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**ARAŞTIRMA MAKALESİ** **RESEARCH PAPER**

# A Preliminary Study For Determination Of Neutrophil To Lymphocyte, Monocyte To Lymphocyte And Platelet To Lymphocyte Ratios In Dogs With Canine Distemper Virus Infection

**Didem PEKMEZCİ*** **Gamze Nur KONAÇOĞLU** **Zeynep Nurselin ÇOLAK**
*Department of Internal Medicine, Faculty of Veterinary Medicine, University of Ondokuz Mayis, TR-55200 Samsun -TÜRKİYE*

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*: https://orcid.org/0000-0003-2072-8165
: https://orcid.org/0000-0002-1883-3428
: https://orcid.org/0000-0002-0631-5471

***Corresponding author's:**
Didem PEKMEZCİ
Department of Internal Medicine, Faculty of Veterinary Medicine, University of Ondokuz Mayis, TR-55200 Samsun–TÜRKİYE
✉: dkazanci@omu.edu.tr

**Abstract:** It is aimed to compare the pretreatment Neutrophil/Lymphocyte (NEU/LYM), Monocyte/Lymphocyte (MONO/LYM), and Platelet/Lymphocyte (PLT/LYM) ratios with whole blood count (WBC) of dogs naturally infected with Canine Distemper Virus (CDV) and healthy individuals within this preliminary study. For this purpose, retrospectively, 22 owned, 3-12 (6.2) months old dogs diagnosed with CDV infection found to be positive with rapid test kits based up on the records of Animal Hospital, Faculty of Veterinary Medicine, constituted the study group. On the other hand, eight owned, 2-10 (8.2) months old, and healthy dogs enrolled in the control group. In the comparison of pre-treatment NEU/LYM, MONO/LYM, PLT/LYM, and WBC parameters between the groups, we found only a statistical difference ($P< 0.05$) between median LYM, EOS, NEU%, LYM%, EOS%, BAS%, MCH, MCHC, RDWCV, PLT with NEU/LYM, MONO/LYM, PLT/LYM ratios. The median NEU/LYM, MONO/LYM, and PLT/LYM levels in the CDV positive group were 31.9, 2.33, and 391.2, respectively, while the same values of the control group were determined as 6.39, 0.37 and 187.7, respectively. The optimized cut-off points of NEU/LYM, MONO/LYM, and PLT/LYM values in estimating CDV patients were also determined as NEU/LYM =7.6, MONO/LYM=0.61 and PLT/LYM=0.852, respectively. In conclusion, this preliminary study suggests that NEU/LYM, MONO/LYM, and PLT/LYM values may be inexpensive, objective, and non-invasive new marker candidates for clinicians to support the diagnosis of CDV infected dogs in suspicious circumstances.

**Keywords:** Canine distemper virus, dog, monocyte to lymphocyte ratio, neutrophil to lymphocyte ratio, platelet to lymphocyte ratio.

# Kanin Distemper Virus İle Enfekte Köpeklerde Nötrofil/Lenfosit, Monosit/Lenfosit Ve Platelet/Lenfosit Oranlarının Belirlenmesine Yönelik Bir Ön Çalışma

***Sorumlu yazar:**
Didem PEKMEZCİ
Ondokuz Mayıs Üniversitesi Veteriner Fakültesi İç Hastalıkları Anabilim Dalı, TR-55200 Samsun–TÜRKİYE
✉: dkazanci@omu.edu.tr

**Öz:** Sunulan ön çalışma ile doğal olarak Kanin Distemper Virüs (KDV) ile enfekte köpeklerin tedavi öncesi Nötrofil/Lenfosit (NEU/LYM), Monosit/Lenfosit (MONO/LYM), ve Platelet/Lenfosit (PLT/LYM) oranları ile tam kan sayımı (TKS) verilerin sağlıklı bireyler ile karşılaştırılması amaçlanmıştır. Bu amaçla retrospektif olarak Veteriner Fakültesi, Hayvan Hastanesi kayıtlarında yer alan 22 adet sahipli, 3-12 (6,2) aylık, KDV enfeksiyonu tanısı almış ve hızlı test kitleri ile pozitifliği saptanan köpekler çalışma grubunu oluşturmuştur. Diğer taraftan, 8 adet sahipli, 2-10 (8,2) aylık ve sağlıklı olduğu tespit edilen köpek ise kontrol grubunu oluşturmuştur. Gruplar arasında tedavi öncesi NEU/LYM, MONO/LYM, PLT/LYM ve TKS parametrelerinin karşılaştırılmasında KDV pozitif grubu medyan NEU/LYM, MONO/LYM, PLT/LYM ile TKS değerlerinden sadece LYM, EOS, %NEU, %LYM, %EOS, %BAS, MCH, MCHC, RDWCV, PLT değerleri arasında istatistiksel fark ($P< 0,05$) bulunmuştur. KDV pozitif grubu medyan NEU/LYM, MONO/LYM ve PLT/LYM düzeyleri ise sırasıyla 31,9, 2,33 ve 391,2 olarak bulunurken kontrol grubunun aynı değerleri sırası ile 6,39, 0,37 ve 187,7 olarak tespit edilmiştir. KDV hastalarının tahmin edilmesinde aynı zamanda NEU/LYM, MONO/LYM ve PLT/LYM değerlerinin optimize edilmiş kesim noktaları ise sırası ile NEU/LYM =7,6, MONO/LYM=0,61 ve PLT/LYM=0,852 olarak tespit edilmiştir. Sonuç olarak, bu ön çalışma NEU/LYM, MONO/LYM ve PLT/LYM değerlerinin KDV ile enfekte köpeklerin şüpheli durumlarda tanılarının desteklenmesinde klinisyenlerce kullanılabilecek ucuz, objektif ve invazif olmayan yeni belirteç adayı olabileceklerini düşündürmektedir.

**Anahtar kelimeler:** Kanin distemper virüs, köpek, monositin lenfosite oranı, nötrofilin lenfosite oranı, plateletin lenfosite oranı.

## INTRODUCTION

Canine distemper Virus (CDV) is a worldwide occurring fatal and highly contagious systemic infectious disease affecting terrestrial carnivores including Canidae, Mustelidae, Ailuridae, Mephitidae, Ursidae, Felidae, Viverridae, and Procyonidae families (Deem et al., 2000; Martinez-Gutierrez et al., 2016). Immune status and the age of the host with the virulence of the strain are the factors affecting the severity of the disease (Loots et al., 2017). Essentially, manifestations of the disease range no-specific symptom to severe clinical signs such as abnormal behavior, incoordination, and convulsions up to paralysis with a 50% mortality rate (Appel, 1970; Krakowka et al., 1980; Moritz et al., 2000). However, fever, pneumonia, and most critically, leukopenia is seen almost in all cases (Martinez-Gutierrez et al., 2016).

Whereby early detection of CDV infection could allow appropriate treatment quickly. Such a test helps in reducing the morbidity and mortality associated with CDV infections to other animals (Dong et al., 2008). Therefore, early diagnosis such lateral flow assays are much important at field level (Desai et al., 2021). On the other hand, false negative results can be obtained in rapid test kits due to insufficient samples or other factors. Molecular based techniques like Reverse transcriptase PCR can be used for the confirmatory diagnosis of CDV infections (Kim et al., 2001; Desai et al., 2021), but it requires costly machine, skills, and longer period (Desai et al., 2021). Although PCR is the most accurate and precise technique, it has disadvantages in terms of qualified personnel, cost, and time. Point of care-based diagnostic aids like lateral flow assay are much important at field level for early diagnosis (Desai et al., 2021). Since early detection of CDV would allow appropriate treatment quickly. Therefore, clinicians need inexpensive and rapid diagnostic methods that will confirm the diagnosis as well as the results of rapid test kits, the presence of the patient's clinical findings.

In this regard, NEU/LYM, MONO/LYM and PLT/LYM ratios as bedside parameters, which are frequently used in cardiovascular diseases (Li et al., 2017), malignancies (Xiang et al., 2017), acute pancreatitis (Wang et al., 2017), autoimmune diseases (Chandrashekara et al., 2017) and chronic obstructive pulmonary disease (Yao et al., 2017) in human medicine, can be used as markers of poor prognosis or major inflammation in dogs with CDV infections in dogs. Nowadays researches on NEU/LYM, MONO/LYM and PLT/LYM in veterinary medicine, have been increasing (Burton et al., 2013; Rejec et al., 2017; Troìa et al., 2017; Hodgson et al., 2018; Çakir & Pekmezci 2019; Benvenuti et al., 2020; Pierini et al, 2020; Becher et al, 2021; Conway et al., 2021; Gori et al., 2021; Neumann, 2021; Pekmezci & Çolak, 2021). Therefore, the main purpose of this study is to investigate whether these easy and cost-effective values can contribute to the clinician's diagnosis of CDV infection after comparing the NEU/LYM, MONO/LYM and PLT/LYM values in dogs with naturally CDV Ag positive and clinical symptoms with healthy dogs.

## MATERIAL AND METHOD

***Study Materials:*** The medical record database at the Veterinary Teaching Animal Hospital was searched for 22 cases of CDV infected dogs and randomly chosen 8 clinically health dogs between March 2020 and March 2022. Although the study was carried out retrospectively through patient records, informed consent was obtained from the patients.

***Study Group:*** Dogs were included if they had a definitive diagnosis of “Canine Distemper” at the presentation. Definitive diagnosis was made based on the clinical manifestations and a positive CDV Ag test result of the cases. CDV Ag results of conjunctival samples were studied with test kits. Pretreatment WBC at the time of admission to the Veterinary Teaching Animal Hospital from the recording software of 22 dogs with their demographic data were then transferred to an Excel file.

***Control Group:*** The medical record database was also searched for patients who brought to the Veterinary Teaching Animal Hospital for a routine checkup or for an elective surgery. Eight clinically healthy dogs were randomly chosen for control group and their whole blood counts at the time of admission to the Veterinary Teaching Animal Hospital from the recording software with their demographic data were also transferred to an Excel file.

***Whole Blood Count Records:*** Whole blood counts were formed before the treatment and controls of the patients in the study group (n=22) and control group (n=8). In our hospital's routine practice, 2 ml blood samples from *vena cephalica antebrachii* collected into tubes with EDTA, and then rotated in the mixer at medium speed for 5 minutes, run with a Veterinary Automatic Hematology Analyzer and the results are transferred to the patient registration program via the digital system.

In this context, all the hemogram records of the above-mentioned 30 patients were accessed completely. NEU/LYM, MONO/LYM, EOS/LYM and PLT/LYM values were obtained by dividing LYM into NEU, MONO, EOS and PLT values separately as previously reported (Rossi et al., 2017).

***Statistical Analyses:*** Statistical analyses for comparing WBC values of the study and control groups were performed using "SPSS 21.0.0. Windows" software. Shapiro-Wilk, the skewness and kurtosis, detrended probability plot, histogram, and coefficient of variance

analyzes were used for checking the normality assumption to decide whether a parametric or non-parametric test needs to be used. Therefore, Mann-Whitney *U t*ests were used to test the statistical significance of differences in median values of categorical variables. The receiver operator characteristics area under the curve was used to assess the optimal cut off values for the NEU/LYM, MONO/LYM and PLT/LYM to differentiate CDV positive dogs from healthy controls with their corresponding sensitivity and specificity values. A P value < 0.05 accepted statistically significant.

## RESULTS

The age of the dogs in the study group was 3-12 (6.2) months, 11 of them were female and 10 of them were male dogs, and the gender record of 1 of them could not be reached. The ages of 8 healthy dogs in the control group were 2-10 (8.2) months, and it was determined that 4 of them were female and 4 of them were male. The data on the age, gender, and breeds of the dogs belonging to the study and control groups are presented in Table 1.

**Table 1:** Breed, age and sexes in the study and control dogs.

| Case Number | Study group (n=22) | | | Control group (n=8) | | |
|---|---|---|---|---|---|---|
| | Breed | Mean Age (6,2) Months | Sex | Breed | Mean Age (8,2) Months | Sex |
| 1 | German shepherd | 9 | Female | French Bulldog | 10 | Female |
| 2 | Kangal shepherd | 6 | Male | Mix breed | 2 | Male |
| 3 | Golden retriever | 6 | Male | Pointer | 4 | Female |
| 4 | Mix breed | 12 | Female | Mix breed | 6 | Male |
| 5 | Maltase terrier | 12 | Male | Mix breed | 4 | Male |
| 6 | Rottweiler | 12 | Male | Terrier | 5 | Female |
| 7 | Mix breed | 2 | Female | Mix breed | 2 | Male |
| 8 | Mix breed | 12 | Male | Cocker spaniel | 4 | Female |
| 9 | Kangal shepherd | 3 | Male | | | |
| 10 | Mix breed | 4 | Female | | | |
| 11 | Mix breed | 7 | Male | | | |
| 12 | German shepherd | 6 | Male | | | |
| 13 | Caucasian shepherd | 9 | Female | | | |
| 14 | Mix breed | 4 | Female | | | |
| 15 | German shepherd | 4 | Male | | | |
| 16 | Pincher | 8 | Female | | | |
| 17 | German shepherd | 3 | Female | | | |
| 18 | Mix breed | 3 | Female | | | |
| 19 | Kangal shepherd | 4 | Female | | | |
| 20 | Terrier | 4 | Unknown | | | |
| 21 | Golden retriever | 5 | Female | | | |
| 22 | Belgian shepherd | 3 | Male | | | |

Clinical symptoms consistent with CDV infection of the dogs in the study group are shown in Table 2.

**Table 2:** Clinical symptoms of the study dogs.

| Case number | Anorexia | Vomiting | Diarrhea | Prulent eye discharge | Prulent nose discharge | Hard pad | Neorological signs |
|---|---|---|---|---|---|---|---|
| 1 | | + | + | + | | | |
| 2 | + | | | + | + | | |
| 3 | + | | | + | | | |
| 4 | | | | | + | + | |
| 5 | + | | | + | | | |
| 6 | | | | | | | N, T, E |
| 7 | | | | + | + | | |
| 8 | | | | + | + | | |
| 9 | | | | | | | T, E |
| 10 | + | | | + | | | |
| 11 | | | | + | + | | T |
| 12 | | | | | | + | T |
| 13 | | | | | + | + | |
| 14 | | + | + | + | + | | |
| 15 | + | | | + | | | |
| 16 | | + | + | + | | | |
| 17 | + | | | + | | | |
| 18 | | | | + | + | | N, T |
| 19 | | | | + | + | | |
| 20 | + | | + | + | + | | |
| 21 | + | | | + | | | |
| 22 | + | + | | + | | | T |

When assessing median values of hemogram and NEU/LYM, MONO/LYM and PLT/LYM ratios, there were significant differences for dogs with CDV infections when compared with the healthy population (Table 3). Median values of hemogram parameters; LYM, EOS, NEU%, LYM%, EOS%, BAS%, MCH, MCHC, RDWCV, PLT values were found statistically different P< 0.05 (Table 3) when comparing the median values between groups.

**Table 3:** Median values of hemogram parameters and NEU/LYM, MONO/LYM, EOS/LYM, PLT/LYM values. * P< 0.05

| Parameter | Groups | | P value |
|---|---|---|---|
| | Study Median (Min.-Max.)(n=22) | Control Median (Min.-Max.) (n=8) | |
| WBC $10^9$/L | 10.13 (5.56-39.14) | 12.59 (6.85-14.71) | .344 |
| NEU $10^9$/L | 8.19 (4.36-35.34) | 9.5 (3.98-11.48) | .730 |
| LYM $10^9$/L | 0.2 (0.07-1.72) | 1.7 81.16-3.47) | .000* |
| MON $10^9$/L | 0.7 (0.05-5.12) | 0.6 (0.45-1.22) | .696 |
| EOS $10^9$/L | 0.14 (0.02-0.67) | 0.41 (0.11-0.72) | .013* |
| BAS $10^9$/L | 0.01(0.0-0.12) | 0.03 (0.0-0.06) | .475 |
| NEU % | 0.86 (0.71-0.95) | 0.78 (0.56-0.83) | .001* |
| LYM % | 0.03 (0.0-0.17) | 0.12 (0.1-0.35) | .000* |
| MON % | 0.07 (0.0-0.6) | 0.06 (0.04-0.08) | .277 |
| EOS % | 0.01 (0.0-0.08) | 0.04 (0.0-05) | .010* |
| BAS % | 0.0 (0.0-0.1) | 0.0 (0.0-0.0) | .001* |
| RBC $10^{12}$/L | 4.5 (2.5-7.45) | 5.3 (4.21-6.46) | .202 |
| HGB g/dL | 11 (6.1-18.9) | 14.5 (9.3-17.3) | .063 |
| HCT % | 30 (17-52) | 37 (25-43) | .202 |
| MCV fL | 65.6 (53.9-75.9) | 67.7 (61.2-71.8) | .420 |
| MCH pg | 24.4 (20-26.9) | 26.3 (22-27.8) | 0.12* |
| MCHC g/L | 365 (337-390) | 387.5 (360-396) | 0.04* |
| RDW-CV % | 15 (12-24) | 12 (11-17) | 0.006* |
| RDW-SD fL | 38.6 (32.6-53.4) | 37.1 (32.9-42.5) | .156 |
| PLT $10^9$/L | 176 (32-595) | 290 (160-497) | .040* |
| MPV fL | 9.9 (7.1-17.6) | 9.4 (8.5-11.9) | .872 |
| PDW % | 15.8 (15-18) | 15.5 (15.2-15.8) | .063 |
| PCT ml/L | 1.84 (0.24-5.96) | 2.97 (1.91-4.24) | .031 |
| NEU/LYM | 31.9 (4.81-167.7) | 6.39 (1.60-7.65) | .000* |
| MONO/LYM | 2.33 (0.03-25.6) | 0.37 (0.17-0.67) | .000* |
| EOS/LYM | 0.40 (0.02-9.57) | 0.22 80.05-0.48) | .142 |
| PLT/LYM | 391.2 (100-4885) | 187.7 (86-331.3) | .003* |

Study median NEU/LYM value (31.9) was significantly higher P< .000 when comparing the control median value (6.39) (Table 3, Figure 1). Study median MONO/LYM level (2.33) found statistically higher (P< .000) than the median MONO/LYM level (0.37) of healthy dogs (Table 3, Figure 1). Likewise, median PLT/LYM value (391.2) of the study group was found statistically (P< .003) higher than the median PLT/LYM value (187.7) of control dogs (Table 3, Figure 1).

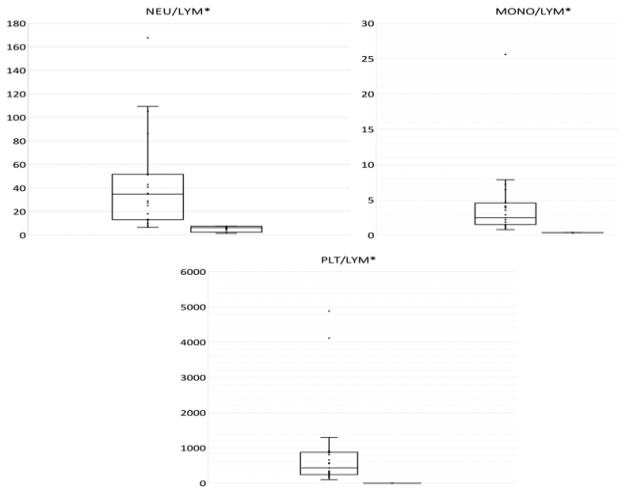


**Figure 1:** Box-and-whiskers plot of pretreatment NEU/LYM, MONO/LYM, and PLT/LYM values in dogs with infected CDV (left box) or healthy controls (right box). The top and bottom of the shaded boxes represent the 75$^{th}$ and 25$^{th}$ percentile, respectively. The median is denoted within each box. The whiskers extend up to the maximum, and down to the minimum. *P< 0.05

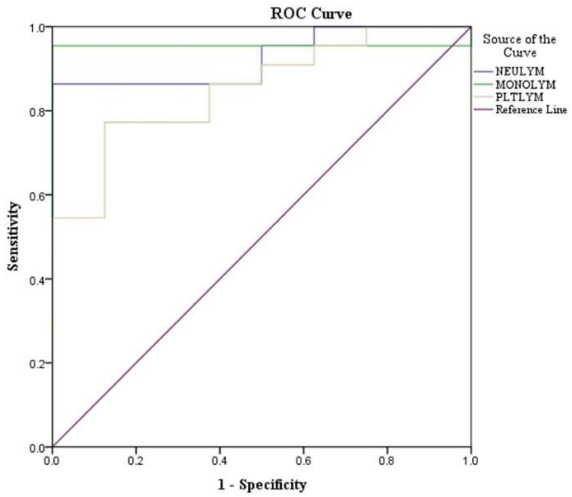


**Figure 2:** Receiver operating characteristic (ROC) curve illustrating the sensitivity and specificity of NEU/LYM, MONO/LYM and PLT/LYM ratio to distinguish dogs with CDV infection and health dogs.

**Table 4:** Areas under the ROC Curve (AUC), Sensitivity and spesifity by the optimized Cut off points for the NEU/LYM, MONO/LYM and PLT/LYM in predicting CDV patients.

| Risk factor | AUC (95%) | Cut off | P value | Sensitivity % | Specifity % |
|---|---|---|---|---|---|
| **NEU/LYM** | 0.926 (0.833-1) | 7.6 | .000 | 86.4 | 87.5 |
| **MONO/LYM** | 0.955 (0.868-1) | 0.61 | .000 | 95.5 | 87.5 |
| **PLT/LYM** | 0.852 (0.712-0.993) | 267 | .004 | 77.3 | 87.5 |

## DISCUSSION AND CONCLUSION

The main purpose of the present study was to evaluate the pretreatment NEU/LYM, MONO/LYM and PLT/LYM ratios in the dogs naturally occurring CDV infection. For this reason, we had to analyze the WBC initially. Interestingly, there are little studies investigated the WBC values of the CDV infected dogs in the literature. There may be several reasons for this. But one of the most important reasons is the belief that the WBC is nonspecific for CDV infection in dogs. In the past, this argument was acceptable, but today we are collecting new data that we can use, this inexpensive, cost- and equipment-free method, more effectively in our CDV-infected patients.

A study which investigates the clinical and molecular aspects of a canine distemper outbreak and vector-borne infections in a group of importing rescue dogs, reported that anemia, leukocytosis, eosinophilia, neutrophilia, and monocytosis were the initial and common WBC findings of the dogs with CDV infection (Willi et al., 2015). Another study which investigated haematologic parameters in the CDV infected dogs showed that there was a remarkable anaemia and lymphopenia in dogs infected with CDV (Ezeibe & Udegbunam, 2008). A decrease in RBC, HGB, MCH and MCHC values and a mild normochromic, normocytic anemia in the pre-treatment haemogram analyzes was also observed in the dogs infected with CDV in a recent study (Daldaban et al., 2021).

Our results showed that there were statistically significant differences in LYM, EOS, NEU%, LYM%, EOS%, MCH, MCHC, RDW-CV%, PLT values between CDV infected and healthy dogs (Table 3). There was a significant anemia found in our CDV infected dogs. Thus, there was a decrease seen in RBC, HGB, MCH and MCHC values in the CDV infected dogs, which shows combability with the other studies (Ezeibe & Udegbunam, 2008; Willi et al., 2015; Daldaban et al., 2021). Anemia observed in the CDV infected dogs could be explained by the over production of inflammatory mediators, which could inhibit erythropoiesis and shorten RBC life span (Meyer and Harvey, 1998).

Moreover, lymphopenia in this study dogs infected with CDV also consisted with another study reported by (Ezeibe & Udegbunam, 2008). On the other hand, our WBC, NEU, MONO and EOS values were in the reference ranges and differed from the study previously done by (Willi et al., 2015). However, our NEU%, LYM%, and EOS% values were increased in the CDV infected dogs. Another important parameter PLT levels were also stated in the reference in our study dogs same as others (Daldaban et al., 2021). PLT has a main role of platelets in inflammation and infection which they modulate leukocytosis function by phagocytosis (Jenne & Kubes, 2015). Daldaban et al. (2021) also reported that PLT has roles in the development of clinical findings in the acute phase of CD and in the formation of immunity against the virus, especially with TLR4 gene expression. Moreover, it is known local disturbance of blood circulation that compromises congestion, edema, thrombosis, and the existence of disseminated intravascular coagulation in CDV infected dogs' grey matter, white matter, and meninges (Pan et al., 2013).

In humans, NEU/LYM, MONO/LYM and PLT/LYM ratios that can be calculated using WBC and is easily accessible accepted as an independent predictive index of morbidity and mortality in sepsis and the results have consistently shown that higher NEU/LYM, MONO/LYM and PLT/LYM ratios are independent diagnostic and prognostic markers (Zahorec, 2021). Today these ratios are also have been started to be studying in various disease of the dog and cat (Burton et al., 2013; Rejec et al., 2017; Troìa et al., 2017; Hodgson et al., 2018; Çakir & Pekmezci 2019; Benvenuti et al., 2020; Pierini et al, 2020; Becher et al., 2021; Conway et al., 2021; Gori et al., 2021; Neumann, 2021; Pekmezci & Çolak, 2021; Park et al., 2022).

This retrospective study suggests that dogs with naturally infected with CDV have an increased NEU/LYM, MONO/LYM and PLT/LYM ratios compared with healthy dogs.

In a study investigating the NEU/LYM ratio in inflammatory bowel disease in dogs, found a moderate correlation between the NEU/LYM ratio and the canine chronic enteropathy clinical activity index, and the mean

NEU/LYM ratio of dogs diagnosed with inflammatory bowel disease was 4.28, while the healthy control group's mean NEU/LYM ratio was 3 (Benvenuti et al., 2020). On the other hand, Pekmezci & Çakır (2019) found NEU/LYM ratio as 7.2 of the dogs infected with *Borrelia burgdorferi* and 5.2 in the healthy group dogs. In another study, in which dogs naturally infected with Parvovirus (PVI) and healthy controls were compared, the researchers found the mean NEU/LYM ratio as 4.3 in the PVI group (Pekmezci & Çolak, 2021). Hodgson et al. (2018) found the mean NEU/LYM as 25.1 in dogs with sepsis and 3.9 in healthy dog population. They also showed that a cutoff value of NEU/LYM equal or above 6 had an 84.39% sensitivity and 86.95% specificity to identify dogs with systemic inflammatory states (Hodgson et al., 2018). Another study conducted by Conway et al. (2021) demonstrates that dogs with pneumonia had median 16.7 NEU/LYM and the NEU/LYM did not differ significantly between the survivors and non-survivors, nor did total WBC count or NEU count. Park et al. (2022) also showed the NEU/LYM could be a biomarker for diagnosing meningoencephalitis of unknown etiology and distinguishing it from other intracranial diseases in dogs. The median NEU/LYM ratio was 6.08 in the meningoencephalitis of unknown etiology (Park et al., 2022). In the present study, we found a median NEU/LYM value as 31.9 in dogs with CDV infection which found significantly higher (P< .000) when comparing the control median value of 6.39. We also found a cutoff value of NEU/LYM = 7.6, area under the curve = 0.926, sensitivity = 86.4%, and specificity = 87.5% is specificity to identify dogs with CDV. Our results consisted with a high NEU/LYM value in the diseases associated with inflammation previously reported (Hodgson et al., 2018). We found 7.6 as the cutoff value for NEU/LYM for the CDV infected dogs for distinguish them from healthy ones, similarly Hodgson et al. (2018). Contrary to other studies (Çakir & Pekmezci 2019; Benvenuti et al., 2020; Pekmezci & Çolak, 2021), our NEU/LYM ratio was found to be high in the control dogs.

Studies found a relationship between NEU/LYM, MONO/LYM, and fibrinogen levels to inflammation and infection in human medicine (Kartal & Kartal, 2017; Huang et al., 2018). On the other hand, the number of studies investigating the MONO/LYM ratios of dogs in various diseases is quite limited, we previously compared the MONO/LYM ratios of naturally infected dogs with *B. burgdorferi* and healthy groups and did not find a statistical difference between the two groups (Çakir & Pekmezci 2019). Meanwhile we found the mean MONO/LYM ratios of the infected dogs with *B. burgdorferi* and control group 0.7 and 0.4, respectively. Although a similar situation was observed in another of previously study, the mean MONO/LYM ratio in the PVI and control group was found to be 1.2, and 0.4, respectively (Pekmezci & Çolak, 2021). Contrary to previously reports (Çakir & Pekmezci 2019; Pekmezci & Çolak, 2021) we found a median MONO/LYM level as 2.33 in the CDV infected dogs. Also, an optimal MONO/LYM = 0.61, area under the curve = 0.955, sensitivity = 95.5%, and specificity = 87.5% cutoff values between CDV infected and healthy dogs determined for the first time within this present study.

In a retrospective study comparing the NEU/LYM, PLT/LYM, and other WBC indices of dogs with periodontitis and oropharyngeal tumors with healthy dogs, researchers found that healthy dogs (n=71) had the lowest mean PLT/LYM values (145.31) when comparing the periodontitis and oropharyngeal indices compared to other groups (Rejec et al., 2017). They also reported that the PLT/LYM values of the periodontitis and oropharyngeal groups were 224 and 290.5, respectively (Rejec et al., 2017). Our median PLT/LYM value 187.7 of healthy dogs in the control group was found to be like those previously reported 145.31 (Rejec et al. 2017), 182.8 (Çakir & Pekmezci 2019), 164 (Neumann, 2021), and 135.26 (Pekmezci & Çolak, 2021). Moreover, our study CDV infected dogs' median PLT/LYM ratio 391.2 is consisted with previously reported studies (Rejec et al., 2017; Pekmezci & Çolak, 2021). There is an optimal PLT/LYM cutoff between CDV infected and healthy dogs determined as PLT/LYM = 0.852, area under the curve = 267, sensitivity = 77.3%, and specificity = 87.5% for the first time within this present study. Platelets are involved in inflammation and infection by modulating leukocytosis function with phagocytosis (Jenne & Kubes, 2015). Therefore, it may be elevated in CDV infected dogs, as in diseases such as PVE, periodontitis and oropharyngeal tumors. However, the evaluation of platelet count alone will of course not be sufficient in acute and chronic infections.

In conclusion, the NEU/LYM, MONO/LYM and PLT/LYM values was able to discriminate dogs with naturally CDV infected from a healthy population in this preliminary study, and these easy, low-cost, objective, and non-invasive parameters could contribute the clinician for a definitive diagnosis under field and shelter population management. Further studies are strictly needed for NEU/LYM, MONO/LYM and PLT/LYM values in the Veterinary field.

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