Hematological ratios and indices in canine large B-cell lymphoma

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Abstract

Background: Canine lymphoma is the most common hematopoietic cancer in dogs. Numerous studies have evaluated the prognostic value of hematological abnormalities and ratios in both humans and dogs with lymphoma.

Aim: To compare hematological parameters and complete blood count ratios between a population of dogs affected by lymphoma and healthy dogs to identify potential prognostic markers for lymphoma.

Methods: This retrospective case-control study compares hematological parameters and complete blood count ratios between a population of 114 dogs affected by multicentric large B-cell lymphoma (LBCL) and 60 healthy dogs.

Results: The study found several statistically significant differences between the hematological indices of LBCL dogs and healthy dogs, but no correlation between these parameters and the survival times of 78 dogs treated with chemotherapy Madison Wisconsin protocol. In addition, hematological alterations were evaluated such as anemia, leukocytosis, and thrombocytopenia.

Conclusion: Hematological ratios have been suggested as potential prognostic markers for canine LBCL but their real prognostic value remains controversial and requires future investigation.

Keywords: Dogs, Lymphoma, CBC, Indices, Ratios.

Introduction

Canine lymphoma is the most prevalent hematopoietic malignancy in dogs. This term encompasses a group of heterogeneous diseases that differ in clinical presentation, immunophenotype, and prognosis (Valli *et al*., 2013; Zandvliet, 2016; Antognoni *et al*., 2021; Magni *et al*., 2023). Canine Lymphoma is a correspondent to non-Hodgkin Lymphoma in humans. Large B-cell lymphoma (LBCL) is the most frequent immunophenotype of multicentric lymphoma in dogs. Typically, lymphoma is diagnosed in middle-aged or dogs older than 10 years. Although no significant gender predisposition has been demonstrated, spayed females appear to have an increased risk (Zandvliet, 2016). The most widely used therapy for canine lymphoma consists of a multidrug combination (CHOP-based protocol) of cyclophosphamide, doxorubicin, vincristine, prednisone, and l-asparaginase without maintenance further therapy. The neoplasm is extremely aggressive, with a median survival time under treatment of approximately 8–10 months in dogs with LBCL (Bienzle and Vernau, 2011; Valli *et al*., 2013; Zandvliet, 2016).

Many prognostic factors have been considered significant for LBCL, including clinical symptoms, stage, histological aspects, grade, proliferation markers, and response to treatment (Zandvliet, 2016; Davies *et al*., 2018). Given the aggressive behavior of LBCL, prognostic markers that allow monitoring the course of the disease in an easy, repeatable, and inexpensive way, are of critical importance. Many studies have focused on hematological parameters as potential prognostic markers. The hematologic examination is a minimally invasive procedure available to all veterinary clinicians, and it is routinely executed before chemotherapy administration. Several hematological parameters both in human and veterinary medicine have been proposed as prognostic markers for many different diseases, including vascular (Balta *et al*., 2016), degenerative (Wan *et al*., 2020), and inflammatory diseases (Neumann, 2021), as well as different types of tumors, such as soft tissue sarcoma (Macfarlane *et al*., 2016), laryngeal carcinoma (Yılmaz *et al*., 2018), acute myeloid leukemia (Zhang *et al*., 2021), and lymphomas (Henriques *et al*., 2021), and others.

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The possibility to use neutrophils/lymphocytes ratio (NLR) as an independent prognostic factor has been investigated in human patients with LBCL. To date, a high NLR value has been found to be associated with poor overall survival and poor progression-free survival. At the time of diagnosis, an NLR cut-off value of 3 has been reported in one study (Wang *et al*., 2017), and 3.5 in another (Porrata *et al*., 2010). This association between a high NLR and poor prognosis may be related to the production of inflammation mediators, resulting in immunosuppression and a reduction in the patient's immune efficacy. In addition, a high platelets/lymphocytes ratio (PLR) appears to be correlated with a worse clinical condition of the patient and a poor prognosis in human LBCL, as reported by a 2019 meta-analysis study (Chen *et al*., 2019). In veterinary medicine, the predictive value of platelets/neutrophils ratio (PNR), NLR, and the level of anemia as prognostic factors in dogs with a diagnosis of LBCL has been suggested. Patients with a higher PNR above 0.032 showed earlier disease progression than others, while patients with NLR above 7.5 showed a higher mortality rate at 180 days (Henriques *et al*., 2021). On the other hand, several studies in both human and veterinary medicine have failed to demonstrate a statistically significant association between NLR and overall survival or progression-free survival (Mutz *et al*., 2015; Azuma *et al*., 2019). In humans, multiple studies have identified higher red blood cell (RBC) distribution width (RDW) as a negative prognostic factor in patients with LBCL (Zhou *et al*., 2017; Ai *et al*., 2018; Hasan and Elmeshhedany, 2021). In dogs, the prognostic value of this index has been investigated for a few diseases (Guglielmini *et al*., 2013, 2021, 2022; Garcia-Arce *et al*., 2022; Miglio *et al*., 2023) and only one study considered LBCL (Martinez *et al*., 2019). Furthermore, to date, the red blood cell distribution width to total serum calcium ratio (RDW/Ca) has been investigated and suggested as a ratio of prognostic relevance only in patients with acute pancreatitis, in both humans (Gravito-Soares *et al*., 2018; Han *et al*., 2021) and canine species (Guglielmini *et al*., 2022).

However, the prognostic value of these hematological ratios in canine LBCL remains controversial and under investigated. Most of the existing studies conducted on this topic lack a control group of healthy patients for comparison.

The main objective of the study is to assess the role of hematological ratios in LBCL by establishing a comprehensive list of dogs with LBCL and comparing them to reference intervals (RIs) in a population of healthy dogs. We also aimed to investigate the possible correlation of these indexes with the survival times of dogs receiving treatment with a chemotherapy Madison Wisconsin protocol. Furthermore, complete blood count (CBC) and Ca modifications compared to the reference range (RR) and the possible correlation of white blood cell (WBC), RBC, and Platelet (PLT) count with survival times were evaluated.

Materials and Methods

Animal recruitment

The medical records of 180 dogs presented at two University Teaching Hospitals (University of Camerino and University of Perugia), between 2012 and 2022 were retrospectively reviewed. Dogs affected by lymphoma were considered eligible if they had a diagnosis of multicentric lymphoma, according to the criteria established by the World Health Organization (WHO) classification (Valli *et al*., 2011)not specialists in hematopathology, in applying the World Health Organization (WHO. Dogs with infiltration of neoplastic cells in peripheral blood and/or bone marrow, detected by cytological observation, were excluded from the study.

The control group included healthy blood donor dogs. Data regarding hematology CBC and available calcium level measurements of both the investigated groups of dogs were obtained from the review of the medical database and subsequently analyzed.

Dogs with LBCL

The first group included a total of 114 dogs affected by LBCL classified in the III–IV stage based on the WHO staging. For all dogs, the diagnostic protocol was similar to those indicated in previous studies (Valli *et al*., 2011; Marconato *et al*., 2015; Mutz *et al*., 2015; Henriques *et al*., 2021). The evaluation was performed by considering history, physical examination, CBC and microscopic blood smear evaluation with differential count (Supplementary Table 1), biochemistry and urinalysis, thoracic radiographs, abdominal ultrasound, and cytology evaluation of fine-needle aspirate (FNA) of enlarged peripheral lymph nodes. FNA of the liver and spleen were included in case of suggested altered ultrasound appearance. Immunophenotyping of lymphoma was performed by lymph node immunohistochemistry (CD21, CD79a, CD45) or flow cytometry (CD3, CD5, CD21, CD34, CD79a). A bone marrow FNA aspirate and cytology were performed in 41 dogs, but no circulating neoplastic cells were observed on the blood smear of all cases by expert veterinary or board-certified clinical pathologists.

Seventy-eight dogs were treated, with prior written informed consent by the owners, with chemotherapy Madison-Wisconsin protocol (L-asparaginase, cyclophosphamide, vincristine, doxorubicin, prednisone). The duration of the administration of chemotherapy lasts 25 weeks.

Healthy dogs

The second group of dogs, defined as being healthy (H), served as a control for the investigated parameters. These dogs were presented for a complete period of screening before blood donation and they underwent an evaluation to accomplish the requirements indicated in the Italian Ministry of Health Guidelines regarding the healthy measures adopted in veterinary blood transfusion (Ministero della Salute Dipartimento della Sanità Pubblica e dell'Innovazione, 2016), i.e.,

history of no clinical signs, regular prophylaxis, and vaccination, physical examination without findings. CBC, blood smear evaluation, total serum protein, and serological titers were also routinely evaluated.

A total of 60 control dogs were enrolled in the study, all of whom were clinically healthy, between 2 and 8 years old, weighed more than 25 kg, and of different breeds and sexes.

Blood collection and analysis

All analyses evaluated in this study were performed on the day of diagnosis for dogs affected by LBCL. All dogs had CBC parameters measured using automated hematology analyzers (ProCyte Dx, IDEXX®, and Sysmex XT-1800-i®), on whole peripheral blood samples collected in 1 ml K_3 ethylenediaminetetraacetic acid tubes. Hematological alterations were calculated based on the reference ranges of each analyzer used (Supplementary Table 2).

Calcium total level was assessed by liquid chemistry (Hitachi 904, Boehringer Mannheim® and Seac Radim® reagent, Biolabo sas, France) in 1 ml serum samples.

Calculation of CBC ratios and indices

NLR, PLR, monocytes/lymphocytes ratio (MLR), and PNR were calculated as ratios of the absolute values of neutrophils, platelets, monocytes to lymphocytes, and the platelet count to neutrophils, respectively. The MPV/PLT ratio was calculated as a ratio of mean platelet volume to platelets. The RDW/Ca ratio was calculated as a ratio of red cell distribution width and Ca levels.

Survival time

The survival time was calculated from the first visit to lymphoma-related death.

Statistical analysis

The evaluation of the normality of parameters was performed with the Shapiro-Wilk test. Data are not normally distributed. Each variable was compared by the Mann-Whitney test. Differences were considered statistically significant when $p < 0.05$. The lymphoma-specific survival (LSS) was evaluated by the Kaplan-Meyer method. In addition, median and standard deviation were determined for each parameter for cases, mean, median, standard deviation, and percentiles (0.025–0.975) for control. A Kaplan-Mayer analysis with a log-rank test was performed to evaluate the correlation between ratio and LSS. A Rank correlation test was also performed by assuming as a risk factor a cut-off greater than the max calculated for ratios and indices in healthy dogs. Statistical analysis was performed using a software package (MedCalc® Version 19.1.3., Ostend, Belgium).

Ethical approval

Not needed for this retrospective study.

Results

One hundred and fourteen cases of LBCL that met the inclusion criteria were included in the study. Of these cases, 78 were treated with the Madison Wisconsin protocol.

The mean age of the cases was 7.95 ± 2.93 (SD) years, with 70 (61.4%) males and 44 (38.6%) females. The most common breeds included 32 (28.07%) mixed breed, 17 (14.91%) German Shepherd, 6 (5.26%) Golden Retriever, 6 (5.26%) Rottweiler, 5 (4.38%) Boxer, 5 (4.38%) Border collie, 5 (4.38%) Labrador retriever, 5 (4.38%) Irish Setter, 4 (4.38%) Dobermann, 4 (4.38%) Bernese Mountain dog, and 25 (21.9%) other breeds.

The mean age of the control dogs was 5.00 ± 2.01 (SD) years, with 33 (55.00%) males and 27 (45.00%) females. The most common breeds included 15 (25.00%) Segugio italiano, 10 (16.66%) mixed breeds, 4 (6.67%) Weimaraner, 4 (6.67%) Ariegeois, 4 (6.67%) Bleu de Gascoigne, 4 (6.67%) Bracco Italiano, 3 (5%) and 16 (26,66%) dogs of various other breeds.

A CBC was performed at the time of diagnosis for all cases, and the results and any alterations are described in Table 1. Serum calcium measurements were evaluated for 48 cases.

The statistical results of the control group's ratios are reported in Table 2. The reference values are expressed by percentiles (0.025/0.975).

Table 3 shows ratios and indexes both in cases and control groups and compared by statistical methods.

None of the ratios and indexes examined can be considered a prognostic factor related to survival in a population of dogs with lymphoma (end point 6 and 12 months; rank correlation test; $p > 0.05$). Also, the rank correlation test performed by assuming as a risk factor a cut-off greater than the mean calculated for ratios and indices in healthy dogs (NLR > 2.54; MLR > 0.13; PNR > 41.49; PLR > 106.89; MPV/PLT > 0.04; RDW > 16.9; RDW/Ca > 1.91) not resulted as a prognostic factor.

The lymphoma-specific median survival (LSS) time (represented in Fig. 1) for the 78 treated cases was 223 days (range 11–1792), with two dogs still alive. Among the dogs, 28 (36.84%) died within 180 days, while 48 (63.16%) survived > 180 days.

The LSS of anemic dogs was 172 days, compared to 247 days for nonanemic dogs, but no statistical significance was found between survival and the presence of anemia. Furthermore, we did not find an association between LSS and neutrophil and platelet count.

Three dogs whose LSS was >1050 days (1074, 1298, and 1792 days) (Marconato *et al*., 2011, 2015; Childress *et al*., 2018; Davies *et al*., 2018; Childress *et al*., 2022; Faroni *et al*., 2021) received additional chemotherapy protocols when, following initial clinical remission, they developed relapse.

Discussion

The aim of the present study was to evaluate a complete list of ratio and hematological indices in

Table 1. Hematological alterations in dogs with DLBCL.

Parameters	Normal (n/%)	Increased (n/%)	Decreased (n/%)
RBC (10^{12}/L)	67/58.77	4/3.51	43/37.72
HCT (%)	73/64.03	2/1.75	39/34.21
HGB (g/dl)	69/60.53	1/0.87	44/38.59
MCV (fl)	94/82.46	11/9.65	9/7.89
MCH (pg)	96/84.21	6/5.26	12/10.53
MCHC (g/dl)	75/65.80	16/14.03	23/20.17
RDW (%)	96/84.21	6/5.26	12/10.53
WBC (10^9/l)	73/64.03	29/25.44	12/10.53
Neu (10^9/l)	76/66.67	30/26.32	8/7.02
Lym (10^9/l)	60/52.63	14/12.28	40/35.09
Mon (10^9/l)	58/50.88	34/29.82	22/19.30
Eos (10^9/l)	71/62.28	0/0.000	43/37.72
PLT (10^9/l)	85/74.56	8/7.02	21/18.42
MPV (%)	97/86.61	4/3.57	11/9.82
[1]Ca (g/dl)	37/77.09	7/14.58	4/8.33

[1]Ca: *n* = 48 cases. Legenda: RBC: erythrocyte; HCT: hematocrit; HGB: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; WBC: leukocyte; Neu: neutrophil; Lym: lymphocyte; Mon: monocyte; Eos: Eosinophil; PLT: platelet; MPV: mean platelet volume.

Table 2. RIs of control dogs' ratios.

	NLR	PLR	MLR	PNR	MPV/PLT	RDW/Ca
Mean	2.79	125.67	0.14	46.68	0.05	1.95
Median	2.54	106.89	0.13	41.49	0.04	1.91
SD	1.22	80.20	0.07	22.92	0.03	0.25
Max	5.73	497.66	0.41	139.08	0.13	2.41
Min	1.07	28.72	0.05	14.19	0.01	1.54
χ0.025	1.12	31.32	0.061	16.68	0.02	1.55
χ0.975	5.31	279.68	0.287	91.16	0.12	2.34

Legenda: SD: standard deviation; χ0.025 percentiles; χ0.975 percentiles; max: maximum value; min: minimum values.

Table 3. Ratio and indexes in healthy and DLBCL dogs.

Median ± SD	NLR	MLR	PNR	PLR	MPV/PLT	RDW	RDW/Ca
Lymphoma	5.50 ± 8.56	0.68 ± 1.74	25.89 ± 55.40	120.57 ± 423.95	0.05 ± 0.09	15.0 ± 2.45	1.54 ± 0.30
Control	2.54 ± 1.22	0.13 ± 0.07	41.49 ± 22.92	106.89 ± 80.20	0.04 ± 0.03	16.9 ± 2.00	1.91 ± 0.25
p	***< 0.0001	***< 0.0001	**= 0.0002	= 0.2292	*p* = 0.0695	*= 0.0320	*** < 0.0001

*Statistical difference *p<0.05, **p < 0.01, ***p < 0.001. SD: standard deviation. RDW/Ca 48 cases.

canine lymphoma, compare them with ratios in healthy dogs, and eventually study the prognostic role of these parameters with LSS.

One hundred and fourteen dogs with LBCL and sixty control dogs were analyzed.

Anemia (predominantly normocytic normochromic or macrocytic hypochromic) was present in about 37% of examined cases with LBCL and, in many studies, has been described as a negative prognostic indicator in dogs treated with CHOP-based chemotherapy protocol (Miller *et al*., 2009; Curran and Thamm, 2016; Henriques *et al*., 2021). We can observe that MCV was increased (macrocytosis) only in 9% of cases, to show that, probably, it is not regenerative. However, in this study, we did not find any statistical association between anemic subjects and LSS, despite having

observed a decrease in survival times, as described by Vos *et al*. (2019). This is probably because the cases of LBCL were limited to the III and IV clinical stages and the anemia observed was always mild or moderate and only in two cases severe. Observed anemia in oncologic disease is mostly associated with chronic disease in conjunction with decreased availability of iron, but also with bone marrow erythroid hypoplasia and immune-mediated hemolytic process, but underlying pathogenesis in veterinary patients is, to date, not fully understood (Mutz *et al*., 2015; Garrett *et al*., 2002). No correlation between platelet counts and leukocyte counts, and LSS was found. Neutropenia, typical of more advanced subjects undergoing chemotherapy, was also present in a reduced number of cases, supporting the lack of bone marrow involvement at this stage (Henriques *et al*., 2021).

The median LSS of LBCL was 223 days, similar to other studies previously reported with the same chemotherapy protocol used (Gavazza *et al*., 2008; Henriques *et al*., 2021; Mutz *et al*., 2015).

The control group was represented by healthy dogs that regularly donate blood to the veterinary blood banks of the University of Perugia and the University of Camerino. This allowed us to calculate a complete list of indices and evaluate RI in healthy dogs. This process begins with a healthy reference population made up of individuals who are initially chosen based on inclusion or exclusion criteria. In some research papers, the value of the hematological ratio from healthy dogs has been partially evaluated to compare them with pathological cases, but the RIs were never calculated for a full variety of ratios. To make medical decisions and to evaluate the patient's conditions the test results are compared with correct Ris (Forget *et al*., 2017; Latimer and Duncan, 2011a). It has been recognized to be essential to evaluate the RIs of ratios with statistical methods in healthy animals to estimate the difference between oncologic and hematological disease (Geffré *et al*., 2009; Friedrichs *et al*., 2012; Miglio *et al*., 2020). So far, NLR is the most investigated index, and we can observe that the median value determined in our study (2.54) is between that determined by Benvenuti *et al*. (2020) (3 median) and Rejec (2017) (2.7 median).

Moreover, the evaluation of RIs in healthy dogs allowed us to make a statistical comparison with the indices calculated in the population affected by lymphoma. Table 3 shows the statistically significant differences in ratio and hematological indexes between the two populations.

Malignancy-associated systemic inflammatory response assessed by CBC indices (NLR, PLR, MLR, MPV/PLT, PLR) has already been shown to correlate with the pathogenesis and prognosis of certain cancers in humans and veterinary (Bhatti *et al*., 2010; Kwon *et al*., 2012; Balta *et al*., 2013; Marconato *et al*., 2015). In the present study, we used, in relation to the risk factor, as a cut-off a value greater than the mean obtained

from indices in healthy dogs to be able to provide a unique value independent from receiver operating characteristic (ROC) curves. None of the variables analyzed showed statistical significance. Below is the description and commentary of the individual indexes. The NLR ratio has been widely investigated in both human and veterinary medicine for oncological and nononcological diseases. The purpose of this study was to compare the body's inflammatory response with the immune response derived from disorders. The systemic inflammatory response in dogs and humans with neoplastic conditions, which interact directly and indirectly with host's inflammatory cells, has been well described (Elinav *et al*., 2013). In our study, NLR in lymphoma was two times greater than in the control group (5.50 versus 2.54; $p < 0.0001$) due to the production of neutrophils in the inflammatory response and tumor-associated immunosuppressive mediators. In humans, NLR > 3.5 was considered a negative prognostic factor for survival (Porrata *et al*., 2010) but no correlation was found in the present study. Then, the NLR was not found to be a significant value for LSS in this study, similar to what was observed by Mutz et al. (2015) but contrary to the results reported by Henriques *et al*. (2021), who found a positive association between survival and NLR > 7.45 at 180 days. The median of patients with NLR < 5.5 (max normal value) was compared with patients with NLR > 5.5 and no statistically significant difference was found. Regarding leukocytosis, and in particular neutrophilia, as a typical value in advanced tumor stages that was associated to inflammation and tissue necrosis, we can observe that, instead, in our study neutrophilia was present in 26% of dogs and lymphopenia in 35% of dogs (Watabe *et al*., 2011).

Significant statistical differences between LBCL subjects and healthy dogs were also observed for MLR (0.68 *vs.* 0.13) and PNR (25.89 *vs.* 41.49). As previously reported, higher MLR was associated with a risk of death in dogs with sepsis (Pierini *et al*., 2020) but lower MLR in nonsurvivors dogs with primary immune-mediated hemolytic anemia was noticed (Alaimo *et al*., 2023). The development of lymphopenia (35%) and monocytosis (29%) was marked in LBCL dogs and could explain higher MLR. Monocytes are an essential component of the innate immune system, which may be elevated in various diseases, generally migrate from blood to various tissues, and secrete a variety of substances that modulate inflammatory response (Latimer and Duncan, 2011b). Dogs with lymphoma that present PNR value above 0.032 have an earlier disease progression but no correlation with LSS has been demonstrated. The interaction between neutrophils and platelets in cancer can cause changes in the PNR index in both humans and dogs, especially where paraneoplastic hypercoagulability syndrome is present. The presence of different mediators in the hypercoagulability syndrome plays a crucial role in

perpetuating inflammation and thus the progression of cancer (Henriques *et al*., 2021). In our study, PNR was lower in dogs with lymphoma compared to healthy dogs because thrombocytopenia is more frequent than thrombocytosis, and neutrophilia is proportionally represented in a higher percentage of cases. However, no correlation between these indices and LSS has been observed but we cannot rule out that it influences the progression of the disease.

The PLR index was not systematically studied in neoplastic diseases. No statistically significant difference between LBCL and healthy dogs (120.57 *vs*. 106.89) for the PLR index was observed in this report. In human patients affected by B cell lymphoma, the high value of PLR was associated with shorter overall survival and progression free survival (Wang *et al*., 2017). Thus far, in dogs diffuse large B cell lymphoma PLR value was associated with Overall Survival but not for the progression of the disease (Henriques *et al*., 2021). A study showed that PLR was not significantly different despite the observation that the highest values of PLR were found in dogs with oropharyngeal tumors when compared to healthy dogs. The results do not support PLR as a systemic biomarkers oropharyngeal tumors disease activity (Rejec *et al*., 2017).

Also, MPV, if increased, indicates an abnormal rate of platelet production and stimulation. Thrombocytosis and increased MPV are indicated to be associated with a worse prognosis in multiple solid tumors in humans (Karaman *et al*., 2011; Kurt *et al*., 2012; Korniluk *et al*., 2019). The study of Rejec *et al*. (2017) does not confirm significant differences in MPV/PLT values between groups of healthy and neoplastic dogs (Rejec *et al*., 2017). Similar to the PLR, unfortunately, no statistically significant difference was observed in this study between dogs with lymphoma and healthy dogs. Therefore, the role of the MPV/PLT ratio remains inconclusive (Rejec *et al*., 2017).

The interpretation of RDW in combination with mean corpuscular volume (MCV) may further aid the subclassification of anemias. High baseline RDW has previously been demonstrated as an independent prognostic marker of poor outcomes in human patients with LBCL (Periša *et al*., 2015). Then, RDW is suggested as a parameter reflecting the level of systemic inflammatory response in dogs and is helpful in predicting its severity and results in acute pancreatitis (Guglielmini *et al*., 2022). Moreover, significantly increased RDW values were noticed in dogs with cardiorespiratory, renal, neoplastic (lymphoma), hepatic, endocrine, and immune-mediated diseases. Despite having found a significant difference between dogs with lymphoma and healthy dogs in our study, surprisingly the RDW of the first is lower than the second. This trend agrees with the results reported by the study of Martinez *et al.* (2019), in which the diagnosis of multicentric lymphoma was based on peripheral lymphadenopathy and cytological or histopathological,

but the clinical staging was missing. In addition, both values are fully within the reference ranges, therefore it does not seem to have prognostic significance unlike in cardiovascular diseases (Martinez *et al*., 2019). Indeed, a recent study showed that RDW can be considered, even within RIs, an independent predictor of poor outcomes in dogs with myxomatous mitral valve disease (Guglielmini *et al*., 2021). Certainly, further evaluation of the prognostic meaning of RDW in cancer, and particularly, in canine, lymphoma is necessary. It will also be useful to evaluate the progress of RDW in the progression of the disease.

RDW/Ca ratio has been shown to be an excellent predictor of acute pancreatitis severity and mortality in humans (Han *et al*., 2021). In dogs with acute pancreatitis, RDW/Ca was significantly higher in nonsurvivors compared to survivors, which suggests that the presence of hypocalcemia can be a concern in dogs with this condition and an RDW of >12.7% had good accuracy in predicting short-term death (Guglielmini *et al*., 2022). However, in dogs with lymphoma, hypocalcemia was rarely observed, but hypercalcemia was more frequently observed in immunophenotype T (Zandvliet, 2016). This may explain the lack of correlation of the RDW/Ca index with the survival times.

The study presents several limitations, including the retrospective nature and some dogs, as described, have received multiple chemotherapy treatments. Also, it is known that the interpretation of the blood and bone marrow smear was strongly operator-dependent but this limit cannot be eliminated. In addition, bone marrow cytology was not performed in all dogs, but they were classified in clinical stage III–IV excluding the presence of circulating neoplastic cells on blood and/or bone marrow smears.

Conclusion

A comprehensive evaluation of hematological ratios and indices was conducted and compared with a healthy dog population. Ratios were economical and easy markers to evaluate immune processes and acute or chronic inflammation. In our study, despite having observed several statistically significant differences between the hematological ratios of LBCL dogs and healthy, no correlation with survival times was found. Then, the prognostic value of hematological ratios is still controversial in canine LBCL also in relation to the method of calculating the cut-offs, and it remains under investigation.

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Supplementary Materials

Supplementary Table 1. Differential blood count.

	RBC (10¹²/l)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)	WBC (10⁹/l)	PLT (10⁹/l)	MPV (fl)
Mean	5.71	13.39	38.00	67.12	23.57	35.49	15.65	14.71	238.85	10.80
Median	5.92	13.90	39.24	67.55	23.70	35.26	15.00	11.87	213.00	10.55
SD	1.34	3.12	8.25	6.25	1.98	6.05	2.45	10.71	142.73	1.97

Supplementary Table 1. RR of hematological parameters.

	RBC (10¹²/l)	HGB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)	WBC (10⁹/l)	PLT (10⁹/l)	MPV (fl)
Sysmex	5.5–8.5	13.2–19	40–55	60–77	21–27	32–36	10.4–24.5	6–12	150–500	8.7–13.2
Idexx	5.65–8.87	13.1–20–5	37.3–61.6	61.6–63.5	21.2–25.9	32–37.9	13.6–21.7	5.05–16.76	148–484	