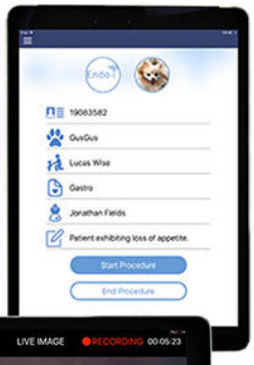
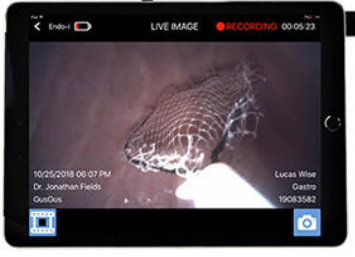
SOMETIMES OLD SCHOOL ISN'T COOL

There's a *Better* Way to Scope...

Endo-i® Wireless HD Endoscopes

- No Bulky Towers
- Easy to Transport
- 3 Models Available (1m, 1.5m, 3m)
- Tablet & App Included





EASE-OF-USE

MANAGE PATIENT INFORMATION

EXPORT PROCEDURAL DATA

MADE IN USA

Patented Technology

STERIS
Animal Health

1.844.540.9810
sterisanimalhealth.com

J Vet Intern Med 2011;25:100–106

Immunophenotype Predicts Survival Time in Dogs with Chronic Lymphocytic Leukemia

S. Comazzi, M.E. Gelain, V. Martini, F. Riondato, B. Miniscalco, L. Marconato, D. Stefanello, and M. Mortarino

Background: Chronic lymphocytic leukemia (CLL) is a hematologic disorder in dogs, but studies on prognostic factors and clinical outcome are lacking. In people, several prognostic factors have been identified and currently are used to manage patients and determine therapy.

Objectives: The aim of the study was to determine if the immunophenotype of neoplastic cells predicts survival in canine CLL.

Design: Retrospective study.

Animals: Forty-three dogs with CLL.

Procedures: Records of dogs with a final diagnosis of CLL were reviewed. For each included dog, a CBC, blood smear for microscopic reevaluation, and immunophenotyping data had to be available. Data on signalment, history, clinical findings, therapy, follow-up, as well as date and cause of death were retrieved.

Results: Seventeen dogs had B-CLL (CD21+), 19 had T-CLL (CD3+ CD8+), and 7 had atypical CLL (3 CD3− CD8+, 2 CD3+ CD4− CD8−, 1 CD3+ CD4+ CD8+, and 1 CD3+ CD21+). Among the variables considered, only immunophenotype was associated with survival. Dogs with T-CLL had approximately 3-fold and 19-fold higher probability of surviving than dogs with B-CLL and atypical CLL, respectively. Old dogs with B-CLL survived significantly longer than did young dogs, and anemic dogs with T-CLL survived a significantly shorter time than dogs without anemia.

Conclusions: Although preliminary, results suggested that immunophenotype is useful to predict survival in dogs with CLL. Young age and anemia are associated with shorter survival in dogs with B-CLL and T-CLL, respectively.

Key words: Blood; Canine; Lymphoproliferative disorders; Oncology; Prognosis.

Chronic lymphocytic leukemia (CLL) is a hematologic disorder occurring in middle-aged to old dogs, although studies on its actual prevalence still are lacking. Also, no extensive description of clinical behavior and prognostic factors is available. Hematologic features of CLL[1–4] and data on clinical outcome have been reported on a limited number of patients.[5,6] In human medicine, 2 widely accepted staging systems (the so-called modified Rai and Binet staging systems) have been developed and routinely applied in the clinical setting.[7,8] Several prognostic factors, including lymphoadenomegaly, splenomegaly or hepatomegaly, anemia, thrombocytopenia, severe lymphocytosis, rapid lymphocyte doubling time, >10% prolymphocytes or selected chromosomal anomalies, have been linked to a worse prognosis, thus requiring a more aggressive therapeutic approach. In people, CLL primarily is a B-cell neoplasm caused by the expansion of a subpopulation of cells with CD5 positivity. However, some cytogenetic similarities have been reported between humans and dogs.[9]

In people, B-CLL tends to be an indolent disease and, even if cure is not possible, at the moment, median survival times often are >150 months. On the other hand, some studies report that T-CLL often follows a more aggressive course.[10] In dogs, T-CLL is the most common disease but, to our knowledge, few studies focusing on immunophenotype-related prognosis have been carried out. According to some authors, T-CLL with large granular lymphocyte (LGL) morphology does not harbor a worse prognosis than B-CLL.[2] A study on neoplastic lymphocytosis has been published,[11] and found no differences in survival based on immunophenotype. An important finding of that study was that dogs with B-cell neoplasms characterized by small circulating cells or with T-cell lymphocytosis with <30,000 cells/μL had long survival times. A limitation of that study was the lack of differentiation between CLL and lymphoma with blood infiltration, being attributable to the unavailability of consensus definitions.

Chemotherapy is not always indicated, because CLL has an indolent course in most cases.[12] In agreement with human oncology, chemotherapy may be indicated in the case of progressive disease with cytopenia, high lymphocyte count, lymphoadenomegaly and splenomegaly, fever, or infections. To date, the efficacy of different therapeutic approaches and outcomes have not been explored in dogs.

The aim of this retrospective study was to determine if immunophenotype predicts survival in dogs with CLL. Additional factors associated with long survival also were evaluated.

From the Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria, Università degli Studi di Milano, Milan, Italy (Comazzi, Gelain, Martini, Mortarino); the Dipartimento di Patologia Animale, Università di Torino, Turin, Italy (Riondato, Miniscalco); the Animal Oncology and Imaging Center, Hünenberg, Switzerland (Marconato); and the Dipartimento di Scienze Cliniche Veterinarie, Università degli Studi di Milano, Milan, Italy (Stefanello).

Corresponding author: Stefano Comazzi, DVM, PhD, Dip-ECVCP, Dipartimento di Patologia Animale, Igiene e Sanità Pubblica Veterinaria, Università degli Studi di Milano, Via Celoria 10, 20133 Milan, Italy; e-mail: stefano.comazzi@unimi.it.

Submitted February 23, 2010; Revised July 20, 2010; Accepted September 29, 2010.

10.1111/j.1939-1676.2010.0640.x

Materials and Methods

Eligibility

The databases of the authors' institutions were reviewed to identify dogs with CLL, blood samples of which were referred for immunophenotyping (September 2006 to January 2010).

To be included in the study, flow cytometry of peripheral blood showing lymphocyte counts $>6\times10^9/L$[12] had to be available. Additionally, immunophenotyping data had to be suggestive of a homogeneous expansion of lymphocytes.[11]

Exclusion criteria included:

(1) morphology suggestive of immature or blast cells in >30% of cells (based on medium to large cells; round or indented, medium to large nucleus with poorly condensed chromatin; presence of nucleoli or some combination of these),
(2) CD34 positivity,
(3) moderate or severe lymphadenomegaly, splenomegaly, or both with nodal, splenic, or both having cytological features compatible with lymphoma. Mild lymphadenopathy or splenomegaly was not considered an exclusion criteria, except for those cases showing cytological features suggestive of specific lymphoma subtypes, and
(4) positive serologic titer for Ehrlichia or Leishmania or any other identifiable cause of lymphocytosis (eg, hypoadrenocorticism, postvaccinal lymphocytosis, stress lymphocytosis).

Dogs that were severely symptomatic or had specific clinical signs attributable to multiorgan infiltration were reevaluated by means of clinical reassessment, cytology of presumptive lesions, and imaging to exclude lymphoma. Any clinical sign attributable to anemia or thrombocytopenia was not considered an exclusion criterion.

For each included case, the following data had to be available: a CBC, at least 1 high quality blood smear for microscopic reevaluation, a complete medical record including signalment, history, clinical findings, therapy, follow-up as well as date and presumptive cause of death (if applicable).

Hematology

CBC was performed on EDTA blood samples with an automated laser analyzer. Automated differentials were validated by microscopic evaluation of blood smears stained with May Grünwald-Giemsa. Blood smears were accurately evaluated, paying particular attention to the percentage of cells with prolymphocytic morphology (ie, medium cells with slightly basophilic cytoplasm and centrally placed, round, or slightly indented nucleus with minimal coarse chromatin and a large, round bluish nucleolus) or with LGL appearance (medium cells with abundant clear cytoplasm and fine cytoplasmic granules, round nucleus with clumped chromatin, and an inconspicuous nucleolus). Anemia was defined by PCV <37% (i.e., lower limit of the reference range). Thrombocytopenia was confirmed if both platelet count was <100,000/mL and blood smear showed low platelet estimation and no evidence of platelet clumping.[13]

Immunophenotype

Flow cytometric immunophenotype was performed as reported previously[14] with a flow cytometer.[a] The following monoclonal antibodies were used: CD45-PE[b] (clone YKIX716.13, Serotec), CD3-FITC (clone CA17.2A12, Serotec, T cells), CD4-FITC (clone YKIX302.9, Serotec, T-helper and neutrophils), CD8-PE (clone YCATE55.9, Serotec, T-cytotoxic/suppressor), CD21-PE (clone CA21D6 Serotec, B cells), CD34-PE (clone 1H6 Pharmingen, precursor cells).[c] Analysis was conducted by specific software (Cell Quest). Based on flow cytometric results, cases were grouped as follows: B-CLL (CD45+ CD21+), T-CLL (CD3+ CD8+), and atypical CLL (other phenotypes).

Statistical Analysis

Data were recorded in a spreadsheet and summarized by descriptive statistics with standard statistical software.[d]

Hematological features of different immunophenotype groups were analyzed by means of the Kruskal-Wallis test for independent samples. To test correlation between different hematologic variables, the Spearman test was performed. Frequency of anemia (PCV <37%), thrombocytopenia, and extreme lymphocytosis (lymphocytes $>50\times10^9/L$) were compared within different phenotypic groups by the Pearson χ^2-test.

To assess whether immunophenotype influenced survival, curves were generated by the Kaplan-Meier method and compared with the log-rank test. Survival time was calculated from the date of diagnosis of CLL to death. Cases were censored if lost to follow-up, if they died or were euthanized because of unrelated causes, or if they were alive at the end of the study.

The following factors were investigated with multivariate Cox's proportional hazard regression analysis to assess association with long survival: immunophenotype, age, PCV, total lymphocyte count. Age, PCV, and total lymphocyte count were analyzed as continuous noncategorical variables. Analysis was performed in 2 steps: initially, all cases were included in the analysis, thereafter only B-CLL and T-CLL were evaluated to define possible variables predicting survival within different phenotypic groups. The Cox proportional hazards regression model assumption was tested by plotting the "log negative log of survival" among cases.

Finally, curves were generated by the Kaplan-Meier method and compared with the log-rank test to verify whether therapy affected survival times. These data were not included in the multivariate analysis because they were not considered independent from some prognostic features (eg, anemia, severe lymphocytosis, organ failure, clinical signs) and because therapy was not started immediately after diagnosis in all cases. Therapeutic approaches were arbitrarily grouped into 3 categories: (A) dogs receiving neither antineoplastic chemotherapy nor corticosteroids, (B) dogs receiving only corticosteroids, and (C) dogs receiving cytotoxic antineoplastic chemotherapy and corticosteroids.

P-values < .05 were considered significant.

Results

Of 272 cases with homogeneous lymphocytosis, 153 were excluded because of clinical and cytological aspects suggestive of lymphoma, whereas 53 were excluded because of CD34 positivity or morphological appearance of precursor blast cells.

Two cases were excluded because low-grade lymphoma and CLL could not be differentiated based on clinical signs (moderate lymphoadenomegaly and splenomegaly) and cytological features (medium or small lymphocytes).

Ten cases with moderate to marked lymphadenomegaly were excluded because cytological smears were absent or of poor quality, thereby precluding the possibility of reevaluation. Seven cases were excluded because of absence of clinical and follow-up data.

Four cases showing a homogeneous expansion of lymphocytes were excluded because of serologic positivity to Ehrlichia (n = 3) or Leishmania (n = 1).

Eight cases showing moderate (PCV < 30%) anemia and 7 cases with thrombocytopenia were included because of the absence of masses or lymph node enlargement. Mild to moderate splenomegaly and lymphadenomegaly were observed in 7 and 11 cases, respectively; these dogs were included based on the presence of a prevalent homogeneous population of small mature lymphocytes.

Forty-three dogs met the inclusion criteria: there were 17 B-CLL (CD21+), 19 T-CLL (CD3+ CD8+), and 7 atypical CLL, including 3 cases with CD3− CD8+ immunophenotype 2 double-negative T-CLL (CD3+ CD4− CD8−), 1 double-positive T-CLL (CD3+ CD4+ CD8+), and 1 biphenotypic CLL (CD3+ CD21+). Median age was 10 years (range, 2–16 years).

Hematology

Results of the main hematological features are shown in Table 1. Twenty-one (48.8%) dogs were anemic (9/17; 9/19; 3/7 in B-, T-, and atypical CLL, respectively); anemia generally was mild (PCV > 31%). Leukocytosis with lymphocytosis was uniformly present and in 22 (51.2%) of 43 cases (6/17; 12/19; 4/7 in B-, T-, and atypical CLL, respectively) lymphocyte count was $>50\times10^9$/L (extreme lymphocytosis). Ten (23.3%) dogs were thrombocytopenic. The percentage of prolymphocytes was <10% in all samples except for 1 B-CLL, 1 T-CLL, and 1 atypical CLL, reaching 20%. LGLs were found in 14/19 T-CLL and in 4 atypical CLL (2 CD3− CD8+, 1 double-negative, and 1 biphenotypic). No statistical differences were found among B-, T-, and atypical CLL for any variable examined, with the exception of platelet count, which was higher in T-CLL ($P = .039$). Pearson's χ^2-test showed no significant difference between frequency of anemia, thrombocytopenia, and extreme lymphocytosis among different phenotypic groups.

Erythrocyte number, PCV, and hemoglobin concentration were directly correlated with platelet count ($P = .012$ $r^2 = 0.384$; $P = .002$ $r^2 = 0.470$; $P = .025$ $r^2 = .344$, respectively) and inversely correlated with lymphocyte count ($P < .001$ $r^2 = -0.539$; $P = .003$ $r^2 = -0.437$; $P = .001$ $r^2 = -0.470$, respectively).

Clinical Outcome

B-CLL. Among the 17 cases with B-CLL, 11 (64.7%) dogs died during the study period. In 1 case only, death was unrelated to CLL and occurred 150 days after diagnosis. In 8 cases, cause of death was attributable to progressive CLL, with deterioration of clinical condition, development of anemia, thrombocytopenia, progressive increase of lymphocytes, or some combination of these. In 2 cases, a B-cell high-grade lymphoma developed, being confirmed by cytology and immunophenotyping. Death occurred after 571 and 292 days, respectively, most likely attributable to hepatic and renal failure because of neoplastic infiltration. Among the dogs that died of CLL, 8 received chemotherapy (chlorambucil and prednisone in 7 cases, doxorubicin and prednisone in 1 case) whereas 2 dogs received no treatment. Six (35.3%) dogs were alive at the end of study, with a median follow-up time of 323 days (range, 99–797 days). These dogs showed stable disease (confirmed by serial CBC follow-ups) without clinical signs. Regarding treatment, 4 dogs received chlorambucil and prednisone, whereas 2 dogs were not treated.

T-CLL. Among the 19 cases with T-CLL, 7 (36.8%) died during the study period whereas 3 dogs were censored because they were lost to follow-up 150, 240, and 300 days after diagnosis. Among the dogs that died, 1 developed a high-grade T-lymphoma and died 270 days after diagnosis, whereas death was because of progressive disease in the other 6. With respect to treatment, 3 dogs received no therapy, 2 received chlorambucil and prednisone, 1 was treated with melphalan and prednisone, and 1 received prednisone only.

Nine (47.3%) dogs were alive at the end of study, with a median follow-up time of 380 days (range, 41–1,101 days). With respect to treatment, 5 dogs received chemotherapy (chlorambucil and prednisone), 1 received prednisone only, and 3 dogs were not treated.

Atypical CLL. Six of the 7 dogs with atypical CLL died during the study period because of progressive disease. Three dogs received no treatment, 2 were treated with chemotherapy (chlorambucil and prednisone in 1 case and L-asparaginase in 1 case), and 1 dog received prednisone alone.

One dog was alive at the end of the study, 356 days after diagnosis and without treatment. This dog had an incidental finding of lymphocytosis, with the cells showing positivity to CD3 and CD21.

Survival Analysis

The Kaplan-Meier survival curve is shown in Figure 1. Based on log-rank test, immunophenotype was signifi-

Table 1. Main hematological features (median) and minimum-maximum intervals (in parenthesis) for each phenotypic group of CLL.

Group	No.	Erythrocyte ($\times10^6$/μL)	Hemoglobin (g/dL)	PCV (L/L)	Platelets ($\times10^3$/μL)	Leukocytes ($\times10^3$/μL)	Lymphocytes ($\times10^3$/μL)	Neutrophils ($\times10^3$/μL)
B-CLL	17	5.50 (2.57–7.70)	12.90 (6.00–16.80)	0.35 (0.20–0.47)	225 (24–576)	48.74 (13–288)	35.98 (8–201)	8.25 (0.74–75.0)
T-CLL	19	5.58 (2.07–7.84)	13.20 (5.20–16.80)	0.38 (0.19–0.51)	405 (50–757)	85.43 (19–419)	75.61 (12–380)	7.45 (3.6–33.0)
Atypical CLL	7	5.66 (3.1–6.61)	14.10 (8.40–14.30)	0.40 (0.30–0.42)	219 (60–280)	73.06 (27–836)	61.30 (19–819)	13.25 (7.0–88.4)

No statistical differences were detected between the groups except for platelets number ($P = .039$).
CLL, chronic lymphocytic leukemia.

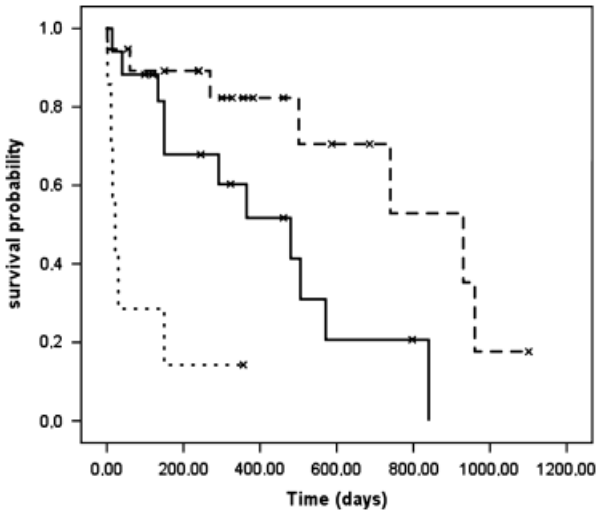


Fig 1. Kaplan-Meier curves for survival times in dogs with CLL with different phenotypes. Dotted line indicates atypical CLL; solid line indicates B-CLL; broken line indicates T-CLL. The difference is significant, $P < .001$. CLL, chronic lymphocytic leukemia.

cantly associated with survival ($P < .001$) . Median overall survival time was 22 days for atypical CLL, 480 days for B-CLL, and 930 days for T-CLL. Therapeutic approach was not associated with survival in either group ($P = .71$).

Results of Cox's multivariate analysis are shown in Table 2. Only immunophenotype was significantly associated with survival. In particular, dogs with atypical CLL had approximately 19-fold higher likelihood of dying compared with dogs with T-CLL, whereas dogs with B-CLL had >3-fold higher likelihood of dying compared with dogs with T-CLL. Degree of anemia and lymphocytosis at diagnosis were not associated with survival. With regard to B-CLL, young age at diagnosis was associated with a poor prognosis ($P = .014$, hazard ratio = 0.65, CI 0.47–0.92). With regard to T-CLL, low PCV was associated with shorter survival ($P = .023$, hazard ratio = 0.72, CI 0.54–0.96).

Discussion

The distinction between CLL and lymphoma with blood involvement is challenging. For our study purposes, we arbitrarily applied the following exclusion criteria: dogs with circulating immature or large lymphoid cells (nuclei ≥2 RBC in diameter), with moderately to severely enlarged lymph nodes or with mild lymph node enlargement with cytology suggestive of a specific lymphoma subtype.[15] Furthermore, dogs with severe clinical signs were accurately reevaluated to eliminate occult lymphoma. Use of these criteria allowed exclusion most cases of stage V lymphoma. Nevertheless, a limited number of stage V small cell lymphoma (lymphocytic subtype), cases may have been included in our cases. However, this subtype occurs quite rarely in dogs, accounting for <1% of all lymphoma cases.[16]

Table 2. Results of Cox's multivariate analysis for analyzed variables in all CLL cases.

Variables	No. of Cases	Hazard Ratio	95% CI	*P*-Value
Phenotype				
B-CLL	17	3.43	1.10–10.68	.034*
Atypical-CLL	7	19.17	4.57–80.35	<.001*
T-CLL	19	Reference	—	—
Age	43	0.91	0.78–1.05	.204
PCV	43	0.96	0.90–1.02	.262
Lympho-cytes (mmc)	43	1.00	0.99–1.01	.695

CI, confidence interval; CLL, chronic lymphocytic leukemia.
*$P < .05$.

According to the REAL/WHO classification system, the nomenclature is linked to the tissue with the greatest volume of tumor, meaning that if the neoplasm is mostly in the bone marrow, it is called leukemia. On the other hand, if the neoplasm is mostly in the peripheral tissues, it is called lymphoma. In practice, CLL and low-grade lymphocytic lymphoma represent different manifestations of the same disease.[17,18]

CD34-positive cases were excluded. Although not all acute lymphoid leukemias are reported to be CD34 positive,[2] cases in which immature or blast cells were the prevalent population also were excluded, thereby eliminating potentially CD34-negative acute leukemias from the cases.

CLL is frequent in dogs, but no studies focusing on prognosis have been published. Some case series have been reported describing hematological features and immunophenotype.[2–4] CLL rarely has been associated with severe cytopenias, with no differences between phenotypes. T-CLL with CD3+ CD8+ phenotype has been reported as the most frequent form, representing 73% of CLL, mainly showing LGL morphology (54%).[2] In the present work, T-CLL represented only 44% of the cases, but this finding may have been biased by the inclusion criteria, as some cases were excluded because of lack of complete clinical data or lymph node cytology for reevaluation. In agreement with previous studies[2,3] our results confirm that there are no statistical differences within phenotypic groups with regard to hematological variables, with the exception of a higher platelet count in T-CLL. Prolymphocytes rarely are found in canine CLL, without differences within the phenotypic groups. In people, prolymphocyte percentage >10% (but <55%) is considered a poor prognostic factor.[19] In the present study, dogs with high prolymphocyte percentages did not have a worse prognosis.

According to our results, LGL morphology was a frequent feature of T-CLL and atypical CLL. However, atypical CLL were of T lineage, except for 1 case that coexpressed CD3 and CD21. LGL morphology also has been reported to be frequent in T-CLL by other authors,[2,3] but no correlation has been found with biological behavior, although extensive survival studies are lacking. In our case series, the presence of LGL was not associated with a specific survival time, and this may be

attributable to the phenotype, as T-CLL had a longer survival time in comparison with atypical CLL.

CLL generally is a disease of old animals,[20] with a median age at diagnosis of 10.5 years.[1] Our results support this finding, with a median age at diagnosis of 10 years. However, 6 dogs (2 with B-CLL, 2 with T-CLL, and 2 with atypical CLL) were younger than 8 years. Among these 6 dogs, 4 harbored a poor prognosis, surviving <40 days. The 2 dogs with T-CLL showed survival times that were similar to those of the older dogs. For B-CLL, young age was statistically associated with shorter survival, suggesting that leukemia in these dogs may have a more aggressive behavior. In dogs with B-CLL, young age therefore may be considered as a possible negative prognostic factor. Conversely, T-CLL may have a more homogeneous clinical behavior with respect to age, whereas the severity of anemia was correlated to a worse prognosis in T-CLL only.

To the best of our knowledge, there is only 1 published study[11] identifying prognostic factors in dogs with neoplastic lymphocytosis. The authors found no difference in survival with regard to phenotype, but the inclusion criteria used were very different from those used in the present study, because no attempt was made to distinguish between CLL and stage V lymphoma. The authors found that small size of cells in B-neoplasms and a lymphocyte count $<30{,}000$ cells/μL in T-cell neoplasms were predictive of longer survival time.

Our study shows that T-CLL generally is associated with very long survival times, although anemia must be considered as a negative prognostic factor. On the other hand, atypical CLL (mainly of T lineage) has a very aggressive course and is associated with a short survival time. The finding of 1 case with biphenotypic CLL that lived more than 1 year without evident clinical signs remains to be elucidated and the different outcome might be related to the particular cell type involved in this very infrequent leukemia.

Short survival times are quite unusual for CLL. CD34 negativity and lack of severe cytopenia allowed excluding acute lymphoid leukemia. All dogs were accurately evaluated (including imaging and cytology) to rule out presence of masses or visceral involvement. However, despite our effort to differentiate CLL from stage V lymphoma, we cannot be certain that we did not include dogs with occult small cell lymphoma in this series. Regardless of the possible misclassification, our results indicate that a more aggressive course is expected in dogs with hematological and clinical signs of CLL showing atypical phenotypes.

B-CLL may be considered an intermediate phenotype, with old dogs surviving quite long, and young dogs showing a more aggressive course. These results partially disagree with those of the study of Williams et al,[12] which may be attributable to the different inclusion criteria. Although no consensus exists on how to differentiate CLL from stage V lymphoma, to the authors' experience, the distinction between these 2 entities is relatively easy in most cases, with the exception of small cell lymphomas. Clinical features and an accurate evaluation of lymph node cytology may be of use in most cases to make this distinction. In the present study, we made an effort to differentiate between stage V lymphoma and CLL based on clinical and cytological presentation.[18]

Williams and colleagues found that lymphocytosis because of small cells (according to an arbitrarily fixed cutoff) were linked to very long survival. In the present study, the low number of samples did not allow us to distinguish leukemias based on cellular size. Furthermore, we found that anemia, but not lymphocytosis, was correlated with survival in T-CLL. Both of these findings disagree with the previously cited study, but the low number of cases may account for such differences. Prospective studies including more dogs and by similar inclusion criteria are warranted.

In our study, 2 dogs with B-CLL and 1 with T-CLL developed high-grade aggressive lymphoma. This particular condition is similar to the well-known Richter's syndrome reported in people, occurring in about 5% of patients with CLL.[21] In most cases, the aggressive lymphoma evolves from the original leukemic cell clone. Conversely, lymphoma may represent a second malignancy.[22] Several molecular predictors of Richter's transformation have been identified in people.[22] To our knowledge, this evolution has been reported previously only in 2 of 22 dogs with CLL,[1] but immunophenotype data were not provided. Additional studies will be useful to clarify analogies between the canine and human forms.

In human CLL, mutational status of the variable region of immunoglobulin heavy chain genes, cytogenetic aberrations, expression level of ZAP-70, CD38, and molecules regulating the process of angiogenesis have been evaluated as possible molecular markers having prognostic value.[23] Recently, telomere length has been proposed as an independent predictor of outcome in human CLL, including overall survival and Richter's syndrome transformation.[24] Taken together, the above findings encourage research efforts aimed at the evaluation of the potential utility of these molecular markers for canine CLL.

Another possible unfavorable evolution of CLL is progressive active disease, being characterized by massive lymphoadenopathy and splenomegaly, increased number of lymphocytes, progressive anemia, and thrombocytopenia. Death generally occurs secondary to organ failure. Indeed, all dogs that were alive at the end of the study showed stable disease with absent or minimal clinical signs; stable erythrocytes, lymphocytes, and platelets; no evidence of massive splenomegaly or lymphadenopathy; and no evidence of organ failure.

With regard to treatment, chronic immunosuppression with chlorambucil and prednisone is indicated in cases with severe hematological abnormalities, bulky or symptomatic lymphadenopathy, or organomegaly.[5,20] Treatment also may be valuable in cases with clinical evidence of disease, including lethargy or anorexia. On the other hand, asymptomatic dogs are followed and receive treatment only if disease progression occurs.

Even in the face of treatment, CLL remains incurable with standard therapies. Affected dogs eventually

relapse, become refractory to treatment, or undergo disease transformation. According to the results obtained here, no benefit in overall survival has been documented when considering treated and untreated dogs. Because of the retrospective nature of this study, however, treatment varied among dogs and different subgroups of CLL, thereby possibly preventing differences from being identified. Additionally, whether treatment was indicated or not was left at the discretion of the referring clinicians, who were not necessarily oncologists, and incorrect decisions may have been made, including providing unnecessary treatments or not treating dogs that actually may have benefited from chemotherapy. Future prospective trials should be carried out to better elucidate the role of chemotherapy in the treatment of specific subsets of canine CLL and to illustrate which populations of dogs will require chemotherapy.

In conclusion, this is the first study focused on prognostic factors associated with survival in canine CLL. Based on our results, determination of flow cytometric immunophenotype is strongly recommended as an accurate approach to canine lymphocytosis, not only because it allows the clinician to identify clonal versus nonclonal expansion in most cases, but also because it permits one to distinguish T-CLL (showing the classic indolent behavior and long survival time), atypical CLL (characterized by very aggressive behavior and short survival), and B-CLL with long survival time, especially in older dogs. Conversely, young animals have shorter survival times. Moreover, we described development of high-grade aggressive lymphoma as a possible negative evolution of canine CLL, similarly to human Richter's syndrome. Additional studies are needed to confirm our results and to identify possible molecular biomarkers associated with different survival times or disease evolution.

Footnotes

[a] FACSCalibur, Becton Dickinson, San Jose, CA
[b] Serotec, Oxford, UK
[c] Pharmingen, BD Bioscience, San Jose, CA
[d] SPSS Statistics 17.0, SPSS Company, Wacker Drive, Chicago, IL

Acknowledgments

The authors thank Dr G. Romanelli and the private practitioners who provided follow-up data. Supported PUR 2008 (Dr Comazzi), University of Milan, PRIN 2008 (Dr Comazzi).

References

1. Leifer CE, Matus RE. Chronic lymphocytic leukemia in the dog: 22 cases (1974–1984). J Am Vet Med Assoc 1986;189:214–217.

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

3. Tasca S, Carli E, Caldin M, et al. LS Hematologic abnormalities and flow cytometric immunophenotyping results in dogs with hematopoietic neoplasia: 210 cases (2002–2006). Vet Clin Pathol 2009;38:2–12.

4. Adam F, Villiers E, Watson S, et al. Clinical pathological and epidemiological assessment of morphologically and immunologically confirmed canine leukaemia. Vet Comp Oncol 2009;7: 181–195.

5. Harvey JW, Terrell TG, Hyde DM, et al. Well-differentiated lymphocytic leukemia in a dog: Long-term survival without therapy. Vet Pathol 1981;18:37–47.

6. Fujino Y, Sawamura S, Kurakawa N, et al. Treatment of chronic lymphocytic leukaemia in three dogs with melphalan and prednisolone. J Small Anim Pract 2004;45:298–303.

7. Rai KR, Sawitsky A. A review of the prognostic role of cytogenetic, phenotypic, morphologic, and immune function characteristics in chronic lymphocytic leukemia. Blood Cells 1987; 12:327–338.

8. Binet JL, Auquier A, Dighiero G, et al. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer 1981;48:198–206.

9. Breen M, Modiano JF. Evolutionarily conserved cytogenetic changes in hematological malignancies of dogs and humans—man and his best friend share more than companionship. Chromosome Res 2008;16:145–154.

10. Hoyer JD, Ross CW, Li CY, et al. True T-cell chronic lymphocytic leukemia: A morphologic and immunophenotypic study of 25 cases. Blood 1995;86:1163–1169.

11. Workman HC, Vernau W. Chronic lymphocytic leukemia in dogs and cats: The veterinary perspective. Vet Clin North Am Small Anim Pract 2003;33:1379–1399.

12. Williams MJ, Avery AC, Lana SE, et al. Canine lympho proliferative disease characterized by lymphocytosis: Immunophenotypic markers of prognosis. J Vet Intern Med 2008;22:596–601.

13. Botsch V, Küchenhoff H, Hartmann K, et al. Retrospective study of 871 dogs with thrombocytopenia. Vet Rec 2009;164:647–651.

14. Comazzi S, Gelain ME, Spagnolo V, et al. Flow cytometric patterns in blood from dogs with non-neoplastic and neoplastic hematologic diseases using double labeling for CD18 and CD45. Vet Clin Pathol 2006;35:47–54.

15. Fournel-Fleury C, Magnol JP, Bricaire P, et al. Cytohistological and immunological classification of canine malignant lymphomas: Comparison with human non-Hodgkin's lymphomas. J Comp Pathol 1997;117:35–59.

16. Ponce F, Marchal JP, Turinelli V, et al. A morphological study of 608 cases of canine malignant lymphoma in France with a focus on comparative similarities between canine and human lymphoma morphology. Vet Pathol 2010;47:414–433.

17. Jacobs RM, Messick JB, Valli VE. Tumors of the hemolymphatic system. In: Meuten DJ., ed. Tumors in Domestic Animals, 4th ed. Ames, IA: Iowa State Press; 2002:177–180.

18. Vail DM, Young KM. Haematopoietic tumors. In: Withrow SJ, Vail DM, eds. Withrow and Mac Ewen's Small Animal Clinical Oncology. St Louis, MO: Saunders; 2007:722–725.

19. Vallespí T, Montserrat E, Sanz MA. Chronic lymphocytic leukaemia: Prognostic value of lymphocyte morphological subtypes. A multivariate survival analysis in 146 patients. Br J Haematol 1991;77:478–485.

20. Helfand SC, Modiano JF. Chronic lymphocytic leukemia. In: Feldman BF, Zinkl JG, Jain NC, eds. Schalm's Veterinary Hematology, 5th ed. Baltimore, MD: Lippincott Williams & Wilkins; 2000:638–641.

21. Tsimberidou AM, Keating MJ. Richter syndrome: Biology, incidence, and therapeutic strategies. Cancer 2005;103: 216–228.

22. Rossi D, Gaidano G. Richter syndrome: Molecular insights and clinical perspectives. Hematol Oncol 2009;27:1–10.

23. Vroblová V, Smolej L, Vrbacký F, et al. Biological prognostic markers in chronic lymphocytic leukemia. Acta Medica (Hradec Kralove) 2009;52:3–8.

24. Rossi D, Lobetti Bodoni C, Genuardi E, et al. Telomere length is an independent predictor of survival, treatment requirement and Richter's syndrome transformation in chronic lymphocytic leukemia. Leukemia 2009;23:1062–1072.