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Chronic lymphocytic leukemia transformation into high-grade lymphoma: a description of Richter's syndrome in eight dogs

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**CHRONIC LYMPHOCYTIC LEUKEMIA TRANSFORMATION
INTO HIGH GRADE LYMPHOMA: A DESCRIPTION OF
RICHTER SYNDROME IN 8 DOGS**

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3 **CHRONIC LYMPHOCYTIC LEUKEMIA TRANSFORMATION INTO HIGH GRADE**
4 **LYMPHOMA: A DESCRIPTION OF RICHTER SYNDROME IN 8 DOGS**
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32 **Running title:** Richter syndrome in 8 dogs
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ABSTRACT

Richter's syndrome (RS) is the rapid development of an aggressive lymphoma in patients with chronic lymphocytic leukaemia (CLL). In humans, RS occurs in 2-20% of CLL and frequently transforms into diffuse large B-cell lymphoma but reports in veterinary medicine are scarce.

This study retrospectively describes 8 dogs with CLL progressing into RS.

From a total of 153 dogs with CLL (93 T CD8+ and 55 B-CLL) RS was demonstrated in 8 cases (5.2%): 2 with T-cell (2.2%) and 6 with a B-cell immunophenotype (10.9%).

When RS occurred, lymphocyte counts were decreased compared to CLL. Mild anaemia was found in 5 dogs and thrombocytopenia in 2 dogs. Clinical signs were frequent and included lymph node swelling, coughing, vomiting, neurological signs and poor clinical conditions. Independently from the therapy, RS was associated with a short survival (median 62 days).

Richter syndrome should be considered as a possible unfavourable evolution in canine CLL.

Keywords: dog, chronic lymphocytic leukaemia, Richter syndrome, high grade lymphoma, transformation, flow cytometry

INTRODUCTION

Chronic lymphocytic leukaemia (CLL) in people is the most common haematological malignancy in western countries and its associations with second lymphoid and non-lymphoid neoplasms is well documented.¹ Richter's transformation or Richter's syndrome- (RS) is a clinico-pathological term used to describe the rapid development of an aggressive lymphoma in patients with CLL. In humans, RS occurs in 2-20% of CLL cases and most frequently (90%) CLL transforms into diffuse large B-cell lymphoma (DLBCL) while more rarely (10%) into Hodgkin lymphoma.²

In contrast the transformation into large T-cell lymphoma in T-CLL patients has been rarely described.^{3,4}

In the pathogenesis of RS the large cells observed may arise from a transformation of the original CLL clone in approximately 80% of cases, due to the accumulation of genetic and/or epigenetic lesions.⁵ In the remaining 20% of cases, RS represents a new neoplasm in which the pathogenetic mechanism is more likely related to host genetic background or microenvironmental dysfunction, enhancing the probability of DLBCL.

CLL is a common haematological disorder in dogs of middle-elderly age, showing an indolent course. Late in the course of disease, dogs with CLL typically experience progressive disease, multiorgan dysfunction, or blastic transformation in acute leukemia.⁶

To our knowledge, only two cases of canine RS have been previously documented.⁷ Recently, the simultaneous presence of T-CLL and DLBCL has been reported in one dog with no evidence of progression from one disease to the other.⁸

Aim of this study was to retrospectively describe 8 cases of CLL in dogs progressing into RS. The clinical and clinico-pathological presentation is compared with the analogous human counterpart.

MATERIAL AND METHODS

The databases of the flow cytometry service of the University of Milan and Turin were interrogated for cases of possible RS. CLL was classified as previously described⁹ whereas the diagnosis of RS

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3 was defined based on the following aspects: 1) documented history of CLL; 2) development of
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5 systemic lymphadenopathy being suggestive of high grade lymphoma based on cytology,
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7 immunophenotype and, if possible, histopathology

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10 Samples were submitted by veterinarians and referral laboratories and shipped within 24 hours from
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12 collection. Flow cytometric immunophenotyping was performed as previously described on
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14 peripheral blood and bone marrow for the diagnosis of CLL⁹, and on lymph node aspirates¹⁰ for the
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16 diagnosis of RS.

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18 For each case, the referring veterinarians were contacted and asked for a complete follow-up,
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20 including serial CBCs, treatment (if any), survival and cause of death. For 2 cases (case 1 and 3),
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22 DNA samples obtained from peripheral blood at the time of CLL diagnosis and later during
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24 transformation into RS were available. On these samples, PARR (PCR for Antigen Receptor
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26 Rearrangements) analysis was performed according to Burnett et al.¹¹ in order to verify whether RS
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28 might be considered as an evolution of the pre-existing leukaemia rather than a second malignancy .
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30 Results were not analysed statistically due to the low number of cases and the extreme
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32 heterogeneity of the data.
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38 RESULTS

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40 Between January 2006 and December 2014, a total of 2443 cases (either peripheral blood, bone
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42 marrow or lymph node aspirates) were received for flow cytometric immunophenotyping of
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44 neoplastic diseases at the two units. A total of 153 cases were diagnosed as CLL, 93 of which
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46 having T CD8+ immunophenotype (60,8%) and 55 having a B immunophenotype (35,9%).

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48 Different atypical phenotypes were identified in the remaining 5 cases (3.3%). RS was
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50 demonstrated in 8 cases (5.2%): 2 having a T-cell immunophenotype (2.2%) and 6 having a B-cell
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52 immunophenotype (10.9%).
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56 Signalment and hematologic data of the 8 cases are summarized in Table 1.
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3 Collection of clinical data, when available, confirmed the presence of only minor symptoms at
4 diagnosis of CLL, whereas clinical signs detected during RS were frequent and variable, including
5 coughing, vomiting, neurological signs and poor clinical conditions.
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9 Regarding CBC, at the time of CLL diagnosis all dogs showed lymphocytosis, ranging from 27,000
10 to > 100,000 lymphocyte/mm³, 1 dog presented with a slight anaemia, 1 had thrombocytopenia and
11 one had thrombocytosis.
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15 When RS occurred, the overall lymphocyte counts were decreased in all cases. In particular, in 4
16 out of 8 cases, lymphocytosis was still present, and in 2 cases leukopenia with lymphopenia was
17 observed. At this time, mild anemia was detectable in 5 dogs and thrombocytopenia in 2 dogs. A
18 double population of small lymphocytes and large blasts was clearly identifiable in peripheral blood
19 and/or bone marrow in 6 cases (figure 1).
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23 Cytology of enlarged lymph node confirmed the presence of large cell high grade lymphoma. Flow
24 cytometry confirmed a decrease of small lymphocyte in peripheral blood, and the presence of a
25 prevalent population of large neoplastic cells indistinguishable from those of a primary large high
26 grade lymphoma in the lymph node (Figure 2).
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30 Treatment and follow-up are summarized in table 2. Five CLL cases were treated with chlorambucil
31 and prednisone, whereas no treatment was pursued in 3 dogs. When RS occurred, a CHOP-based
32 chemotherapy regimen was administered in 4 dogs, while 2 dogs still received the previous therapy
33 (chlorambucil + prednisone). In 2 cases the owners elected euthanasia.
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37 Independently from the duration of CLL phase, RS was associated with a short survival time (range,
38 1 to 83 days, median 62 days).
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42 PARR was tested only in two samples one with T cell CLL/RS and one with B cell CLL/RS (Table
43 1, no 1 and 3 respectively) (Figure 3). In both cases, a similar antigen receptor rearrangement was
44 found with PCR products at the same molecular weight.
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49 50 51 52 53 54 55 56 57 58 **DISCUSSION** 59 60

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3 RS is a clinical condition in which indolent CLL evolves into a more aggressive high-grade
4 lymphoma. The present study describes clinical and clinico-pathological features of a series of 8
5 dogs presenting with CLL, eventually evolving into RS.
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9 In human CLL patients, RS transformation is characterized by the rapid onset of systemic
10 symptoms with sudden clinical deterioration, lymphadenomegaly, hepatomegaly and/or
11 splenomegaly. Frequently, the neoplastic population arises from lymph nodes or bone marrow
12 eventually spreading to other organs. Less commonly, RS involves extranodal sites such as
13 gastrointestinal tract, central nervous system, skin, lungs or kidneys¹². Occasionally, circulating
14 blast cells may be present together with anemia, neutropenia and thrombocytopenia, which can be
15 also related to the underlying CLL. The final diagnosis of RS requires a lymph node biopsy and the
16 morphological identification of large lymphoma cells, thereby allowing the distinction from CLL
17 progression or CLL refractory to therapy¹³. In some cases, small lymphocytes from CLL and large
18 blasts from DLBCL may coexist as a composite lymphoma, while less frequently some patients
19 may develop Hodgkin disease¹⁴. In very few cases, a T-cell lymphoma may arise from a T-CLL³ or
20 B-CLL patients may develop a high grade T-cell lymphoma¹⁵
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36 To the authors' knowledge, RS in dogs has been previously described only in 2 cases out of a series
37 of 22 CLL, but no data on phenotype, follow up and evolution of the solid lesions were reported⁷
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40 In the present study, 6 cases of RS of B phenotype are described. They represent more than 10% of
41 the total number of B-CLL included in our records. This percentage is similar to what reported in
42 human medicine, as in the B-CLL population, the prevalence of RS development ranges from 2-to
43 20%,^{2,16}. On the contrary, in our records only 2 cases of T-CLL, which is the most common CLL
44 subtype in dogs, experienced RS transformation. Although the number of cases is too low to define
45 a precise prevalence, these data confirm that RS should be considered as a possible evolution of
46 CLL also in dogs. In both species (humans and dogs), RS occurs more often in B-CLL than T-CLL
47 cases, although T-CLL is by far less common than B-CLL in humans, thus possibly biasing the
48 comparative aspects. In humans the prognosis of RS transformation is generally poor. However, the
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3 pattern of survival is not homogeneous and may be predicted by some prognostic factors by which
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5 phenotype, with RS developing from T-CLL possibly harbouring a better outcome.¹⁴ The reason for
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7 this different behaviour, both in the prevalence of RS evolution and in the response to therapies, is
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9 still unknown.

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11 Hematologic and clinical features of CLL undergoing RS are not different to those previously
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13 reported in dogs with CLL.¹⁷ Severe cytopenias are uncommon and only minimal anaemia and
14
15 thrombocytopenia may occur.

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17 From the present study emerges that clinically, patients with Richter transformation show an
18
19 aggressive disease course, with rapidly enlarging lymph nodes and hepato-splenomegaly.
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21 Interestingly, Richter transformation occurred in concomitance with a decrease of lymphocyte
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23 number in all cases and with a decrease of erythrocytes leading to a mild anaemia in most cases.
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25 Lymphocyte count in some cases may return within reference intervals or even fall below lower
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27 reference limit. This suggests that clinicians should carefully interpret a rapid decrease of
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29 lymphocyte number and the onset of anaemia in dogs with CLL as a possible sign of evolution
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31 towards a more aggressive phase.
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37 In agreement with the human literature¹⁴, prognosis was poor in dogs as well, with a median
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39 survival of 41 days. Notably, response to treatment was scarce and no remotely comparable to the
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41 response commonly obtained in primarily occurring high grade lymphomas.^{18,19}
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44 The majority of human RS cases are clonally related to the original CLL clone, and only rarely they
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46 represent a high grade lymphoma developing de novo likely due to immunologic derangements or
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48 genetic background of the CLL host.^{2,14}

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50 To our knowledge, to date no similar molecular biology studies have been performed in dogs. In the
51
52 present study, we succeeded in recovering frozen DNA from a couple of cases in which samples
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54 from blood in the CLL phase and aspirates from lymph node in RS had been harvested in our tissue
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56 bank. PARR showed an identical B-cell receptor rearrangement in CLL and RS phase, thus
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58 suggesting that they represent an evolution of the same disease. Unfortunately, PARR was not
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3 carried out in most cases. We can't completely rule out that, similarly to human medicine, some
4 cases develop a second malignancy. This may be true particularly for case #2, in which a double
5 negative CD4-CD8- high-grade lymphoma developed from a primary CD8+ CLL.
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9 The main limit of the present study is the low number of cases due to the low prevalence of both B-
10 CLL and RS in the canine population. In addition, data were retrospectively collected from the
11 database of the flow cytometry service of the two units: thus, prevalence of RS transformation
12 might have been underestimated in the present study, since it is possible that owners or referring
13 veterinarians did not repeat sampling and immunophenotyping at time of transformation,
14 particularly because of the disregarded nature of RS in veterinary medicine. The real percentage of
15 CLL undergoing RS should be better evaluated by a prospective study.
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19 Another pitfall is the lack of a complete follow-up in some cases as well as a lack of a standardized
20 therapeutic approach, which are inherent to the retrospective nature of the study.
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24 Finally, it would be interesting to confirm the data on PARR with a higher number of cases in
25 which DNA from the CLL phase and during SR are available.
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29 In conclusion, our findings suggests that RS may be considered a possible transformation of CLL,
30 mainly of B-phenotype, also in dogs. Similarly to human medicine, RS is linked to the onset of
31 clinical signs and lymphadenomegaly with a cytological aspects of a high grade large cell
32 lymphoma. During RS, lymphocyte counts generally decrease and anemia may occur. Prognosis is
33 generally poor with a scarce response to chemotherapy. Further studies on the possible predisposing
34 factors to RS are needed.
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37 38 39 40 41 42 43 44 45 46 47 **Acknowledgement**

48
49 The authors wish to thank Dr Angelo Capasso and the referring clinicians for providing follow-up
50 data.
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Table 1

No	breed	gender	age	lymph/mm ³ CLL	CBC CLL	phenotype CLL	lymph/mm ³ RS	CBC RS	phenotype RS
1	golden retriever	f	9 y	75,600	no alterations	T CD8+	49,938	anemia (Ht 30), circulating blasts	T CD8+
2	carlino	fs	13 y	27,790	anemia (HT 20)	T CD8+	430	anemia (HT 24), circulating blasts	T CD4- CD8-
3	boxer	f	10 y	27,000	no alterations	B	400	no alterations	B
4	WHWT	fs	9 y	48,450	no alterations	B	1,300	anemia (HT 26), circulating blasts	B
5	mongrel	m	16 y	28,500	thrombocytosis	B	7,230	anemia (HT 30), circulating blasts	B
6	English setter	m	13 y	>100,000	no alterations	B	29,230	anemia (HT 25), thrombocytopenia circulating blasts	B
7	beagle	fs	8 y	110,000	thrombocytopenia	B	4,280	no alterations	B
8	dobermann	fs	8 y	29,485	no alterations	B	11,570	thrombocytopenia circulating blasts	B

Table 2

No	therapy for CLL	time to progression	therapy for RS	cause of death	survival with RS	overall survival
1	chlorambucile + prednisone	210 d	Wisconsin Madison + cytosine	high grade lymphoma, progressive disease	60 d	270 d
2	no therapy	303 d	no therapy (euthanasia)	euthanasia	1 d	304 d
3	no therapy	488 d	CHOP	high grade lymphoma progressive disease + hepatic adenocarcinoma	83 d	571 d
4	chlorambucile + prednisone;	89 d	CHOP	high grade lymphoma, progressive disease + renal failure	24 d	113 d
5	no therapy	250 d	COP	high grade lymphoma, progressive disease + renal failure	14 d	264 d
6	chlorambucile + prednisone;	112 d	no therapy (euthanasia)	euthanasia	1 d	113 d
7	chlorambucile + prednisone;	266 d	chlorambucile + prednisone + lomustine	high grade lymphoma progressive disease, euthanasia	58 d	324 d
8	chlorambucile + prednisone	63 d	chlorambucile + prednisone	high grade lymphoma progressive disease, euthanasia	62 d	125 d

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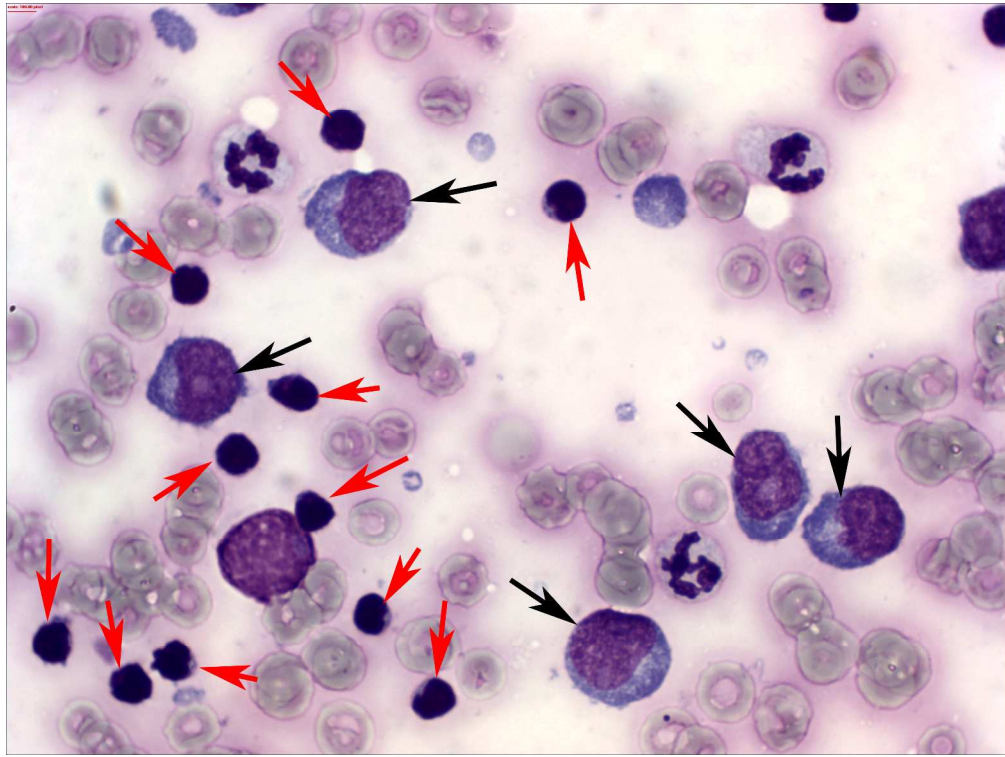


Figure 1: bone marrow slide from case no. 6. Large blast cells (black arrows) are identifiable together with many small lymphocytes (red arrows). May Grünwald-Giemsa stain, 100x 295x221mm (300 x 300 DPI)

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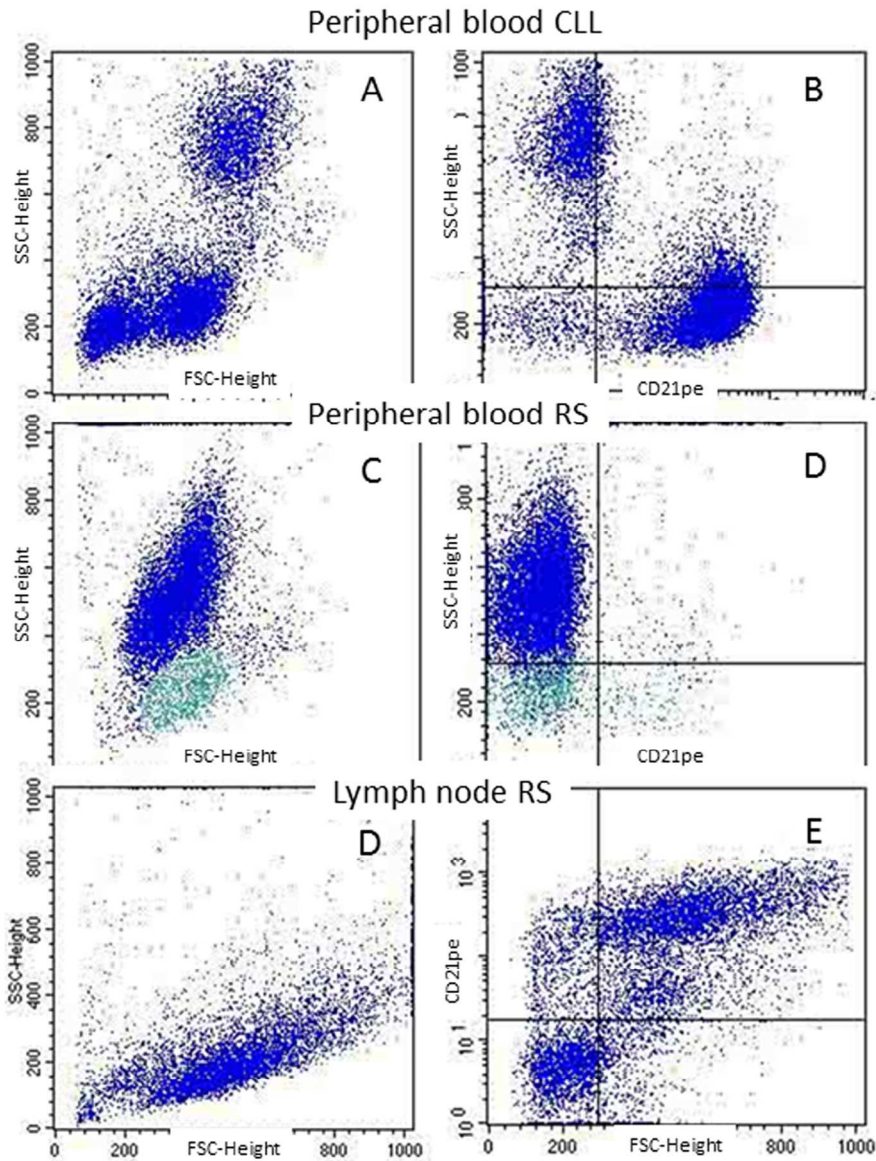


Figure 2: Flow cytometric scattergrams of peripheral blood (A,B,C,D) and lymph node (E,F) from dog no.3. At time of diagnosis of CLL (A,B) an abundant population of small B lymphocytes was found. During Richter transformation B lymphocytes decreased in peripheral blood (C,D) and large B lymphoid blast cells appeared in the lymph node aspirate (E,F)
147x190mm (300 x 300 DPI)

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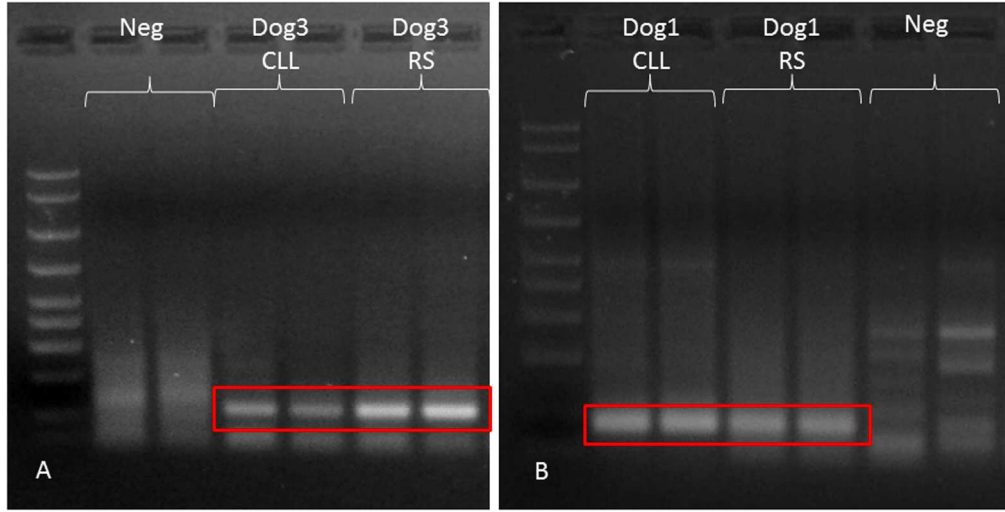


Figure 3: PARR for BCR (A) and TCR (B) on dog #3 and #1 with B and T CLL/RS respectively. Clonal bands at the same molecular weight were detected on blood in CLL phase and in lymph node in RS. Samples were run in duplicate.
232x118mm (300 x 300 DPI)

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3 **Legends for illustrations**
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7 **Table 1:** signalment, hematologic and immunophenotypic features of 8 dogs with chronic lymphocytic
8 leukemia undergoing Richter transformation.
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10 **Table 2:** therapy and follow up data of 8 dogs with chronic lymphocytic leukemia undergoing Richter
11 transformation.
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13 **Figure 1:** bone marrow slide from case no. 6. Large blast cells (black arrows) are identifiable together with
14 many small lymphocytes (red arrows). May Grünwald-Giemsa stain, 100x
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17 **Figure 2:** Flow cytometric scattergrams of peripheral blood (A,B,C,D) and lymph node (E,F) from dog no.3.
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23 **Figure 3:** PARR for BCR (A) and TCR (B) on dog #3 and #1 with B and T CLL/RS respectively. Clonal bands at
24 the same molecular weight were detected on blood in CLL phase and in lymph node in RS. Samples were
25 run in duplicate.
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