The impact of peripheral blood cell ratios in dogs with diffuse B-cellsmall lymphocytic lymphoma treated with CHOP protocol

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ABSTRACT

In this study, pre-chemotherapy hematological values of 14 dogs diagnosed with diffuse B-cell small lymphocytic lymphoma were compared with the hematological data of 26 healthy dogs. Neutrophil/lymphocyte ratio (NLR), lymphocyte/monocyte ratio (LMR), platelet/lymphocyte ratio (PLR), and platelet/neutrophil ratio (PNR) were evaluated between two groups. Anemia and an increased total leukocyte count were observed in dogs with lymphoma compared to healthy ones. The PNR value was found to be significantly lower in dogs with lymphoma. It was concluded that more comprehensive studies are needed to clearly understand the diagnostic and prognostic importance of hematological parameters in B-cell small lymphocytic lymphoma of dogs.

Introduction

B-cell lymphoma has been defined asthe most common lymphoma histotype affecting dogs (25, 28). Diffuse small B-cell lymphocytic lymphoma (DSLL) is also very rare (<1%) among all canine lymphomas (8, 24).

Although B-cell lymphoma is a highly chemoresponsive neoplasm, several variations have been published in recent years with different outcomes (16, 33). Therefore, studies have focused on the prognostic markers such as stage, substage, immunophenotype, anatomical localization, hypercalcemia, histological type, and cell morphology (6, 14, 22, 27). Many of these prognostic factors are costly, difficult to perform, and data are not easy to evaluate. It is very important to determine cheap and easily applicable prognostic information before treatment in veterinary medicine.

In medicine, it is well-known the association of inflammation in lymphomagenesis and tumor progression (3, 12). So the evaluation of immune cell subsets from peripheral blood may reflect the inflammation and host-tumor interaction. In the veterinary literature, some studies showing prognostic importance of neutrophil-lymphocyte (NLR), lymphocyte-monocyte (LMR), platelet-neutrophil (PNR), and platelet-lymphocyte (PLR) ratio in dogs with lymphoma have been described (7, 11, 18). Nevertheless, B-cell DSLL is very rare, and more studies in dogs with B-cell DSLL are still required.

The purpose of the current study was to determine thepre-treatment immune cell subsets in dogs with B-cell DSLL that may be useful to manage the life quality of dogs compared to healthy individuals.

Materials and Methods

Animals: A total of 14 client-owned dogs with histopathologically confirmed B-cell DSLL diagnosed and treated at Ankara University Veterinary Training Hospital were included in this study. Inclusion criteria in dogs were a confirmed histopathological diagnosis of B-cell DSLL, available pre-treatment haematological data and, evaluation of WHO stage III/IV determined by full clinical examination, thoracic radiographs, abdominal ultrasonography and peripheral blood smears. Exclusion criteria were administration of previous chemotherapy or corticosteroids and central nervous system, cutaneousor leukemic involvement of B-cell lymphoma, and dogs having WHO stage I/II or V. Twenty-six clinically healthy dogs (control group) were used in the present study. No dogs in control group had also other inflammatory, infectious, immune-mediated, or neoplastic diseases. None of the dogs had receive medical or surgical treatment at least 3 months before CBC analysis.

Study Design: Clinical procedures including clinical examination, blood analyses, urinalysis, and imaging (abdominal and thoracic radiography and ultrasonography) were performed in all dogs. Medical data of signalment, history, tumor histopathological type, and hematological data were also recorded. CBC indices such as neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), and platelet-to-neutrophil ratio (PNR) were calculated using absolute monocyte, neutrophil, lymphocyte and platelet values. Complete blood counts (CBC) were performed on an Exigo Eos Veterinary Hematology Analyzer with whole blood in EDTA. World Health Organization (WHO) classification of lymphoma was used in staging the dogs clinically (20). Histopathological examination was routinely performed from a lymph node extirpated totally in each dog (34). Lymphoma chemotherapy consisted of 19-week CHOP protocol including vincristine (0.5 mg/m^2 IV), cyclophosphamide (200 mg/m^2 IV), doxorubicin (30 mg/m^2 IV), and prednisolone (2 mg/kg BID with tapering for 4 weeks). Written consent was also obtained from the owners.

Statistical Analysis: All statistical analyses were performed using Stata 12/MP4 and MedCalc Version 9.2.0.1. The variables were examined with the Shapiro-Wilk test and Levene test as parametric test assumptions. The differences in hematological parameters among the two groups were analyzed using the Student t-test when parametric test assumptions were met and the Mann-Whitney U test otherwise. ROC analysis was used to determine a predicted threshold for the identification of disease (Table 3). ROC curves for the detection of B-cell lymphoma were obtained for each hematological parameter. Sensitivity, specificity and area under the curve (AUC) were calculated for each variable. All data were presented as mean ± standard deviation (SD) and median. Differences with $P<0.05$ were considered statistically significant.

Results

Data were collected from 40 clint-owned dogs. The group of 14 dogs with B-cell DSLL consisted of mixed breed (n:5, 35.7 %), Husky (n:2, 14.4 %), Labrador Retriever (n:1, 7.1 %), Golden Retriever (n:4, 28.6 %), Rotweiler (n:1, 7.1 %) and Kangal (n:1, 7.1 %). The reference population also consisted of 26 healthy dogs including Labrador Retriever (n:2, 7.7 %), Golden Retriever (n:5, 19.2 %), mixed (n:3, 11.5 %), terrier types (n:8, 30.8 %), Akbas Shepperd Dog (n:2, 7.7 %), Pekingese (n:1, 3.8 %), Pointer (n:1, 3.8 %), Pug (n:1, 3.8 %), Cavalier King Charles (n:1, 3.8 %), English Setter (n:1, 3.8 %) and Cocker Spaniel (n:1, 3.8 %). The mean age, weight and gender distributions of dogs in groups were shown in Table 1. Hematology profiles in each groups were also presented in Table 2. The most common clinical signs were generalized lympadenopathy (92.85%), anorexia (57.14%), fever (14.28%) and weigth loss (57.14%). Most dogs presented with a combination of clinical sings but no dominant combination was apparent.

Table 1. Characteristics of Dog Population with Diffuse B-cell Small Lymphocytic Lymphoma.

Characteristics	Dogs with B-cell DSLL (n:14)	Healthy Dogs (n:26)
Age (year)	7.7 ± 2.88	7.9 ± 3.63
Weight (kg)	12.3 ± 2.46	14.9 ± 4.61
Gender (n,%)	14 (100)	26 (100)
Male	8 (57.1)	18 (69.2)
Female	6 (42.9)	8 (30.8)

DSLL: B-cell Diffuse Small Lymphocytic Lymphoma; Who Stage in B-cell DSLL: II, n:1 (7.1 %); III, n:7 (50 %); IV, n:6 (42.9 %); Who Substage: a, n:11 (78.6 %); b, n:3 (21.4 %).

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Table 2. Hematology profiles in groups.

Variables	Dogs with B-cell DSLL		Healthy Dogs		P
	mean ± SD	median	mean ± SD	median	
WBC *(10^9/L)*	14.36 ± 9.31	11.89	8.13 ± 1.96	7.40	**0.002**
RBC *(10^{12}/L)*	5.65 ± 0.97	5.89	6.65 ± 0.65	6.61	**0.003**
HGB *(g/dL)*	13.35 ± 2.72	13.50	16.23 ± 1.96	16.25	**0.002**
PCV *(%)*	37.86 ± 6.56	38	45.67 ± 4.48	44.65	**< 0.001**
MCV *(fl)*	68.26 ± 4.04	68.1	69.7 ± 2.76	68.5	0.435
MCH *(pg)*	23.17 ± 3.11	23.3	25 ± 1.39	24.95	0.06
MCHC *(g/dL)*	33.72 ± 4.56	35	36 ± 0.89	35.95	0.32
RDW *(%)*	17.55 ± 6.29	16	13.17 ± 0.65	13.1	**< 0.001**
PLT *(10^9/L)*	247.15 ± 119.9	210	292.38 ± 90.06	282	0.06
PCT *(%)*	0.24 ± 0.12	0.2	0.24 ± 0,07	0.23	0.67
NLR	13.14 ± 21.33	4.75	4.49 ± 2.75	3.88	0.69
LMR	6.91 ± 10.84	2.74	3.08 ± 0.92	3.10	0.87
PLR	251.90 ± 337.01	102	214.53 ± 111.68	189.08	0.15
PNR	36.86 ± 34.07	30.06	54.41 ± 21.15	49.4	**0.011**

WBC: White Blood Cell; RBC: Red Blood Cell; HGB: Hemoglobin; PCV: Packed Cell Volume; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW: Red Blood Cell Distribution Width; PLT: Platelet; PCT: Plateletcrit; NLR: Neutrophil-to-Lymphocyte Ratio; LMR: Lymphocyte-to-Monocyte Ratio; PLR: Platelet-to-Lymphocyte Ratio; PNR: Platelet-to-Neutrophil Ratio; DSLL: Diffuse Small Lymphoctytic Lymphoma.

Table 3. The ROC curves of the hematological parameters for the development of B-cell lymphoma.

Variables	Threshold	Sensitivity	%95 Cl for Se	Specificity	%95 Cl for Sp	AUC	P
WBC	>10.9	53.85	25.2 - 80.7	96.15	80.3 - 99.4	0.796	**< 0.001**
RBC	<=5.98	69.23	38.6 - 90.7	80.77	60.6 - 93.4	0.790	**< 0.001**
HGB	<=14.6	69.23	38.6 - 90.7	84.62	65.1 - 95.5	0.800	**< 0.001**
PCV	<=39.5	69.23	38.6 - 90.7	100	86.7 - 100.0	0.839	**< 0.001**
RDW%	>13.8	76.92	46.2 - 94.7	88.46	69.8 - 97.4	0.874	**< 0.001**
PLT	<=210	53.85	25.2 - 80.7	84.62	65.1 - 95.5	0.685	**0.034**
MPV	>8.8	76.92	46.2 - 94.7	69.23	48.2 - 85.6	0.768	**0.002**
NLR	>4.05	61.54	31.6 - 86.0	61.54	40.6 - 79.7	0.541	0.678
LMR	>5.29	38.46	14.0 - 68.4	100	86.7 - 100.0	0.482	0.858
PNR	<=30.09	61.54	31.6 - 86.0	92.31	74.8 - 98.8	0.749	**0.002**
PLR	<=110.61	61.54	31.6 - 86.0	88.46	69.8 - 97.4	0.645	0.112

Se: Sensitivity; Sp: Specificity; AUC: Area under the curve.

Discussion and Conclusion

B-cell lymphomas delineated as diffuse or nodular pattern sare the most common lymphoma histotype (60-70%) in dogs (24, 28). Several B-cell lymphoma subtypes such as marginal zone, mantle cell, follicular and small lymphocytic lymphoma have been defined previously (35). B-cell DSLL has also been reported to account for <1 % of all canine lymphomas (8, 24).

B-cell lymphocytic lymphoma and chronic lymphocytic leukemia (CLL) are considered the same disease in humans (31). In veterinary medicine,authors have attributed the rarer diagnosis of small lymphocytic lymphoma to less preference for lymph node biopsies in the diagnostic phase of CLL (13). There is also no consensus on the distinction between leukemia and the leukemic phase of canine lymphoma in veterinary literature (1). Therefore, inclusion criteria differed on B-cell chronic lymphocytic leukemia in previous studies (2, 5). The common opinion in these studies is that it is difficult to differentiate small cell lymphoma from chronic lymphocytic leukemia. The retrospective nature of all studies and the inadequacy of histopathological examination made it difficult to reach a consensus.

A study in dogs with B-cell DSLL suggested that the aggressive progression and mitotic count of B cell-DSLL are more similar to mantle cell lymphoma in humans than small lymphocytic lymphoma (SLL)/chronic lymphocytic leukemia (CLL) (13). In the same study, it was emphasized that flow cytometry was insufficient in the diagnosis of B-cell lymphoma, and histopathology is required for a definitive diagnosis. In thepresented study, while WHO classification of lymphoma was used in

staging the dogs clinically (20), histopathological classification was performed from a lymph node extirpated totally in each dog (34). Although CLL is also characterized by circulating small lymphocytes, we prefer to use the term of B-cell DSLL based on the histopathological classification and the dogs with lymphadenopathy, liver or spleen involvement.

In consistent with the studies considering that the incidence of lymphoma mostly affects medium and largebreed dogs (38), in our study, all dogs with lymphoma were also largebreed dogs. It is thought that the reason for this situation may be related to genetic susceptibility rather than growth hormone (38). Although no gender predisposition has been reported, lymphoma is less common in female dogs because of the protective effect of endogenous estrogens (36). In the study here, the majority of lymphoma dogs (57.1%) were male dogs compatible with the results previously described (9, 18, 23, 32).

In medicine, it is well-known the association of inflammation in lymphomagenesis and tumor progression (3, 12). Necrotic and infectious processes associated with neoplasia have also caused inflammation related to leukocytosis (18, 37). Therefore, the immune cell subsets from peripheral blood may directly reflect the inflammation and host-tumor interaction (7, 11, 18). In our study, remarkably increased leukocyte levels in dogs with lymphoma compared to healthy individuals were consistent with reports previously described (19, 22).

Few studies revealed the anemia rate in dogs with lymphoma as 57%, 48%, 41%, and 53%, respectively (9, 15, 19, 21). Although the anemia pathogenesis in dogs with lymphoma remains unknown, lots of processes including shortening of erythrocyte lifespan, auto-immune hemolysis, abnormal iron metabolism, decreased production of erythropoietin, interleukins and hepcidinplay an important role in the mechanism of anemia (11, 21). Anemia (defined as PCV<%39) was also remarkable in lymphoma dogs in the present study.

In human medicine, an increase in NLR has been reported as a negative prognostic indicator of prognosis in lymphoma patients (10, 17). Rejec et al. (2017), found a higher NLR value in dogs with oral tumors (26). In this study, it was reported that this value was higher in tumors with high malignancy. The increased levels of neutrophils in dogs with cancer may be caused by acute or chronic inflammation, tissue necrosis, and stres (4). Causes of the reduction in lymphocytes seen in dogs with cancer include decreased lymphocyte production or suppression of maturation, increased peripheral destruction, generalized lympholysis, or altered circulation patterns (18). In our study, no significant difference of NLR we determined in lymphoma dogs before chemotherapy compared to healthy ones. Mutz et al. (2015), obtained similar results in their research on dogs with lymphoma as well and the researchers recommended further investigation of the correlation of lower or higher NLR values associated with less or more aggressive biological behavior (18). Lymphocyte/monocyte ratio (LMR) hasprognostic importance in lymphoma patients in humans (29). LMR has previously been reported as prognostic significance for survival in canine multicentric centroblastic diffuse large B-cell lymphoma and canine cutaneous mast cell tumors (7, 30). In our study no significant changes of LMR we defined in two groups of dogs. We think that this is due to the difference in tumor type and the LMR value being affected by non-specific etiologies. Henriques et al. have reported the unrelated situation of PLR on prognosis (11). In the study here, although lower platelet level we defined in lymphoma dogs, no statistically significant differences of PLR were possible in consistent with the reports previously defined. Although the reason for the decrease in platelet count is not fully understood, it may be the result of upregulation of inflammatory markers, bone marrow involvement, autoimmune destruction and systemic inflammatory conditions, similar to humans. Contrary to our study, in a study including animals with oropharyngeal tumors and healthy ones, authors have observed higher PNR levels in dogs with tumors (26). We think this discrepancy is related to different tumor types and tumor stages. Henriques et al reported that dogs with large diffuse B-cell lymphoma with a PNR above a certain threshold tended to have earlier lymphoma progression. (11). The data obtained from the results of the current study have shown thatPNR can be used as a marker to distinguish between lymphoma dogs and healthy ones (Figure 1).

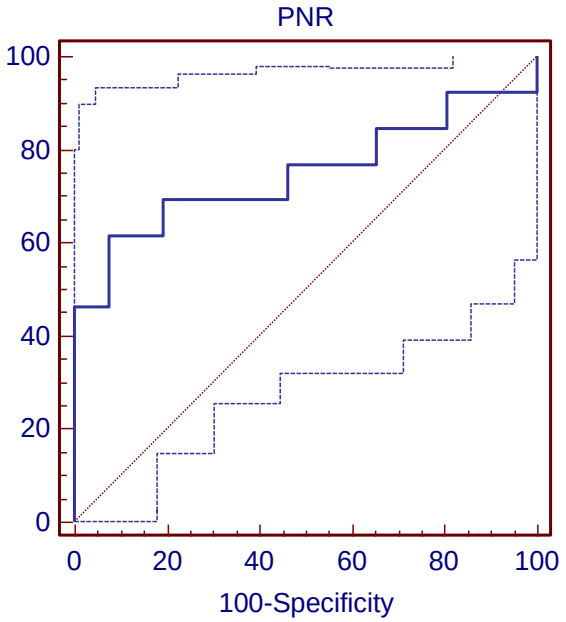

Figure 1. Display of ROC curve for threshold of PNR.

As a conclusion, in dogs with diffuse B-cell small lymphocytic lymphoma, total leukocyte count, hematocrit, and PNR values obtained from whole blood evaluation were different from those in healthy dogs. However, we also believe that more comprehensive studies are needed to understand the diagnostic and prognostic values of the mentioned parameters.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study does not present any ethical concerns.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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