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Full Research Article

SELECTED HEMATOLOGY RATIOS IN CATS WITH NON-SEPTIC EFFUSIONS HIGHLY SUSPECTED OF FELINE INFECTIOUS PERITONITIS

Aleksandar KOPILOVIĆ[1], Dragan GVOZDIĆ[2], Milena RADAKOVIĆ[2], Kristina SPARIOSU[2], Nenad ANDRIĆ[2], Jelena FRANCUSKI ANDRIĆ[2*]

[1]Small Animal Veterinary Practice "ZooHome", Belgrade, Serbia;

[2]University of Belgrade, Faculty of Veterinary Medicine, Department of Pathophysiology, Belgrade, Serbia

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Abstract

In veterinary medicine, knowledge about hematologic ratios (neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and mean platelet volume-to-platelet ratio (MPV/PLT)) is limited, particularly in cats. While the roles of these ratios have been proven in oncology, systemic inflammation with or without systemic inflammatory response syndrome (SIRS), and sepsis, information is lacking about their alterations in non-septic effusions, like feline infectious peritonitis (FIP).

This study aimed to describe whether NLR, PLR, and MPV/PLT were changed and whether they correlated with routine hematologic and biochemical parameters in 16 cats with non-septic effusions, highly suspected to be the effusive form of FIP without SIRS, compared to nine clinically healthy cats.

The NLR was calculated as the absolute count of neutrophils divided by the absolute count of lymphocytes, PLR by calculating the absolute platelet divided by the absolute lymphocyte count, and MPV/PLT by dividing mean platelet volume by absolute platelet count.

The NLR, MPV, and MPV/PLT ratios were higher in cats with non-septic effusions suspected to be FIP, but PLR did not differ, when compared to healthy cats. Correlation

*Corresponding author – e-mail: jelenaf@vet.bg.ac.rs

analysis did not show any association between the selected ratios and hematological and biochemical parameters.

In the absence of leukocytosis, increased NLR could help us to confirm the presence of systemic inflammation in cats with non-septic effusions indicative of FIP. However, a high MPV/PLT ratio should be interpreted with caution, especially in cats.

Key Words: inflammation, mean platelet volume-to-platelet ratio, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio

INTRODUCTION

Effusions are non-specific clinical findings that are ultimately caused by abnormal accumulation of fluid in the body cavities. For effusion classification, total nucleated cell count (TNCC), biochemical (e.g., total proteins, glucose, lactate, creatinine, total cholesterol, triglycerides), and cytological analyses are all required (Thompson and Rebar, 2016; Dempsey and Ewing, 2011). Effusions are classified as protein-rich or protein-poor transudate, modified transudate, septic or non-septic exudate, effusions resulting from vascular or viscus disruption, and those caused by cell exfoliation. Exudate formation is caused by inflammatory reactions caused by microorganisms, vessel injury, neoplasia, urine, pancreatic enzyme leakage, bile salts, or immune complexes. Septic exudates can be separated from non-septic exudates by the use of biochemical parameters and the presence of bacteria and degenerative neutrophils in cytology smears (Thompson and Rebar, 2016; Dempsey and Ewing, 2011).

Most inflammatory effusions are cytologically non-specific in terms of etiologic diagnostics. The effusive form of feline infectious peritonitis (FIP) is distinct among inflammatory effusions, due to its high protein concentration and low cellularity. FIP, especially the non-effusive form, is also one of the most challenging cat diseases to diagnose *ante mortem* due to this disease's unspecific clinical signs and clinico-pathological findings. Tasker et al. (2023) provided an overview of factors that could be used to determine highly suspected FIP, to make FIP diagnosis very likely, or to confirm a precise FIP diagnosis. They emphasized the importance of finding new parameters and biomarkers that could raise the likelihood of FIP diagnosis.

In human medicine, calculated ratios from complete blood count (neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and mean platelet volume-to-platelet ratio (MPV/PLT)) are sensitive markers of inflammation, infection, systemic inflammatory response syndrome (SIRS), sepsis, and stress (Zahorec, 2021). Their significance in the clinical pathology of cats is rarely discussed in studies (Yin et al., 2021), especially in inflammatory diseases characterized by effusion formation. In cats, the NLR increases in response to inflammation and chronic renal disease (Tsouloufi et al., 2021), mammary tumors (Naito et al., 2021), and hypertrophic cardiomyopathy (Fries et al., 2022). Pancreatitis causes increases in the NLR and PLR (Neumann, 2021), but there is no information on how the MPV/PLT ratio varies in cats.

This study aimed to describe whether NLR, PLR, and MPV/PLT were changed and whether they correlated with routine hematologic and biochemical parameters in cats with non-septic effusions suspected of being FIP without the presence of SIRS, compared to healthy cats.

MATERIALS AND METHODS

Case selection

This retrospective study included cases from database provided by the ZooHome small animal veterinary practice, Belgrade, Serbia. Medical records of cats with abdominal, pleural, and both abdominal and pleural effusions who had: 1) signalment, 2) parameters from physical examination, 3) abdominal ultrasound and/or thoracic radiographs, 4) complete blood count (CBC), 5) serum biochemistry analyses, 6) serum agarose gel electrophoresis, 7) point-of-care tests for *Toxoplasma gondii* (immunochromatography, Megacor, Austria), feline leukemia virus (FeLV), feline immunodeficiency virus (FIV) (ELISA, Ingenasa, Spain) and feline pancreas-specific lipase immunoreactivity (fPLI test, Idexx, USA), 8) effusions with gross, biochemical (Rivalta's test, total proteins, albumin, A/G ratio, glucose, lactate), TNCC, microbiological and cytological analyses, were chosen and processed according to the inclusion and exclusion criteria listed in Table 1.

Table 1. Exclusion and inclusion criteria for cats with non-septic effusions highly suspected of being the effusive form of FIP (Thompson and Rebar, 2016; Tasker et al., 2023).

Exclusion criteria		
A. *Effusion characteristics*	B. *Differential diagnoses*	C. *SIRS criteria*
TP < 25g/L	Presence or history of cardiac and neoplastic disease	Body temperature less than 37.8°C or higher than 39.7°C
TNCC > 5×10^9/L	Evidence of enlarged lymph nodes and any changes in the intestine, liver and/or pancreas detected by abdominal ultrasound examination or radiographs	Heart rate under 140 or above 225 beats/min
pH < 7	Regenerative anemia	Respiratory rate above 40 breaths/min
Concentration of glucose < 50 mg/dL	Lymphocytosis	WBC count less than 5×10^9/L or higher than 19.5×10^9/L
Concentration of lactate > 5.5 mmol/L	Positive point-of-care tests for *Toxoplasma gondii*, FIV/FeLV and fPLI	More than 5% band neutrophils
Blood-effusion glucose concentration > 20 mg/dL	White and opaque effusions, yellow-brown to green-brown and ones that stay red after centrifugation	
Presence of bacteria and degenerative neutrophils in effusion cytology smears, or marked neutrophilia	Neoplastic cells or marked lymphocytosis on effusion cytology smears	
Positive microbiology results		

cont. Table 1.

Inclusion criteria
Age less than 2 years
Clinical signs of fever below 40°C that was refractory to antibiotics, lethargy, anorexia, weight loss, swollen abdomen and dyspnea
Serum biochemistry characterized by hyperglobulinemia and A/G ratio less than 0.6
Increased serum α_2 and γ-globulins
Yellow, viscous abdominal and/or pleural effusion that tested positive on Rivalta's test, TNCC $< 5\times10^9$/L, TP > 35 g/L
Presence of non-degenerative neutrophils and macrophages in the effusion cytology smears

Table 2. Clinical examination findings, serum agarose gel electrophoresis and effusion analyses of cats with non-septic effusions suspected of being FIP (FIP susp.) and healthy cats (control).

	FIP susp. (n=16)	Control (n=9)
Signalment		
Median age (months)	18 (min-max: 12-24)	48 (min-max:12-96)
Males	12/16	5/9
Females	4/16	4/9
FIP susp. (n=16)		
Clinical signs		
Fever below 40°C	16/16	
Lethargy	16/16	
Anorexia	16/16	
Weight loss	16/16	
Swollen abdomen	9/16	
Dyspnea	7/16	
Physical examination		
Body temperature (°C)	39.8 ± 0.12	
Heart rate (beats/min)	203 ± 3	
Respiratory rate (breaths/min)	38.1 ± 3.92	
Effusion analyses		
Abdominal	9/16	
Pleural	1/16	
Abdominal and pleural	6/16	
Yellow, viscous	16/16	
Positive Rivalta's test	16/16	
Total nucleated cell count ($\times10^9$/L)	0.8 ± 0.20	
Total proteins (g/L)	62.27 ± 7.37	
Albumin/globulin ratio	0.25 ± 0.03	
Serum agarose gel electrophoresis		
Increased α_2 globulins	16/16	
Polyclonal gammopathy	16/16	

Legend: Results are expressed as mean ± SEM or as the number of positive cats/total number of cats.

In order to select cases of protein-rich non-septic effusions, we first excluded all cats with protein-poor and septic effusions (Thompson and Rebar, 2016). Secondly, following exclusion criteria by Tasker et al. (2023), we excluded non-septic effusions that corresponded to differential diagnoses of FIP (Table 1).

To select cats with evidence leading to FIP being highly suspected, we used the far more likely (+++) and extremely likely (++++) inclusion criteria given by Tasker et al. (2023), according to our database (Table 1).

Table 3. Complete blood cell count and serum biochemistry analyses results in cats with non-septic effusions suspected of being FIP (FIP susp.) and healthy cats (control).

	FIP susp. (n=16)	Control (n=9)	Reference range
Complete blood cell parameter (unit)			
WBC ($\times 10^9$/L)	13.24±1.62**	6.63±0.36	5.5-19.5
Ne ($\times 10^9$/L)	10.04±1.68**	3.51±0.37	2.5-12.8
Ly ($\times 10^9$/L)	2.62±0.75	2.52±0.21	1.5-7.0
Mo ($\times 10^9$/L)	0.22±0.08	0.17±0.03	0.0-1.4
Eos ($\times 10^9$/L)	0.28±0.20	0.33±0.05	0.0-1.5
Bas ($\times 10^9$/L)	0.71 ± 0.69	0.02±0.002	0.0-0.5
RBC ($\times 10^{12}$/L)	6.14±0.43**	8.82±0.49	5.9-11.2
HCT (%)	28±2**	38±2	25-45
Hgb (g/L)	90.0±5.79**	131.20±5.40	80-150
MCV (fL)	45±1.4	44±1.3	39-52
MCH (pg)	15±0.49	15±0.42	12.5-17.5
MCHC (g/L)	331±8.0	345±2.9	300-370
PLT ($\times 10^9$/L)	229±44	285±26	150-500
MPV (fL)	24±2***	15±1	5-20
Blood biochemistry parameter (unit)			
TP (g/L)	76.98±2.87	70.98±2.22	57-94
Alb (g/L)	25.53±1.42**	32.79±1.39	25-56
Glob (g/L)	55.90±4.80*	38.19±1.60	24-47
A/G	0.49±0.22	0.86±0.06	0.8-2.2
Urea (mmol/L)	7.21±0.74*	8.34±0.43	5.5-11.1
Creatinine (mmol/L)	71.18±8.60*	102.50±6.97	0.0-168
ALP (U/L)	14.01±2.78	21.86±2.57	12-65.1
ALT (U/L)	50.83±8.58	43.54±3.02	8.3-52.5
AST (U/L)	61.28±7.40**	25.80±2.00	9.2-30
GGT (U/L)	0.48±0.14**	1.69±0.26	1.8-12
TCa (mmol/L)	2.21±0.04*	2.39±0.07	2.3-3.0
iCa (mmol/L)	1.26±0.02	1.30±0.03	1.1-1.4

Legend: Results are expressed as mean±SEM. *p<0.05; **p<0.01; ***p<0.001.

We were not able to perform analyses which could increase the diagnostics of FIP, such as polymerase chain reaction (PCR) and immunocytochemistry (ICH), or to confirm diagnosis of FIP using immunohistochemistry (IHH), because the owners declined additional diagnostics, treatment, or *post mortem* examination. Since we excluded all causes of non-septic effusions that did not correspond to the diagnosis of FIP, we then described the cats finally included in the study as having non-septic effusions suspected of being FIP.

These cats were then evaluated for SIRS criteria following Alves et al., (2020). Cats that did not meet three of the four criteria for SIRS were included in the study group (Table 1). Sixteen cats satisfied all inclusion and exclusion criteria (Tables 1 and 2). The control group included nine clinically healthy cats that were presented to the clinic for regular health control and that had no changes in CBC, biochemical analyses, and/or abdominal ultrasound and radiographs (Tables 2 and 3).

Cat owners signed informed consent that the residual samples and the obtained results could be used for scientific purposes. The research was approved by the Ethical Committee at the Faculty of Veterinary Medicine, University of Belgrade, Serbia, and based on the Serbian Law of Animal Welfare, permission was acquired from the Ministry of Agriculture, Forestry and Water Management, Republic of Serbia (permission number: 323-07-07930/2022-05/1).

Hematology, biochemistry, and agarose gel electrophoresis

The CBC was performed using an Advia 120 Siemens, USA. Within one hour, blood smears were made and stained using a Bio-Diff kit (Biognost, Croatia) to assess the presence of band neutrophils and platelet clumping. The NLR was calculated as the absolute count of neutrophils divided by the absolute count of lymphocytes, PLR by calculating the absolute platelet count divided by the absolute lymphocyte count, and MPV/PLT by dividing mean platelet volume by absolute platelet count.

Biochemistry parameters were measured by commercial kits, using an automated analyzer (Olympus AU400, USA): total proteins (TP), albumin (Alb), albumin/globulin ratio (A/G ratio), urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), γ-glutamyl transferase (GGT), total and ionized calcium (TCa, iCa) and phosphate.

Agarose (1%) gel serum electrophoresis was performed to identify the following fractions: albumin, α1-, α2-, β- and γ-globulins, as previously reported by Milanović et al. (2017).

Effusion evaluation

Total proteins were determined by refractometer, TNCC by using a hemocytometer, and cytology smears using the sedimentation method.

Statistical analysis

Complete blood count and biochemistry analysis data (Table 3) were tested for homogeneity, using D'Agostino & Pearson omnibus normality test (GraphPad Prism 5.03), and an unpaired t-test was performed on all data sets having a normal distribution. Mann Whitney U-test was used for testing differences between medians if data sets did not pass normality testing. Additionally, correlations between selected hematological ratios were also determined. In all of the statistical analyses, $P \leq 0.05$ was considered significant.

RESULTS

Hematology and biochemistry results for the cats with non-septic effusions suspected of being FIP and for the healthy control cats are displayed in Table 3. In the cats with effusions, and despite the absence of anemia, red blood cell count (RBC), hematocrit (HCT), and hemoglobin concentration (Hgb) were at the lower range of the referent intervals (RI) and showed significant differences when compared to the values in healthy cats. Biochemical analyses of sera from cats with non-septic effusions suspected of being FIP showed no changes in TP and Alb concentration, but Alb concentration was at the lower range of RI and was significantly different from that of healthy cats. Also, despite the absence of azotemia, the concentration of urea and creatinine in cats with effusions were significantly different in comparison to the levels in healthy cats, TCa and GGT were significantly decreased, while AST activity was significantly increased compared to the healthy cat control group (Table 3).

Cats with non-septic effusions suspected of being FIP had a higher NLR (8±3) than did healthy cats (1.5±0.2) (Figure 1A) and a higher MPV/PLT ratio (0.23±0.06) than

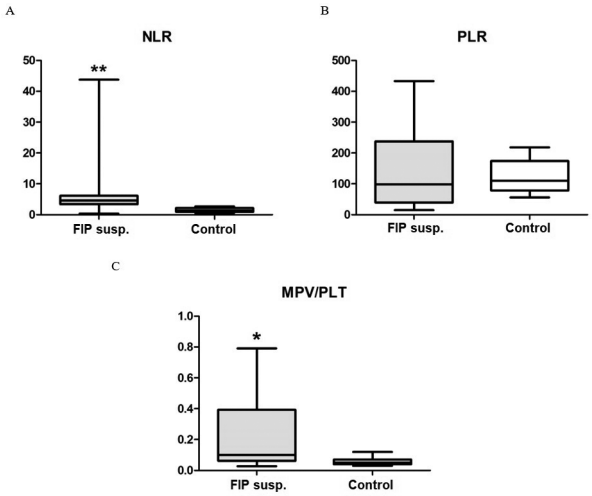

Figure 1. NLR, PLR, and MPV/PLT ratio in cats with non-septic effusions suspected of being FIP (FIP susp., n=16) and control cats (control, n=9); **p<0.01; median with interquartile range (IQR, boxes), minimum to maximum (whiskers). NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; MPV/PLT, mean platelet volume to platelet ratio.

healthy cats (0.06±0.008) (Figure 1C). PLR did not differ between the two groups of cats (137±31 and 123±18, respectively, for healthy cats and those with effusions) (Figure 1B).

Examination of the blood smears did not reveal the presence of micro- or macro-aggregation of platelets. Correlation analysis of cats with suspected FIP and healthy cats revealed no association between NLR, PLR, and MPV/PLT ratios with hematological and biochemical parameters, nor between these ratios (data not shown).

DISCUSSION

We found that, compared to healthy cats, the NLR was increased five-fold in cats with non-septic effusions suspected of being FIP without SIRS, and the MPV/PLT ratio was increased four-fold. The PLR was unchanged between the two groups of cats.

The significance of studying the NLR is shown in its links to inflammation, SIRS, and sepsis in both human and veterinary medicine. To appropriately assess NLR changes in cats with inflammation, reference intervals for NLR in healthy cats need to be established. Currently, the small number of publications giving such data is insufficient to provide conclusive NLR ranges in healthy cats, just as it has been for human medicine (Zahorec, 2021). Recently published research revealed that the NLR in healthy cats ranges from 1.5 (Neumann, 2021), as we found in our study, to 1.9 (Fries et al., 2022; Petrucci et al., 2021; Gori et al., 2021). Even though the stress leukogram is common in both healthy and cats with systemic inflammation, the influence of stress on NLR values has not been investigated, as is the situation in human medicine.

The NLR in cats with systemic inflammation ranges from 2, in cats with a low level of inflammation, as observed in tumors (Petrucci et al., 2021) and hypertrophic cardiomyopathy at stage B (Fries et al., 2022), to 5, in the conditions characterized with a higher degree of inflammation, such as hypertrophic cardiomyopathy at stage C (Fries et al., 2022), and pancreatitis (Neumann, 2021). In cats with SIRS and sepsis, NLR mean values are much higher and reached 8.9 and 9, respectively (Gori et al., 2021).

In the present study, cats with non-septic effusions suspected of being FIP had a NLR of 8, without the presence of an inflammatory leukogram. These cats had chronic inflammation, indicated by the existence of an abdominal/pleural effusion that required weeks to form, and by the presence of increased α_2 and γ-globulins. At the presentation, a balance was established between increased bone marrow production and consumption of leukocytes, which led to an unchanged leukocyte count at that time. Also, cats in this study did not meet the necessary criteria for SIRS at presentation. Although Gori et al. (2021) recommended a cut-off value of 4.53 for the NLR in SIRS and sepsis, their recommendation does not match the findings of this study, and in the future, the NLR should be investigated more in terms of clinical use in cats. Based on our results, we could only suggest the NLR can help to confirm the presence of chronic inflammation, especially in the absence of leukocytosis.

In addition to the NLR, we also investigated MPV and the MPV/PLT ratios in cats with non-septic effusions suspected of being FIP. Since platelets participate in inflammation and interact with other cells, including neutrophils and lymphocytes, and changes in platelet count and size could be seen (Margraf and Zerbock, 2019), it was reasonable to combine MPV with PLT and investigate possible alterations. Moreover, previous studies in septic patients showed that MPV and the MPV/PLT ratio are indicators of clinical severity and mortality in sepsis in the first 72 hours of serial measurements (Vélez-Páez et al., 2022). However, a study in dogs with periodontitis and oropharyngeal tumors did not find any links between MPV, MPV/PLT, and inflammation (Rejec et al., 2017). Since cats with FIP commonly develop thrombocytopenia as a consequence of the disseminated intravascular coagulation, this highlights the importance of analyzing the MPV and the MPV/PLT ratio (Hartmann, 2005). However, evaluation of increased MPV in cats is challenging, because it is difficult to conclude whether the increase is a physiological phenomenon or a pathological outcome. In healthy cats, MPV could be raised because platelets fluctuate in size more than in other species, and it is harder to distinguish them visually in a blood smear. In response to systemic inflammation, MPV increases as a result of the production of large platelets in bone marrow, caused by cytokine and thrombopoietin stimulation. Moreover, cats' platelets frequently clump on blood sample collection and become swollen with storage in EDTA tubes, which could lead to incorrect readings by hematology analyzers (Schaefer, 2022).

Given that the platelet count in cats with non-septic effusions suspected of being FIP did not change and that no micro- or macro-aggregation was detected in the blood smears, the high MPV and the MPV/PLT ratio in these animals should be interpreted with caution in the light of their changes caused by the presence of chronic inflammation in these cats. The MPV/PLT ratio should be further investigated until the clinical significance of MPV is proven, and cut-off values for MPV/PLT need to be defined.

CONCLUSION

In cats with non-septic effusions suspected of being FIP, an increased NLR could indicate the presence of inflammation, especially in the absence of leukocytosis. When diagnosing inflammation, the NLR is more helpful than individual measures. Increases in the MPV/PLT ratio should be interpreted with caution, especially in cats. In the future, all ratios should be stated in terms of their prospective, predictive value, and clinical use.

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Authors' contributions

JFA designed the study; AK, MR, and KS collected the samples; MR, KS, and JFA performed the analyses; DG performed and interpreted the statistical analyses; NA edited the manuscript. All authors interpreted the data, wrote and critically revised the manuscript for important intellectual contents, and approved the final version.

Conflict of interests

The authors declare that they do not have any financial or personal conflicts of interest that could bias the study.

Animal welfare statement

Cat owners signed informed consent that the residual samples and the obtained results could be used for scientific purposes. The research was approved by Ethical Committee at the Faculty of Veterinary Medicine, University of Belgrade, Serbia, and based on the Serbian Law of Animal Welfare, permission was acquired from the Ministry of Agriculture, Forestry and Water Management, Republic of Serbia (permission numbers: 323-07-07930/2022-05/1).

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EVALUACIJA ODABRANIH HEMATOLOŠKIH ODNOSA KOD MAČAKA SA NESEPTIČNIM IZLIVIMA SUMNJIVIM NA INFEKTIVNI PERITONITIS

Aleksandar KOPILOVIĆ, Dragan GVOZDIĆ, Milena RADAKOVIĆ, Kristina SPARIOSU, Nenad ANDRIĆ, Jelena FRANCUSKI ANDRIĆ

Kratak sadržaj

U veterinarskoj medicini, poznavanje hematoloških odnosa: odnos neutrofila i limfocita (NLR); odnos trombocita i limfocita (PLR); odnos prosečne zapremine trombocita i broja trombocita (MPV/PLT), je limitirano, posebno kod mačaka. Dokazana je njihova uloga u onkologiji i sistemskoj inflamaciji sa ili bez prisustva sindroma sistemskog inflamatornog odgovora (*Systemic Inflammatory Response Syndrome* – SIRS) i sepse, ali nedostaju informacije o tome kako se ovi hematološki odnosi menjaju kod neseptičnih izliva, kao što je infektivni peritonitis mačaka (*Feline Infectious Peritonitis* – FIP).

Ova studija je imala za cilj da opiše da li su NLR, PLR i MPV/PLT promenjeni i da li su u korelaciji sa rutinskim hematološkim i biohemijskim parametrima kod 16 mačaka sa neseptičnim izlivima sumnjivim na FIP bez SIRS-a, i devet klinički zdravih mačaka.

Odnosi su dobijeni deljenjem: apsolutnog broja neutrofila sa apsolutnim broja limfocita (NLR), apsolutnog broja trombocita sa apsolutnim brojem limfocita (PLR) i prosečne zapremine trombocita sa apsolutnim brojem trombocita (MPV/PLT).

Odnosi NLR, MPV i MPV/PLT su bili veći kod mačaka sa neseptičnim izlivima sumnjivim na FIP i nisu uočene promene PLR u odnosu na zdrave mačke. Korelaciona analiza nije pokazala međusobnu povezanost između odabranih odnosa niti u odnosu na hematološke i biohemijske parametare.

Kod mačaka sa neseptičnim izlivima sumnjivim na FIP povećanje NLR može ukazivati na prisustvo inflamacije naročito kada ne postoje promene u ukupnom broju leukocita. Povećanje odnosa MPV/PLT treba tumačiti oprezno, posebno kod mačaka.

Ključne reči: inflamacija, odnos neutrofila i limfocita, odnos trombocita i limfocita, prosečan odnos zapremine trombocita i trombocita.