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Breed Distribution and Clinical Characteristics of B Cell Chronic Lymphocytic Leukemia in Dogs

J.L. Bromberek, E.D. Rout, M.R. Agnew, J. Yoshimoto, P.S. Morley, and A.C. Avery

Background: B-cell chronic lymphocytic leukemia (B-CLL) is the most common hematopoietic malignancy in humans in the developed world and the primary risk factor is genetic. Dogs also develop B-CLL, but there is no systematic description of the disease in dogs. Understanding the epidemiology of B-CLL in dogs may help practitioners recognize the disease and position the dog as a model for future genetic studies.

Objectives: To describe B-CLL presentation in dogs, its clinicopathologic findings, and breed predisposition.

Animals: Four hundred and ninety-one dogs with B-CLL and 5,673 control dogs with suspicion of a lymphoproliferative disorder (LPD).

Methods: Retrospective cross-sectional study of dogs for which samples were submitted to the Colorado State University Clinical Immunology Laboratory for immunophenotyping between 2010 and 2014. To assess breed predilection, dogs with B-CLL were compared to those with suspicion of other LPDs using logistic regression.

Results: The median age was 11 years with no sex predilection. Half of the dogs presented with peripheral lymphadenopathy or splenomegaly and 26% had anemia. Eleven small-breed dogs had significantly increased odds of B-CLL. In addition, English Bulldogs had an increased risk and a unique presentation: these dogs were diagnosed at a median of 6 years and expressed lower class II MHC and CD25.

Conclusions: B-cell chronic lymphocytic leukemia is overrepresented in small-breed dogs. Future genetic studies of these breeds may identify genetic risk factors. The unique presentation of English Bulldogs provides evidence of multiple forms of this disease. Additional studies are necessary to determine whether presenting signs are associated with survival.

Key words: Dog; Epidemiology; Immunophenotyping; Lymphoma; Leukemia; Oncology.

B-cell chronic lymphocytic leukemia (B-CLL) is the second most common leukemia affecting people in the United States.[1] This disease is well characterized and a strong genetic risk has been identified.[2] Dogs also develop B-CLL and likely have associated genetic risk factors. Because of the discrete genetic pools maintained by selective breeding, the dog may be a useful model for studying genetic risk factors for this disease. Little is known, however, about the population of dogs that develop B-CLL. Our study aims to further describe B-CLL in dogs by providing a systematic description of the typical patient presentation and clinical signs as well as exploring potential breed predisposition for the disease.

There are no consensus criteria for diagnosis of B-CLL in dogs, nor has it been determined if there is a solid tissue equivalent for this disease. Neoplasms involving canine B-cells do not express CD5, which is used to distinguish B-CLL from other B-cell lymphoproliferative disorders (LPDs) in people.[3] Prior studies used a variety of criteria for CLL diagnosis, including the use of morphologic appearance, immunophenotype, or both, with some studies excluding cases with substantial lymphadenopathy.[3–5] However, lymphadenopathy and splenomegaly are part of the staging criteria used in humans,[6] and excluding these cases may exclude patients with advanced stage B-CLL. Because of the subjective nature of cytologic assessment of lymphocytes and the severity of lymphadenopathy, we previously carried out an analysis of B-cell leukemia characterized by small cells based solely on flow cytometric (FC) features to determine if objective criteria alone could be used to diagnose and predict prognosis in cases of B-cell leukemia.[7] Lymphadenopathy and splenomegaly were noted in 40 and 29% of cases, respectively, but these were not used as exclusion criteria. The results indicated that small cell size predicted an indolent clinical outcome in cases of B-cell leukemia and lymphoma. Although we did not specifically label this disease B-CLL, the indolent clinical course is consistent with this diagnosis.

Abbreviations:

LPD	lymphoproliferative disorder
FC	flow cytometry
PE	physical examination
B-CLL	B-cell chronic lymphocytic leukemia
CSU-CI	Colorado State University Clinical Immunology
MFI	median fluorescence intensity
OR	odds ratio
CI	confidence interval

From the Department of Microbiology, Immunology and Pathology, (Bromberek, Rout, Agnew, Yoshimoto, Avery); and the Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO (Morley).

Corresponding author: A. C. Avery, Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523; e-mail: anne.avery@colostate.edu.

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In people, some patients experience a more progressive form of CLL, which has been associated with cell surface phenotype and IgV_H mutational status.[8,9] Prior studies have shown cell surface phenotype, including class II MHC expression, is predictive of prognosis in dogs with B-cell lymphomas.[7,10] However, no research has directly assessed B-CLL in dogs. Because an unmutated phenotype is predictive of poor survival in people,[8] expression of surface markers indicative of B-cell maturation and activation, such as class II MHC and CD25, may have prognostic relevance in dogs with B-CLL.[11–13]

The goal of our study was to describe the clinical, immunophenotypic and breed characteristics of a large cohort of dogs with B-CLL. Our findings provide a basis on which to establish the dog model of B-CLL, and may provide clinicians and clinical pathologists with information that aids in the diagnosis of this disease. In addition, our findings can be used as a prelude for future studies evaluating genetic risk factors as well as identifying discrete subtypes of B-CLL that may have prognostic relevance.

Materials and Methods

Study Population

A retrospective cross-sectional study was performed using samples submitted to the Colorado State University Clinical Immunology (CSU-CI) Laboratory. The CSU-CI laboratory (http://csu-cvmbs.colostate.edu/academics/mip/ci-lab/Pages/default.aspx) receives samples from dogs with suspicion of LPD that are submitted by reference laboratories and clinicians. For this study, all dogs were included that had samples submitted to the CSU-CI laboratory for immunophenotyping between September 17, 2010 and June 10, 2014. These samples included blood as well as aspirates of lymph nodes, bone marrow, masses, and cavity fluid. If multiple samples were received for a given dog, only the first diagnostic sample was included.

Flow Cytometry

Flow cytometry was carried out as previously described.[14] Samples acquired before May 11, 2012 were analyzed using the antibody combinations listed in panel 1 (Table 1) using a single laser Coulter XL.[a] Samples submitted after May 11, 2012 were analyzed with the antibody combinations listed in panel 2 (Table 1) using a 3-laser Coulter Gallios.[a] All data analysis was carried out using Kaluza Analysis Software.[a] Level of class II MHC expression on B-cells was determined by the median fluorescence intensity (MFI) of staining on gated B-cells in tube 3, panel 2 (Fig S1). The CD21 and CD25 antibodies were not together in the same staining reaction, and CD25 expression level was determined by gating on small lymphocytes in tube 2, panel 2, and excluding cells that expressed CD3, CD4, CD5, and CD8 (Fig S2).

Case Definition

The B-CLL cases were identified by FC of peripheral blood. All patients were required to have a CBC performed within 48 hours of sample acquisition. Only samples with >5,000 lymphocytes/μL were included. The FC criteria included homogeneous expansion (>60%) of "small" CD21+ lymphocytes. Cells were considered "small" if the ratio of the geometric mean of forward scatter of B-cells to neutrophils was <0.55. Percentage of CD21+ lymphocytes was calculated as the number of CD21+ cells divided by the total number of B and T (CD4+ and CD8+) cells in the sample. Dogs that met this case definition were shown in a prior study to have an indolent disease course consistent with B-CLL.[7] No cases with expansion of CD34+ cells were included in this study. In addition to FC classification, a subset of B-CLL cases had a cytology report reviewed by a board-certified clinical pathologist. These cases were considered "cytology-confirmed" if the report described the majority of cells as "mature", "small", having "condensed chromatin", noted "definitive or possible CLL", or some combination of these descriptors.

Table 1. Antibody panels used for immunopheno typing.

Tube	Antibody Specificity and Fluorochrome
Panel 1 (2 color)	
1	None
2	M[a] IgG1-FITC/CD45-PE
3	CD18-FITC/M IgG1-PE
4	CD4-FITC/CD8-PE
5	CD5-FITC/CD21-PE
6	CD3-FITC/CD45-PE
7	CD4-FITC/CD14-PE
8	Class II MHC-FITC/CD34-PE
Panel 2 (Multicolor)	
1	M IgG1-FITC/M IgG1-PE/M IgG1-Alexa 647/M IgG1-Alexa 700/M IgG1-PE-Alexa-750/M IgG1-Pacific Blue
2	CD3-FITC/CD25-PE/CD5-APC/CD8-Alexa 700/CD4-Pacific Blue
3	Class II MHC-FITC/CD22-PE/CD21-Alexa 647
4	Class II MHC-FITC/CD34-PE/CD5-APC - CD14-PE-Alexa 750
5	Class II MHC-FITC/CD18-PE/CD5-APC/CD14 PE-Alexa 750/CD4-Pacific Blue
6	CD5-FITC/CD45-PE/CD21-Alexa 647

[a]M, mouse.

Unless otherwise noted, all antibodies were purchased from AbD Serotec Raleigh, NC 27609. Clones are as follows: CD45 = YKIX716.13, CD18 = YFC118.3 (human CD18), CD4 = YKIX302.9, CD8 = YCATE 55.9, CD5 = YKIX322.3, CD21 = CA2.1D6, CD22 = RFB4 (human CD22, purchased from AbCam Cambridge, MA 02139), CD3 = CA17.2A12, CD14 = UCHM (human, used in panel 1) and CD14 = TUK4 (human, used in panel 2), class II MHC = YKIX334.2, CD34 = 1H6, CD25 = P2A10 (purchased from eBiosciences San Diego, CA 92121).

Clinical Variables

As part of the standard submission form for the CSU-CI laboratory, signalment, physical examination (PE), and laboratory findings were obtained. Signalment included breed, sex, and age at diagnosis (rounded to the nearest year). PE findings included the presence of peripheral lymphadenopathy, visceral lymphadenopathy, splenomegaly, hepatomegaly, or mediastinal mass. Laboratory findings included the presence of hypercalcemia or hyperglobulinemia. All were categorized as present, absent, or unknown (either not evaluated or not indicated on the form) as denoted by the clinician completing the form. Additional data abstracted from the CBC included hematocrit and absolute counts of lymphocytes, neutrophils, platelets, and reticulocytes. Because cell counts are

coded into the database without specific cross-reference to the submitting laboratory's normal reference range, conservative cut points were derived from those used by the most common submitting laboratories. Neutrophil totals were categorized as neutropenic (<2,000/μL), normal (2,000–12,000/μL), and neutrophilic (>12,000/μL). Platelet totals were categorized as thrombocytopenic (<175,000/μL with no clumps noted), normal (175,000–500,000/μL), and thrombocytosis (>500,000/μL). When the platelet count was below the minimum cut-off and platelet clumps were described, these cases were excluded from analysis of platelet numbers. Hematocrit was used to define anemia (<36%). Automated reticulocyte enumeration was utilized in the majority of cases and regeneration was defined as >100,000 reticulocytes/μL.[15]

Statistical Analysis

Patient Presentation. For all dogs with B-CLL, continuous and interval (cell count) variables were evaluated for normality, the frequency distributions of categorical data were summarized, and descriptive statistics were calculated. Dogs diagnosed with B-CLL using cytology and FC were compared to dogs diagnosed by only FC using chi-squared or Fisher's exact tests for categorical variables and Wilcoxon rank sum test for continuous variables. Data for PE findings marked as "unknown" were excluded from analyses. As a secondary analysis, patient presentation was evaluated for all dogs with board-certified clinical pathologist-reviewed cytology reports, comparing reports that were considered cytology-confirmed (as described above) to those for which the cytology report did not meet our criteria for a cytologic diagnosis of B-CLL. For these comparisons, Wilcoxon rank sum test and Fisher's exact test were used.

Immunophenotype. Data on CD25 and class II MHC expression were evaluated for dogs with B-CLL. These data only were available for samples received after May 11, 2012 (357 dogs) because of the change in flow cytometer. The percentage of B-cells expressing CD25 was categorized into tertiles because of an underlying U-shaped distribution. Class II MHC expression was measured as the MFI on gated B-cells and categorized based on the median MFI for all dogs with B-CLL.

Breed. For breed analyses, dogs reported to be a specific breed were assumed to be purebred unless otherwise noted. All "designer" breeds (eg, "Goldendoodles") were placed in the "mixed-breed" category. Only breeds with ≥30 total submissions during the study period were considered for analysis. All breeds with <30 submissions, or where breed was not indicated, were combined as "other." Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using a simple logistic regression model comparing B-CLL cases to all other immunophenotyping submissions suspected of LPDs by breed. For breeds with no B-CLL cases, Fisher's exact test was used. Separate analyses were conducted using mixed breeds and Labrador Retrievers as the reference group. The reference breeds were chosen a priori, with mixed breeds believed to be representative of all breeds combined, and Labrador Retrievers chosen as a well-defined and more homogeneous reference group.

From the breeds with significantly increased odds of B-CLL, the 6 with the highest number of B-CLL cases were further examined to evaluate breed-specific patient presentation. Because mixed-breed B-CLL cases were most similar to B-CLL cases overall with respect to signalment, PE findings and CBC findings, they were used as the reference group for statistical analysis (Table S3). Only variables related to signalment, PE, and laboratory findings for which ≥50% of B-CLL cases had data were evaluated. Wilcoxon rank sum test and Fisher's exact test were calculated as appropriate. All statistical analyses were conducted using SAS 9.3.[b]

Results

Patient Presentation

A total of 6,164 unique clinical samples were submitted to the CSU-CI laboratory for immunophenotyping by FC because of the suspicion of LPD during the period from September 17, 2010 to June 10, 2014. Submissions represented 48 states (ME and SD not represented), 1,046 clinics, 23 veterinary teaching hospitals, and 15 reference laboratories. Four-hundred and ninety-one dogs (8%) met our case definition for B-CLL, leaving 5,673 dogs in our reference population. Board-certified pathologist-reviewed peripheral blood cytology reports were available for 333 dogs with B-CLL (68%), and 83% of these fit our criteria for cytology-confirmed B-CLL. Of the remaining 17% (55 cases), 10 were described as "acute leukemia" by the reviewing pathologist. The remaining cases did not include a description of blast cells, but because cells were intermediate in size or the chromatin was described as immature, these cases did not meet our strict definition of B-CLL. Dogs with cytology reports not suggestive of B-CLL were significantly more likely to have peripheral lymphadenopathy (60 versus 41%; $P = .04$; data not shown) than dogs with cytology reports confirming B-CLL. Otherwise, there were no significant differences between dogs with and without cytology reports confirming B-CLL with regard to patient signalment or presenting signs.

When comparing cytology-confirmed B-CLL cases to cases diagnosed exclusively by FC, no clinically meaningful significant differences were noted when evaluating breed, signalment, or presenting signs (Table 2). Lymphocyte total differed significantly between the 2 groups, but both groups had a median substantially above the reference range. In addition, all 5 neutropenic dogs were in the cytology-confirmed group, leading to a statistically significant difference in neutrophil count.

Table 2 shows signalment, PE, and CBC findings for all 491 B-CLL cases in the study. Overall, there was a 1 : 1 ratio of males to females, with 242 males (24 intact, 212 neutered, 6 unknown) and 245 females (3 intact, 239 neutered, 3 unknown). The median age at diagnosis was 11 years (IQR, 9–13; range, 2–17). Data on peripheral lymphadenopathy were available for 63% of dogs; of these, 141 (46%) had peripheral lymphadenopathy. Of the dogs for which PE or imaging findings were reported, 51% had splenomegaly, 29% had hepatomegaly, 3% had a mediastinal mass, and 23% had visceral lymphadenopathy. Veterinarian-reported biochemical data were available for approximately 60% of dogs. Twenty-six percent of dogs had hyperglobulinemia and 5% had hypercalcemia.

The B-CLL case definition required a minimum lymphocyte count of 5,000/μL and >60% B-cells. Among dogs with B-CLL, the median lymphocyte count was 24,600/μL (IQR, 14,000–49,370; range, 5,000–812,544) and the median percentage of lymphocytes that were B-cells was 94% (IQR, 89–97%). Overall, neutropenia and thrombocytopenia were rare (1 and 7% of cases,

Table 2. Signalment, physical examination findings, and laboratory findings for 491 cases of B-CLL. Findings are compared between cases that are confirmed by a board-certified clinical pathologist cytology review of peripheral blood and those diagnosed by flow cytometric immunophenotyping only.

	Dogs with Available Data (%)	Total CLL Cases (n = 491)		Cytology-Confirmed Cases (n = 278)		Flow Cytometry Only Cases (n = 213)	
		n	%	n	%	n	%
Signalment							
Sex							
Male (intact or neutered)	100	242	49.7	142	51.3	100	47.6
Female (intact or neutered)		245	50.3	135	48.7	110	52.4
Age, Median (IQR)	98.4	11.0	(9–13)[a]	11	(9–13)	11	(9–13)
Veterinarian-reported Physical Exam and imaging Findings							
Peripheral Lymphadenopathy	62.7	141	45.8	68	41.2	73	51.0
Splenomegaly	46.0	115	50.9	63	52.5	52	49.1
Hepatomegaly	41.1	58	28.7	32	29.1	26	28.3
Mediastinal Mass	34.0	5	3.0	3	3.2	2	2.7
Visceral Lymphadenopathy	35.8	41	23.3	23	23.5	18	23.1
Veterinarian-reported Laboratory Findings							
Hyperglobulinemia	62.7	81	26.3	50	27.3	31	24.8
Hypercalcemia	58.9	13	4.5	7	4.1	6	5.0
CBC findings							
Neutrophil Total*							
Neutropenia (<2,000/μL)		5	1.0	5	1.8	0	0.0
Normal (2,000–12,000/μL)		394	80.6	223	80.5	171	80.7
Neutrophilia (>12,000/μL)	99.6	90	18.4	49	17.7	41	19.3
Platelet total							
Thrombocytopenia (<175,000/μL and no clumps noted)		28	6.9	16	5.8	12	5.7
Normal (175,000–500,000/μL)		310	76.7	179	64.6	131	61.8
Thrombocytosis (>500,000/μL)	82.3	66	16.3	38	13.7	28	13.2
Anemia							
Hematocrit <36	99.4	127	26.0	68	24.5	59	28.1
Lymphocyte Total**, Median cells × 10^3/μL (IQR)	100	24.6	(14.0–49.4)	27.0	(16.1–51.4)	19.9	(12.6–45.8)

[a]Values for age and lymphocyte total are given as median (IQR).

*$P < .05$ comparing cytology-confirmed cases to flow cytometry cases using Fisher's exact test.

**$P < .05$ comparing cytology-confirmed cases to flow cytometry cases using Wilcoxon rank sum test.

respectively), but anemia was found in 26% of dogs. When present, thrombocytopenia generally was mild (median, 137,000/μL; IQR, 89,000–161,000/μL). Among the 128 anemic dogs, hematocrit ranged from 11–35% (median, 30%; IQR, 26–34%; Table S1). Reticulocyte counts were available for half of the anemic dogs (67/128); of these, 31% (21/67) had regenerative anemia (>100,000/μL reticulocytes). Most anemic dogs were not neutropenic or thrombocytopenic (98 and 85%, respectively).

Breed

We compared the proportion of breeds in the B-CLL population to the proportion in the population of all dogs with suspected LPD using logistic regression. Thirty-seven breeds had ≥30 submissions for suspected LPD (Fig 1; Table S2). Together, these breeds represented >80% of both total submissions (5108/6164) and B-CLL cases (401/491). Of the breeds with ≥30 submissions, 12 had significantly increased odds of B-CLL compared to mixed breeds. This analysis included 10 small breeds (Bichon Frise, Boston Terrier, Cairn Terrier, Cocker Spaniel, Dachshund, Jack Russell Terrier, Maltese, Pomeranian, Shih Tzu, and Yorkshire Terrier) and 2 larger breeds (English Bulldog and Pit Bull). All breeds that were significantly different from mixed breeds remained significant when compared to Labrador Retrievers (Fig 1; Table S2); 4 additional breeds were significantly different from Labrador Retrievers (Boxer, Chihuahua, West Highland White Terrier, and Doberman).

Six large breeds had significantly decreased odds of B-CLL compared to mixed breeds: Bernese Mountain Dog, German Shepherd, Golden Retriever, Labrador Retriever, Rottweiler and Standard Poodle. Welsh Corgis also were at decreased risk (Fig 1; Table S2). Notably, although Golden Retrievers comprised the majority of purebred submissions, only 3 cases of B-CLL were identified.

Breed-specific patient presentation for the top 6 breeds with statistically significant increased odds of B-CLL was compared to mixed breeds. These breeds were Shih Tzu (n = 28), Pit Bull (n = 15), Cocker Spaniel (n = 14), English Bulldog (n = 23), Jack Russell Terrier (n = 14), and Dachshund (n = 14). No signifi-

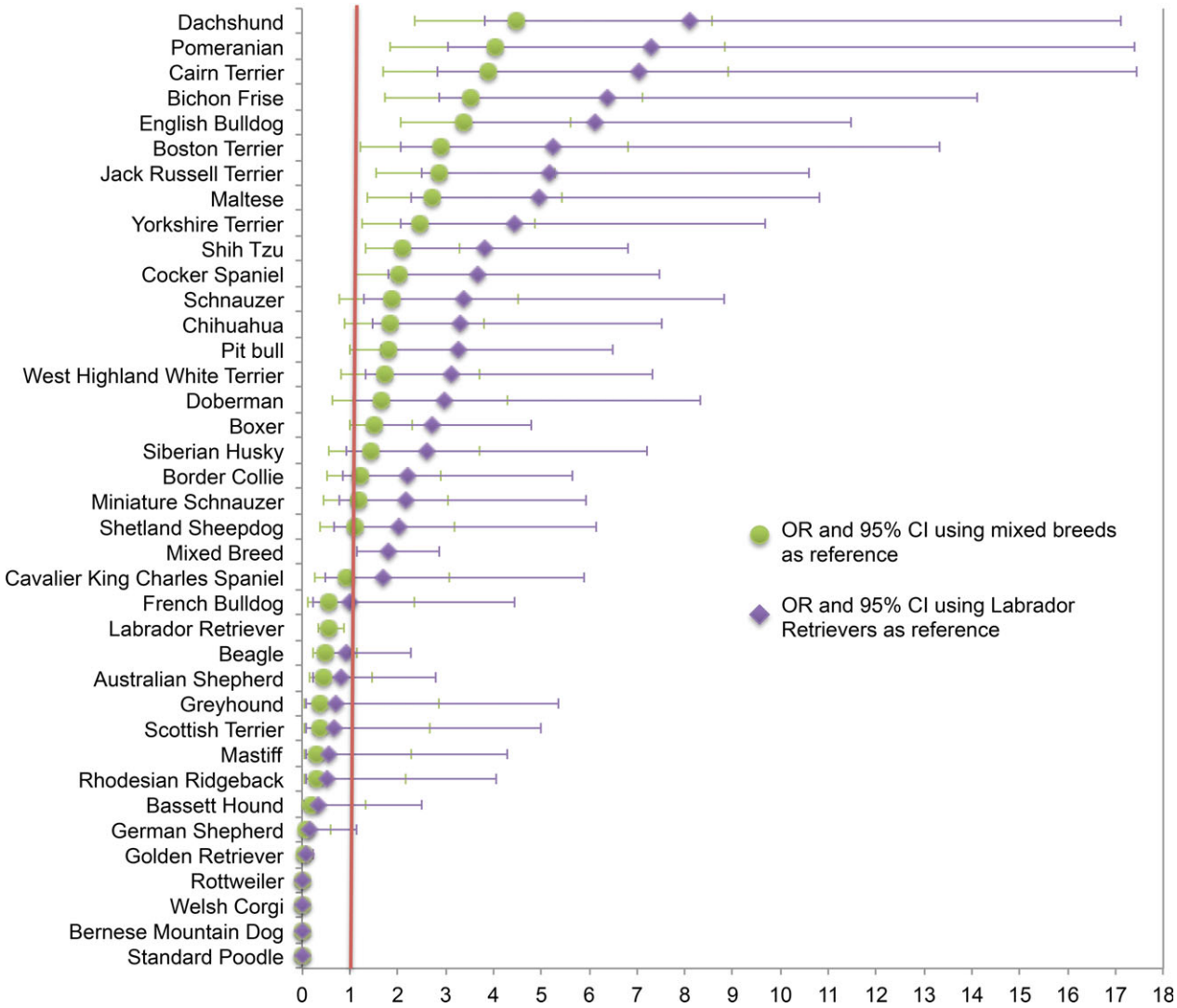

Fig 1. Odds ratios and 95% confidence intervals comparing B-CLL among each breed to all other lymphoproliferative disorders, using mixed breeds and Labrador Retrievers as a reference. Only breeds with at least 30 overall submissions were considered (37 breeds). Twelve breeds had significantly increased odds of B-CLL when compared to both mixed breeds and Labrador Retrievers: Dachshund, Pomeranian, Cairn Terrier, Bichon Frise, English Bulldog, Boston Terrier, Jack Russell Terrier, Maltese, Yorkshire Terrier, Shih Tzu, Cocker Spaniel, and Pit Bull. In addition, six breeds had significantly decreased odds of B-CLL compared to both mixed breeds and Labrador Retrievers: German Shepherd, Golden Retriever, Rottweiler, Welsh Corgi, Bernese Mountain Dog, and Standard Poodle.

cant differences were noted for most variables (sex, lymphocyte count, neutropenia, thrombocytopenia, and anemia; data not shown). We did not evaluate the frequency of splenomegaly, hepatomegaly, mediastinal mass, and hypercalcemia because data were available for <50% of B-CLL cases. English Bulldogs, however, presented at a significantly younger age (median 6 versus 11 years for mixed breeds; $P < .001$; Fig 2). In contrast, Cocker Spaniels were significantly older when diagnosed (median, 13.5 years; $P = .012$) and were significantly less likely to present with peripheral lymphadenopathy (11 versus 53% for mixed breeds; $P = .03$; data not shown). Half of the English Bulldog B-CLL cases had data available on hyperglobulinemia. Of those, 55% were hyperglobulinemic (versus 23% among mixed breeds; $P = .06$).

Flow Cytometric Characterization

Among cases, we examined the expression of 2 antigens, CD25 and class II MHC, on canine B-CLL cells, comparing the top 6 breeds (as described above) to mixed-breed dogs using Fisher's exact test. Significant differences were only noted for English Bulldogs and Shih Tzus. The level of CD25 expression was measured as the percentage of B-cells expressing CD25 (Fig S2). Overall, the median percentage of CD25-positive B cells was 58% (IQR, 13–88%; data not shown). Tertile cutpoints were used to categorize the percentage of CD25-positive B cells into low (<29% of B-cells CD25-positive), medium (29–80%), and high (>80%) groups. Compared to mixed breeds, English Bulldogs were more likely to fall into the lowest tertile (have a low percent-

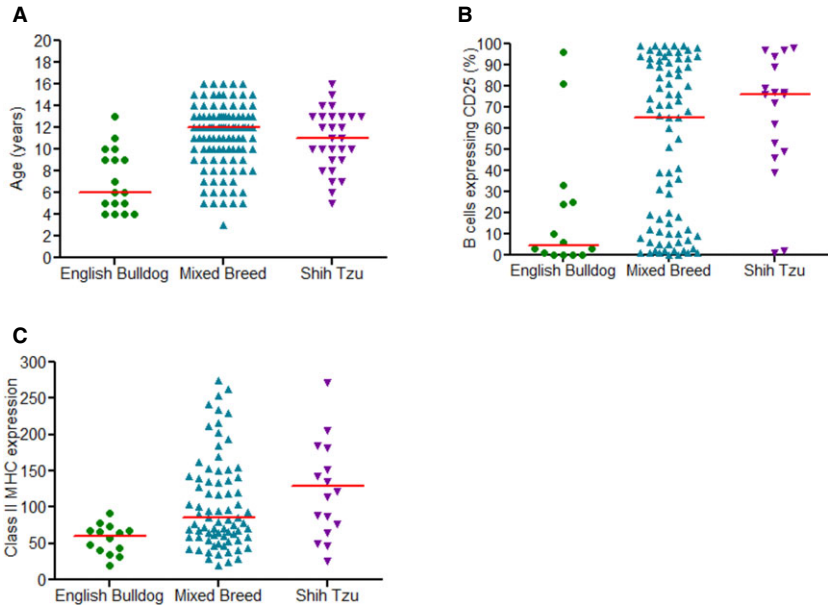

Fig 2. Median age (**A**), percent of CD25-positive B-cells (**B**), and median class II MHC expression (**C**) were compared for the top six breeds with significantly increased odds of B-CLL (Shih Tzu, Pit Bull, Cocker Spaniel, English Bulldog, Jack Russell Terrier, Dachshund) versus mixed-breed dogs. Significant differences were noted for English Bulldogs and Shih Tzus with B-CLL, and are displayed in the figure. Compared to mixed-breed dogs, English Bulldogs presented at a significantly younger age, with lower percent of CD25-positive B-cells, and decreased class II MHC expression. Shih Tzus had no significant difference in age or class II MHC expression, but were significantly less likely to have a low percentage of CD25-positive B cells.

age of CD25-positive B-cells), with a median of 4.5% CD25-positive B-cells ($P = .012$; Fig 2). In contrast, Shih Tzus had a median of 76% CD25-positive B cells, making them less likely to fall into low category ($P = .023$). All cases expressed class II MHC at varying levels. English Bulldogs were significantly more likely to have class II MHC expression below the median (94 versus 51%; $P < .001$; median, 54.5%; Figs 2 and S1). More Shih Tzus had class II MHC expression above the median (72%), but this difference was not statistically significant ($P = .116$).

Discussion

Overall, 8% of samples submitted for immunophenotyping because of suspicion of LPD were diagnosed as B-CLL. Our study demonstrated that B-CLL in dogs shows strong breed-specific risk relative to the population of dogs with suspected LPD. Ours is the first published study to evaluate this association, and represents the largest population of dogs with B-CLL in the veterinary literature. Several breeds with an increased prevalence of other LPDs (Golden Retriever, German Shepherd, Rottweiler) are rarely diagnosed with B-CLL (Fig 1). With the exception of English Bulldogs and Pit Bulls, all of the breeds with significantly increased odds of B-CLL were small-breed dogs. This finding could be because of a shared genetic risk in small-breed dogs[16,17] or differences in the average lifespan of small versus large-breed dogs. Small-breed dogs tend to live longer, allowing them a prolonged opportunity to develop clinical disease. In contrast, many larger breed dogs are nearing the end of their lifespan around the average age of B-CLL diagnosis and the vague clinical signs of B-CLL could be misinterpreted as signs of old age or could be masked by more severe health problems. One resistant breed, however, the Golden Retriever, is highly represented among T-zone lymphoma, which also is a disease that affects older dogs (median age, 10.5 years).[14]

By evaluating breed-specific presentation, we determined that certain breeds have a unique presentation of B-CLL (Fig 2). Compared to mixed-breed dogs, English Bulldogs presented at a significantly younger age, and with decreased class II MHC and CD25 expression. Prior research has shown that CD25 is expressed on mature or memory B cells,[12,13] Thus, it is plausible B-CLL in English Bulldogs arises from a naïve, unactivated B cell. The low level of class II MHC expression in English Bulldogs also supports this notion, and suggests that further investigation of the activation state of these cells is warranted. Thus, our results suggest that English Bulldogs exhibit a unique B-CLL phenotype. Additional study of the B cells in this breed, including IgV_H mutation status, is underway.

We found that B-CLL generally is a disease of older dogs with no sex predilection (Table 2). Where reported, approximately half of the dogs had peripheral lymphadenopathy or splenomegaly and a quarter had hepatomegaly, visceral lymphadenopathy, or hyperglobulinemia. Hypercalcemia and mediastinal mass were rare, suggesting that it would be prudent to seek an alternative cause for these clinical signs if they are present in a dog with B-CLL. Serum protein electrophore-

sis data were not available in our population, but a previous study found 68% of dogs with hyperglobulinemia had a monoclonal gammopathy.[18] With the exception of lymphocytosis, the majority of dogs had normal CBC findings. Approximately 26% of dogs were anemic on presentation, and the majority (68%) were classified as having nonregenerative anemia (Tables 2 and S1). However, only 15% were thrombocytopenic and 1% were neutropenic. Of 8 dogs with bone marrow aspirates available, all but 1 had increased small, mature lymphocytes, with infiltration ranging from 28–65% of nucleated cells. However, only 2 of these dogs had associated cytopenias (anemia and thrombocytopenia). In humans with B-CLL, anemia attributed to autoimmune destruction is found in 3–37% of cases.[19] Because the majority of anemic dogs in our study had non-regenerative anemia with no comments about spherocytes, we suspect most had anemia of chronic disease. Of the 21 dogs with regenerative anemia, 10 had clinical pathologist-reviewed cytology reports. Only 1 of these reports mentioned spherocytes; all reviews either did not mention saline agglutination tests or mentioned that saline agglutination tests were negative (n = 3). Thus, only 1 of these 10 dogs had suspicion for immune-mediated anemia.

The typical patient signalment seen in this study parallels that of previous studies.[3,4,18,20] However, as previously mentioned, our inclusion and exclusion criteria differed from prior studies. For example, diagnostic criteria for 2 studies[4,5] included morphologic appearance of cells and excluded cases with moderate to severe lymphadenopathy, which may have skewed findings toward less advanced cases. In contrast, another study[3] required cases to have a lymphocytosis of >50,000 cells/μL, which may have skewed findings toward advanced-stage cases. Importantly, many prior studies had small sample sizes and included both B and T-CLL, making it difficult to make accurate comparisons with our cases.

One limitation of our study is that, in cases with nodal involvement, we were unable to distinguish B-CLL from other forms of B-cell LPDs that include a circulating component of small, mature B-cells. Such a distinction is not possible without histology and immunohistochemistry, which were unavailable for our study participants because of the retrospective nature of our study. It is therefore possible our dataset includes mantle cell lymphoma, nodal marginal zone lymphoma, splenic marginal zone lymphoma, or follicular lymphoma with leukemic involvement.[21] However, these histologic subtypes appear to be rare in dogs,[22–24] and we suspect their influence is minimal and that the majority of our cases truly are B-CLL. In addition, lymphocytosis was not found in any of the 15 cases of marginal zone lymphomas confirmed by H&E staining and IHC in another study.[25] Taken together, we believe it is likely that most of these cases were B-CLL. In ongoing studies, we are recommending lymph node biopsies from these dogs to further explore the extent to which other histologic subtypes may be represented.

Although 17% (n = 55) of dogs with cytology reports did not meet our strict definition of cytology-confirmed B-CLL, the majority (71%) were consistent with CLL. Only 10 cases had cytology reports that contradicted a B-CLL diagnosis. When we compared patient presentation between dogs with cytology reports confirming B-CLL and the 17% that were not confirmatory, the only relevant difference noted was a higher frequency of peripheral lymphadenopathy among dogs without a confirmed diagnosis. Therefore, we do not expect this finding to substantially bias the patient presentation findings we reported, with the exception of potential overrepresentation of the frequency of peripheral lymphadenopathy.

It is currently unclear whether FC or cytologic evaluation is the more accurate method for diagnosing this disease and predicting outcome. In this study, we found no substantial differences in clinical presentation between cytology-confirmed B-CLL cases and those diagnosed with FC only (Table 2). Because cytologic interpretation can differ among clinical pathologists, we favor moving toward more objective criteria, such as FC, in the classification of B-CLL and other forms of leukemia.

A second limitation of the study is that the reference population for breed calculations is drawn from dogs with a suspicion of LPD. A breed predilection for lymphomas has been reported.[26,27] Therefore, the breed distribution in our study population is likely skewed toward these breeds and may not be representative of the overall breed distribution of dogs in the United States. We chose mixed-breed dogs as our primary comparison group for breed calculations because we believe they represent the genetic diversity of all dogs. Furthermore, they closely represented the overall patient presentation seen in our B-CLL population (Table S3). However, they are not an ideal reference group because they are an unknown group of dogs that may vary considerably across populations. In order to have a better-defined comparison group, we also used Labrador Retrievers, a more homogeneous group that is more likely to be comparable across populations. However, Labrador Retrievers represented a smaller population of dogs with B-CLL (n = 23) and had a lower frequency of B-CLL than the overall population (5 versus 8%). Therefore, the associated ORs for these calculations were skewed toward larger values than when comparing to mixed-breed dogs. The overall trend was the same for both comparisons, increasing our confidence that B-CLL occurs more commonly in small-breed dogs (Fig 1).

Taken together, the results from this large population of dogs suggest that B-CLL in dogs shares many features with B-CLL in people. Dogs are gaining recognition as models for naturally occurring human cancers, and thereby may be useful for studying risk factors and treatments for B-CLL in humans. Furthermore, the strong breed-specific risk identified in this study poses an opportunity to study genetic risk factors for B-CLL and to identify unique subpopulations of B-CLL.

Footnotes

[a] Beckman Coulter, Inc, Brea, CA
[b] SAS 9.3, SAS Institute Inc, Cary, NC

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Conflict of Interest Declaration: Authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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Supporting Information

Additional Supporting Information may be found online in Supporting Information:

Fig S1. Gating strategy for evaluation of class II MHC levels on B cells in peripheral blood.

Fig S2. Gating strategy to detect CD25 expression on B cells.

Table S1. Detailed CBC findings for 128 anemic dogs with B-CLL.

Table S2. Number of cases, total number of submissions, and percent of submissions diagnosed as B-CLL for each breed.

Table S3. Signalment, physical examination findings, and laboratory findings for 491 cases of B-cell chronic lymphocytic leukemia (B-CLL).