLL is confirmed by cytologic, histologic, and/or immunophenotypic examination of blood, bone marrow, and infiltrated organs. Neoplastic lymphocytes are morphologically similar to the cells seen in the peripheral blood (Fig. 1), bone marrow, spleen (Fig. 2), and liver in this hyena.[5,6,9,10] This appearance contrasts with the larger lymphoblasts seen in cases of acute lymphocytic leukemia.[5,6,10]

Immunophenotyping complements morphologic evaluation and enables differentiation of T-lymphocytic and B-lymphocytic CLL.[10] Antibodies used to differentiate T- and B-lymphocytes react with the lymphocyte surface receptor complexes, as used in this case against the T-lymphocyte CD3 epitope and

the B-lymphocyte CD79 epitope. Traditionally, CLL was thought to be of bone marrow origin; however, recent literature indicates that T-lymphocytic CLL originates in the spleen with subsequent late progressive involvement of the bone marrow.[8] More specific immunophenotypic characterization of neoplastic T-lymphocytes and additional cases of CLL in hyenas are needed to discern whether the CLL of T-lymphocytes originates in the spleen, bone marrow, or other organ.

Chronic lymphocytic leukemia is often slowly progressive and subclinical; therefore, chemotherapeutic intervention is not always advised, but may become necessary as the disease progresses.[6,10] Indications that treatment is necessary include clinical signs of disease, lymphadenopathy, splenomegaly, anemia or other cytopenias, lymphocyte counts >100,000 cells/μl, and a lymphocyte doubling interval of <6 mo.[10] There is no cure for CLL, so the goal of treatment is to control the disease.[6,10] Treatment of this hyena was initiated because the lymphocyte count was >300,000 cells/μl, the lymphocyte count quadrupled in 1 mo, and neoplastic lymphocytes infiltrated the spleen and liver. Although not always recommended, splenectomy may have prevented the splenic rupture that led to hemoabdomen and euthanasia in this case.

In dogs and humans, the most effective therapy for CLL is chlorambucil, used alone or in combination with prednisone.[6,10] These agents are frequently used together because antitumor activity has been shown to be superior to the use of chlorambucil alone in humans.[1] The dosage of chlorambucil should be adjusted based on clinical response and bone marrow tolerance.[10] Response to therapy can take several months.[6]

Survival times of 1–2 yr may be expected in dogs with CLL, both with and without treatment.[6,10] Cats with CLL may survive 1–2 yr when treated with chlorambucil.[7,10] This spotted hyena survived for 2 yr with combination treatment using chlorambucil, prednisone, and a single dose of L-asparaginase. The improvement in hematologic parameters suggests that chemotherapy prolonged survival time.

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LITERATURE CITED

1. Han, T., E. Z. Ezdinli, K. Shimaoka, and D. V. Desai. 1973. Chlorambucil vs combined chlorambucil-corticosteroid therapy in chronic lymphocytic leukemia. Cancer 31: 502–508.

2. International Species Information System. 1999. Physiological Data Reference Values. CD-ROM. International Species Inventory System, Apple Valley, Minnesota.

3. Nobel, T. A., U. Klopfer, and B. Yakobson. 1983. Lymphosarcoma in a spotted hyena, *Crocuta crocuta*. J. S. Afr. Vet. Assoc. 54: 209.

4. Porter, R. E., Jr., R. H. Russon, and R. C. Cambre. 1987. Intestinal and intraocular lymphosarcoma in a striped hyena (*Hyaena hyaena*). J. Zoo Wildl. Med. 18: 159–161.

5. Raskin, R. E., K. S. Latimer, and H. Tvedten. 2004. Leukocyte disorders. *In:* Willard, M. D., and H. Tvedten (eds.). Small Animal Clinical Diagnosis by Laboratory Methods, 4th ed. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 86–87.

6. Vail, D. M., E. G. MacEwen, and K. M. Young. 2001. Hematopoietic tumors: canine lymphoma and lymphoid leukemias. *In:* Withrow, S. J., and E. G. MacEwen (eds.). Small Animal Clinical Oncology, 3rd ed. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 558–590.

7. Vail, D. M., and E. G. MacEwen. 2001. Hematopoietic tumors: feline lymphoma and leukemias. *In:* Withrow, S. J., and E. G. MacEwen (eds.). Small Animal Clinical Oncology, 3rd ed. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 590–611.

8. Vernau, W., and P. F. Moore. 1999. An immunophenotypic study of canine leukemias and preliminary assessment of clonality by polymerase chain reaction. Vet. Immunol. Immunopathol. 69: 145–164.

9. Weiss, D. J. 2005. Differentiating benign and malignant causes of lymphocytosis in feline bone marrow. J. Vet. Intern. Med. 19: 855–859.

10. Workman, H. C., and W. Vernau. 2003. Chronic lymphocytic leukemia in dogs and cats: the veterinary perspective. Vet. Clin. N. Am. Small Anim. Pract. 33: 1379–1399.

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