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Dogs

Evaluation of White Blood Cell Ratios in Newly Diagnosed Multicentric Lymphoma in Dogs

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ABSTRACT

Background and Objective: Lymphoma is one of the most common malignancies in dogs. In human medicine, complete blood count (CBC)-derived inflammatory ratios, such as the neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR) and platelet-to-lymphocyte ratio (PLR), have been investigated as potential biomarkers in cancer. However, studies evaluating these parameters in canine lymphoma are limited. This study aimed to compare NLR, MLR and PLR values between dogs diagnosed with lymphoma and healthy controls.

Methods: A total of 26 dogs with lymphoma and 10 clinically healthy dogs were included. CBC parameters were analysed, and NLR, MLR and PLR were calculated.

Results: NLR and PLR values were significantly higher in dogs with lymphoma compared to healthy dogs, whereas MLR decreased.

Conclusion: These findings indicate that CBC-derived inflammatory ratios, particularly NLR and PLR, are elevated in dogs with lymphoma and may reflect tumour-associated systemic inflammation. Further studies are needed to evaluate their potential clinical utility.

1 | Introduction

Canine lymphoma is recognized as the most common hematopoietic neoplasm in dogs, comprising a diverse group of malignancies that primarily affect lymphoid tissues. This disease is characterized by its heterogeneity, with various subtypes including B-cell and T-cell lymphomas, each exhibiting distinct biological behaviours and clinical outcomes (Marconato et al. 2012). The clinical presentation of canine lymphoma often involves advanced stages at diagnosis, with many cases exhibiting aggressive progress and requiring immediate intervention (Marconato et al. 2012). Furthermore, the molecular and genetic parallels between canine and human non-Hodgkin lymphoma (NHL) underscore the relevance of canine lymphoma as a model for studying disease mechanisms and therapeutic strategies in humans (Gardner et al. 2018; Ito et al. 2014). Overall, the study of canine lymphoma not only contributes to veterinary oncology but also provides valuable insights into the pathogenesis and treatment of similar malignancies in humans.

White blood cell–derived inflammatory ratios, including the neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR) and platelet-to-lymphocyte ratio (PLR), have gained increasing attention as readily accessible and cost-effective biomarkers in human medicine. These indices reflect the balance between systemic inflammation and immune response and have

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been associated with disease severity and progression in various inflammatory, infectious and neoplastic conditions. In parallel with advances in human medicine, interest in inflammation-based haematological markers has also increased in veterinary medicine. In recent years, alterations in these parameters have been investigated in a range of diseases in dogs, including inflammatory disorders, cardiovascular diseases and neoplasia. Among malignant conditions, canine lymphoma represents one of the most commonly diagnosed hematopoietic tumours and is characterized by marked immune dysregulation and tumour-associated inflammation, making it a relevant model for evaluating inflammation-related haematological indices. Previous studies in veterinary medicine have evaluated these hematologic ratios in dogs with lymphoma and have reported associations with disease characteristics and treatment response (Park et al. 2024; Gavazza et al. 2024). However, further evaluation of these parameters, particularly at the time of diagnosis and in comparison with healthy controls, remains warranted. Therefore, the aim of the present study was to evaluate NLR, PLR and MLR in dogs diagnosed with lymphoma at the time of diagnosis and to compare these parameters with those of healthy dogs.

2 | Methods

A total of 36 dogs included in this study, comprising 26 dogs diagnosed with lymphoma and 10 healthy control dogs. The health status of the control group was confirmed through clinical examination, complete blood count (CBC) and biochemistry profile, ensuring that they were free from any underlying diseases that could affect the haematological parameters. The diagnosis of lymphoma was confirmed through cytological examination and clinical evaluation. In addition, the blood biochemistry levels are within normal parameters in all dogs for the same reason as the control group. Dogs that had undergone initial chemotherapy protocols before the CBC analyses were excluded from the present study.

All patients underwent same diagnostic procedures, including routine blood analyses, radiography and abdominal ultrasonography. Blood samples were collected from all dogs to perform CBC and biochemistry analyses. Each blood sample (approximately 2 mL) was withdrawn from the cephalic or jugular vein and processed within 1 h of collection to ensure the integrity of the samples. The CBC was performed using an automated haematology analyser (IDEXX ProCyte Dx), which provided counts for neutrophils, lymphocytes, monocytes and platelets. The NLR, MLR and PLR were calculated using the following formulas:

- NLR = neutrophil count/lymphocyte count
- MLR = monocyte count/lymphocyte count
- PLR = platelet count/lymphocyte count

To further support the diagnosis of lymphoma, cytological evaluation was conducted on fine-needle aspirates (FNA) collected from palpable, enlarged peripheral lymph nodes. Aspirates were obtained using a 22-gauge needle via non-aspiration technique to preserve cellular architecture, and the aspirated material was immediately smeared onto pre-cleaned glass slides. Slides were air-dried, subsequently stained using the May–Grünwald–Giemsa (MGG) method, and evaluated under an Olympus BX51 light microscope. Cytological interpretation focused on criteria such as cellularity, overall cell morphology, nuclear size and contour, chromatin distribution, nucleolar prominence, mitotic activity and the presence of lymphoglandular bodies. Lymphoma was diagnosed based on the presence of a monomorphic population of large lymphoid cells with high nuclear-to-cytoplasmic (N/C) ratios, open chromatin patterns, prominent nucleoli and frequent mitotic figures, which are consistent with high-grade malignant lymphoid neoplasia (Figure 1).

Statistical analyses were conducted to compare the haematological ratios between the lymphoma group and the healthy control group. Descriptive statistics were used to summarize the data, and the Mann–Whitney *U* test was employed to assess differences in NLR, MLR and PLR between the two groups. A p value of <0.05 was considered statistically significant. All analyses were performed using statistical software (SPSS).

3 | Results

In this study, healthy dogs and dogs diagnosed with lymphoma were compared regarding breed, age and gender distribution. Among the 10 healthy dogs, the breeds included 4 Cocker Spaniels, 3 Golden Retrievers, 1 Rottweiler, 1 Terrier and 1 mixed breed. The average age of these dogs was 9.7 years (ranging from 3 to 16 years). Gender distribution was equal, with five females and five males. In contrast, the 26 dogs diagnosed with lymphoma included 8 Golden Retrievers, 6 mixed breeds, 2 Terriers, 2 Pinschers, 2 German Shepherd Dogs, 1 Doberman, 1 Beagle, 1 Pug, 1 Boxer, 1 Cocker Spaniel and 1 Rottweiler. Their average age was 8.6 years (ranging from 3 to 15 years). Gender distribution in this group showed 10 females and 16 males. All dogs with lymphoma are mulcentric and WHO stage III.

The comparison of erythrocyte, leukocyte and platelet parameters between healthy dogs and dogs with lymphoma is presented in Tables 1 and 2. Significant alterations were observed in several haematological variables in dogs diagnosed with lymphoma. RBC counts were significantly higher in dogs with lymphoma compared to healthy dogs ($p < 0.001$). In contrast, HGB concentrations were significantly lower in the lymphoma group ($p < 0.001$), despite values remaining within the reference interval. HCT values did not differ significantly between groups. Erythrocyte indices showed mild but statistically significant differences. MCV and MCH were slightly increased in dogs with lymphoma compared to healthy dogs ($p < 0.05$). MCHC was also significantly higher in the lymphoma group ($p < 0.001$). WBC counts were significantly elevated in dogs with lymphoma compared to healthy controls ($p < 0.05$). Conversely, PLT counts were significantly lower in dogs with lymphoma ($p < 0.01$), although values remained within the established reference range. In addition to Table 1, Table 2 shows that dogs with lymphoma exhibited significantly higher neutrophil counts compared to healthy dogs ($p < 0.05$). Similarly, monocyte counts were significantly increased in the lymphoma group ($p < 0.05$). Although lymphocyte counts were numerically higher in dogs with lymphoma, this difference did not reach statistical significance. In contrast, eosinophil counts were significantly lower in dogs with lymphoma compared to healthy controls ($p < 0.001$).

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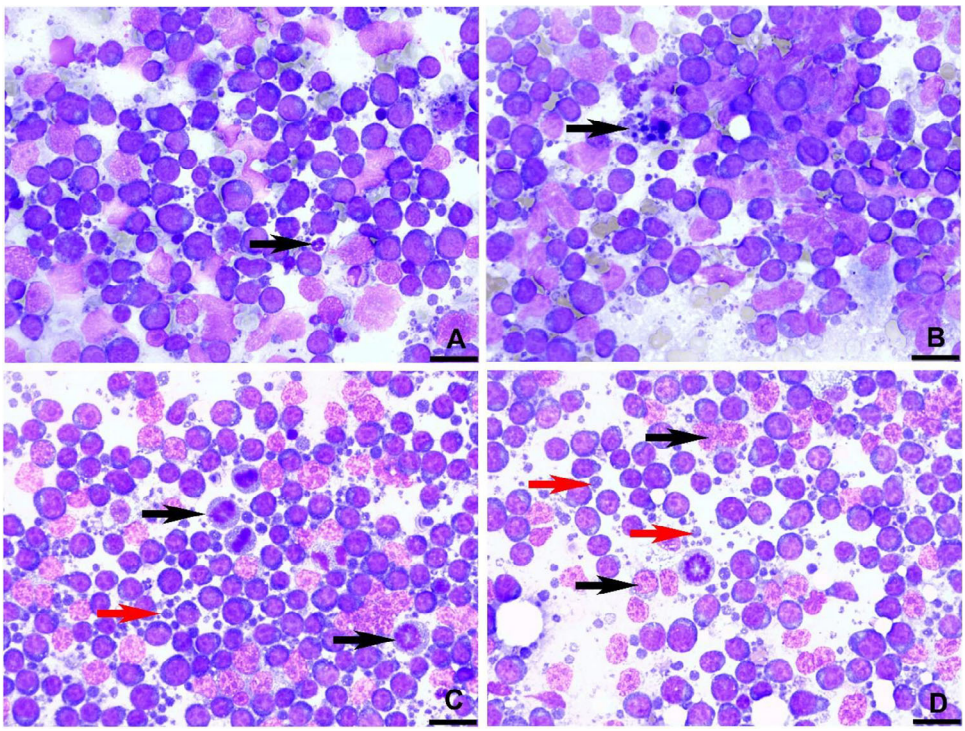


FIGURE 1 | Cytological smear from a fine-needle aspirate of an enlarged peripheral lymph node from a dog with multicentric lymphoma, MGG stain. (A) Clusters of large atypical lymphoid cells with marked anisocytosis and anisokaryosis consistent with a high grade lymphoma. Mature lymphocytes and neutrophils (arrow) proofing the neoplastic lymphocytes are really large size and cellular debris in the back ground, Bar = 10 μm. (B) A tingible body macrophage (arrow) among the neoplastic large lymphocytes, Bar = 10 μm. (C) Numerous mitotic figures (black arrows) and lymphoglandular bodies (red arrow) in the back ground, Bar = 10 μm. (D) Large atypical lymphoid cells with prominent nucleoli, irregular nuclear contours, and high nuclear-to-cytoplasmic ratios, observed within a background of lymphoglandular bodies (red arrows) and degenerating cells (black arrows), Bar = 10 μm.

TABLE 1 | Complete blood count (CBC) of the groups.

	Healthy dogs	Dogs with lymphoma	Normal values
RBC	9.7 ± 0.5	20.6 ± 4.1***	5.10–8.50
HCT	28.4 ± 5	31 ± 1.7	36.00–56.00
HGB	34.4 ± 5	12.9 ± 1.1***	11.00–19.00
MCV	66.2 ± 0.6	65.6 ± 0.4*	62.00–78.00
MCH	23.7 ± 0.3	24.4 ± 0.3*	21.00–28.00
MCHC	35.8 ± 0.2	37.5 ± 0.4***	30.00–38.00
WBC	9.6 ± 0.5	20.6 ± 4.2*	6.00–17.00
PLT	281.5 ± 25.5	193.1 ± 24.7**	117.00–460.00

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

TABLE 2 | Formule leukocyte of the animals.

	Healthy dogs	Dogs with lymphoma	Normal values
Neutrophil	6.6 ± 0.5	11.8 ± 2.9*	3.62–11.32
Lymphocyte	2.6 ± 0.4	4.4 ± 1.8	0.83–4.69
Monocyte	1 ± 0.1	4.1 ± 1.7*	0.14–1.97
Eosinophil	0.4 ± 0.1	0.2 ± 0.03***	0.04–1
Basophil	0.04 ± 0.02	0.03 ± 0.009	0.00–0.12

*$p < 0.05$.
***$p < 0.001$.

Inflammation-based haematological indices, including the NLR, PLR and MLR, are presented in Table 3. Dogs with lymphoma exhibited a significantly higher NLR compared to healthy dogs ($p < 0.05$). Similarly, the PLR was significantly increased in the lymphoma group ($p < 0.05$). In contrast, the MLR was significantly lower in dogs with lymphoma when compared to the control group ($p < 0.01$).

TABLE 3 | Neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and monocyte-to-lymphocyte ratio (MLR) results of the healthy dogs and dogs with lymphoma.

	Healthy dogs	Dogs with lymphoma
NLR	2.7 ± 0.5	8.8 ± 2.3*
PLR	129.3 ± 22.8	165.2 ± 29.9*
MLR	1.89 ± 0.4	0.4 ± 0.08**

*$p < 0.05$.
**$p < 0.01$.

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4 | Discussion

In general, studies conducted on animals have shown that total leukocyte counts often remain within normal limits despite the presence of disease. This has prompted researchers to investigate new and cost-effective parameters that could assist clinicians in making more accurate diagnoses and characterizing disease-associated changes better. Although Sevim et al. (2024) reported markedly elevated leukocyte counts in their study, in our investigation, the leukocyte values were only slightly above the upper reference limit. In contrast, the findings of this study highlight the significant role of haematological ratios, particularly NLR and PLR, between dogs with lymphoma and healthy controls.

In human medicine, elevated NLR has been associated with disease severity and adverse outcomes in various malignancies, reflecting an imbalance between inflammatory and immune responses (Mutz et al. 2013; Sánchez et al. 2019). This imbalance is typically characterized by neutrophilia, indicating systemic inflammation, and lymphopenia, suggesting impaired immune surveillance (Benvenuti et al. 2020; Sánchez et al. 2019). Similar associations have been reported in veterinary studies, where NLR has been evaluated in dogs with lymphoma, particularly in relation to disease characteristics and treatment response (Henriques et al. 2020; Park et al. 2024). In the present study, NLR values were significantly higher in dogs with lymphoma at the time of diagnosis compared to healthy dogs, supporting the presence of tumour-associated systemic inflammation.

NLR is potential prognostic marker in canine lymphoma, particularly in diffuse large B-cell lymphoma (DLBCL), which is the most prevalent hematopoietic tumour in dogs (Henriques et al. 2020; Mutz et al. 2013). Research indicates that elevated NLR values correlate with poorer outcomes in canine lymphoma, similar to findings in human oncology (Park et al. 2024). Studies have shown that a high NLR is associated with decreased lymphoma-specific survival and time-to-progression in dogs undergoing chemotherapy, particularly with the CHOP protocol (Henriques et al. 2020; Mutz et al. 2013). The underlying mechanism for the prognostic significance of NLR in canine lymphoma may relate to the balance between inflammatory and immune responses (Park et al. 2024).

In previous studies, NLR has been reported to range between 2.32 and 5.54 in humans and between 7.45 and 9.44 in animals (Park et al. 2024). In the study by Park et al. (2024), it was determined as 3.764. Our results were similar to the other researchers. The difference with the Park et al. (2024) study may be due to the fact that they used small breed dogs in their study. Differences between studies may be related to variations in study populations, including breed, disease stage and inclusion criteria.

Similarly, the MLR has emerged as a valuable marker, with higher values indicating a more pronounced inflammatory state that may contribute to tumour progression. Research has also indicated that MLR can serve as a diagnostic marker for lymphoma. In human studies, elevated MLR has been linked to the presence of malignancies, and similar trends have been observed in canine populations (Kamiya et al. 2022; Manachai et al. 2022). For instance, a study reported that dogs with lymphoma exhibited significantly higher MLR compared to healthy controls, suggesting its utility in distinguishing between reactive lymphadenopathy and lymphoma (Tezol et al. 2020; Shi et al. 2016). In addition, elevated MLR values have been associated with poorer clinical outcomes, reflecting the complex interplay between the immune system and tumour progression. In veterinary oncology, studies have demonstrated that a higher MLR correlates with decreased overall survival and progression-free survival in dogs diagnosed with lymphoma (Perry et al. 2010; Henriques et al. 2020). The significance of MLR in canine lymphoma can be attributed to the roles of monocytes and lymphocytes in the tumour microenvironment. Monocytes are known to contribute to tumour progression through various mechanisms, including promoting angiogenesis and suppressing anti-tumour immune responses (Becher et al. 2021; Perry et al. 2010; Manachai et al. 2022; Park et al. 2024). In contrast, lymphocytes, particularly T-cells, are crucial for mounting effective immune responses against tumours. A decrease in lymphocyte counts, often seen in conjunction with elevated monocyte counts, can indicate an immunosuppressive environment conducive to tumour growth (Tagawa et al. 2019; Manachai et al. 2022).

The PLR, although less extensively studied, has also been associated with inflammatory status in neoplastic conditions. Elevated PLR has been linked to adverse outcomes in various cancers, including canine lymphoma, suggesting that it may reflect an increased inflammatory response that could facilitate tumour growth and metastasis (Templeton et al. 2014). The interplay between platelets and lymphocytes in the context of cancer is complex, as platelets can secrete pro-inflammatory cytokines that may enhance tumour progression, whereas lymphopenia can indicate a compromised immune response (Templeton et al. 2014; Shafique et al. 2012). Therefore, the integration of PLR with other haematological ratios may provide a more comprehensive picture of the systemic inflammatory response in canine lymphoma patients (Cristóbal et al. 2022). In canine lymphoma, studies have shown that higher PLR correlates with advanced disease stages and reduced survival times, similar to findings in human oncology (Henriques et al. 2020). The underlying mechanism for this association may involve the role of platelets in promoting tumour growth and metastasis through the secretion of pro-inflammatory cytokines and growth factors, which can enhance the survival and proliferation of malignant cells (Tezol et al. 2020). Furthermore, lymphopenia, often seen in lymphoma patients, can exacerbate the inflammatory environment, leading to an increased PLR (Henriques et al. 2020). In the present study, PLR values were significantly higher in dogs with lymphoma compared to healthy controls, further supporting the presence of systemic inflammation at the time of diagnosis.

Recent research has highlighted the potential of PLR as a complementary biomarker when used alongside other haematological ratios, such as NLR and MLR, to provide a more comprehensive assessment of the inflammatory status and immune response in dogs with lymphoma (Henriques et al. 2020). This multifactorial approach to evaluating blood cell ratios underscores the complexity of canine lymphoma and the need for further studies to establish standardized a criteria based on these haematological markers.

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The limitation of this study is that it could not be distinguished as B or T cell lymphoma. Immunophenotyping is essential for accurate subclassification of lymphomas, as B-cell and T-cell variants exhibit distinct biological behaviours, therapeutic responses and prognostic outcomes. In veterinary pathology, immunohistochemistry (IHC) using markers such as CD3 for T cells and CD79a or CD20 for B cells is commonly employed to differentiate these subtypes (Valli et al. 2011). Flow cytometry has also been increasingly used to analyse surface antigen expression in canine lymphoma, providing valuable information regarding immunophenotype and clonal expansion (Seelig et al. 2014). For instance, studies have demonstrated that B-cell lymphomas tend to respond better to chemotherapy and are associated with longer survival times compared to their T-cell counterparts (Marconato et al. 2015). However, the present study lacked access to these diagnostic modalities across all cases, limiting the ability to classify the lymphomas more precisely. Future research incorporating immunophenotypic and molecular techniques is recommended to enable more refined classification and to facilitate improved prognostic and therapeutic decision-making in canine lymphoma cases.

5 | Conclusions

In conclusion, the evaluation of NLR, MLR and PLR in canine lymphoma patients may reflect tumour-associated systemic inflammation in canine lymphoma. Future studies should aim to validate these findings in larger cohorts and explore the potential of these ratios as part of a multimodal approach to treatment and monitoring in canine lymphoma. Additionally, further research is warranted to elucidate the underlying mechanisms by which these haematological indices influence tumour behaviour and patient outcomes.

Author Contributions

Lora Koenhemsi conceived the study idea and was primarily responsible for writing the manuscript. Sinem Yaren Güzelbakan captured the pathology-related images and contributed to the writing and revision of the manuscript. Askarbek Tulobaev performed the statistical analyses, interpreted the results, and contributed to manuscript revisions. Funda Yıldırım selected the clinical cases and contributed to the editorial revisions of the manuscript. All authors read and approved the final version of the manuscript.

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The authors have nothing to report.

Ethics Statement

This study was conducted as a retrospective analysis of previously recorded clinical data retrieved from computerized medical records. As no prospective animal involvement, experimental procedures, or owner interventions were undertaken, approval from an institutional ethics committee was not required. Data were collected and analysed in accordance with institutional and data protection guidelines, and all patient information was anonymized.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Our data and material are available when requested.

Peer Review

For transparency, the peer review documents associated with this article are available at https://doi.org/10.1002/vms3.70974.

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