A case of T-cell chronic lymphocytic leukemia progressing to Richter syndrome with central nervous system involvement in a dog

Running header title: Richter syndrome with CNS involvement in a dog

Elizabeth A Conway[1], Elspeth M Waugh[2], Clare Knottenbelt[1]

[1]Small Animal Hospital, School of Veterinary Medicine, College of Medical, Veterinary, and Life Sciences, University of Glasgow, Glasgow, United Kingdom, [2] School of Veterinary Medicine, College of Medical, Veterinary, and Life Sciences, University of Glasgow, Glasgow, United Kingdom

1) Corresponding Author:

Elizabeth A. Conway, Small Animal Hospital, School of Veterinary Medicine, College of Medical, Veterinary, and Life Sciences, University of Glasgow, Glasgow, United Kingdom.

Email: e.conway.2@research.gla.ac.uk

Abstract

An 8-year-old neutered Beagle dog was presented with polyuria and polydipsia. Routine clinicopathologic testing showed a significant lymphocytosis and proteinuria. Lymphocytes were of small to intermediate in size with a mature morphology. Infectious disease screening was negative. PCR for antigen receptor gene rearrangements (PARR) showed a clonal T-cell receptor (TCR) rearrangement consistent with T-cell chronic lymphocytic leukemia (CLL). Bone marrow cytology showed <30% lymphocytes, while the proportion in splenic fine-needle aspirate cytology was considered increased. The dog was initially monitored but started on prednisolone and chlorambucil therapy two months later due to worsening clinical signs and progressive lymphocytosis. After an additional two weeks, the dog developed multifocal spinal pain and single-node lymphadenomegaly. Cytology of the lymph node showed a monomorphic population of large lymphoblasts consistent with lymphoma. Cytology of a cerebrospinal fluid sample also showed large lymphoblasts. PARR at both sites showed a clonal TCR rearrangement of the same molecular size as in the initial leukemic cells. The dog was diagnosed with a transformation of the CLL to Richter syndrome with involvement of the central nervous system (CNS). Therapy was started with L-asparaginase and an increased dose of prednisolone; however, the dog was euthanized due to progressive clinical signs. To our knowledge, this is the first report of canine Richter syndrome (RS) with direct involvement of the CNS.

Keywords: Canine, Cerebrospinal Fluid, CLL, Lymphoma, PARR

Case presentation

An 8-year-old, 11.7kg female neutered Beagle was initially referred to the University of Glasgow Small Animal Hospital, with a 6-week history of polyuria and polydipsia (PUPD). A peripheral lymphocytosis (32.9 $x10^9$/l) had been detected by the referring veterinarian. No medications had been administered prior to referral.

The initial clinical examination was unremarkable, with no evidence of peripheral lymphadenomegaly, a normal body condition score, and normal temperature. The dog was reported to be well aside from the PUPD. Results of a CBC (Advia 120, Siemens, Frimley, UK) showed a mild lymphocytosis (8.72 $x10^9$/L), but the dog was otherwise normal (Table 1). A blood smear examination revealed a monomorphic population of lymphocytes, which were small to intermediate in size with clumped chromatin and scant to moderate amounts of pale cytoplasm, which often contained a few fine magenta granules (Figure 1). A biochemistry panel showed mild hypoalbuminemia (26 g/l) and hypophosphatemia (0.92 mmol/l) (Table 2). Electrolytes, including calcium, were within reference intervals (WRI). The urinalysis showed an inactive sediment, but an increased urine protein:creatinine ratio (UPC) of 4.27 with a urine specific gravity (USG) of 1.002. The urine culture was negative. An abdominal ultrasound revealed no abnormalities, nor did the thoracic computed tomography (CT). A further investigation into the cause of the lymphocytosis was performed with point-of-care serology for *Anaplasma Phagocytophilium*, *Anaplasma Platys*, *Borrelia Burgdorferi*, *Ehrlichia canis*, *Ehrlichia ewingii* plus a Dirofilaria antigen (SNAP 4Dx Plus, IDEXX, UK) that were all negative.

Clonality of the lymphocytosis was evaluated by PCR for antigen receptor gene rearrangement (PARR), performed on the peripheral blood as previously described[1]. A clonal TCR rearrangement was detected; however, there was evidence of DNA degradation within the sample, and this result was regarded with caution. As such, the dog returned a week later for a repeat hematology panel, which showed a more marked lymphocytosis of 30 $x10^9$/L, and PARR was repeated at this time. The

second analysis demonstrated a clonal TCR rearrangement of the same molecular size (66 bp; Figure 2A). Amplification with immunoglobulin primer sets yielded only small polyclonal distributions (presumed to be a residual normal B-cell population). Based on these findings, a diagnosis of T-cell chronic lymphocytic leukemia (CLL) with concurrent proteinuria was made.

To help determine the source of the circulating leukemic cells, fine-needle aspirates (FNAs) were obtained from the rib bone marrow and spleen. Cytology of the rib bone marrow showed an M:E ratio of 2.1:1 with all cell lines present and intact progression of all lineages. Lymphoid cells made up 17% of the nucleated cell count, with the majority being small to intermediate in size with a mature morphology. Splenic cytology showed mainly small- to intermediate-sized, mature lymphoid cells, with occasional lymphoblasts and plasma cells. In dogs, CLL is considered to be primarily of bone marrow origin when the marrow contains >30% small mature-appearing lymphocytes. Given the comparatively lower lymphocyte percentage found within the marrow sample, in this case, and the predominance of small lymphocytes within the spleen, the spleen was considered the most likely source of the peripheral leukemia. This hypothesis is consistent with the presumed CLL phenotype: while B-cell CLL is usually of marrow origin, T-cell CLL is commonly thought to be of splenic origin [2,3].

Supportive therapy for the proteinuria was initiated with benazepril (0.5 mg/kg SID), plus clopidogrel (3 mg/kg SID) as thromboprophylaxis. No specific CLL therapy was started at this stage, as the dog was clinically well. At subsequent revisits, the dog remained clinically stable, with a non-changing lymphocytosis that lasted until two months after the initial visit. At that point the PUPD worsened, and the lymphocytosis was persistently increased (44 $x10^9$/L). Clinical examination remained normal, with no detectable lymphadenomegaly. Biochemistry and electrolytes remained WRI. The proteinuria had initially improved with benazepril but increased again (UPC 4.43). The decision was made to start therapy for CLL using a prednisolone-chlorambucil protocol therapy (chlorambucil 4 mg/m^2/24h prednisolone 40 mg/m^2/24h for the first 7 days, reduced by half thereafter). Two weeks later, the owner reported that the dog was more lethargic, and was showing stiffness, an

intermittent right forelimb lameness, and a stilted gait. Clinical examination found thoracic and lumbosacral spinal pain, plus an enlargement of the right retropharyngeal lymph node (RPLN). The rest of a full neurologic exam was unremarkable, and the orthopedic exam identified no focus of pain on the right forelimb. CT of the neck and lumbar spine were unremarkable. Hematology showed a reduction in the lymphocytosis to $11.4x10^9$/L, but a leukemic blood picture with small to intermediate lymphocytes was still apparent (Table 1). FNAs were taken for cytology from the RPLN, and the dog was discharged for the weekend with analgesia (paracetamol 10 mg/kg/12h) pending results. RPLN cytology demonstrated a monomorphic population of intermediate to large lymphoblasts admixed with lower numbers of small lymphocytes. The neoplastic cells had a scant to moderate band of deeply basophilic cytoplasm, with a few fine pink granules and small perinuclear clearing occasionally seen. The nucleus contained finely stippled chromatin with multiple indistinct nucleoli. An occasional hand-mirror morphology was observed, and several mitotic figures were present (Figure 3).

Given these results, the dog returned to the hospital two days later for repeat staging of her lymphoid neoplasia. Her owners reported ongoing spinal pain and worsening lethargy. Repeat hematology (Table 1) showed the lymphocyte count had normalized, and occasional large lymphoblasts were now present in circulation (Figure 4). The biochemistry panel indicated persistence of the mild hypoalbuminemia, and the urinalysis showed the proteinuria had worsened markedly (UPC 18.3). On abdominal ultrasound, the liver was enlarged and diffusely hyperechoic with rounded margins; these changes were consistent with chronic corticosteroid administration. There were no changes to the spleen or the abdominal lymph nodes. No overt abnormalities were observed on splenic cytology, although the sample size was small. Thoracic radiographs showed no concerns for intrathoracic lymphadenomegaly. Magnetic resonance imaging (MRI) of the whole spine in T1, T2, and STIR sequences showed no structural changes to explain the spinal pain; however, a contrast agent was not used due to concerns regarding the potential for pre-existing renal disease.

Cisternal cerebrospinal fluid (CSF) analysis revealed an increased protein concentration (1200 mg/l), occasional red cells (15/ul), and a nucleated cell count of 895/ul. Cytocentrifuged preparations showed that the majority of cells were a monomorphic population of large lymphoblasts. These cells had finely stippled chromatin, multiple nucleoli, and basophilic cytoplasm, often with a perinuclear clearing. Frequent mitotic figures were observed. Rare small mature lymphocytes and plasma cells were also present (Figure 5). Morphologically, the neoplastic population in the lymph node and CSF appeared highly similar. PARR was performed on both and demonstrated a clonal TCR rearrangement of the same molecular size (66 bp), like that seen previously in the peripheral blood (Figure 2B and 2C). This was interpreted as consistent with Richter syndrome (RS), a transformation of the previous CLL into a clonally related high-grade lymphoma in both the RPLN and CNS.

Therapy was started with L-asparaginase (10,000 IU/m^2 s/c), and the prednisolone was increased back to 1.5 mg/kg/24h, with a plan to continue with a lomustine- or a cytosine arabinoside-based protocol after that. Chlorambucil was discontinued. Analgesia was increased with the addition of tramadol (2 mg/kg/8h) and gabapentin (10 mg/kg/8h). The dog's comfort improved markedly, but she remained quiet, and developed tonic-clonic seizures within 24 h. These were successfully managed with the addition of phenobarbitone (1 mg/kg/12h). At re-examination, three days after the change in chemotherapy, the dog remained quiet and comfortable and had had no further seizures and maintained a good appetite. The hematology results showed the development of a mild poorly regenerative anemia (HCT 31%), lymphopenia (0.8 x10^9/L), and mature neutrophilia (18 x10^9/l). The platelet count was also mildly reduced (132 x10^{12}/L). The owners elected to continue palliative care only and discontinue the chemotherapy. The dog was euthanized two days later due to worsening lethargy. The survival time of this dog was 111 days from the first detection of the lymphocytosis by the referring veterinarian, and 12 days from the detection of the enlarged lymph node consistent with transformation to RS.

Discussion

The finding of a clonal TCR rearrangement of the same molecular size in the large lymphoblasts of the CSF and RPLN as in the initial peripheral intermediate-sized leukemic cells was consistent with a malignant transformation of the initial CLL. This transformation of CLL into high-grade lymphoma is known as RS in humans. Canine RS has been rarely reported, with only 10 cases previously documented in the literature[4,5]. While neurologic signs were described at the point of transformation in some of those cases, the development of confirmed CNS lymphoma as part of RS has never been reported in dogs.

In human medicine, RS occurs in 3.3 to 10.6% of patients with CLL[6,7]. Most commonly, the transformed neoplastic population arises from lymph nodes or bone marrow and then spreads to other organs. Asymmetric lymphadenomegaly, or lymphadenomegaly at a single site, as seen in this case, is common[6]. The mechanisms by which CLL transforms into RS are poorly understood, but viral infections such as Epstein-Barr virus, and chromosomal abnormalities such as Trisomy 12 have been implicated in some patients [6,8,9]. The development of a second new hematopoietic neoplasm of different lineage in human patients with CLL can also occur and has been reported once in a dog[6,10]. In human medicine, cases of RS with clonally-unrelated de novo lymphomas have a better prognosis than those with the classic clonally-related lymphomas, leading to suggestions that the former should not be considered a true Richter transformation[6].

Development of CNS lymphoma as part of human RS is very rare but has been reported both alongside nodal enlargement as the most common variant and as isolated RS in the CNS[7,9,11]. There are, however, several different mechanisms described by which human CLL patients could develop neurologic signs in addition to developing CNS RS. In one study of CLL patients that developed neurologic signs, only 19% of the cases were caused by direct neoplastic involvement of the CNS, with the majority being due to other etiologies such as infections or autoimmune/inflammatory conditions[7]. In those cases, where there is direct CNS involvement, illnesses could have been due to

extramedullary CLL, where the CSF analyses revealed the same population of small/intermediate lymphocytes as in the blood samples, or as CNS RS, where the population of neoplastic lymphocytes is different between those in the CNS and those in the blood. In that same study, these two forms were seen in roughly equal proportions, of around 10% of the cases[7]. CNS RS can, in some cases, only affect the leptomeninges, and in other cases, present as a mass lesion affecting the brain parenchyma[7,11]. There was also one case with suspected spinal cord involvement[12]. It is also noteworthy that up to 70% of human CLL patients show asymptomatic CNS CLL involvement[7]. While some human CNS RS cases can be confirmed on CSF analysis alone, the majority require a tissue biopsy for diagnosis[7]. In the dog of this study, we diagnosed CNS RS based on the presence of a clonal population of large lymphoblasts in the CSF. These lymphoblasts showed the same T-cell clonal rearrangement as those seen in the peripheral lymph node. Alternative explanations for the presence of the lymphocytes in the CSF sample could have included contamination of the sample by peripheral blood and the introduction of peripheral leukemic cells. We feel these are unlikely for a number of reasons. First, only occasional erythrocytes were seen in the sample, suggesting minimal blood contamination. Secondly, on the cytologic exam, the lymphoid cells were large lymphoblasts of the same morphology of those seen in the RPLN and not small to intermediate CLL lymphocytes, as had been seen in the peripheral blood smears. Thirdly, the dog was no longer leukemic by the time the CSF sample was taken, so blood contamination should not have introduced a significant number of peripheral lymphocytes into the CSF sample. Thus, we feel that the presence of the lymphocytes in the CNS corresponds with CNS RS in this dog, and not simply with blood contamination or extramedullary CLL. Interestingly, on MRI, no changes suspicious for CNS lymphoma were seen. While CNS RS has not been reported in dogs previously, canine CNS lymphoma is also only rarely seen, and as such, limited cases have been described in the literature [13–17]. CNS lymphoma has been reported in dogs both as a primary tumor and as part of the progression of multicentric lymphoma[13,14,17]. Although most reported canine CNS lymphoma cases had MRI changes on plain T2 sequences, all have been described as highly contrast enhancing[15,16]. A

limitation in this case report is that contrast was not used because of concerns about the dog's renal function based on the pre-existing persistent proteinuria. Thus, there could have been undetected contrast-enhancing changes in the spine or leptomeninges. Additionally, the brain was not imaged as the neurologic signs were only localized to the spinal cord at the time of MRI. Since the dog presented with seizures 24 h later, it is also possible that an undetected brain lesion was present. Notably, however, in human medicine, a significant proportion of human CLL cases with CNS involvement do not have changes on MRI [7].

Interestingly, in a previously reported case series of canine RS, all dogs had a decrease in lymphocyte counts at the point of transformation with two developing lymphopenia[5]. Additionally, six of the eight cases began to show lymphoblasts in circulation at the same timepoint. The dog reported here showed both of these changes, with a sudden lymphocyte count normalization around the time that the lymphadenomegaly developed, which then progressed to a lymphopenia with occasional circulating lymphoblasts. Changes to peripheral CLL lymphocyte counts have not been reported in humans with RS, while only occasional cases have shown circulating lymphoblasts, [6] which suggests that it is prudent to closely monitor canine CLL cases with a sudden drop in lymphocyte counts, as this could be an early sign of RS.

In this case, PARR indicated both the initial CLL and subsequent RS were of T-cell type. Of the previously reported canine RS cases, two were not immunophenotyped, while two were T-cell CLL and six B-cell CLL [4,5]. In human medicine, RS occurs in 2-20% of B-cell CLL cases, with T-cell CLL transformation into lymphoma being only rarely described[6]. Unlike in dogs, however, B-cell CLL is the most common immunophenotype in people, so it is perhaps unsurprising that B-cell RS is also the most common type. Canine CNS lymphoma has been described as both T- and B-cell immunophenotypes; however, due to the rare nature of the disease, it is still unclear whether one immunophenotype is more common than the other[13]. Classically, T-cell CLL is considered to have a better prognosis in dogs compared with B-cell CLL; however, a recent study showed that a

proportion of dogs with 'atypical' T-cell CLL, based on flow cytometric immunophenotyping, had a very poor prognosis[3,18]. Immunophenotyping was not performed in this case; instead, the diagnosis was made by the detection of clonality using PARR. It is possible that flow cytometry could have provided further prognostic information. This dog survived only 111 days from the initial CLL diagnosis, and 12 days from RS detection with the symptoms of RS being the deciding factors for euthanasia. In the previous canine case series, the median survival was similarly poor, at 41 days from the development of RS[5]. In human medicine, the prognosis for RS is likewise considered poor, with a median survival of less than 6 months[6,8]. The limited number of CNS RS cases also followed this pattern, and in one study, 80% of cases with CNS RS had died within 12 months of diagnosis[7]. There is no consensus on the best therapeutic approach, with various combinations of chemotherapy and immunotherapy attempted; however, the response rates were also poor, ranging from 5-43% [6,8]. Local irradiation has also been used for cases with brain involvement [7,9,11]. This dog did not show significant improvement with the initial therapy of L-asparaginase and an increased prednisolone dose; however, further chemotherapy was not pursued. No consensus in veterinary medicine has been proposed for CNS lymphoma therapy, with chemotherapeutic agents, such as cytosine arabinoside, methotrexate, and lomustine, plus local irradiation and surgery for focal lesions, all being reported [13]. However, the prognosis for CNS RS in dogs should be considered guarded based on the dog of this study and the previously reported cases of canine RS and canine CNS lymphoma.

In conclusion, this report outlines a case of canine T-cell CLL transforming to RS, presenting with both single-node lymphadenomegaly and multifocal spinal pain. CNS involvement was shown by the presence of significant numbers of large lymphoblasts on CSF cytology that had a clonal TCR rearrangement of the same molecular size as in the cells in the lymph node and those in the blood at the initial CLL diagnosis. To our knowledge, this is the first case report of canine RS with direct CNS involvement.

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Tables

Table 1: Serial Hematologic data from a dog with T-cell chronic lymphocytic leukemia progressing to Richter syndrome.

	unit	RI	Day 1	Day 15	Day 79	Day 95	Day 98	Day 105
RBC	$x10^9/l$	5.5-8.5	6.61	8.05	7.95	7.45	6.19	4.7
HCT	%	37-55	47.6	58.4	55.1	49.3	40.5	31
Hb	g/dl	12-18	15.1	18.3	17.9	17	13.5	11
MCV	fl	60-77	72	72.6	69.4	66.2	65.4	65.9
MCHC	g/dl	32-36	31.8	31.3	32.5	34.4	33.3	35
WBC	$x10^9/l$	6-12	16.1	37.5	50.6	18.9	11.7	20.6
band neutrophils	$x10^9/l$		0	0	0	0	0	0.4
neutrophils	$x10^9/l$	3-11.8	6.8	6.4	5.1	6.8	9.3	18.2
lymphocytes	$x10^9/l$	1-4.8	8.7	30	44	11.4	1.8	0.8
monocytes	$x10^9/l$	0.15-1.35	0.3	0.8	1	0.8	0.6	0
eosinophils	$x10^9/l$	0.1-1.25	0.3	0.4	0.5	0	0	1.2
basophils	$x10^9/l$	0	0	0	0	0	0	0
PLT	$x10^9/l$	200-500	380	420	433	403	346	132

Reference intervals (RIs) are from the laboratory at the Veterinary Diagnostic Services of the University of Glasgow. PCV, packed cell volume; Hb, hemoglobin; MCV, mean cell volume; MCHC, mean corpuscular hemaglobin; PLT, platelet count.

Table 2: Biochemical data from from a dog with T-cell chronic lymphocytic leukemia progressing to Richter syndrome on the initial presentation.

	unit	RI	Day 1
total protein	g/l	50-78	59
albumin	g/l	29-36	26
globulin	g/l	28-42	33
sodium	mmol/l	136-159	144.1
potassium	mmol/l	3.4-5.8	4
chloride	mmol/l	95-115	112.6
calcium	mmol/l	2.34-3	2.54
phosphate	mmol/l	1.29-2.9	0.92
urea	mmol/l	2.5-8.5	2.7
creatinine	umol/l	45-155	66
total bilirubin	umol/l	<10	2
ALT	U/l	<90	19
ALKP	U/l	<230	62

Reference intervals (RIs) are from the laboratory at the Veterinary Diagnostic Services of the University of Glasgow. ALT, alanine aminotransferase; ALKP, alkaline phosphatase.

Figures:

Figure 1: A photomicrograph of a blood smear from a dog with T-cell chronic lymphocytic leukemia progressing to Richter syndrome. A lymphocytosis was present that consisted of predominantly small- to intermediate-sized lymphocytes. A few fine magenta cytoplasmic granules were seen in some cells (arrow). ×200 objective, May Grünwald Giemsa stain

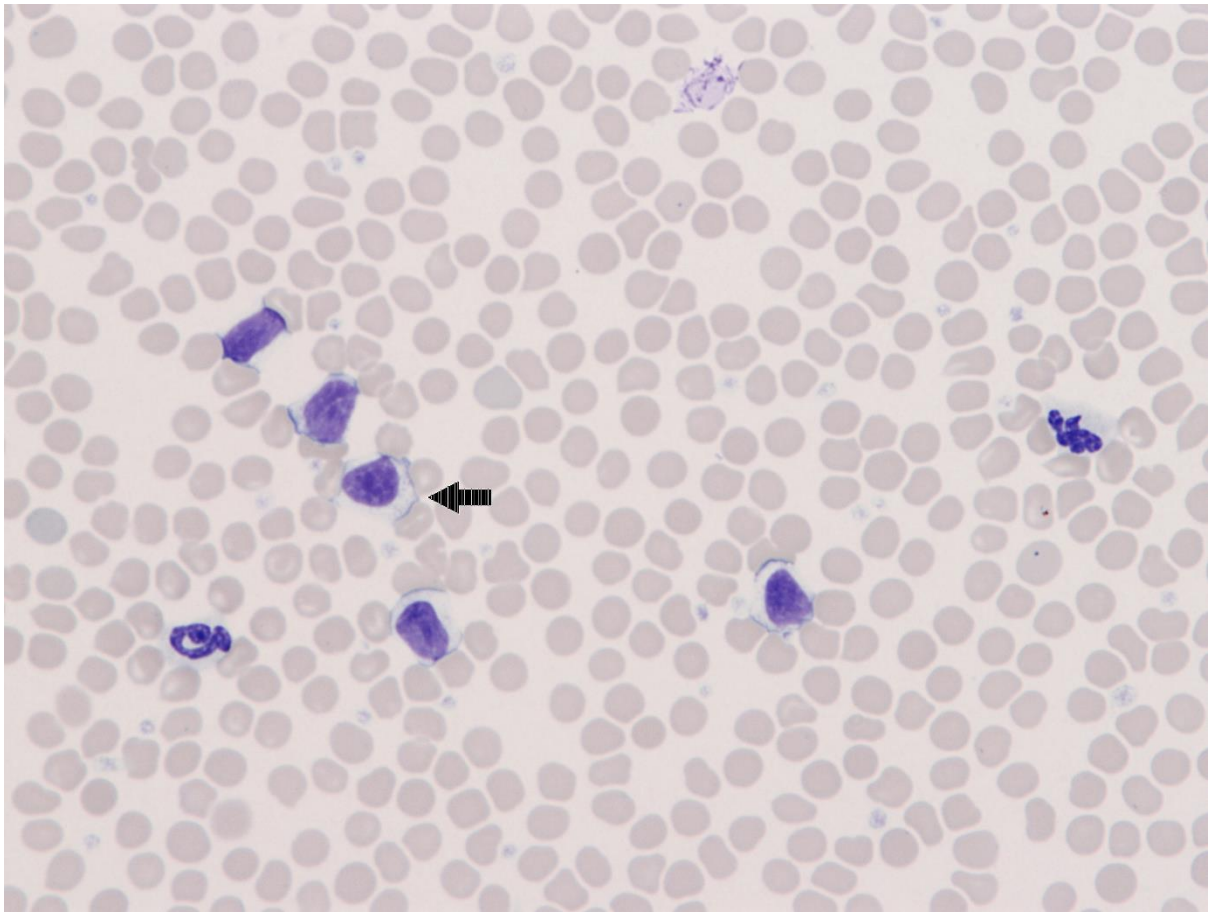

Figure 2: Electropherograms of PCR for antigen receptor gene rearrangements from a dog with T-cell chronic lymphocytic leukemia progressing to Richter syndrome. A, peripheral blood, B, the retropharyngeal lymph node, and C, cerebrospinal fluid. Products were amplified using a T-cell receptor gamma primer set, as previously described (Waugh et al 2016). Size markers are in red (GeneScan 500 ROX, Fisher Scientific, Loughborough). A clonal peak (blue) of approximately 66 base pairs was amplified from all three samples. The lower amplitude of the peak in sample C (CSF) reflects the lower quantity of input DNA available from this sample.

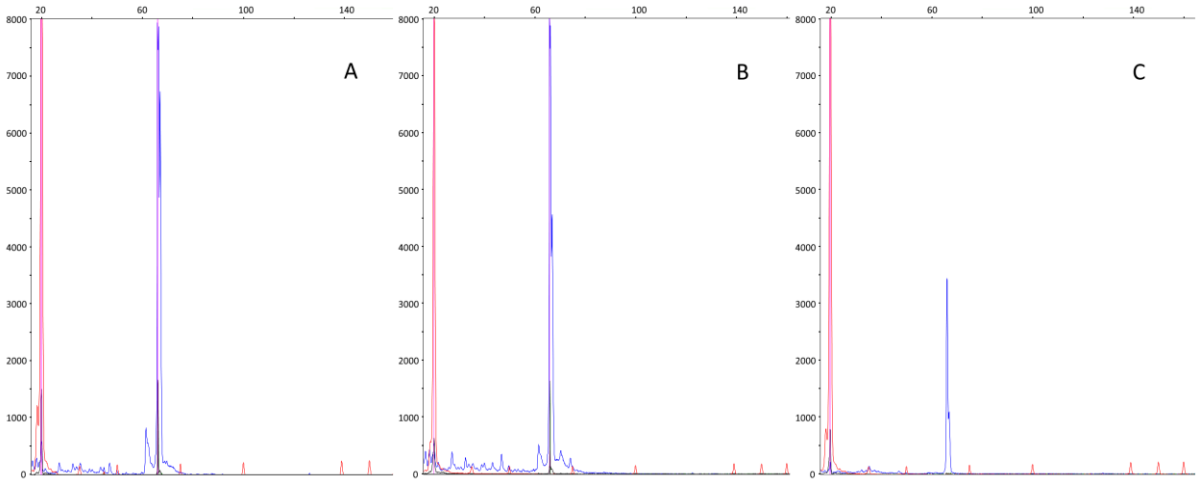

Figure 3: Photomicrographs of a fine-needle aspirate from the right retropharyngeal lymph node of a dog with T-cell chronic lymphocytic leukemia progressing to Richter syndrome. A predominance of large lymphoblasts can be seen. A few small lymphocytes are also present (black arrow). Occasional cells show a hand-mirror morphology (white arrow), and occasional mitotic figures were observed (gray arrow). ×400 objective, May Grünwald Giemsa stain

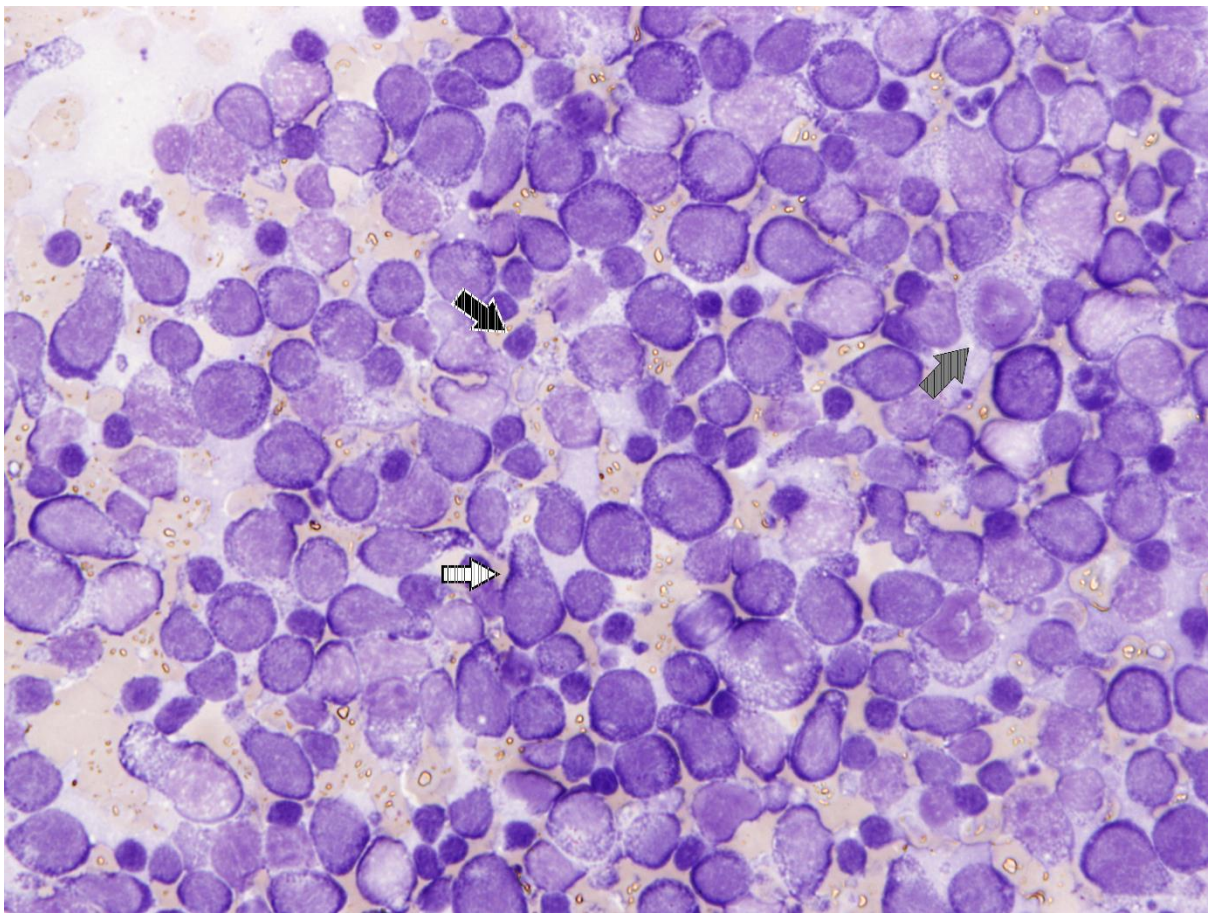

Figure 4: A photomicrograph of a blood smear from a dog with T-cell chronic lymphocytic leukemia progressing to Richter syndrome. Lymphocyte numbers were within reference intervals and were predominantly small, with occasional large lymphoblasts observed (arrow). ×400 objective, May Grünwald Giemsa stain

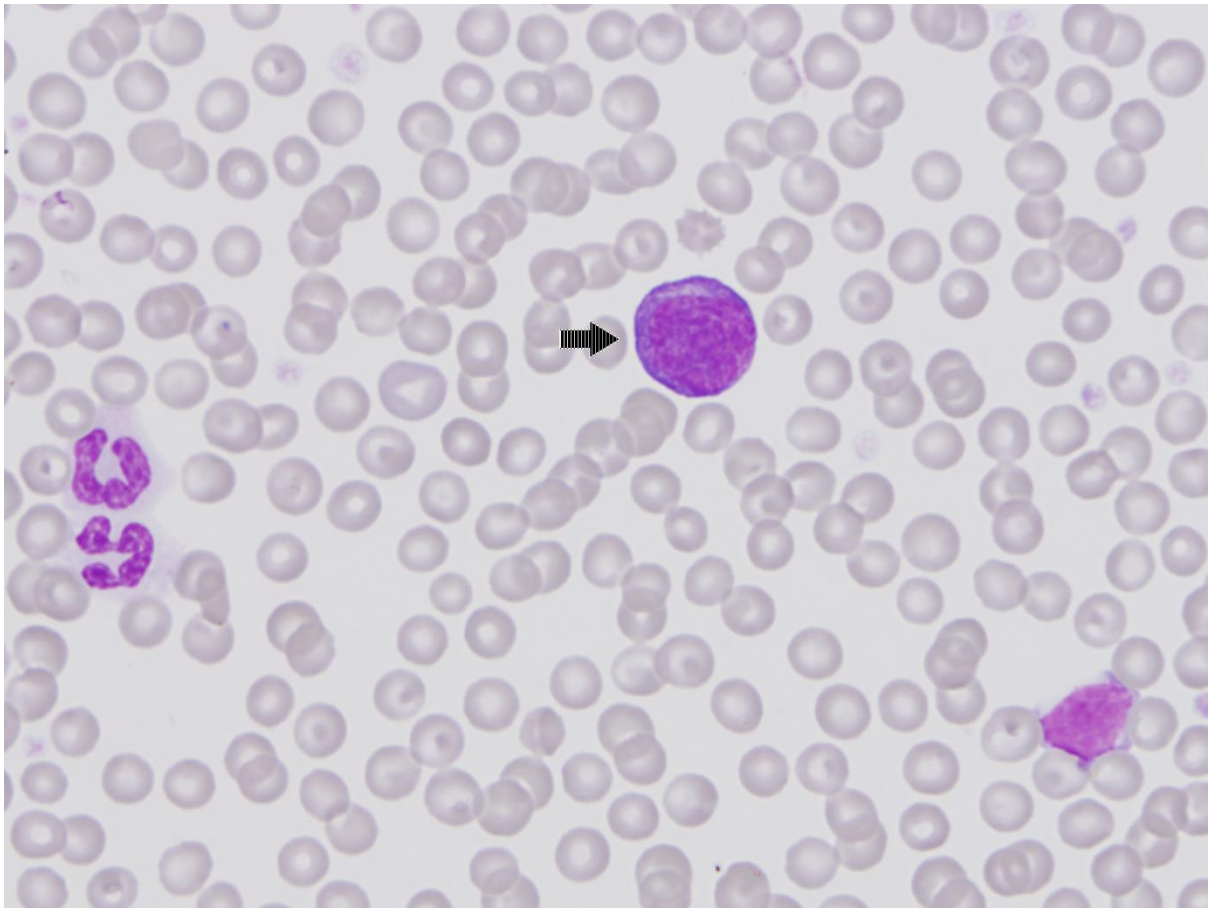

Figure 5: A photomicrograph of cerebrospinal fluid (cisternal) from a dog with T-cell chronic lymphocytic leukemia progressing to Richter syndrome. A Predominance of large lymphoblasts are seen, often showing perinuclear clearing. Rare small lymphocytes (black arrow) and several mitotic figures (white arrow) were also noted. ×400 objective, May Grünwald Giemsa stain