

# Large granular lymphocytic leukemia in a dog

## *Granulaire lymfocyttaire leukemie bij een hond*

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### ABSTRACT

The present case study describes a 14-year-old female intact Staffordshire with large granular lymphocytic (LGL) leukemia with an aggressive clinical course. This is a rare lymphoproliferative disorder that is unique due to the morphology of the neoplastic lymphocytes, which are characterized by the presence of large round to irregularly shaped azurophilic cytoplasmic granules. The diagnosis was made on the basis of a combination of clinical signs (lethargy, anorexia, vomiting and diarrhea), routine hematology (anemia, thrombocytopenia, and circulatory neoplastic cells), cytology, histopathology (involvement of liver, spleen and bone marrow) and immunophenotyping. Neoplastic lymphocytes were characterized as T-cells likely expressing the gamma/delta receptor. This condition closely resembles hepatosplenic lymphoma in humans.

### SAMENVATTING

Granulaire lymfocyttaire leukemie met een agressief klinisch verloop werd gediagnosticeerd bij een 14-jaar oude Staffordshire teef. Dit is een zeldzame lymfoproliferatieve aandoening waarbij de tumorale lymfocyten gekenmerkt worden door de aanwezigheid van grote ronde tot onregelmatige azuurblauwe cytoplasmatische granulen. De diagnose werd gesteld aan de hand van de klinische symptomen (lethargie, anorexie, braken en diarree), routinehematologie (anemie, trombocytopenie, circulatoire neoplastische cellen), cytologie, histopathologie (aantasting van lever, milt en beenmerg) en immunofenotypering. De neoplastische lymfocyten waren T-cellen met de gamma/delta-receptor. Deze aandoening vertoont sterke gelijkenissen met een lever-miltlymfoma bij de mens.

### INTRODUCTION

Large granular lymphocytes (LGL) are a heterogeneous cell population that is able to lyse certain tumors or virus-infected cells and has possible immunoregulatory functions (Trinchieri, 1989). They constitute a morphologically recognizable lymphoid subset with abundant cytoplasm containing azurophilic granules, and they represent only a minority (around 10%) of normal canine peripheral blood lymphocytes (Ghernati *et al.*, 2000; McDonough and Moore, 2000). The granules in LGL resemble lysosomes and contain substances that mediate cell lysis, including perforin, a pore-forming protein unique to cytotoxic T lymphocytes and NK cells (Wellman, 2006). Studies have demonstrated that most large granular lymphocytes in dogs are of T-cell origin, with the minority of natural killer (NK) cell origin (Vernau and Moore, 1999; McDonough and Moore, 2000).

Canine LGL lymphocytosis may be either transient (reactive) or persistent (reactive, preneoplastic or neoplastic)

(McDonough and Moore, 2000). Clinical disease associated with LGL lymphocytosis occurs only rarely. Reactive LGL lymphocytosis can be found in some dogs with chronic ehrlichiosis (Weiser *et al.*, 1991). Large granular lymphocyte lymphocytosis can also be a rapidly progressive and aggressive malignancy (Wellman *et al.*, 1989; McDonough and Moore, 2000).

There are two subsets of LGL: a CD3+ T cell subtype (T-LGL) and a CD3- natural killer subtype (NK-LGL) (Ghernati *et al.*, 2000). In humans, both T-cell derived and NK-cell derived forms exist, but T-LGL leukemias are the most frequent (Loughran, 1993).

Descriptive case reports of LGL leukemias in dogs are limited. One report describes three cases of canine LGL leukemias (Wellman *et al.*, 1989). Two of these dogs had a rapidly progressive form of the disease, with hepatosplenomegaly or mediastinal mass, anemia, and thrombocytopenia. The third had only mild enlargement of peripheral lymph nodes, and lymphocytosis was the only abnormality on complete blood count. In this third case the

clinical course was prolonged over more than 4 years. Another study reports a single case of LGL leukemia associated with cutaneous lymphoma (Helfand *et al.*, 1995). Most cases of LGL leukemia originate outside of the bone marrow and are thus considered lymphoma. LGL expressing gamma/delta TCR and CD11d are believed to be of splenic origin. Infiltration of the bone marrow subsequently can occur, and patients will present with a leukemic blood picture (McDonough and Moore, 2000). In humans, T-LGL leukemias that express CD8 and gamma/delta T-cell receptors have common immunophenotypic features with hepatosplenic T-cell lymphoma (Hertling *et al.*, 2004), making classification of the disorder uniformly difficult.

The present case is an acute LGL leukemia of an old dog diagnosed via hematology, biochemistry, cytology, autopsy, histopathology and immunophenotyping. In contrast to the case described by Fry *et al.* (2003), this dog shows a prominent circulatory presence of neoplastic cells.

## CASE REPORT

### Case history

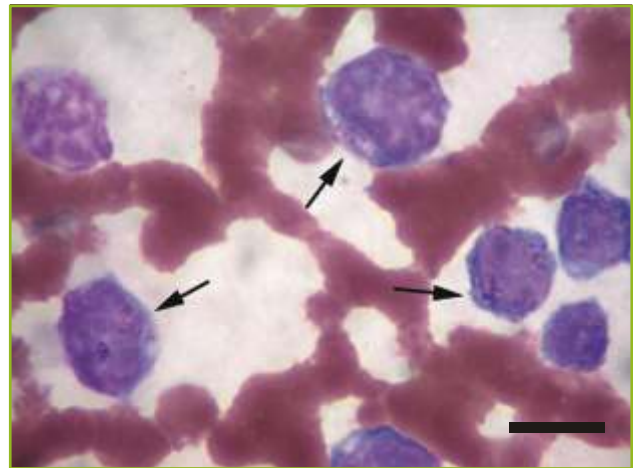
A 14-year-old intact female Staffordshire was presented with lethargy, partial anorexia, vomiting and diarrhea, which it had had over the past several weeks. The dog had not traveled abroad and the presence of ticks was not observed by the owners. Clinical examination revealed a distended abdomen due to organomegaly. A complete blood count and serum biochemistry profile were performed. The blood analysis showed a moderate leukocytosis with a left shift, neutropenia, moderate lymphocytosis with 32% lymphoblasts and a reduced number of normal lymphocytes and thrombocytopenia. There was a moderate increase of AST, ALT and bile acids, and a marked increase of alkaline phosphatase (Table 1).

Ehrlichiosis is the most important differential diagnosis of severe lymphocytosis, therefore a qualitative rapid one-step test (Speed®EHRLI, Bio Veto Test, France) based on the immunochromatographic sandwich principle was used to test for *Ehrlichia canis* infection. The dog tested negative.

Meanwhile the dog was treated with antibiotics and corticosteroids. There was a mild to moderate response to this treatment, i.e. weight gain and return of appetite. However, after 2 weeks the condition of the dog deteriorated severely to almost complete lethargy. A second blood sample (EDTA tube) was analyzed. This analysis showed a very marked leukocytosis with neutropenia, severe lymphocytosis with 92% lymphoblasts, thrombocytopenia and non-regenerative anemia (Table 1). The owners refused further treatment due to the severe deterioration of the dog's condition, and euthanasia was requested.

### Blood cytology

In the first blood smear (Modified Wright stain, Sigma Aldrich) there were 32% atypical lymphocytes present.



**Figure 1.** Microphotograph of blood smear with atypical lymphocytes characterized by a very large cellular size, with abundant lightly basophilic cytoplasm containing azurophilic granules (arrows) and sometimes cytoplasmic vacuoles and round or reniform nuclei (Modified Wright stain – Bar = 20 µm).

In the second blood smear, 2 weeks later, this percentage increased to 92%. The atypical lymphocytes were characterized by a very large size, abundant lightly basophilic cytoplasm with azurophilic cytoplasmic granules and sometimes cytoplasmic vacuoles and round or reniform nuclei (Figure 1). These granules were generally prominent, but the granularity varied among the cells. The atypical lymphocytes were infrequently granulated but otherwise had the cytologic characteristics of large granular lymphocytes.

### Autopsy

At autopsy, the general body condition was very good, despite the anorexia. The spleen was markedly, diffusely enlarged (approximately 3-4 times the normal size), swollen and firm with irregular borders and a mottled surface. The liver was also enlarged, had rounded edges and was palely colored. Lymph nodes were not enlarged. No gross abnormalities were observed in other organ systems. Samples of the liver, spleen, and bone marrow were collected for histopathology/cytology.

### Cytology and histopathology

Cytological examination (Modified Wright stain) of bone marrow, prelevated at autopsy, showed severe myelosuppression, with diminished erythropoiesis and megakaryocytopoiesis, diffuse lymphocytic infiltration and the presence of large granular lymphocytes.

The diagnosis of LGL leukemia was based on the symptoms, the findings in the blood smears and blood analysis. Further characterization of cell origin was done via flow cytometry.

Histopathology (Hematoxylin-eosin staining) of the liver revealed moderate to severe centrilobular to midzonal vacuolar degeneration hepatocytes, diffusely spread moderate amounts of bile in the hepatocytes, dilated sinusoids severely filled with lymphocytic and lympho-

**Table 1. Results of hematology, biochemistry and serology.**

Hematology	Blood analysis 1		Blood analysis 2		Units	References
erythrocytes	7.13		3.73	-	x 10 <sup>12</sup> /l	5.5 – 7.5
leucocytes	16.8	+	113.8	+	x 10 <sup>9</sup> /l	6.0 – 12.0
% banded neutro	8	+	0			3 – 6
% segmented neutro	44	-	5	-		50 – 63
% lympho	12	-	3	-		13 – 30
% atypical lympho	32	+	92	+		
% mono	4		0			3 – 7
% eos	0		0			0 – 3
% baso	0		0			0 – 1
hemoglobin	10.5		6.8	-	mmol/l	8.5 – 12.0
hematocrit	493		300	-	ml/l	420 – 540
MCV	69		80		fl	67 – 80
MCH	14		17		fmol	13 – 16
MCHC	21		22		mmol/l	20 – 23
platelets	38	-	71	-	x 10 <sup>3</sup> /μl	200 – 400
% reticulocytan			0.29		%	< 2
# reticulocytan			19.8			
<b>Biochemistry</b>						
AST (GOT)	79	+	not analyzed		U/l	15 – 30
ALT (GPT)	94	+	not analyzed		U/l	25 – 55
Alk. Phosph.	557	+	not analyzed		U/l	10 – 50
bile acids	26	+	not analyzed		μmol/l	< 20
lipase	156		not analyzed		U/l	< 500

blastic cells, and atrophy of the hepatocytes. The spleen showed a loss of normal architecture, the presence of large amounts of hemosiderin and a large diffuse lymphocytic and lymphoblastic red pulp infiltration. Erythofagocytosis was not observed. In both organs, a very large population of atypical lymphoid cells was present.

### Immunophenotyping

Immunophenotyping was performed by four color analysis on a Coulter Epics XL-MCL flow cytometer with Expo 32 acquisition and analysis software (Beckman-Coulter). All reagents and conjugated antibodies were obtained from Beckman-Coulter (IOTest product line). The following CD markers gave a weak positive signal: CD3, CD8, CD7, CD25, CD57 and CD45. The markers - CD19, surface immunoglobulins (kappa and

lambda light chains), CD4, CD2, CD5, CD1a, TCR and TCR $\beta$ , CD16, CD56 and HLA-DR – tested negative.

However, negative results should be interpreted with some caution. First, the antisera used are validated for the analysis of human samples. Nevertheless, some of these antisera can be used in different species because of their crossreactivity with the antigenic epitopes of other animals, however this crossreactivity is not documented for the antisera used in the present case. Second, at the time of immunophenotyping, the sample was already 5 days old, which is not ideal.

### DISCUSSION

In the literature there appear to be different terminologies for the same disorder, namely hepatosplenic lymphoma, large granular lymphocytic/lymphoblastic leukemia, lym-



phoproliferative disease of granular lymphocytes, or large granular lymphocytosis (Semenzato *et al.*, 1997; Lau *et al.*, 1999; McDonough and Moore, 2000; Fry *et al.*, 2003; Cienava *et al.*, 2004).

Large granular lymphocyte proliferative disorders have been described in many species, including humans, cats, horses, rats, ferrets, birds, cat and dogs (Turinelli *et al.*, 2005). "Chronic" LGL leukemia has been described mainly in dogs (Vernau and Moore, 1999; Ghernati *et al.*, 2000; McDonough and Moore, 2000; Valli *et al.*, 2002). However, it can also appear as a rapidly progressing disease (Ghernati *et al.*, 2000; McDonough and Moore, 2000; Turinelli *et al.*, 2005), as in the present case. Interestingly, this disorder is also described in a dog as hepatosplenic lymphoma (Fry *et al.*, 2003). The symptoms most commonly noted are lethargy, pyrexia, anemia, thrombocytopenia and hepato-splenomegaly (Ghernati *et al.*, 2000; McDonough and Moore, 2000; Fry *et al.*, 2003; Turinelli *et al.*, 2005), which correspond very well with our case.

Distinctive pathologic findings of T-LGL leukemia in dogs involve the bone marrow, spleen and liver, whereas kidney, lung, gastro-intestinal tract, tonsils and lymph nodes are uncommon sites (Fry *et al.*, 2003). Most cases show a diffuse lymphocytic infiltration of bone marrow, spleen and liver, as in the present case (Vernau and Moore, 1999; Ghernati *et al.*, 2000; McDonough and Moore, 2000; Valli *et al.*, 2002). The anemia, thrombocytopenia and abnormal white blood cell count and formula are a consequence of the LGL leukemia. Morbidity is related to the degree of the cytopenias rather than the tumor burden (McDonough and Moore, 2000). The mechanism of neutropenia is not completely understood (Loughran, 1993). Bone marrow lymphocytic infiltration is usually sparse and accompanied by erythroid hypoplasia and myeloid maturation arrest (Loughran and Starkebaum, 1987). However, the degree of lymphocytic infiltration cannot account for the neutropenia alone. There is also some evidence for autoimmune neutropenia (Loughran and Starkebaum, 1987) and decreased neutrophil survival (Starkebaum *et al.*, 1983). Anemia and thrombocytopenia can be explained by a direct inhibitory effect of the cytokines produced by neoplastic LGL cells (Oshimi, 1988; Fry *et al.*, 2003). The increased AST, ALT, AF and bile acids levels are due to liver cell degeneration/necrosis, secondary to neoplastic infiltration.

Examination of a blood smear is critical in establishing the diagnosis of LGL leukemia (Loughran, 1993). The presence of increased numbers of LGL, usually identified by a size greater than normal lymphocytes, with abundant pale cytoplasm and prominent azurophilic granules (3 or more) is the characteristic finding (Walden *et al.*, 2003). However, these features may vary, even among cells of the same patient (Loughran, 1993). Granulation can range from fine to coarse, and some cells may have otherwise characteristic features but lack granules (Alleman Rick, personal communication, 2006) or the cytoplasmic granules may be difficult to discern on hematoxylin and eosin-stained tissue sections. This was also observed in the present case, though large granular

lymphocytes may nevertheless be present in the liver and spleen.

CD3+ LGLs are of the T-cell subtype and are thought to be in vivo activated cytotoxic T-cells (Loughran *et al.*, 1988; Wellman, 1997). Other T-cell markers were also present: CD8, CD7, CD25 and CD45 (pan-leucocytair). Although CD57 is a NK cell marker, CD57 expression is specific for gamma/delta T-cell LGL leukemias and expression of this antigen may be associated with a more indolent clinical course (Ahmad *et al.*, 2005). CD3, CD7, CD45 and CD8 are expressed in gamma/delta T-cells. Cells from most acute leukemias, irrespective of their subtype (T, common or nonlymphoid leukemias), as well as T-cell lymphoblastic lymphomas and peripheral T-cell lymphomas, expressed only the p70-75 beta subunit of the IL-2 receptor CD25, though CD8+ T-cells express only low levels of CD25 (Rosolen *et al.*, 1989).

Other T-cell markers like CD4, CD2, CD5, CD1a, TCRalpha/beta and TCRgamma/delta tested negative. The neoplastic cells were also negative for certain B-cell and NK-cell markers, namely CD19, kappa, lambda and CD16, CD56, respectively. Wilkerson *et al.* (2005) assigned a number of canine lymphoma cases to the T-cell category because they expressed the early common thymocyte markers, CD7 and CD1a, but not the B-cell markers CD19 and CD22.

Immunophenotyping for gamma/delta TCR was negative. This could be due to the assay, since the antisera were validated for the analysis of human samples and crossreactivity with the canine antigenic epitopes is not documented for the antisera used in the present case. Furthermore, at the time of analysis the sample was 5 days old, which made it less suitable for immunophenotyping. Specimen aging might lead to increased granulocyte contamination of lymphocyte gates and false positive staining or non-specific staining of dead cells (Sun, 2002). In our case, however, the granulocytes were vastly outnumbered by the leukemic cells, and the immunoreactivity pattern observed was consistent with cell morphology and previously published phenotypes.

Nevertheless, since CD3, CD7, CD8, CD45 and CD57 were expressed on neoplastic cells, it can be assumed that they were of gamma/delta T-cell origin. (CD8 is expressed on gamma/delta T-cells, and CD57 is specific for gamma/delta T-cell LGL leukemias).

Leukemia is classically defined as malignant neoplasia of hematopoietic cells in the bone marrow with spillover in the blood (Cotran *et al.*, 1989). However it is believed that LGL leukemias are not primarily diseases of the bone marrow, but rather are extramedullary in origin. The high frequency of splenomegaly and  $\delta 2$  expression associated with LGL lymphocytosis suggests splenic red pulp involvement, an environment normally highly enriched with  $\delta 2$  cells of diverse lineages (McDonough and Moore, 2000). The expression of  $\delta 2$  is highly restricted in normal dogs to the red pulp of the spleen on macrophages (Danilenko *et al.*, 1995) and diverse lineages of T-cells and perhaps NK cell lymphocytes (McDonough and Moore, 2000). However, LGL leukemia in

dogs may also arise from gamma/delta T-cells (McDonough and Moore, 1996), which also have nearly exclusive splenic red pulp localization (McDonough and Moore, 2000). Immunohistological studies of the spleen have revealed diffuse infiltration of the splenic red pulp, while other lymphoid organs, including bone marrow, have been less involved (McDonough and Moore, 2000). Although leukemias can invade the spleen as a secondary site, it seems likely that canine LGL lymphocytosis arises from  $\alpha_2$  or gamma/delta lymphocytes of the splenic red pulp (McDonough and Moore, 2000). In humans, analysis of DNA content has shown that the proliferating LGLs are located in the spleen (Palutke *et al.*, 1983; Semenzato *et al.*, 1987).

Patients should be monitored weekly with a complete blood count and physical examination. Patients that are asymptomatic can become symptomatic with little warning. However, rapid increase in LGL cells, infiltration of multiple organs and the development of hematological abnormalities are poor prognostic indicators, a fact which has also been observed in the present case. Treatment with prednisone and chlorambucil is effective in LGL neoplasms in dogs. However, standard combination chemotherapy protocols for lymphoma may also be tried. One study showed that longevity could be greater than three years with prednisone and chlorambucil administration alone. Some acutely aggressive forms did not respond to combination chemotherapy (Loughran, 1993). Immunophenotyping is necessary for accurate classification, though it does not correspond to the clinical prognosis (McDonough and Moore, 2000).

In conclusion, the present case of LGL leukemia of gamma/delta T-cells is consistent with the human and canine hepatosplenic T-cell lymphoma cases (Fry *et al.*, 2003; Turinelli *et al.*, 2005). In this case, the circulatory presence of neoplastic lymphocytes was prominent compared to the hepatosplenic involvement described in another canine T-cell lymphoma (Fry *et al.*, 2003).

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