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Diarrhea in Cats Infected with *Cystoisospora* spp. - Evaluation of the Neutrophil-to-Lymphocyte Ratio and Monocyte-to-Lymphocyte Ratio

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ABSTRACT

***Background*:** *Cystoisospora* spp. is considered a potential pathogen to cause diarrhoea in cats, and the disease occurs clinically, especially in young, weak and immunocompromised cats. The present study aimed to evaluate the clinical utility and diagnostic roles of neutrophil-to-lymphocyte ratio (NLR) and monocyte-to-lymphocyte (MLR) ratios in diarrheal cats infected with Cystoisospora spp.

***Materials, Methods & Results*:** Twenty-five cats with diarrhea due to infection caused by *Cystoisospora* spp. and 15 healthy cats were included in this study. The anamnesis, clinical and laboratory findings of all cats were recorded. A clinical severity score was generated for diarrheal cats, which included activity, appetite, presence of vomit, and faecal consistency. Also, cats infected with *Cystoisospora* spp. were classified as systemic inflammatory response syndrome (SIRS) positive (+) and SIRS (-) group according to the presence of at least 3 of the SIRS criteria. The *Cystoisospora* spp. oocysts were diagnosed by direct smear and simple faecal flotation. Cats found positive for other parasitic and protozoal agents by faeces examination were excluded from the study. Complete blood counts (CBC) were performed with an automated blood cell counter for all cats. Blood smears were prepared (with May-Grunwald Giemsa) from samples, and the CBC results were confirmed microscopically (obj.100x). White blood cell (WBC), neutrophil, lymphocyte and monocyte counts were recorded directly from the CBC. The neutrophil-to-lymphocyte ratio was calculated as the ratio of the absolute neutrophil count to the absolute lymphocyte count. The monocyte-to-lymphocyte was calculated as the absolute monocyte count ratio to the absolute lymphocyte count. White blood cell ($P = 0.015$), neutrophil ($P = 0.001$), monocyte ($P = 0.033$), NLR ($P = 0.000$) and MLR ($P = 0.042$) of diarrheal cats infected with *Cystoisospora* spp. were significantly higher than in the healthy group. There was no significant difference between groups in the lymphocyte counts ($P > 0.05$). SIRS (+) group had statistically significantly higher WBC ($P = 0.014$), neutrophil counts ($P = 0.000$), NLR ($P = 0.000$) and MLR ($P = 0.037$) than the healthy group. Also, neutrophil counts and NLR of the SIRS (+) group were statistically significantly higher than the SIRS (-) group ($P = 0.032$; $P = 0.001$, respectively). However, there was no significant difference SIRS (+), SIRS (-), and healthy groups regarding lymphocyte and monocyte counts ($P > 0.05$). The clinical severity of the disease positively correlated with NLR and MLR. Also, the best cut-off value of NLR to predict SIRS was > 1.67, with 92.86% sensitivity and 84.62% specificity. MLR was not a useful predictor for SIRS.

***Discussion*:** NLR and MLR are parameters derived from complete blood count. In recent years, they have been used in human and veterinary medicine as a biomarker in the diagnosis, prognosis and treatment follow-up of various diseases and provide valuable information about inflammation. In cats, there are limited studies on NLR, and to our knowledge, there are no studies on MLR. The current study suggests that NLR and MLR may be used to determine the severity of the inflammatory condition in diarrheal cats infected with *Cystoisospora* spp., and NLR is a useful marker for predicting SIRS status in these cats. In addition, this study also will contribute to larger-scale studies in the future.

Keywords: cats, complete blood count, *Cystoisospora*, diarrhoea, NLR, MLR, SIRS.

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INTRODUCTION

Diarrhoea is defined as an increase in the amount of fluid in the stool, its volume and the frequency of defecation [24]. Although it arises from multiple causes and pathophysiological mechanisms, infectious causes such as endoparasites, protozoans, and viral and bacterial agents are believed to play an important role in cats [4,24]. *Cystoisospora* species, one of the coccidia group's infectious agents, are considered a potential pathogen to cause diarrhoea in cats [13,23]. *Cystoisospora* spp. is host-specific, and *Cystoisospora felis* and *C. rivolta* species are responsible for the occurrence of the disease in cats [13,23].

The neutrophil-to-lymphocyte ratio (NLR) and monocyte-to-lymphocyte ratio (MLR) are rapidly performed, low-cost, and biomarkers that can be calculated from the complete blood count (CBC). NLR and MLR are obtained by dividing the total number of neutrophils (segmented and band neutrophils) and monocytes by the number of lymphocytes, respectively. They have recently been increasingly used in human and veterinary medicine to assess inflammatory responses [3,31,32,37]. There is a limited number of studies on NLR in cats. These studies were conducted on mammary tumours [25,29], feline injection-site sarcoma [11], and acute pancreatitis [26]. The diagnostic and prognostic importance of NLR has been demonstrated in these studies. Also, to our knowledge, there is no study on MLR. Therefore, this study aimed to evaluate the clinical utility and diagnostic roles of NLR and MLR in diarrheal cats infected with *Cystoisospora* spp.

MATERIALS AND METHODS

Cats and inclusion criteria

This study was conducted from December 2021 through May 2022 at the Department of Internal Medicine of Aydın Adnan Menderes University Veterinary Faculty and some private veterinary clinics and animal hospitals in Aydın, İzmir, Manisa, Denizli and Muğla provinces. Forty client-owned cats were enrolled in this study. Twenty-five diarrheal cats infected with *Cystoisospora* spp. and 15 healthy cats were included. The anamnesis, clinical and laboratory findings of all cats were recorded. Inclusion criteria included having a stool score of ≥ 6 (Nestle Purina Fecal Scoring System), the presence of only *Cystoisospora* spp. oocyst in microscopy, anamnesis findings related to immunosuppression, younger than 6 months of age, and not using any medication before.

Laboratory analysis

Blood samples were collected from cats into tubes with anticoagulants to calculate haematological parameters (ethylenediaminetetraacetic acid). CBC was performed with an automated blood cell counter[1]. White blood cell (WBC), neutrophil, lymphocyte and monocyte counts were recorded directly from the CBC. Blood smears were prepared (with May-Grunwald Giemsa) from samples, and the CBC results were confirmed microscopically (100x). NLR was calculated as the ratio of the absolute neutrophil count to the absolute lymphocyte count. MLR was calculated as the absolute monocyte count ratio to the absolute lymphocyte count. Also, cats were tested for Feline immunodeficiency virus (FIV)[2], Feline leukaemia virus (FeLV)[2] and Feline coronavirus (FCov)[3] with commercial test kits. Cats positive for these diseases were not included in the study.

The faecal samples of cats were collected fresh to evaluate the presence of worm eggs and protozoal oocysts. The *Cystoisospora* spp. oocysts were diagnosed by direct smear and simple faecal flotation [16]. Slides were scanned using a light microscope (10x) for the presence of oocysts, and *Cystoisospora* spp. was identified based on egg morphology (40x) [Figure 1] [38]. Samples found positive for other parasitic and protozoal agents by faeces examination were excluded from the study.

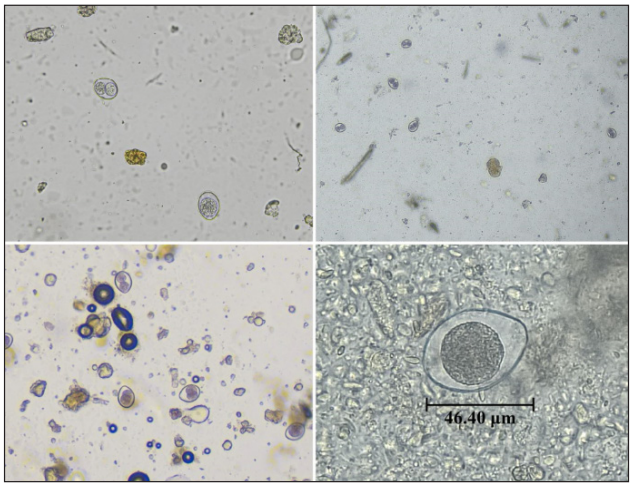

Figure 1. *Cystoisospora* spp. oocysts from cats feces (sporulated and unsporulated).

A clinical severity score was generated for cats, which included activity, appetite, presence of vomit, and faecal consistency (Table 1). Diarrheal cats infected with *Cystoisospora* spp. were classified as systemic inflammatory response syndrome (SIRS) positive (+) and SIRS (-) group according to the presence of at least 3 of the SIRS criteria [8]. SIRS criteria included abnormal rectal temperature (≥ 39.7°C or ≤ 37.8°C), a heart rate (≥ 225 beats/min or ≤ 140 beats/min), a respiratory rate (≥ 40 breaths per min) and a WBC count (≥ 19.500/μL or ≤ 5000/μL).

Table 1. A clinical severity score system of diarrheal cats infected with *Cystoisospora* spp.

	0: Normal	1: Mild	2: Moderate	3: Severe
Attitude	Normal	Depressed	Lethargic	Comatose
Appetite	Normal	Mildly decreased	Moderately decreased	Severely decreased
Vomiting (times/day)	0	1	2	> 3
Stool	diarrhoea	Watery diarrhoea	Mucus diarrhoea	Hemorrhagic diarrhoea

Statistical analysis

Although the data showed normal distribution according to the Shapiro-Wilk normality test results, non-parametric tests were used considering the sample size. For this reason, the Mann-Whitney U test was used to compare 2 groups, and the Kruskal-Wallis test was used to compare 3 groups. Post-hoc Tukey test was used to investigate the differences between groups. Correlations between mean NLR, MLR and disease severity were evaluated with the Spearman correlation coefficient (rho). Diagnostic cut-off values were determined to predict SIRS by receiver operating characteristic (ROC) curve analysis. MedCalc[4] and SPSS[5] statistical software were used for all statistical procedures. Differences in $P < 0.05$ value were considered statistically significant.

RESULTS

Fifteen cats were deemed healthy according to anamnesis, clinical findings, laboratory findings and stool examination. The mean age of healthy cats was 3.10 ± 1.32 months (min-max: 1.5 - 6). Eight cats were male, and 7 were female. The healthy group included several breeds: crossbreed (n = 5), British Shorthair (n = 4), Persian (n = 3) and Siamese (n = 3).

The mean age of diarrheal cats infected with *Cystoisospora* spp. were 3.60 ± 1.34 months (min-max: 1.5 - 6 months) and included 15 male and 10 female cats. The 25 cats with *Cystoisospora* spp. included 6 breeds. The most common breed was crossbreed (n = 8), followed by British short hair (5), Scottish Fold (4), Persian (4), Siamese (3) and Russian Blue (1). According to the SIRS criteria reported for cats [8], 14 cats were SIRS (+), and 11 cats were SIRS (-). The clinical severity score was 3.91 ± 1.38 in SIRS (-) cats and 6.79 ± 1.72 in SIRS (+) cats.

White blood cell ($P = 0.015$), neutrophil ($P = 0.001$), monocyte ($P = 0.033$), NLR ($P = 0.000$) and MLR ($P = 0.042$) of diarrheal cats infected with Cystoisospora spp. were significantly higher than in healthy cats (Table 2). There was no statistical significance between the groups ($P > 0.05$) in the lymphocyte counts (Table 2). The SIRS (+) group had a significantly higher WBC ($P = 0.014$), neutrophil count ($P = 0.000$), NLR (P = 0.000) and MLR (P = 0.037) than the healthy group (Figures 2 & 3). Also, neutrophil counts and NLR of the SIRS (+) group were statistically significantly higher than the SIRS (-) group ($P = 0.032$; $P = 0.001$, respectively). There was no significant difference between SIRS (+), SIRS (-), and healthy groups regarding lymphocyte and monocyte counts ($P > 0.05$) [Figure 2]. The clinical severity of the disease positively correlated with NLR (rho = 0.760; $P = 0.000$) and MLR (rho = 0.425; $P = 0.034$). Also, the best cut-off value of NLR to predict SIRS was > 1.67, with 92.86% sensitivity and 84.62% specificity ($P = 0.000$; AUC 0.912; 95% Cl 0.779 - 0.978) [Figure 4]. MLR was not a useful predictor for SIRS ($P = 0.32$).

DISCUSSION

This study aimed to investigate the clinical and diagnostic usability of NLR and MLR in diarrheal cats infected with *Cystoisospora* spp. The present study demonstrated that NLR and MLR were higher in diarrheal cats infected with *Cystoisospora* spp. than in healthy cats ($P = 0.000$; $P = 0.042$, respectively). In addition, there was a good positive correlation between disease severity and NLR (rho = 0.760; $P = 0.000$) and a weak positive correlation with MLR (rho = 0.425; $P = 0.034$). It has also shown that NLR can be used to predict SIRS.

Table 2. Some complete blood count variables in cats with diarrhea caused by *Cystoisospora* spp. and their statistical significance compared to the healthy cats.

Parameters		Healthy	*Cystoisospora* spp.	*P*
WBC (×10³/µL)	Mean (± SD)	13.46 ± 3.86	20.00 ± 9.40	0.015
	Median	13.82	21.32	
	IQR	11.13 - 16.63	13.19 - 28.23	
	Min - Max	5.58 - 18.31	2.01 - 33.32	
Lymphocyte (×10³/µL)	Mean (± SD)	6.20 ± 2.17	6.10 ± 2.79	0.906
	Median	5.83	6.05	
	IQR	4.55 - 8.39	4.06 - 8.08	
	Min - Max	2.37 - 9.67	0.65 - 10.77	
Monocyte (×10³/µL)	Mean (± SD)	0.6 ± 0.48	1.13 ± 0.84	0.033
	Median	0.66	0.98	
	IQR	0.10 - 0.95	0.27 - 1.88	
	Min - Max	0.04 - 1.35	0.02 - 2.67	
Neutrophil (×10³/µL)	Mean (± SD)	6.06 ± 2.41	12.24 ± 6.68	0.001
	Median	6.34	12.25	
	IQR	4.11 - 7.50	6.22 - 17.96	
	Min - Max	2.35 - 10.59	1.32 - 23.99	
NLR	Mean (± SD)	1.04 ± 0.44	2.06 ± 0.86	0.000
	Median	0.98	1.98	
	IQR	0.66 - 1.54	1.26 - 2.80	
	Min - Max	0.49 - 1.78	0.77 - 3.97	
MLR	Mean (± SD)	0.11 ± 0.09	0.19 ± 0.13	0.042
	Median	0.07	0.19	
	IQR	0.01 - 0.17	0.08 - 0.25	
	Min - Max	0.01 - 0.28	0.01 - 0.49	

IQR: interquartile range; Max: maximum; Min: minimum; MLR: monocyte-to-lymphocyte ratio; NLR: neutrophil-to-lymphocyte ratio; SD: standard deviation; WBC: White blood cells.

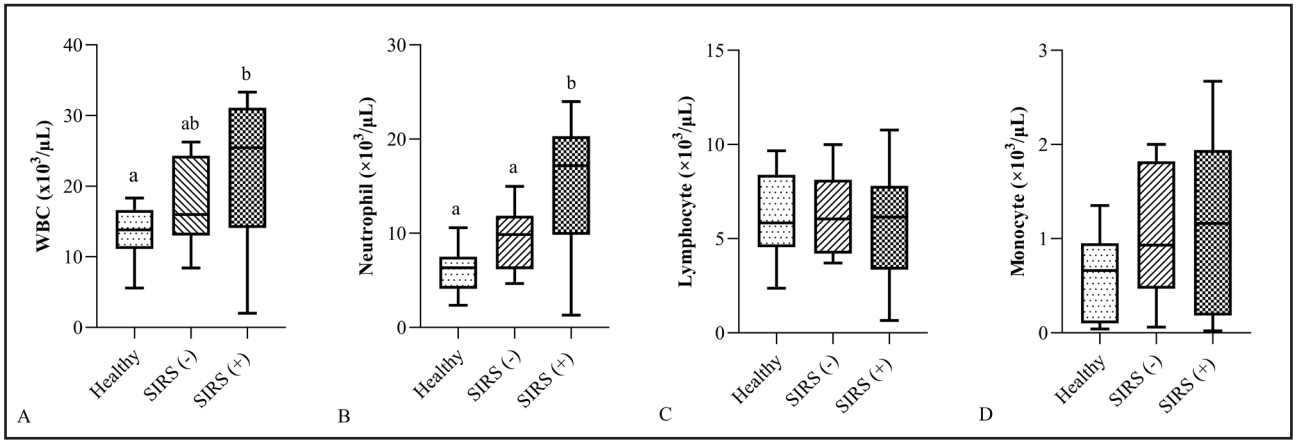

Figure 2. Comparisons of WBC (A), neutrophil (B), lymphocyte (C) and monocyte (D) in healthy, SIRS (+) and SIRS (-) cats with box plot graphs. [The different letters indicate statistical significance ($P < 0.05$)].

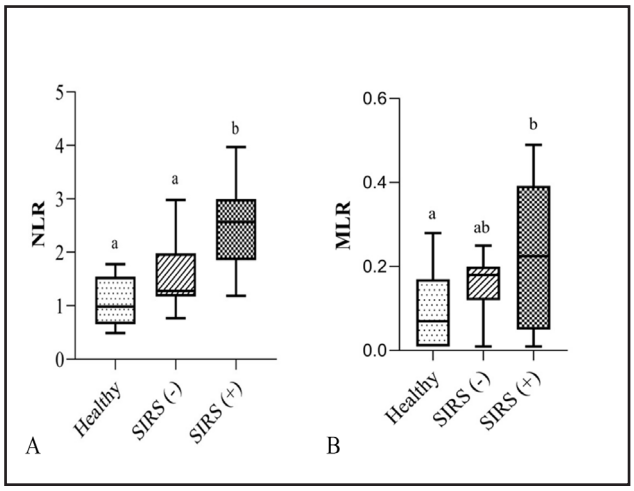

Figure 3. Comparison of NLR (A) and MLR (B) in healthy, SIRS (+) and SIRS (-) cats with box plot graphs. The different letters indicate statistical significance ($P < 0.05$). [NLR: neutrophil-to-lymphocyte ratio; MLR: monocyte-to-lymphocyte ratio; SIRS: systemic inflammatory response syndrome].

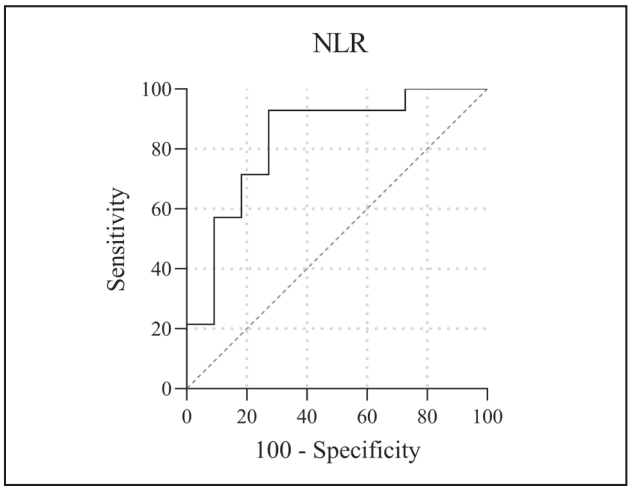

Figure 4. The receiver operating characteristic of NLR for predicting the SIRS in the diarrheal cats infected with *Cystoisospora* spp. AUC = 0.912; 95% CI 0.779 - 0.978. [NLR: neutrophil-to-lymphocyte ratio].

Cystoisospora species cause clinical disease, especially in young, debilitated and immunosuppressed cats. Vomiting, abdominal pain, loss of appetite and watery diarrhoea, sometimes containing blood, are typical clinical signs of *Cystoisospora* spp. infection in cats [13,23,33]. Consistent with this, all cats infected with *Cystoisospora* spp. in this study were younger than 6 months, and there were various clinical signs accompanying diarrhoea. In addition, these cats had stress factors causing immunosuppression, such as separation from their mother, being taken from pet shops or cat catteries and travelling. However, since the clinical findings are not specific in cats infected with *Cystoisospora* spp., the diagnosis needs to be confirmed by laboratory techniques. In this study, the definitive diagnosis of *Cystoisospora* spp. infection was made by direct stool examination and simple faecal flotation [16]. *Cystoisospora* spp. oocysts were defined considering their morphological features. In addition, cats that were positive for other endoparasites and protozoa during these laboratory examinations were also excluded from the study.

In recent years, NLR has been used in human and veterinary medicine as a biomarker in the diagnosis, prognosis and treatment follow-up of various diseases and provides valuable information about inflammation [9,30]. There are limited studies on NLR's diagnostic and prognostic role in animals with diarrhoea in veterinary medicine [5,7,12,28]. The clinical utility of NLR in cats with diarrhoea has not been evaluated in feline medicine. Studies conducted on dogs with acute [12,28] and chronic diarrhoea [5,7] with different etiologies reported that dogs with diarrhoea had higher NLR than the control groups. Similar to the studies mentioned, the NLR values of diarrheal cats infected with *Cystoisospora* spp. were significantly higher than in the control group in our study. Mature and endogenous developmental stages of *Cystoisospora* spp. primarily invade the intestinal epithelial lining and connective tissue under the mucosa, causing diarrhoea. Also, severe diarrhoea characterised by inflammation, oedema and villous necrosis is seen depending on the strain of parasites and concurrent diseases [13]. These situations may lead to an increase in inflammatory markers, resulting in systemic effects. The increase in NLR value may be associated with this inflammatory condition. Also, stress is widespread among cats, and many physiological and pathological conditions lead to stress responses [2,34]. The stress response system includes behavioural, physiological and immunological responses. In response to stress, catecholamines and glucocorticoids are released into the blood. Increasing glucocorticoid levels in the blood of cats redistribute lymphocytes from the blood to the interstitium and cause an increase in circulating neutrophils [34]. The increase in neutrophil count due to stress may also have caused a rise in NLR.

Monocyte-lymphocyte ratio is a parameter derived from complete blood count, like NLR. Recently,

it has been used in human medicine as an inflammatory marker in various conditions such as tumours, heart disease, central nervous system disease, mental illness and inflammatory diseases [10,18,36]. There are limited studies on MLR in veterinary medicine [1,27,31,37], and to our knowledge, there are no studies on MLR in cats. MLR has been studied in dogs with pyometra [37], immune-related hemolytic anaemia [1], sarcoptic mange [27], SIRS and sepsis [31]. These studies demonstrated the diagnostic (dogs with pyometra) and prognostic (dogs with sepsis and IMHA) role of MLR [1,31,37]. This study determined a statistically significantly higher MLR in diarrheal cats infected with *Cystoisospora* spp. compared to the healthy group. Monocytes are an essential component of the innate immune system and may be elevated in various problems, such as bacterial viral, protozoa and fungal diseases [6]. Also, during inflammation, monocytes circulate through the blood and extravasate into inflamed tissues [22]. In this study, increased MLR is associated with increasing monocytes due to the inflammatory condition in diarrheal cats infected with *Cystoisospora* spp.

Systemic inflammatory response syndrome describes a clinical condition characterised by widespread inflammatory system activation secondary to a sterile inflammatory disease or infectious insult [21]. During SIRS, there is an increase in WBC, neutrophils and monocytes and a simultaneous reduction in lymphocytes and eosinophils [20,30]. In this study, SIRS (+) cats' mean WBC and neutrophil counts were significantly higher than in SIRS (-) cats. In addition, Although not statistically significant, the mean monocyte count of SIRS (+) cats was relatively higher than that of SIRS (-) cats. Therefore, SIRS (+) cats had significantly higher MLR and NLR values than SIRS (-) cats. The present study is similar to the previous study findings in cats and dogs with various diseases that suffer from SIRS/sepsis [15,17,30,37]. Gori et al. [15] reported that an NLR value of ≥ 4.53 had a sensitivity of 76% and a specificity of 93.4% in identifying cats with SIRS+sepsis. Similar to the abovementioned study, NLR was a useful marker for predicting SIRS in the current study and the cut-off value for NLR for the diarrheal cats infected with Cystoisospora spp. suffering from SIRS was > 1.67 with 92.86% sensitivity and 84.62% specificity ($P = 0.000$; AUC 0.912; 95% Cl 0.779 - 0.978) [Figure 4]. Different NLR cut-off values in the 2 studies may be related to the fact that the demographic characteristics and diseases of the study groups were other, and therefore the magnitude of the inflammatory response changed. In addition, we think that the 2 studies' preanalytical, analytical and post-analytic variables also affected the results.

Different clinical score systems have been used to determine the clinical severity of diseases in cats with diarrhoea [14,19,35]. In this study, the severity of the disease was determined by modifying the scoring system of Ferri *et al*. [14] in cats with parvovirus. Four clinical parameters were combined to calculate the clinical score of cats with diarrheal cats infected with *Cystoisospora* spp. (Table 1). In this context, activity, appetite, vomiting and stool were evaluated. Since a score of 5 can be expected when calculating the stool score in kittens, scores of 6 and above were considered diarrhoea. No studies evaluate the relationship between the clinical score system and NLR and MLR in cats with diarrhoea. However, different studies on dogs with acute and chronic diarrhoea have shown a positive correlation between the diarrhoea severity index (acute diarrhoea severity index and chronic enteropathy clinical activity index, respectively) and the NLR [7,12]. Similar to these studies, our study determined a positive correlation between the clinical score system and NLR (rho = 0.802; $P = 0.000$). There was also a weak positive correlation between MLR and clinical severity (rho = 0.425; $P = 0.034$).

The current study had some limitations. Firstly, the number of cats in groups is relatively small. Second, although these cats were checked for FIV, FeLV, FCov and other parasitic causes of diarrhoea, some cats might have suffered from other bacterial, viral and mycotic agents causing diarrhoea. Third, complete blood count results were checked with a blood smear, but it should still be considered that preanalytical, analytical and post-analytic factors may affect the results.

CONCLUSION

The present study results have shown that the NLR and MLR are elevated in diarrheal cats infected with *Cystoisospora* spp. compared with healthy cats, and there was a positive correlation between the severity of the disease and these 2 parameters. It has also shown that NLR can be a useful marker for estimating SIRS. To the best of our knowledge, this is the first study to demonstrate the MLR level in cats and the NLR levels of cats with diarrhoea. Nevertheless, large-scale studies are needed to warrant these parameters' clinical usability and diagnostic significance.

MANUFACTURERS

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***Ethical approval*.** Aydın Adnan Menderes University Animal Research Ethics Committee has reviewed and approved all study procedures under protocol number 64583101/2022/7.

***Declaration of interest*.** The author reports no conflicts of interest and is responsible for the content and writing of the paper.

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