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Evaluation of Haematological Indices in Relation to the Severity of the Disease in Cats with Feline Infectious Peritonitis

Kerim Emre YANAR [1, a]

[1] Atatürk University, Faculty of Veterinary Medicine, Department of Veterinary Internal Medicine, Erzurum, TÜRKİYE

[a] ORCİD: 0000-0001-7302-7077

The aim of this study was to evaluate the levels of haematological indices in relation to clinical severity in the cats with feline infectious peritonitis (FIP). Fifteen cats with FIP were included in the study, and cats with an albumin-to-globulin ratio between 0.8 and 0.6 were classified as early FIP groups (n = 8), while those with the ratio below 0.6 were classified as late FIP groups (n = 7). The results of the study indicated that elevated neutrophil count (NEU) could be an important marker in the evaluation of both FIP and FIP severity, whereas neutrophil to lymphocyte ratio (NLR) could only be used to identify cats with FIP and did not change with FIP severity. Finally, this study found that platelet count (PLT) was an important marker in detecting cats only with late FIP. In conclusion, this study demonstrated that the cats with FIP can have different haematological responses depending on the inflammation and severity of the inflammation, and that haematological indices can be used to monitor inflammation. In particular, NEU, NLR, and PLT counts were found to be promising indicators in this regard.

Key Words: *Cat, FIP, haematological parameter, indices*

Feline Enfeksiyöz Peritonitisli Kedilerde Hastalığın Şiddeti ile İlişkili Olarak Hematolojik İndekslerin Değerlendirilmesi

Bu çalışmanın amacı, feline enfeksiyöz peritonitisli (FIP) kedilerde klinik şiddetle ilişkili olarak hematolojik indis düzeylerini değerlendirmektir. FIP'li 15 kedi çalışmaya dahil edilmiş ve albümin-globulin oranı 0.8 ile 0.6 arasında olan kediler erken dönem FIP grupları (n= 8) olarak sınıflandırılırken, albümin-globulin oranı 0.6'nın altında olan kediler geç dönem FIP grupları (n= 7) olarak sınıflandırılmıştır. Çalışmanın sonuçları, yüksek nötrofil sayısının (NEU) hem FIP hem de FIP şiddetinin değerlendirilmesinde önemli bir belirteç olabileceğini, nötrofil/lenfosit oranının (NLR) ise yalnızca FIP'li kediler için kullanılabileceğini ve FIP şiddetiyle değişmediğini göstermiştir. Son olarak, bu çalışma trombosit sayısının (PLT) sadece geç FIP'li kedilerin tespitinde önemli bir belirteç olduğunu ortaya koymuştur. Sonuç olarak, bu çalışma FIP'li kedilerin inflamasyona ve inflamasyonun şiddetine bağlı olarak farklı hematolojik yanıtların oluşabileceğini ve hematolojik indekslerin inflamasyonu izlemek için kullanılabileceğini göstermiştir. Özellikle NEU, NLR ve PLT sayıları bu konuda umut verici göstergeler olarak bulunmuştur.

Anahtar Kelimeler: *Kedi, FIP, hematolojik parametre, indisler*

Introduction

Coronaviruses, known to cause respiratory and gastrointestinal disorders in several species, include two biotypes in cats: feline enteric coronavirus (FECV) and feline infectious peritonitis virus (FIPV) (1, 2). Indeed, a minority of cats infected with feline coronavirus develop a severe condition known as feline infectious peritonitis (FIP), characterized by vasculitis (3). Although identification of the effusive form of the disease is relatively straightforward, diagnosis of FIP in its non-effusive form can be challenging due to the many potential clinical signs, many of which are non-specific (such as anorexia, lethargy, weight loss and fever), and limited accessibility of fluid samples for testing (4). At this point, albumin-to-globulin ratio (A:G) plays a very important role in the diagnosis of FIP and a positive predictive value of 93% has been reported for A:G 0.8. This rate is 94% for 0.7, 95% for 0.6 and 96% for 0.5, respectively (5). Furthermore, earlier research highlighted an A: G ratio <0.6 as highly indicative of an inflammatory process, primarily associated with FIP (6).

The neutrophil/lymphocyte ratio (NLR), which is the absolute count of neutrophils (NEU) divided by the absolute count of lymphocytes (LYM), is a marker of systemic inflammation obtained from a complete blood count. Typically, as an inflammatory disease progresses, the neutrophil count in the blood rises while the lymphocyte count, which indicates the patient's immune status, tends to fall (7, 8). The platelet-lymphocyte ratio (PLR), a haematological marker of inflammation, is determined by dividing the total platelet count (PLT) by the LYM. It has been proven to be another inflammatory marker in human studies, often complementing the NLR (9, 10).

Recent research indicates that mean platelet volume (MPV) holds promise as a diagnostic indicator for inflammatory conditions (11, 12). As a marker of activated platelets, MPV has shown associations with various inflammatory diseases (13). In

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Yazışma Adresi
Correspondence

Kerim Emre YANAR
Atatürk University,
Faculty of Veterinary Medicine,
Department of Veterinary Internal Medicine,
Erzurum – TÜRKİYE

emre.yanar@atauni.edu.tr

addition, the ratio of mean platelet volume to platelet count (MPV/PLT) is used in human medicine to diagnose and evaluate the prognosis of various infectious and inflammatory conditions (14, 15).

In light of these considerations, it was hypothesised that haematological indices – including NLR, PLR and MPV/PLT – vary according to the clinical severity of feline infectious peritonitis (FIP) and may serve to determine the severity of the disease. Furthermore, the study aimed to evaluate the levels of haematological indices in relation to clinical severity in cats with FIP. Haematological indices, which are widely used in human medicine and relatively new in veterinary medicine, offer several advantages in the assessment of disease prognosis. These include ease of measurement and cost-effectiveness. Given these advantages, haematological indices may offer the potential for seamless integration into veterinary clinics and hospitals to determine disease severity in cats with FIP. In conclusion, in addition to determining the severity of disease in cats with FIP, haematological indices have the potential to play a role in determining the prognosis of the disease in further studies and to have prognostic significance for survival or mortality during hospital admission.

Materials and Methods

Research and Publication Ethics: The study was approved by the Local Ethics Committee of Atatürk University (Ethics Committee Decision No: 2024/07).

Animals: The study material consisted of cats admitted to the Small Animal Clinic of the Faculty of Veterinary Medicine, Atatürk University. The diagnosis of FIP was based on clinical signs (fever, vomiting, malaise), detection of coronavirus antigen in septic or non-septic abdominal-thoracic fluid using an ELISA test kit (Asan Easy Test FCoV, Korea), and cats with an A:G ratio of less than 0.8 were also tested for possible parasitic infections associated with FIP. Cats (n=8) with an A:G ratio between 0.8 and 0.6 were classified as early stage FIP, while those with an A:G ratio below 0.6 (n=7) were classified as late stage FIP. The healthy group consisted of clinically healthy cats presented for vaccination and antiparasitic treatment.

Blood sampling and laboratory analysis: Before treatment, blood samples were meticulously obtained through careful venepuncture, employing 21-gauge needles inserted into the jugular vein. The acquired blood was then divided into serum and anticoagulant (EDTA) tubes for further analysis. Complete blood count (CBC) analyses were conducted using an automated haematology analyser (Abacus Junior Vet5®, Hungary). The NLR, PLR, and MPV/PLT were calculated using the following formulas:

NLR = absolute counts of neutrophils/absolute counts of lymphocytes

PLR = absolute counts of platelets/absolute counts of lymphocytes

MPV/PLT = mean platelet volume/absolute counts of platelets.

Statistical Analysis: The study data were subjected to statistical analysis using a one-way ANOVA followed by post hoc tests to compare NEU, LYM, NLR, PLT, PLR, MPV, and MPV/PLT between healthy and early and late-stage FIP groups. Prior to analysis, a normal distribution was confirmed using the Kolmogorov-Smirnov test. Statistical analysis was performed using SPSS 27.0 with a significance level of $P < 0.05$.

Results

Table 1 presents the demographic characteristics of the cats included in the study. The study comprised a total of 15 cats, each infected with feline infectious peritonitis (FIP). Among these, average age of the cats diagnosed as the early stage was6.1 months, while their counterparts in the late stage were 6.8 months. The control group was selected to follow the age and sex distribution of the FIP cohorts.

Haematological analysis revealed notable findings: the neutrophil count in cats with FIP exhibited a statistically significant increase compared to the control group. In addition, within the FIP cohort, the neutrophil count increased significantly in the late stage compared to the early stage, with statistical significance highlighted. Conversely, the PLT count showed a decreasing trend as the severity of the infection progressed. It was particularly notable that a significant decrease in platelet count compared with the control group was only observed in the late stages of the disease. Among the haematological indices, NLR demonstrated a statistically significant increase in response to FIP. However, it is important to note that NLR levels remained unchanged throughout the severity of the disease (Table 2).

Table 1. Demographic findings of cats in the control, FIP (Early stage) and FIP (Late stage)

Group	n	Age (month) Means±SE	Sex (M/F)	Weight (Kg) Means±SE
Control	10	6.2 ± 0.44	4/6	3.2 ±0.2
FIP (Early stage)	8	6.1 ± 0.51	5/3	3.1 ±0.14
FIP (Late stage)	7	6.8 ± 0.67	4/3	2.9 ±0.21

SE: Standart error

Table 2. Results of the haematological analyses and haematological indices according to disease severity in cats with FIP

Parameters	Control Means±SE	FIP (Early stage) Means±SE	FIP (Late stage) Means±SE	P Value
NEU	4.82±.52[a]	11.98±0.38[b]	16.30±.57[c]	P<0.001
LYM	2.93±.35	1.94±.30	2.37±.28	P=0.112
NLR	1.9±0.34[a]	7.51±.93[b]	7.63±1.41[b]	P<0.001
PLT	340.3±34.04[a]	254±24.19[ab]	230.85±25.28[b]	P=0.035
PLR	130.54±20.03	150.63±27.32	106.18±16.9	P=0.420
MPV	10.28±.43	9.82±.62	9.58±1.03	P=0.748
MPV/PLT	0.03±.004	0.04±.007	0.04±0.004	P=0.445

LYM: Lymphocyte, MPV: Mean platelet volume, MPV/PLT: Mean platelet volume-to-platelet ratio, NEU: Neutrophil, NLR: Neutrophil-to-lymphocyte ratio, PLT: Platelet, PLR: Platelet-to-lymphocyte ratio. SE: Standart error. [a,b,c] :The means shown in different lowercase letters between the groups (on the line) are statistically significant.

Discussion

The aim of this study was to evaluate haematological indices commonly used as inflammatory markers in human medicine in relation to disease severity in cats diagnosed with FIP. In cats suffering from FIP, we observed a marked increase in neutrophil counts that correlated with the severity of the disease, a finding that was statistically significant. This is in contrast to the neutropenia typically induced by viral agents including feline panleukopenia (16), feline leukemia virus (17) and feline immunodeficiency virus (18). Notably, this deviation is consistent with recent human studies reporting neutrophilia in severe acute respiratory syndrome caused by coronaviruses (19, 20), highlighting its unique manifestation among coronaviruses. In addition, research suggests that cytokines delay the apoptosis of neutrophils in FIP cats, thereby prolonging their lifespan within lesions. It has also been suggested that neutrophilia in cats with FIP is probably related to the infiltration of neutrophils into granulomatous lesions (21). The increase in neutrophil count with increasing disease severity in FIP cats reflects these findings. Consequently, neutrophilia emerges as a significant inflammatory marker in coronavirus-induced infections in cats. Consequently, further large-scale studies are highly recommended to further investigate neutrophilia in cats with FIP.

We observed a trend of lower lymphocyte counts in cats with FIP compared to the control group, although this decrease did not reach statistical significance. This finding is in aggrement with the previous studies on feline viral infections (22, 23) and FIP (24), which have reported lymphopenia as a common symptom. Previous research proposes a model for FIP pathogenesis wherein virus-induced T-cell depletion and antiviral T-cell responses act as opposing forces, with the efficacy of early T-cell responses crucially influencing infection outcomes (25, 26). While this study did not analyse lymphocyte subtypes, study findings are consistent with this model. However, it is important to note the lack of statistical significance, possibly attributable to the small sample size. Therefore, conducting larger-scale studies to further investigate lymphopenia in cats with FIP is strongly recommended in this context.

The increase in NLR levels observed in cats with FIP, as compared to healthy cats, highlights its central role in signalling inflammation, similar to recent breakthroughs in human studies infected with coronavirus infection (27, 28). This increase in NLR may be related to the immune response in cats, characterised by a paradoxical combination of neutrophilia and lymphopenia, as revealed in these studies. In contrast to viral infections characterised by haematological responses typically associated with neutropenia (feline panleukopenia virus, feline immunodeficiency virus, and feline leukemia virus), the unexpected neutrophilia in FIP, due to the nature of coronaviruses, highlights the remarkable efficacy of NLR as an important haematological marker for monitoring infection in coronavirus-induced infections.

The count of PLT exhibited a notably lower statistical significance in felines afflicted by late-stage FIP when compared with their healthy counterparts. Nevertheless, this decrease did not reach statistical significance in cats with early stage FIP compared to the control cohort. This observation mirrors the occurrence of thrombocytopenia in human research, particularly in cases of coronavirus-induced lung injury in the critically ill patients (29). Furthermore, human studies highlight thrombocytopenia as a hallmark of critical illness, signalling severe organ dysfunction and the onset of intravascular coagulopathy, often culminating in disseminated intravascular coagulation (30, 31). In this framework, it is rational to posit thrombocytopenia as a late marker of inflammation to measure the severity of inflammation in cats with FIP. However, unexpectedly, there was a lack of variation in PLR both in cats affected by FIP and in relation to the severity of FIP. We did not observe any statistical variance in PLR levels between groups, probably due to lymphocyte responses rather than PLT counts. Based on study results, it is conceivable that PLR may not serve as a reliable marker for monitoring inflammation or its severity in FIP-affected cats.

In the present study, we observed a decrease in MPV levels attributed to FIP. Furthermore, this decrease persisted with worsening severity of FIP. We also observed an increase in the MPV/PLT ratio associated

with FIP, but neither of these findings reached statistical significance. The reduction in MPV was consistent with findings from previous research (32, 33). In addition, consistent with studies highlighting the MPV/PLT ratio as a key metric in infection surveillance (34), we documented an increase in the MPV/PLT ratio in cats with FIP. It's reasonable to attribute this to a decreased platelet count rather than an escalation in MPV. However, the statistically insignificant variation identified in analysis cautions against using MPV and the MPV/PLT ratio as robust indicators for monitoring infection in cats with FIP. Further investigation is essential to resolve this question. The major limitation of the study is that other viral agents that may affect A:G could not be tested in all cats due to economic constraints.

In conclusion, this study investigated the potential benefits of haematological indices in monitoring inflammation and its severity in cats infected with FIP. In particular, the NEU, NLR and PLT count emerged as promising metrics in this regard. Given the cost-effectiveness, accessibility, and widespread use of haematological indices, they hold the promise of becoming key parameters in monitoring inflammation and its severity in cats with FIP.

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