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RESEARCH ARTICLE

Evaluation of Serum MMP-9, IL-8, and Novel Inflammatory Indices (SIRI, AISI, SII) as Prognostic Markers in Feline Infectious Peritonitis with and without Seizure

Zeynep Nurselin KOT [1], Umit OZCAN [1(*)], Samil Bugra KOSE [1], Tunahan KURT [1], Zulal Feyza EKER [1]

[1] Ondokuz Mayıs University, Faculty of Veterinary Medicine, Internal Medicine Department, TR-55200 Samsun - TÜRKİYE

(*) **Corresponding author:**
Umit Ozcan
Phone: +90 362 312 1919-1545
Cellular phone: +90 539 546 99 88
E-mail: umit.ozcan@omu.edu.tr

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Abstract

This study aimed to evaluate serum matrix metalloproteinase-9 (MMP-9), interleukin-8 (IL-8), and hematology-derived inflammatory indices as potential prognostic markers in cats with FIP, with particular emphasis on seizure activity. Forty-eight client-owned cats diagnosed with FIP and 14 healthy control cats were included. Cats with FIP were categorized into three groups: effusive FIP (EFIP, n=11), non-effusive FIP (NFIP, n=30), and non-effusive FIP with seizures (NFIP-S, n=7). Hematological parameters were analyzed to calculate inflammatory indices including dLNR, SIRI, AISI, SII, NLR, and related ratios. Serum MMP-9 and IL-8 concentrations were measured using ELISA. Serum MMP-9 concentrations were significantly higher in cats with NFIP-S group compared with other groups ($P<0.05$). IL-8 levels were higher in the seizure group but did not differ significantly between groups. Hematology-derived inflammatory indices were significantly elevated in EFIP and NFIP groups compared with controls ($P<0.05$), whereas NFIP-S group showed values comparable to controls. MMP-9 levels were positively correlated with IL-8 (Spearman's $\rho=0.44$, $P=0.001$). MMP-9 levels were significantly associated with seizure occurrence (OR=1.13, $P=0.016$). Survival analysis demonstrated significantly shorter survival in the NFIP-S group ($P=0.042$). Elevated serum MMP-9 in cats with non-effusive FIP and seizures suggests a potential role of MMP-9 mediated blood-brain barrier disruption and neuroinflammation in seizure pathogenesis. MMP-9 may serve as a useful biomarker for neurological involvement and prognosis in FIP.

Keywords: Feline infectious peritonitis, MMP-9, IL-8, Seizure, Neuroinflammation, Blood-brain barrier

Introduction

Feline infectious peritonitis (FIP), caused by a mutated form of feline coronavirus (FCoV), is a viral disease characterized by immune-mediated vasculitis and granulomatous inflammation in its pathogenesis. FIP presents with a wide spectrum of clinical manifestations, including abdominal signs such as ascites, neurological signs such as seizures, and ocular findings such as corneal edema, and is classified into two clinical forms: effusive and non-effusive [1]. In classical models explaining the development of these clinical forms, a strong humoral (antibody) response combined with an insufficient or impaired cellular immune response leads to immune complex-mediated vasculitis and the accumulation of protein-rich serous effusions, resulting in the effusive form of the disease. In contrast, when a partially effective cellular immune response is present, widespread effusion does not occur; instead, pyogranulomatous or granulomatous lesions develop in various organs, representing the non-effusive form. These two forms should be considered as part of a disease continuum, as the pathology in an individual cat may include both effusion and granulomatous lesions [2,3].

Matrix metalloproteinase-9 (MMP-9) is a zinc-dependent endopeptidase and a member of the matrix metalloproteinase family, produced by macrophages, neutrophils, endothelial cells, and astrocytes. In FIP, intense monocyte/macrophage activation leads to increased MMP-9 activity, which degrades type IV collagen in the vascular basement membrane, thereby disrupting vascular integrity and contributing to the development of vasculitis [4,5]. In humans and laboratory animals, increased expression and activation of MMP-9 have been shown to disrupt blood–brain barrier integrity, leading to blood-brain barrier dysfunction and contributing to the

development of seizures [6,7]. Interleukin-8 (IL-8), a member of the CXC chemokine family primarily produced by monocytes and macrophages, is a proinflammatory cytokine that plays a key role in the recruitment and activation of neutrophils at sites of inflammation, and its levels have been reported to vary during the progression and remission phases of FIP alongside other proinflammatory mediators such as TNF-α, IL-1β, and IL-6 [8,9].

Because multiple inflammatory pathways are involved in the pathophysiology of FIP, the severity and duration of the disease may vary depending on the host immune response and the pathogenicity of the virus. Following the therapeutic success of nucleoside analogues in the treatment of FIP, increasing attention has been directed toward identifying factors that may help predict treatment response and prognosis [10,11]. In human medicine, several hematology-derived inflammatory markers, including the neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII), have been identified as predictive indicators in severe inflammatory diseases such as COVID-19 and have also been investigated in cats with FIP [12]. However, the levels of several other hematology-derived inflammatory parameters-including the derived neutrophil-to-lymphocyte ratio (dNLR), the systemic inflammation response index (SIRI), an indicator of systemic inflammation and immune activation, the aggregate index of systemic inflammation (AISI), a composite inflammation score derived from multiple hematological parameters, the platelet-to-neutrophil ratio (PNR), the neutrophil-to-monocyte ratio (NMR), and mean corpuscular volume (MCV)—have not yet been investigated across different clinical forms of FIP.

Seizures represent one of the neurological manifestations that may develop in cats with non-effusive FIP. These seizures may persist despite antiviral treatment and may require antiepileptic therapy [13]. In some cases, persistent seizure activity may even lead to euthanasia decisions [14,15]. Previous studies have suggested that the occurrence of seizures in FIP indicates widespread systemic inflammation and structural brain damage and may therefore serve as an unfavorable prognostic indicator [14,16]. This study aimed to evaluate serum MMP-9 and IL-8 concentrations and to investigate their associations with hematology-derived inflammatory indices in cats with FIP-associated seizure activity.

Material and Methods

Ethical Statement

This study was approved by the Local Ethics Committee for Animal Experiments at Ondokuz Mayıs University on June 30, 2025, under decision number 2025/38.

Animals and Study Desing

A total of 48 cats that fulfilled the diagnostic criteria for FIP, as described by Thayer et al. [1], were included in the study. The diagnosis of FIP was based on the presence of typical clinicopathological findings. These included history of anorexia and weight loss; clinical signs including uveitis and persistent fever; ultrasonographic evidence of effusion and enlargement of abdominal lymph nodes; hematological abnormalities such as anemia; and biochemical alterations including hypoalbuminemia, hyperglobulinemia, and an albumin-to-globulin ratio below 0.4. In effusive cases, the characteristics of the effusion, a positive Rivalta test, and a high macrophage cell density in the effusion were used as key diagnostic criteria. The presence of FCoV RNA was confirmed using quantitative RT-PCR performed on whole blood samples at a commercial diagnostic laboratory according to previously described protocols [17].

Based on the clinical classification, 11 cats with effusive FIP were assigned to the EFIP group. Among cats with non-effusive FIP, 30 cats without seizure activity were assigned to the NFIP group, whereas 7 cats with seizure activity were assigned to the NFIP-S group. Seizure activity was defined as episodic focal or generalized motor events characterized by tonic, tonic–clonic, or myoclonic activity. The presence of seizures was confirmed either during clinical examination or by review of owner-provided video recordings. In addition, 14 clinically healthy cats presented for routine check-up examinations and showing no abnormalities on clinical, hematological, or biochemical evaluation were included as the healthy control (HC) group.

Blood samples were collected from the vena cephalica antebrachii of each cat. Samples for hematological analysis were obtained into EDTA-containing tubes, while samples for serum biochemistry and MMP-9 and IL-8 measurements were collected into plain tubes. Serum samples were stored at −80 °C until the analysis of MMP-9 and IL-8 concentrations. Following blood sample collection, all cats received antiviral therapy with the adenosine nucleoside analogue molnupiravir at a dosage of 18 mg/kg orally twice daily (PO BID) for 60 days [14,18]. The survival status of the cats, including death, was monitored during follow-up examinations up to day 60.

Hematological Analyses and Hematology-Derived Inflammatory Indices

Blood samples collected for hematological analysis were analyzed using a Mindray BC-60R Vet Hematology Analyzer (Mindray, China). Based on the absolute hematological parameters obtained from the complete blood count, several hematology-derived inflammatory indices were calculated, including the derived neutrophil-

to-lymphocyte ratio (dNLR; N/(WBC-N)), systemic inflammation response index (SIRI; (N×M)/L), aggregate index of systemic inflammation (AISI; (N×M×P)/L), systemic immune-inflammation index (SII; (N×P)/L), neutrophil-to-lymphocyte ratio (NLR; N/L), neutrophil-to-monocyte ratio (NMR; N/M), lymphocyte-to-monocyte ratio (LMR; L/M), platelet-to-lymphocyte ratio (PLR; P/L), platelet-to-neutrophil ratio (PNR; P/N), and platelet-to-monocyte ratio (PMR; P/M).

Serum Biochemical Analyses

Serum total protein and albumin concentrations were measured as part of routine baseline laboratory analyses. Globulin concentration was calculated by subtracting albumin from total protein, and the albumin-to-globulin (A/G) ratio was subsequently determined. Serum biochemical analyses were performed using an automated analyzer (DRI-CHEM NX600; Fujifilm, South Korea).

Measurement of MMP-9 and IL-8

Serum MMP-9 and IL-8 concentrations were determined using species-specific quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (Cat IL-8 ELISA Kit, Cat. No: E0052Cat, Lot No: 202509003; Cat MMP-9 ELISA Kit, Cat. No: E0082Cat, Lot No: 202503007; Bioassay Technology Laboratory, China).

Briefly, microplate wells precoated with specific antibodies against feline MMP-9 or IL-8 were incubated with standards and serum samples. After incubation, unbound components were removed by washing, and a biotin-labeled detection antibody was added to each well. Following further incubation and washing steps, horseradish peroxidase (HRP)-conjugated streptavidin was added to form an antibody-antigen-antibody complex. Subsequently, tetramethylbenzidine (TMB) substrate solution was added to produce a color reaction proportional to the concentration of the analyte. The reaction was terminated with stop solution, and optical density (OD) was measured at 450 nm using a microplate reader. Concentrations of MMP-9 and IL-8 were calculated from standard curves generated using known concentrations of the respective standards.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 21.0 (IBM Corp., Chicago, IL, USA). The normality of data distribution was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests. For parameters showing normal distribution (neutrophil, monocyte, lymphocyte, NMR, MPV, PNR, PMR, total protein, and albumin), intergroup comparisons were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. For parameters that did not follow a normal distribution, comparisons among groups were conducted using the Kruskal-Wallis test, followed by Dunn's post hoc test with Bonferroni correction.

The relationship between MMP-9 and IL-8 levels was evaluated using Spearman's rank correlation analysis. Kaplan–Meier survival analysis was used to estimate survival functions of the groups. Time-to-event was defined as the time from baseline to death, with event status coded as 0 = alive and 1 = death. Differences between survival curves were assessed using the log-rank test. The predictive performance of inflammatory parameters for survival was evaluated using receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) and its 95% confidence interval (CI) were calculated to determine the discriminatory ability of the parameters. The optimal cut-off value was determined using the Youden index (J = sensitivity + specificity – 1). Cox proportional hazards regression analysis was performed to evaluate the association between MCV and mortality. The association between seizure occurrence and MMP-9 levels was assessed using binary logistic regression analysis.

Descriptive statistics were presented as mean ± standard deviation (SD) for normally distributed variables and median (minimum-maximum) for non-normally distributed variables. A P-value <0.05 was considered statistically significant.

Results

Demographic Characteristics

The breed distribution of the cats included in the study showed that the HC group consisted of Domestic Shorthair (n=6), Scottish Fold (n=2), British Shorthair (n=3), Siamese (n=2), and Angora (n=1) cats. All cats in the NFIP-S group (n=7) were Domestic Shorthair. The NFIP group consisted predominantly of Domestic Shorthair cats (n=28), with one British Shorthair and one Scottish Fold. In the EFIP group, nine cats were Domestic Shorthair and two were British Shorthair.

Baseline demographic characteristics and mean survival times are summarized in *Table 1*, with no statistically significant differences observed among the groups. Seizure semiology in the NFIP-S group revealed generalized tonic-clonic seizures in five cats and focal seizures in two cats.

Hematological Parameters

Neutrophil and monocyte counts were significantly higher, while lymphocyte counts were significantly lower in the NFIP and EFIP groups compared with the HC group (P<0.05). In contrast, cats in the NFIP-S group showed intermediate values for neutrophils, lymphocytes,

Table 1. *Demographic characteristics and mean survival time of the groups*

Group		HC	NFIP	NFIP-S	EFIP	P-value
Mean Survival Time (days)		- (not applicable)	22.14±25.86 7 (7-60)[a]	48.56±21.18 60 (3-60)[b]	40.63±23.80 60 (2-60)[ab]	
Age (months)		40.21±25.32	23.71±15.33	27.93±15.72	35.54±22.84	0.17
Sex	Male	10	5	13	7	0.23
	Female	4	2	17	4	

Values are expressed as mean ± standard deviation (median, min-max); [ab] Different superscript letters indicate significant differences between groups (P<0.05); HC: healthy control; NE: non-effusive FIP; NES: non-effusive FIP with seizures; E: effusive FIP

and monocytes, which were higher than those of the HC group but lower than those of the NFIP and EFIP groups, without reaching statistical significance. A significant increase in WBC count was observed only in the EFIP group compared with the HC group (P<0.05).

In Group EFIP, the neutrophil-to-lymphocyte ratio (NLR) and neutrophil-to-monocyte ratio (NMR) were 8.38 (1.97-63.20) and 26.14±26.48 (16.54, 8.49-93.52), respectively, and only the values in Group EFIP were significantly higher than those of the HC group (P<0.05). The LMR and PNR values were lower in all three FIP groups compared with the HC group; however, no significant differences were observed among the FIP groups (P>0.05).

Mean corpuscular volume (MCV) values were higher in the NFIP-S group than in the other groups; however, this difference was statistically significant only when compared with the HC group. Conversely, mean corpuscular hemoglobin concentration (MCHC) was significantly lower in the NFIP-S group compared with all other groups.

Inflammatory Indices

Systemic inflammatory indices, including dNLR, SII, and AISI, were significantly elevated in both the EFIP and NFIP groups compared with the HC group (P<0.05), indicating a more pronounced systemic inflammatory response in cats with effusive and non-effusive FIP.

In Group NFIP-S, the median dNLR and SII levels were higher than those in the HC group and lower than those in the other FIP groups; however, the differences were not statistically significant. Although SIRI values were higher in all groups compared with the HC value of 0.20 (0.08-1.14), statistical significance was observed only in Group EFIP, where the median value was 7.00 (0.50-41.08). Similarly, AISI values were significantly higher in Groups NFIP and EFIP compared with the HC group (P<0.05). Hematological parameters and hematology-derived inflammatory indices are presented in *Table 2*.

Biochemical and Cytokine Findings

Intergroup comparison of total protein levels showed that the TP concentration in Group NFIP was 9.72±1.97 mg/dL, while the value in Group NFIP-S was 8.74±1.60 mg/dL. The mean TP level in Group NFIP was significantly higher than those of the other groups except for Group NFIP-S. Albumin levels were significantly lower in Groups EFIP and NFIP compared with the HC group *Table 3*.

Correlation Analysis

The mean serum MMP-9 concentration in Group NFIP-S was 19.32±8.91 ng/mL, which was significantly higher than those of Groups HC, EFIP, and NFIP *(Table 3, Fig. 1)*. Although the mean IL-8 concentration in Group NFIP-S (1.75±2.61 pg/mL) was higher than those observed in the other groups (HC: 0.46±0.92 pg/mL; NE: 0.98±1.53 pg/mL; E: 0.20±0.69 pg/mL), no statistically significant differences were detected among the groups *(Table 3, Fig. 2)*.

A moderate positive correlation was observed between IL-8 and MMP-9 concentrations (Spearman's rho= 0.44, P=0.001) *(Fig. 3)*.

Survival and Prognostic Analyses

During the 60-day follow-up period, death was recorded in five cats in the NFIP-S group, nine cats in the NFIP group,

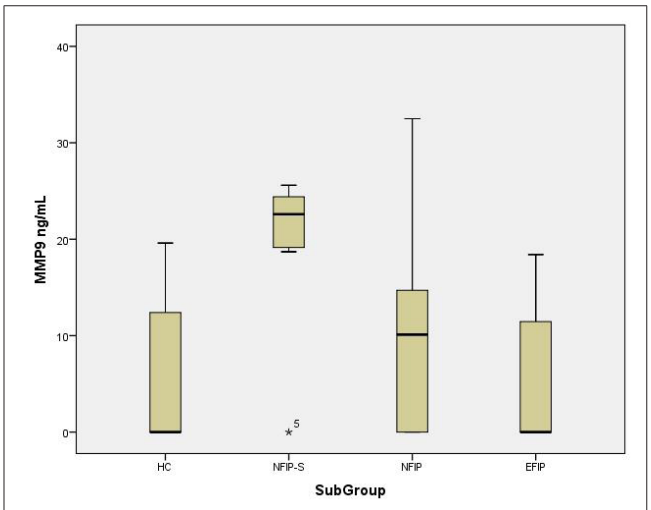

Fig 1. Distribution of serum MMP-9 concentrations among the study groups. Box plots represent the median and interquartile range, while whiskers indicate the minimum and maximum values. Circles and asterisks represent outliers. Serum MMP-9 concentrations were significantly higher in the NFIP-S group compared with the HC, NFIP, and EFIP groups (P<0.05)

Table 2. *Comparison of hematological parameters and derived inflammatory indices among groups*

Group Parameter	HC	NFIP-S	NFIP	EFIP
dNLR	0.99±0.68[a] 0.68 (0.32-2.52)	3.85±3.68[ab] 1.739 (0.22-9.10)	4.13±3.05[b] 3.83 (0.96-16.10)	6.96±5.52[b] 5.61 (1.50-20.19)
SIRI	0.35±0.30[a] (0.20, 0.08-1.14)	5.89±6.59[ab] (1.08, 0.20-14.03)	7.16±9.07[b] (4.16, 0.26-40.84)	13.40±14.09[b] (8.97, 1.00-41.08)
AISI	70.64±78.73[a] 59.19 (2.45-314.04)	1240.87±1628.53[ab] 249.07(25.30-4405.44)	1937.03±2222.86[b] 1237.75 (36.67-8092.78)	2087.05±2175.76[b] 1303.54 (291.41-6326.32)
SII	243.55±180.03[a] 212.74 (5.57-592.53)	1676.46±1967.78[ab] 436.96 (31.62-5244.57)	2343.91±2146.60[b] 1655.80 (174.64-11086.00)	3503.34±3445.88[b] 1676.09 (555.71-9732.80)
WBC (×10³/μL)	7.79±2.52[a] 7.85 (2.80-11.58)	12.54±5.76[ab] 10.16 (7.60-22.43)	12.67±6.09[ab] 11.16 (4.60-26.22)	15.45±7.20[b] 13.71 (6.31-27.34)
NEU (×10³/μL)	3.44±1.70[a] 2.99 (1.48-7.26)	8.69±5.98[ab] 7.66 (1.40-20.21)	9.51±4.59[b] 9.19 (2.75-22.92)	12.15±6.73[b] 9.73 (4.33-21.79)
LYM (×10³/μL)	3.66±1.93[a] 3.42 (0.96-6.52)	2.99±2.37[ab] 3.54 (0.37-6.33)	1.73±1.40[b] 1.45 (0.38-7.12)	1.57±1.05[b] 1.85 (0.10-2.98)
MON (×10³/μL)	0.30±0.15[a] 0.23 (0.12-0.53)	0.64±0.22[ab] 0.57 (0.29-0.94)	0.76±0.51[b] 0.64 (0.06-2.22)	0.73±0.62[b] 0.54 (0.15-2.24)
NLR	1.33±0.99[a] 0.79 (0.38-3.39)	8.24±8.75 [ab] 1.90 (0.25-20.70)	8.45±7.22[ab] 6.93 (1.30-28.34)	16.95±19.35[b] 8.38 (0.10-63.20)
NMR	12.13±6.40[a] 11.30 (5.04-31.00)	14.37±7.96[ab] 15.01 (1.75-24.05)	20.02±33.19[ab] 13.21 (5.18-192.20)	26.14±26.48[b] 16.54 (8.49-93.52)
LMR	13.40±5.88[a] 13.70 (4.61-21.43)	5.58±4.88[b] 6.21 (0.55-12.27)	3.79±5.32[b] 2.26 (0.39-26.47)	2.48±1.96[b] 2.09 (0.15-4.94)
PLT (×10³/μL)	235.21±129.12[a] 235.00 (3.00-452.00)	191.85±68.46[a] 155.00 (122.00-314.00)	301.83±158.90[a] 238.00 (88.00-736.00)	240.36±158.44[a] 168.00 (42.00-578.00)
MPV (fL)	12.10±1.08[a] 11.80 (10.60-14.00)	13.05±2.09[a] 12.70 (9.20-15.60)	12.31±1.34[a] 12.15 (9.90-15.30)	11.45±0.55[a] 11.60 (10.50-12.20)
PLR	78.20±55.03[a] 72.59 (1.40-192.70)	162.65±156.38[a] 71.75 (22.59-408.10)	242.83±206.50[a] 185.70 (45.12-1150.00)	387.15±494.83[a] 179.06 (25.50-1540.00)
PNR	85.29±50.60[a] 94.88 (0.75-152.70)	85.29±50.60[b] 94.88 (0.75-152.70)	37.63±23.27[b] 32.27 (8.55-105.10)	24.54±19.31[b] 24.36 (2.07-66.74)
PMR	960.33±559.56[a] 858.00 (6.81-1766.66)	338.36±178.35[a] 296.07 (152.50-665.51)	772.12±1488.41[a] 391.47 (108.88-8352.94)	497.43±464.59[a] 334.04 (32.55-1494.73)
MCV (fL)	37.59±5.11[a] 39.15 (26.50-45.30)	43.95±4.85[b] 42.10 (37.30-50.70)	38.70±4.93[ab] 38.00 (29.50-49.30)	38.64±4.17[ab] 39.30 (32.30-45.30)
MCHC (g/dL)	381.28±34.48[a] 365.50 (344.00-455.00)	284.02±111.03[b] 322.00 (35.20-347.00)	350.63±18.87[a] 353.00 (298.00-412.00)	350.72±18.37[a] 349.00 (325.00-381.00)

Values are expressed as mean ± standard deviation (median, min-max); [ab] *Different superscript letters indicate significant differences between groups (P<0.05); dNLR: derived neutrophil-to-lymphocyte ratio; SIRI: the systemic inflammation response index; AISI: aggregate index of systemic inflammation; SII: systemic immune-inflammation index; WBC: White blood cell; LYM: Lymphocytes; MON: Monocytes NLR: neutrophil-to-lymphocyte ratio; NMR: Neutrophil-to-monocyte ratio; LMR: lymphocyte-to-monocyte ratio; PLT: Platelets; MPV: Mean platelet volume; PLR: platelet-to-lymphocyte ratio; PNR: platelet-to-neutrophil ratio; PMR: Platelet-to-monocyte ratio; MCV: mean corpuscular volume; MCHC: Mean corpuscular volume concentration; HC: healthy control; NE: non-effusive FIP; NES: non-effusive FIP with seizures; E: effusive FIP*

and six cats in the EFIP group. A significant difference in survival times among the three groups during the 60-day treatment period was observed according to the Kaplan-Meier analysis (log-rank test, χ^2=6.34, df=2, P=0.042). The mean survival time was 22.14 days (95% CI: 4.40-39.88) in the NFIP-S group, 48.56 days (95% CI: 41.11-56.02) in the NFIP group, and 40.63 days (95% CI: 27.22-54.04) in the EFIP group. Cats in the NFIP-S group exhibited significantly shorter survival times compared with the other groups.

The predictive ability of MCV for survival was evaluated using ROC curve analysis. The area under the curve (AUC) was 0.696 (95% CI: 0.546-0.845), indicating a statistically significant discriminatory ability (P=0.022) *(Fig. 4)*. At a cut-off value of 39.05 fL, MCV predicted survival with 80% sensitivity and 61% specificity. Cox regression analysis demonstrated a significant positive association between MCV levels and mortality. Each one-unit increase in MCV was associated with a 10.6% increase in the risk of death (HR = 1.106, 95% CI: 1.003-1.219, P=0.043).

Table 3. *Comparison of biochemical parameters and cytokine levels among the study groups*

Group Parameter	HC	NFIP-S	NFIP	EFIP
TP mg/dL	7.52±0.59[a] 7.59 (6.20-8.19)	8.74±1.60[ab] 9.30 (6.40-10.40)	9.72±1.97[b] 9.27 (7.00-15.84)	7.47±2.29[a] 6.97 (4.39-11.00)
ALB mg/dL	3.40±0.47[a] 3.00 (2.40-3.70)	3.01±0.47[ab] 3.00 (2.40-3.70)	2.80±0.47[b] 2.80 (1.90-3.90)	2.48±0.46[b] 2.70 (1.90-3.20)
AGR	1.19±1.45[a] 0.81 (0.53-6.00)	0.59±0.31[ab] 0.53 (0.31-1.27)	0.44±0.17[b] 0.43 (0.16-1.02)	0.55±0.17[ab] 0.61 (0.30-0.76)
MMP-9 ng/mL	6.67±8.38[a] 0 (0-19.6)	19.32±8.91[b] 22.60 (0-25.60)	9.00±10.08[a] 10.10 (0-32.50)	7.60±7.52[a] 10.40 (0-18.40)
IL-8 pg/mL	0.46±0.92[a] 0 (0-2.40)	1.75±2.61[a] 0 (0-6.90)	0.98±1.53[a] 0 (0-4.70)	0.20±0.69[a] 0 (0-2.30)

Values are expressed as mean ± standard deviation (median, min-max); [ab] *Different superscript letters indicate significant differences between groups (P<0.05); TP: Total Protein; ALB: Albumin; AGR: Albumin to Globulin Ratio; MMP-9: Matrix Metalloproteinase-9; IL-8: Interleukin 9; HC: healthy control; NE: non-effusive FIP; NES: non-effusive FIP with seizures; E: effusive FIP*

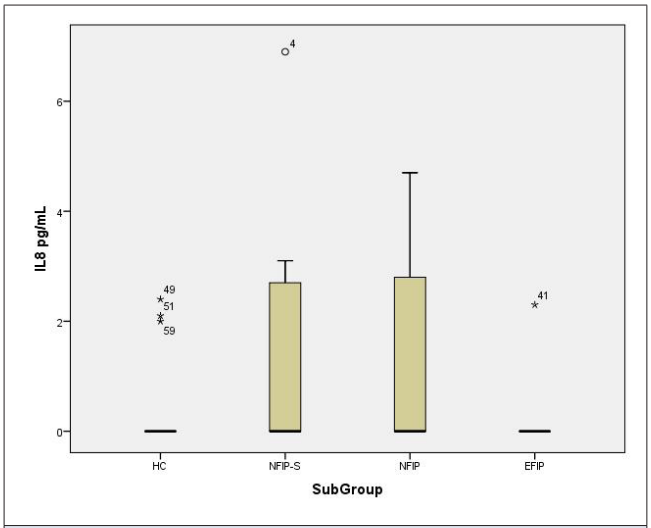

Fig 2. Distribution of serum IL-8 concentrations among the study groups. Box plots represent the median and interquartile range, while whiskers indicate the minimum and maximum values. Circles and asterisks represent outliers. No statistically significant differences were observed among the groups (P>0.05)

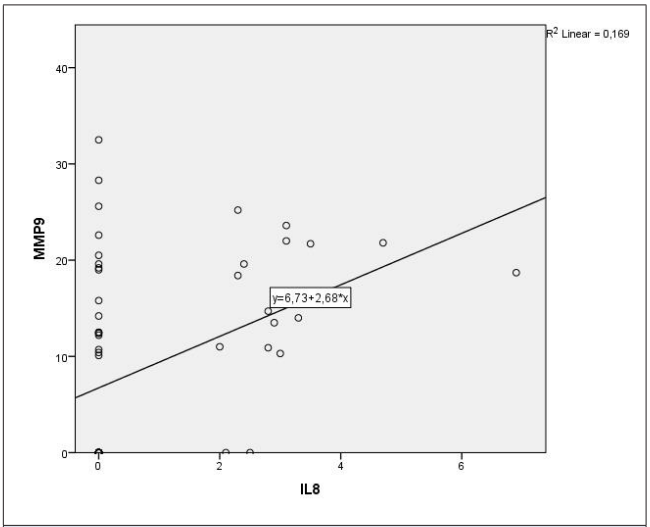

Fig 3. Correlation between serum IL-8 and MMP-9 concentrations in cats included in the study. Scatter plot showing the relationship between IL-8 and MMP-9 levels. The solid line represents the linear regression model (y = −6.73 + 2.68x). A moderate positive correlation was observed between IL-8 and MMP-9 concentrations (Spearman's rho = 0.44, P=0.001).

The relationship between seizure occurrence and MMP-9 levels was evaluated using binary logistic regression analysis. An increase in MMP-9 levels was found to significantly increase the likelihood of seizure occurrence (OR = 1.13, 95% CI: 1.023-1.252, P=0.016). The Hosmer-Lemeshow test indicated a good model fit (P=0.53) *(Fig. 5)*.

Discussion

The most important finding of this study was the markedly elevated MMP-9 levels observed in cats with non-effusive FIP presenting with seizures compared with healthy cats and the other groups (non-effusive and effusive FIP).

Feline infectious peritonitis is an immune-mediated disease triggered by feline coronavirus infection. The development of the effusive (wet) or non-effusive (dry) form of the disease is thought to depend on the complex interaction between the replication rate of the virus in monocytes and the host immune response. The pathogenesis of effusive FIP is associated with a severely impaired cellular immune response in affected cats [1]. Infected macrophages release large amounts of vascular endothelial growth factor (VEGF), which markedly increases vascular permeability and leads to the accumulation of protein-rich effusions within body cavities [19].

The non-effusive form occurs in situations where the host is able to mount a partial cellular immune response [4]. In this case, the virus cannot be completely eliminated, but its dissemination is partially controlled. As a result, pyogranulomatous lesions composed of macrophage and B-cell accumulations develop in various organs, including the kidneys, liver, eyes, and brain [4,5].

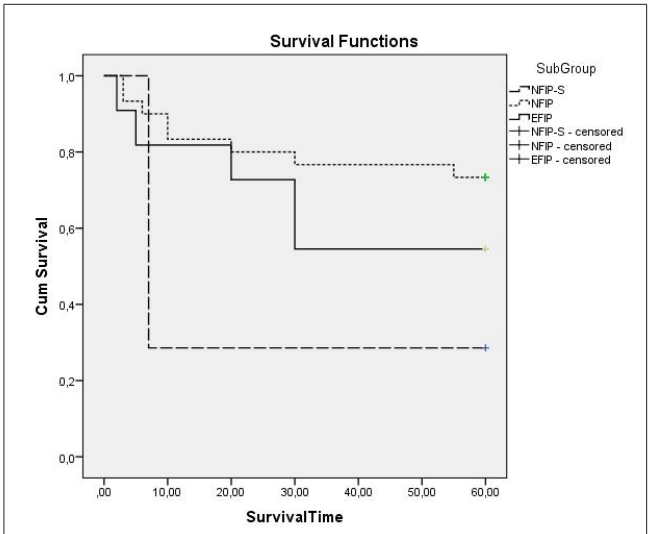

Fig 4. Kaplan-Meier survival curves of cats with different forms of FIP. Kaplan-Meier analysis illustrating the cumulative survival probabilities of cats with non-effusive FIP with seizures (NFIP-S), non-effusive FIP (NFIP), and effusive FIP (EFIP) during the 60-day follow-up period. Cross symbols indicate censored observations

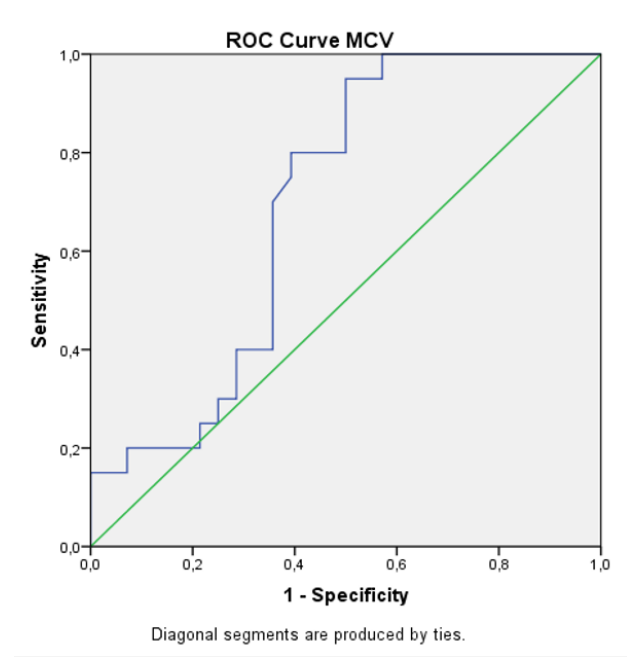

Fig 5. Receiver operating characteristic (ROC) curve analysis of MCV for predicting survival in cats with FIP

Excessive activation of infected monocytes/macrophages, which plays a central role in the pathogenesis of the disease, leads to the release of matrix metalloproteinase-9 (MMP-9). This enzyme degrades type IV collagen in the vascular basement membrane, resulting in increased vascular permeability and the development of effusions in different body compartments in cats [19]. In addition, experimental animal studies have demonstrated that elevated MMP-9 levels are closely associated with epileptogenesis and contribute to neuronal damage and neuroinflammation. Increased MMP-9 protein expression and activity have been shown to induce blood–brain barrier disruption during glutamate-mediated epileptic seizures in humans and rats [6,20]. In the present study, the markedly higher MMP-9 levels observed in the NFIP-S group, particularly compared with the EFIP group, support the hypothesis that FIP may induce seizures in cats through a more specific pathophysiological mechanism.

Furthermore, the observation that the inflammatory indices SII, AISI, SIRI, and dNLR were markedly elevated in the effusive and non-effusive FIP groups but remained comparable to control levels in cats with seizures suggests that seizure development and mortality may not primarily result from a systemic cytokine storm [21]. Instead, these findings support the presence of a more localized inflammatory process involving the central nervous system and blood-brain barrier disruption. Evaluation of albumin, globulin, and AGR values also showed that the NFIP-S group did not differ significantly from the healthy control group, whereas significant alterations were observed in the effusive and non-effusive FIP groups. Although the relatively short survival time of cats with seizures may have limited the progression of systemic inflammatory responses, the markedly elevated MMP-9 levels ($P<0.001$), together with a trend toward increased IL-8 concentrations ($P=0.058$), suggest that localized inflammatory mechanisms may contribute to seizure development. IL-8 signaling has been reported to play an important role in neutrophil activation and degranulation during inflammatory responses, and cats with FIP have been shown to exhibit higher IL-8 levels compared with healthy controls [9]. Increased MMP-9 activity has also been demonstrated to contribute to epileptogenesis through extracellular matrix degradation and blood–brain barrier disruption [6,7]. In the present study, the positive correlation between IL-8 and MMP-9 further supports the involvement of neutrophil-mediated inflammatory pathways in the pathophysiology of seizure development. Taken together, these findings suggest that seizures in cats with non-effusive FIP may be associated with MMP-9-mediated extracellular matrix degradation and neutrophil-driven blood-brain barrier damage, indicating that seizure development in FIP may involve secondary neuroinflammatory mechanisms in addition to viral replication. These findings collectively support the hypothesis that neuroinflammatory mechanisms involving MMP-9 and neutrophil activation may contribute to seizure development in cats with FIP.

Biochemical findings also partially support the pathophysiological differences observed in the NFIP-S group. In FIP, the most frequently reported hematological and biochemical alterations include typical changes

reflecting the systemic inflammatory nature of the disease, such as non-regenerative anemia, lymphopenia, neutrophilia, and hyperglobulinemia [5,22]. In particular, an albumin-to-globulin ratio (AGR) below 0.4 is considered one of the most important biochemical parameters that increases the suspicion of FIP [1,23]. While decreased albumin levels in the NFIP and EFIP groups compared with the healthy control group are consistent with systemic inflammation and protein loss, the observation that albumin, total protein, and AGR values in the NFIP-S group were closer to those of the healthy control group suggests that the systemic biochemical profile in FIP cases presenting with seizures may not fully conform to the typical biochemical pattern observed in FIP.

The aggregate index of systemic inflammation (AISI) has been considered one of the most reliable indices for predicting systemic inflammation during the COVID-19 pandemic in human medicine [24]. The systemic inflammation response index (SIRI), which incorporates monocyte counts in its formula, may be particularly relevant for monitoring the systemic manifestations of FIP, given the central role of monocytes in the pathophysiology of the disease. Hematology-derived inflammatory indices such as NLR, LMR, PLR, PNR, and SII have been reported to show significant alterations in cats suspected of having FIP compared with healthy cats, and they are considered sensitive indicators for detecting systemic inflammation even in cases without marked leukocytosis [12,25]. Similarly, the mean platelet volume-to-platelet ratio (MPV/PLT) has been reported to be elevated in FIP-suspected cases due to inflammatory activity, whereas the platelet-to-lymphocyte ratio did not show a statistically significant difference for disease discrimination [25]. These findings are consistent with the results observed in the NFIP and EFIP groups in the present study. The use of these indices provides a cost-effective approach that adds depth to routine hemogram data for evaluating the severity of cytokine-driven inflammation and monocyte/neutrophil activation, which play critical roles in the pathogenesis of FIP [9,24,26,27].

In FIP, lesions affecting the brain and meninges may develop, resulting in various neurological manifestations. These clinical signs may include ataxia, anisocoria, postural abnormalities, behavioral changes, and seizures [1]. Kunzel et al. [28] reported that FIP was diagnosed in 48% of cats with meningoencephalitis. Rissi [29], reported that seizures were present in 5 of 26 cats (19%) diagnosed with FIP, noting that neurological signs occurred more frequently in the non-effusive form and that, in some cases, seizures may represent the only clinical manifestation of the disease. In another study, seizures were observed in 14 of 55 cats (25%) diagnosed with FIP, and a positive association was identified between forebrain inflammation and enlargement and the occurrence of seizures. Accordingly, the presence of seizures in FIP indicates extensive brain damage and may therefore be considered an unfavorable prognostic sign [30]. It has also been reported that cats with epilepsy associated with structural brain lesions have shorter survival times compared with cats with epilepsy without detectable brain lesions [27]. Furthermore, Rissi [29] demonstrated that among five cats presenting with seizures, three had hydrocephalus and one had ventricular fibrin accumulation. In light of these findings, the significantly shorter survival observed in the NFIP-S group compared with the NFIP and EFIP groups in the Kaplan-Meier analysis is consistent with previous reports identifying seizures as an unfavorable prognostic indicator in FIP.

In the present study, the finding that MCV demonstrated a significant discriminatory ability for predicting survival, and that each unit increase in MCV was associated with an increased risk of mortality, suggests that MCV may represent not only a hematological parameter but also a potential biomarker reflecting disease severity. Mean corpuscular volume (MCV) is a parameter routinely included in complete blood count analysis and is commonly used in the classification of anemia. Previous studies in humans have demonstrated an association between increased MCV values and malignant conditions [31]. Moreover, several studies have reported that elevated MCV values are associated with poorer prognosis in chronic diseases, findings that are consistent with the results of the present study [32]. Therefore, MCV may contribute to clinical evaluation as a potential prognostic biomarker in cats with FIP, particularly in the presence of neurological involvement. However, further prospective and multivariable studies are required to clarify the underlying causal mechanisms of this association.

Increased MMP-9 activity may contribute to inflammation of the blood–brain barrier, which can subsequently lead to neurological manifestations. Neurological conditions such as uncontrolled seizures, structural brain abnormalities, and inflammatory brain diseases have been shown to influence survival rates in humans, dogs, and cats. In particular, uncontrolled seizures have been reported to increase mortality in humans and dogs [33] as well as in cats [27]. In cases where seizures cannot be adequately controlled, euthanasia decisions are frequently made [14,15], and seizures have therefore been recognized as an unfavorable prognostic indicator [30]. Furthermore, studies investigating epileptic cats with structural brain lesions have highlighted the involvement of different clinicopathological pathways in seizure development, which may provide alternative perspectives for disease management, including potential therapeutic approaches targeting MMP-9 activity. Notably, the present study demonstrated that each unit increase in MMP-9 concentration was associated with a 13%

increase in the likelihood of seizure occurrence. Therefore, therapeutic strategies combining antiepileptic treatment with approaches targeting MMP-9 activity may represent a potential area for future investigation in cats presenting with seizures.

This study has several limitations. The relatively small number of cats in the NFIP-S group represents an important limitation. Nevertheless, the finding that MMP-9 levels differed significantly between groups supports the robustness of the observed association. Another limitation is the absence of advanced neurological imaging (MRI) and cerebrospinal fluid (CSF) analysis for the characterization of intracranial pathologies. However, the inclusion of cases based on standardized diagnostic criteria and consistent clinical evaluation procedures partially mitigates this limitation.

In conclusion, the marked increase in serum MMP-9 levels observed in cats with non-effusive FIP presenting with seizures suggests that MMP-9-mediated blood-brain barrier disruption and neuroinflammation may contribute to both seizure development and poorer prognosis in these cases. Interestingly, the absence of marked increases in hematology-derived systemic inflammatory indices in the seizure group, despite significantly elevated MMP-9 levels, supports the hypothesis that seizures in FIP may reflect a distinct neuro-immunopathological phenotype characterized by localized rather than generalized inflammation. Collectively, these findings suggest that MMP-9 may represent not only a prognostic biomarker of neurological involvement and poor outcome, but also a potential therapeutic target in the neuroinflammatory manifestations of the disease.

Declarations

Availability of Data and Materials: The data sets generated and/or analyzed during the current study are available from the corresponding author (UO) upon reasonable request.

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Ethical Statement: This study was approved by the Local Ethics Committee for Animal Experiments at Ondokuz Mayıs University on June 30, 2025, under decision number 2025/38.

Competing Interests: The authors declared that there is no conflict of interest.

Declaration of Generative Artificial Intelligence (AI): The authors declare that the article, tables and figures were not written/created by AI and AI-assisted Technologies.

Author Contributions: Methodology, study design and writing - original draft: ZNK; Conceptualization, methodology, data analysis, interpretation, funding acquisition and writing - review and editing: UO; Methodology and sample collection: SBK; Field examinations and sample collection: YTK; Field examinations and sample collection: ZFE. All authors read and approved the final manuscript.

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