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PROGNOSTIC ROLE OF NON-NEOPLASTIC LYMPHOCYTES IN LYMPH NODE ASPIRATES FROM DOGS WITH DIFFUSE LARGE B-CELL LYMPHOMA TREATED WITH CHEMO-IMMUNOTHERAPY

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Abstract

Dogs with Diffuse Large B-Cell Lymphoma (DLBCL) benefit from the addition of active immunotherapy to traditional chemotherapy. We hypothesized that immune cells within neoplastic lymph nodes (LNs) may play a role in the tumor pathobiology and treatment response.

The present study describes the composition and prognostic role of non-neoplastic lymphocytes in LNs of 59 dogs with treatment-naive DLBCL receiving chemo-immunotherapy.

The percentage of small non-neoplastic cells and of CD5+, CD21+, CD4+ and CD8+ small cells was recorded via flow cytometry. CD4+/CD8+ and CD5+/large CD21+ cell ratios were calculated.

The likelihood of progression significantly diminished with increasing percentage of small cells, CD5+ and CD8+ small cells, and CD5+/large CD21+ cell ratio, with decreasing CD4+/CD8+ ratio and in non-anemic dogs.

Active immunotherapy is more effective in dogs with higher percentage of non-neoplastic lymphocytes at diagnosis. We lay the ground for future studies assessing the role of the immune system in the pathobiology of canine DLBCL.

Keywords dog; DLBCL; T-cells; flow cytometry; chemo-immunotherapy; prognosis

Introduction

A great body of evidence in human medicine has highlighted the role of the immune system in cancerogenesis . Indeed, the presence of immune cells is in the spotlight of the most updated cancer research because of their potential role in tumor surveillance and progression (Kumar and Xu, 2018; Sasada and Suekane, 2011). The diversity, composition and activation status of tumor infiltrating lymphocytes (TILs) are known to affect prognosis in patients with different neoplasms (Badalamenti et al., 2018; Lee et al., 2018; Stanton and Disis, 2016; Xu et al., 2001).

Diffuse Large B-Cell Lymphoma (DLBCL) is the most common lymphoma histotype in dog and systemic polychemotherapy approaches with or without immunotherapy represent nowadays the principal standardized treatment options in this species (Marconato et al., 2014). However, despite the improvement in treatment options, DLBCL in incurable in the majority of dogs, which eventually relapse and die for their disease. Thus, official guidelines have been published to standardize the criteria to assess the response to therapy in dogs affected by multicentric lymphoma (Vail et al., 2010). Under these guidelines, an increase in the size of target and non-target lesions or the appearance of new lesions is named disease progression (PD), whereas the time laps between initiation of treatment and PD is named Time To Progression (TTP).

In DLBCL, normal nodal architecture is completely effaced by the diffuse growth of centroblasts and immunoblasts (Valli et al., 2011) but small multifocal groups of non-neoplastic cells are generally present within the neoplastic population. The origin of these cells is unknown, but they may represent residual lymphoid population still present and compressed aside by tumor growth, reactive immune cells (including TILs) supporting an anti-tumor activity, or a mixture of both. The aim of the present study was to assess the prognostic role of non-neoplastic lymphocytes in lymph nodes (LNs) of dogs with treatment-naive DLBCL and undergone to chemo-immunotherapy. We hypothesize that these cells may have a favorable influence on TTP: on one side, the presence of a larger amount of residual cells may reflect an earlier diagnosis or a less aggressive behavior of the tumor; on the other side, the presence of reactive TILs may be of aid in the control of tumor relapse, prolonging TTP.

Materials and methods

Case inclusion

Cases were retrospectively extracted from the database of the Flow Cytometric Service of the Department of Veterinary Medicine (University of Milan, Milan, Italy) and the Veterinary Teaching Hospital (University of Turin, Turin, Italy) from January 2012 to June 2018. To be recruited, dogs had to fulfill the following inclusion criteria: 1) being diagnosed with DLBCL based on histopathology and immunohistochemistry (Aresu, 2016); 2) being treatment-naïve at the time of FC (corticosteroid were not permitted); 3) having received a standardized chemo-immunotherapeutic protocol; 4) having raw FC data of a LN aspirate available for review.

All dogs were treated at the same referral oncologic center (Centro Oncologico Veterinario, Sasso Marconi, Bologna, Italy) and underwent a standardized and accurate staging work-up consisting of history, physical examination, hematology, serum biochemistry (including Lactate Dehydrogenase activity, LDH), thoracic radiographs and abdominal ultrasound, cytological evaluation of liver and spleen irrespective if their sonographic appearance, cytological and FC evaluation of an aspirate obtained from an enlarged peripheral LN and peripheral blood (PB) and bone marrow (BM) samples. The LN analyzed by cytology and FC was then surgically removed for a definitive histopathological and immunohistochemical diagnosis.

The treatment protocol was in keeping with approved standards. All dogs received the following 19-week dose-intense chemotherapy regimen, consisting of L-Asparaginase (week 1), Vincristine (week 2, 3, 4, 13), Cyclophosphamide (week 2, 13), Doxorubicin (week 7, 16), Lomustine (week 10, 19), and prednisone (week 1 through 19). They also received an intradermal injection of 0.5 ml autologous vaccine on weeks 4, 5, 6, 7, 12, 16, 20, and 24, as previously described (Marconato et al., 2014).

Response was classified as complete remission (CR), partial remission (PR), stable disease (SD), or progressive disease (PD) based on previously published criteria (Vail et al., 2010). Responses were required to last for ≥ 28 days.

All dogs were privately owned and sampled with the informed consent of the owner for diagnostic purposes. Thus, according to the regulations of our Institutions, a formal approval of the Ethical Committee was not required (EC Decision 29 October 2012, renewed with the protocol n°02-2016).

Flow cytometry

FC was performed on LN aspirates collected into RPMI tubes and on PB and BM samples collected into EDTA tubes, using a multicolor approach. Samples were delivered to the laboratory and processed within 24 hours as previously described (Gelain et al., 2008; Marconato et al., 2013). The antibody panel varied according to the preferences of the operator, but a minimum panel of three mAbs was applied to all LN samples, including CD45 (clone YKIX716.13, all leukocytes), CD21 (clone CA2.1D6, B-cells) and CD5 (clone YKIX322.3, T-cells). Additional antibodies were: CD4 (clone YKIX302.9, T-helper cells), CD8 (clone YCATE55.9, T-cytotoxic cells) and MHC II (clone YKIX334.2, all lymphocytes). All antibodies were provided by Bio-Rad (formerly AbD Serotec, Oxford, UK). Samples were acquired with a BD FACScalibur or an Accuri C6 and analyzed with the specific software CellQuest or CFlow Plus (all provided by BD Becton Dickinson, san Josè, CA, USA).

Raw FC data obtained from LN aspirates were re-analyzed for the study purposes. A first gate (R1) was set on a morphological scattergram to exclude platelet and debris. R1 cells were visualized in a second scattergram according to their size and CD45 expression, and a gate (R2) was set to include only CD45+ cells. Thereafter, cells included in both R1 and R2 were visualized in a morphological scattergram and a third gate (R3) was drawn to derive the percentage of small cells (having FSC value lower than mean FSC value of PB neutrophils from the same subject). For each case, the percentages of R3 (small) cells staining positive for CD5, CD21 and possibly CD4 and CD8 were recorded, and the CD4+/CD8+ ratio was calculated. Finally, the percentage of CD5+ cells and of large CD21+ cells out of total CD45+ cells were recorded and coupled to obtain a CD5+/large CD21+ cell ratio as an index of the proportion between T-lymphocytes and neoplastic cells. The gating strategy is shown in Figure 1.

Statistical analyses

TTP was defined as the interval between initiation of treatment and PD or relapse (Vail et al., 2010). Dogs were censored for TTP analysis if they died for lymphoma-unrelated causes or were lost to follow-up before disease progression, and if still alive and in CR at data analysis closure. Univariate Cox's proportional hazard regression analysis was performed to determine possible associations between TTP and the following variables: breed (mixed or pure), sex (male or female), age (≤ or > 7 years), weight (< or ≥10 kgs), stage (I to V), substage (a or b), anemia (present or absent), thrombocytopenia (present or absent), LDH activity (normal or increased), MHC II expression on large B cells (high or low (Rao et al., 2011)), lymphocytes/monocytes ratio (LMR, ≤1.2 or >1.2 (Marconato et al., 2015)), small cells (%), CD5+ small cells (%), CD21+ small cells (%), CD4+ small cells (%), CD8+ small cells (%), CD4+/CD8+ ratio, CD5+/large CD21+ cell ratio. Variables with p≤0.3 at univariate analysis were then included in a backward elimination multivariate analysis. Kaplan-Meier curves were drawn and compared by log-rank test to assess possible differences in median TTP according to categorical variables.

Results

Study population

Fifty-nine dogs fulfilled the inclusion criteria: 9 (15.3%) were mixed-bred, whereas the remaining 50 (84.7%) dogs represented 26 different breeds, including 8 (13.6%) Rottweiler, 5 (8.5%) German shepherd , 5 (8.5%) Doberman, 3 (5.1%) Golden retriever, 3 (5.1%) Beagle, 2 (3.4%) Pit-bull, 2 (3.4%) English setter, 2 (3.4%) Bernese Mountain dog, 2 (3.4%) Border collie, 2 (3.4%) American Staffordshire terrier, and other 16 breeds (1 dog each, 1.7%). There were 35 (59.3%) females (27 spayed) and 24 (40.7%) males (9 neutered). Mean age at diagnosis was 7.8±3.1 years (median 7 years, min-max 3-15 years). Mean weight at diagnosis was 28.7±11.7 kg (median 30.0 kg, min-max 5.3-53.8 kg).

Three (5.1%) dogs had stage III substage a disease, 18 (30.5%) stage IV (13 substage a, 5 substage b), and 38 (64.4%) stage V (27 substage a and 11 substage b). At diagnosis, 11 (18.6%) dogs were anemic, 10 (16.9%) were thrombocytopenic, and 31 (52.5%) had an increased LDH activity. MHC II expression was tested in 19

cases, being high in 13 (68.4%) and low in 6 (31.6%) dogs. LMR was ≤1.2 in 22 (40.7%) dogs and >1.2 in 32 (59.3%).

FC results on LN aspirates are listed in Table 1. Concerning the composition of the small non-neoplastic lymphoid cell population, CD21+ cells predominated in 35 out of 59 (59.3%) cases and CD5+ cells in 24 (40.7%) cases; CD4+ cells outnumbered CD8+ cells in 29 out of 32 cases (90.6%) and vice versa in 3 (9.4%).

All dogs received the same chemo-immunotherapeutic protocol. Fifty-one dogs (86.4%) obtained clinical CR, 6 (10.2%) PR, 1 (1.7%) had SD and 1 (1.7%) progressed.

Survival analyses

Overall median TTP was 274 days (range 1-1617 days). Forty-three (72.9%) dogs progressed during the study period, whereas 16 (27.1%) were censored for TTP analysis: 10 (16.9%) died for lymphoma-unrelated causes before disease progression, 5 (8.5%) were still alive and in CR at data analysis closure after 112, 195, 1020, 1257 and 1617 days, respectively, and 1 (1.7%) dog was lost to follow-up after 258 days still being in CR for lymphoma.

Based on univariate Cox analysis, the likelihood of progression significantly diminished with increasing percentage of small cells (p=0.014, HR=0.972) and of CD5+ and CD8+ cells within the small cell population (p=0.039, HR=0.986 and p=0.021, HR=0.923, respectively), and with increasing CD5+/large CD21+ cells ratio (p=0.019, HR=0.084). These variables were not categorized, in order not to introduce arbitrary cutoffs with uncertain biological relevance. However, median TTP for dogs under and over the mean value of each parameter is reported in Table 2. Based on multivariate analysis, the likelihood of progression significantly increased with increasing CD4+/CD8+ ratio and with the presence of anemia (p=0.033 and p=0.002, respectively). Detailed results of TTP analysis are shown in Table 3.

Discussion

To the authors' knowledge, this is the first study describing the composition of non-neoplastic lymphoid population in LN aspirates from dogs with DLBCL treated with chemo-immunotherapy, and highlighting its prognostic role in this clinical landscape.

Based on our results, increasing percentages of non-neoplastic T-cells was correlated with a longer TTP. One possible explanation beyond this result might be related to active immunotherapy, which acts by enhancing the patient's immunity and by inducing specific and non-specific cellular immune responses. Briefly, the autologous vaccine administered to the dogs consisted of hydroxylapatite ceramic powder and Heat Shock Proteins (HSPs) purified from the dogs' tumors. After intradermal injection, the complex HSPs-hydroxylapatite behaves like a foreign body on one side, thereby attracting monocytes and macrophages to the injection site, and allows HSPs to be released into antigen-presenting cells and presented to the immune system on the other side, thereby enhancing the immune response (Marconato et al., 2014). Based on our results, a higher percentage of T-cells within the tumor might predict the immune response and be associated to an improved efficacy of treatment response, at least considering the duration of the remission. Whether non-neoplastic T-cells play a prognostic role in dogs receiving traditional chemotherapy only remains currently unexplored and wasn't an aim of this study.

The better prognosis for dogs having higher percentages of non-neoplastic lymphoid cells may also be linked to a more limited spread of neoplastic cells within the LNs. In particular, this may explain why an increased CD5+/large CD21+ cell ratio significantly improved TTP. Still, the composition of the non-neoplastic lymphoid population seems to be more relevant from a clinical point of view than the proportion between non-neoplastic and neoplastic cells: among FC parameters, indeed, only the CD4+/CD8+ ratio resulted to be an independent prognostic factor. Prolonged remission times are linked to lower values of CD4+/CD8+ ratio, which in turn may be due to both a decrease of CD4+ T-helper cells, and an increase of CD8+ T-cytotoxic cells. CD4+ cells outnumber CD8+ cells about 3-folds in normal LNs in dogs (Rütgen et al., 2015). The presence of neoplastic cells is expected to elicit a cytotoxic immune response, thus increasing the proportion of CD8+ cells, resulting in a decrease or even in an inversion of the CD4+/CD8+ ratio. This may explain the better outcome of dogs with low CD4+/CD8+ ratios.

The immune system has the innate ability to recognize and kill cells undergoing neoplastic transformation, being attributable to the formation of tumor-specific neo-antigens. In particular, this effect appears to be mediated by T- cells (Jochems and Schlom, 2011; Sasada and Suekane, 2011). Unfortunately, cancer cells can escape the immune surveillance. Studies in human medicine have highlighted many peculiar strategies adopted by lymphoma and leukemia cells. As an example, molecules involved in antigen presentation can be lost or downregulated on the surface of neoplastic cells, causing them to become "invisible" to the immune cells. This is the case of Major Histocompatibility Complex class II (MHC II), whose expression is lost in 20% of human DLBCL resulting in a poor outcome (Tada et al., 2016). Diminished expression of MHC II has been correlated to a worse prognosis in canine B-cell lymphoma as well (Rao et al., 2011). This was not confirmed in the present study, most likely because of the small number of cases in which this antigen was tested.

Additionally, lymphoid neoplastic cells can express on their surface inhibiting molecules that reduce the anti-tumor activity of T-cells. The programmed cell death protein 1 (PD-1) and its ligand (PD-L1) are commonly involved in this mechanism (Laurent et al., 2015) and have been extensively studied in human medicine because of their potential role as therapy target (Constantinidou et al., 2018). A recent study highlighted a possible role of these molecules in the pathobiology of canine B-cell lymphoma, as well (Hartley et al., 2018). Data of gene expression profiling obtained by our research group also support a prognostic value for both PD-1 and PD-L1 in canine DLBCL (Aresu et al., 2018), in disagreement with another study documenting no prognostic relevance for PD-L1 transcript amount in dogs with DLBCL (Ambrosius et al., 2018). Recently, Tagawa and co-authors described the proportion of CD4+ and CD8+ lymphocytes expressing the immune checkpoints molecules PD-1 and CTLA-4 in LNs and PBMC from dogs with high-grade B-cell lymphoma (Tagawa et al., 2018). Unfortunately, the expression of these molecules was not tested in the current series, impeding us to draw any conclusion about the functional state of nodal T-cells.

Beside the composition of non-neoplastic lymphoid population, in the present study we evaluated the effect of many different parameters on TTP, possibly having prognostic relevance. Surprisingly, most of the

factors that are frequently reported in the literature as being associated with poor outcome were not shown to be significant in the current study. Many reasons may account for this discrepancy.

First, the endpoint of the current study was TTP rather than survival, which, conversely, is a common endpoint of the majority of veterinary studies. This choice was influenced by the fact that TTP mostly relies on biological factors (including the interaction of tumor cells with TILs and microenvironment), whereas survival is also influenced by non-biological parameters, including financial issues or considerations about quality of life.

Second, our inclusion criteria were very restrictive, including only dogs with histopathologically confirmed DLBCL,receiving no drugs prior to FC analysis, undergoing full staging and being treated with a standardized chemo-immunotherapy regimen. Unfortunately, most published studies refer to lymphoma as a general entity, thereby enrolling animals with a huge spectrum of histotypes, do not limit enrolment to dogs that had never been treated before, have very often incomplete staging information and different chemotherapeutic protocols, leading to fragmented and unreliable results (Marconato et al., 2017). Also, the exclusion of dogs pre-treated with corticosteroids may have biased the population toward asymptomatic dogs or dogs with minimal clinical signs (which did not require compelling drug administration): this may account for the lack of significance of substage, which is a major prognostic factor in dogs with lymphoma.

Third, the low number of dogs tested for MHC II expression may have affected the results obtained for this specific parameter.

Fourth, the active immunotherapy administered in the present study may have different efficacy in dogs with different conditions at diagnosis, possibly nullifying the role of prognostic factors identified in dogs treated with chemotherapy alone.

The retrospective nature of the present study represents its major pitfall, although it may be partially amended by the inclusion of a highly standardized population of dogs. However, the procedures for FC immunophenotyping and the antibody panel varied between the two laboratories and over time. In

addition, the technique used to evaluate the lymphoid population in the present study has an intrinsic weak point, as it can only be conducted on tissue aspirates and, therefore, the percentage of small cells may vary depending on the precise site of aspiration within the neoplastic LN. However, even if this may affect the percentage of detected non-neoplastic cells, it is less likely to affect the composition of the population itself, including the CD4+/CD8+ ratio, which maintained a prognostic significance on multivariate analysis in our study.

Also, we could not assess whether the T-cells that were found in the LN aspirates represented true TILs rather than resident non-neoplastic cells. Further prospective studies including in-vitro expansion of T-cells prior and after administration of immunotherapy are necessary to solve this point.

In conclusion, the present study describes the non-neoplastic lymphoid population in LN aspirates from dogs with DLBCL, suggesting a prognostic role in animals treated with active immunotherapy. The complex network of interactions between lymphoma cells, non-neoplastic lymphocytes and the microenvironment still has to be investigated, and studies assessing at the same time TILs composition and expression of key molecules such as PD-1, PD-L1 and CTLA-4, as well as defining a biologically relevant cut-off, are warrant.

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Declaration of interest

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of the paper

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Figure 1 flow cytometric scattergrams showing the gate strategy used to evaluate non-neoplastic lymphocytes in lymph node (LN) aspirates from 59 dogs with Diffuse Large B-Cell Lymphoma (DLBCL). **A:** all events acquired are shown; a gate (R1) was set to exclude platelet and debris. **B:** only R1 events are shown; a gate (R2) was set to include only CD45-positive cells. **C:** only events included in both R1 and R2 are shown; a gate (R3) was set to include only small (non-neoplastic) cells. **D:** only events included in R1, R2 and R3 are shown; CD5-positive cells are in the lower right quadrant and CD21-positive cells are in the upper left quadrant. **E:** only events included in both R1 and R2 are shown; CD5-positive cells are in the upper right quadrant. **F:** only events included in both R1 and R2 are shown; large CD21-positive cells are in the upper right quadrant.

Table 1 Composition of non-neoplastic lymphoid population assessed via flow cytometry (FC) in lymph node (LN) aspirates from 59 dogs with Diffuse Large B-Cell Lymphoma (DLBCL)

PERCENTAGES			
Cells population	**Mean±standard deviation (%)**	**Median (%)**	**Min-max (%)**
Small cells	19.5±16.5	13.3	1.5-79.5
CD5+	41.5±21.8	38.9	3.3-85.3
CD21+	46.4±19.7	47.1	7.9-92.6
CD4+	29.2±16.6	31.3	5.1-60.3
CD8+	13.1±7.8	10.2	3.9-32.8
RATIOS			
Ratio	**Mean±standard deviation**	**Median**	**Min-max**
CD4+/CD8+	2.61±1.55	2.34	0.40-7.00
CD5+/large CD21+	0.20±0.45	0.06	0.00-3.17

Table 2 Time To Progression (TTP) of 59 dogs with Diffuse Large B-Cell Lymphoma (DLBCL) treated with chemo-immunotherapy, according to the percentage of non-neoplastic lymphocytes detected by flow cytometry in lymph node aspirates.

Variable		Number of dogs (%)	Time To Progression (days)	
			Median	Range
Small cells				
	≤19.5%	39 (66.1)	250	1-988
	>19.5%	20 (33.9)	348	14-1257
CD5+				
	≤41.5%	34 (57.6)	250	33-988
	>41.5%	25 (42.4)	312	1-1257
CD21+				
	≤46.4%	29 (49.2)	302	1-1257
	>46.4%	30 (50.8)	274	33-988
CD4+				
	≤29.2%	16 (50.0)	250	1-988
	>29.2%	16 (50.0)	317	14-1257
CD8+				
	≤13.1%	18 (56.3)	217	14-988
	>13.1%	14 (43.8)	376	1-1257
CD4+/CD8+				
	≤2.61	21 (65.6)	302	1-1257
	>2.61	11 (34.4)	162	14-988
CD5+/large CD21+				
	≤0.20	46 (78.0)	250	1-988
	>0.20	13 (22.0)	Not reached	14-1257

Table 3 Time To Progression (TTP) of 59 dogs with Diffuse Large B-Cell Lymphoma (DLBCL) treated with chemo-immunotherapy, according to different variables

Variable (number of dogs)	Median TTP in days (range)	P-value			Hazard ratio (95% CI)
		Univariate analysis	Log-rank test	Multivariate analysis	
Breed		0.696	0.694		
Pure (50)	246 (1-1617)				1.167 (0.537-2.537)
Mixed (9)	348 (1-588)				Ref
Sex		0.443	0.439		
Male (24)	312 (1-1257)				Ref
Female (35)	246 (1-1617)				1.274 (0.686-2.365)
Age		0.532	0.529		
≤7 years (31)	246 (1-1617)				1.215 (0.660-2.235)
>7 years (28)	312 (1-1257)				Ref
Weight		0.620	0.616		
≤10 kgs (3)	246 (37-385)				1.349 (0.414-4.396)
>10 kgs (56)	274 (1-1617)				Ref
Stage		0.778	0.774		
III (3)	217 (49-1257)				Ref
IV (18)	312 (1-1020)				1.352 (0.288-6.348)
V (38)	274 (40-1617)				1.580 (0.373-6.701)
Substage		0.268	0.263	0.326	
a (43)	312 (13-1617)				0.685 (0.351-1.338)
b (16)	217 (1-1020)				Ref
Anemia		0.144	0.137	0.002[§]	

Present (11)	217 (1-493)				Ref
Absent (48)	302 (1-1617)				0.597 (0.298-1.192)
Thrombocytopenia		0.516	0.511		
Present (10)	312 (1-385)				Ref
Absent (49)	274 (1-1617)				0.772 (0.354-1.684)
LDH activity		0.139	0.133	0.255	
Normal (28)	317 (1-1617)				0.628 (0.339-1.162)
Increased (31)	250 (1-1020)				Ref
MHC II expression		0.707	0.705		
High (13)	312 (1-1257)				Ref
Low (6)	376 (1-1020)				1.293 (0.338-4.942)
LMR		0.836	0.835		
≤1.2 (22)	302 (35-1020)				Ref
>1.2 (32)	312 (1-1617)				0.932 (0.480-1.812)
Small cells (59)		0.014[§]		0.152	0.972 (0.950-0.994)
CD5+ (59)		0.039[§]		0.745	0.986 (0.974-0.999)
CD21+ (59)		0.094		0.121	1.011 (0.998-1.025)
CD4+ (32)		0.178		0.958	0.983 (0.958-1.008)
CD8+ (32)		0.021[§]		0.241	0.923 (0.862-0.988)
CD5+/large CD21+ ratio (59)		0.019[§]		0.126	0.084 (0.011-0.661)
CD4+/CD8+ ratio (32)		0.142		0.033[§]	1.312 (0.913-1.885)

[§]significant result

highlights

Dogs with Diffuse Large B-Cell Lymphoma (DLBCL) benefit from active immunotherapy

We describe non-neoplastic lymphocytes in canine DLBCL lymph nodes at diagnosis

Higher percentages of T-cells are associated with lower likelihood of progression

Chemo-immunotherapy is more effective in dogs with higher percentages of T-cells