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Evaluation of selected inflammatory markers in cats with feline infectious peritonitis before and after therapy

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Abstract

Background Feline infectious peritonitis (FIP), once considered fatal disease with a mortality rate approaching 100%, has experienced a new therapeutic breakthrough in recent years. The aim of our study was to evaluate selected clinicopathological parameters before and after GS- 445124-based treatment of FIP in cats, which could serve as potential candidates for predicting treatment success and monitoring treatment progress.

Results Pre-treatment haematological parameters in 32 treated cats showed moderate leukocytosis, neutrophilia, lymphopenia and anaemia, which normalised post-treatment. Pre-treatment values of haemogram-derived inflammatory markers (ratio of neutrophils to lymphocytes, platelets to lymphocytes, lymphocytes to monocytes and the systemic immune-inflammatory index) differed significantly from those in the healthy cats and between patients with effusive and non-effusive disease ($p < 0.05$). Post-treatment, only the ratio of lymphocytes to monocytes remained higher; the other three markers were comparable to the control group. The biochemical results showed characteristic abnormalities (e.g. hyperproteinaemia, hypoalbuminemia, hypergammaglobulinemia, hyperbilirubinemia), which normalised with treatment. Lactate dehydrogenase activities did not differ significantly before and after treatment, except in cats with a relapse and one non-responder, which had markedly elevated values at the time of diagnosis. Analysis of archived blood samples using ELISA revealed significant differences in concentration of acute-phase protein haptoglobin ($p = 0.004$) and pro-inflammatory cytokine tumour necrosis factor-α ($p = 0.028$) before and after therapy. Therapy didn't elicit any statistically significant changes in concentrations of ferritin, interleukin- 1β and interleukin- 6.

Conclusions Our findings demonstrate that successful treatment of FIP leads to highly significant changes in most clinicopathological parameters, including haemogram-derived inflammatory markers. The latter could offer a simple, inexpensive and readily available alternative to the more commonly used acute phase proteins for monitoring FIP treatment. Successful therapy leads to a significant decrease in haptoglobin and an increase in tumour necrosis factor-α. In our study, cats with an unfavourable outcome showed a marked increase in lactate dehydrogenase activity before therapy, suggesting that this parameter could be a promising prognostic factor in larger studies.

Keywords Cats, FIP, Inflammatory response, Haemogram-derived inflammatory markers, Acute phase proteins

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Background

Feline infectious peritonitis (FIP) is a unique infectious disease in cats, caused by the feline coronavirus (FCoV). There are two different biotypes of FCoV: the low-virulence feline enteric coronavirus and the highly-virulent feline infectious peritonitis virus [1–3]. The majority of cats overcome the FCoV infection asymptomatically or exhibit transient upper respiratory or mild gastrointestinal clinical signs [4]. Only a small percentage (up to 10%) of infected cats develop fulminant systemic disease with systemic vasculitis and subsequent multiorgan involvement [2]. The mutation, which is responsible for this scenario, occurs spontaneously in a certain region of the FCoV genome, leading to a change in cell tropism from enterocytes to monocytes/macrophages [5]. The factors contributing to the mutation of the virus and causing systemic disease are not entirely elucidated and likely involve a combination of virus mutations and individual characteristics of each cat, including genetic predisposition and specific immune response.

Non-specific clinical signs commonly observed in the early stages of the disease include lethargy, anorexia, fever refractory to antibiotics, lymphadenopathy and weight loss [2, 5]. However, in some cases, cats may retain their body condition [2, 5]. There are two clinical forms of FIP: wet/effusive and dry/non-effusive, with the former being easier to diagnose [5]. The most obvious manifestation of the wet form is ascites; however, thoracic and pericardial effusion can also occur, leading to dyspnoea and tachypnoea. Abdominal organs may be affected by pyogranulomatous lesions and masses [2, 4]. Cats can present with gastrointestinal signs, including vomiting and diarrhoea. Ocular and neurological manifestations are also common in cats with FIP [2, 4].

Until recently, FIP was considered an incurable disease with an extremely poor prognosis, with a mortality rate close to 100% [2]. However, in 2019, researchers from UC Davis published ground-breaking results from a study on the experimental antiviral drug GS- 441524, a nucleoside analogue [6]. The study demonstrated an 80% efficacy rate in treating 31 naturally infected cats. The possibility of effectively treating a previously untreatable disease, combined with the drug's lack of registration for veterinary use, has led to the unique phenomenon of so-called crowd-sourced antiviral GS- 441524-like therapy [7]. In the last two years, several studies have been published, indicating that despite the unlicensed and uncontrolled production of the drug, it is possible to achieve pronounced clinical effect, leading to up to 94% remission rate [8–15].

With the advent of a completely new therapy offering an effective long-term cure, it seems necessary to determine the predictive and prognostic factors as well as parameters that could be used for monitoring the effect of the therapy. FIP shares some features with COVID-19: both are caused by a coronavirus infection, and in both, the infection can either lead to a life-threatening/fatal form or resolve spontaneously with milder clinical signs. Numerous predictive factors are known for the fulminant form of COVID- 19, including neutrophil-to-lymphocyte ratio (NLR); lymphocyte-to-monocyte ratio (LMR); platelet-to-lymphocyte ratio (PLR); systemic inflammatory index (SII); lactate dehydrogenase (LDH) enzyme activity; concentration of cytokines such as interleukin- 6 (IL- 6), interleukin- 1ß (IL- 1ß) and tumour necrosis factor-α (TNF-α); and acute-phase proteins (APPs) such as ferritin and C-reactive protein [16]. Despite the successful treatment of numerous cats with GS- 441524-based therapies in the last half decade, only a few prognostic factors indicating a favourable treatment outcome have been described. These include a good appetite and/or activity level, a lower bilirubin concentration, lower LDH activity [17] and normalisation of alpha-1-acid glycoprotein (AGP) and serum amyloid A (SAA) concentrations [11, 18]. Other parameters that can be used to assess the progress of treatment are the resolution of lymphopenia and anaemia and the normalisation of the albumin to globulin (A/G) ratio [5, 11].

The purpose of this study was to evaluate selected clinicopathologic parameters (hematological parameters including haemogram-derived inflammatory markers and biochemical parameters including concentration of acute phase proteins and cytokines), before and after treatment of FIP in cats, which could serve for monitoring the progress of the treatment and as possible candidates for predicting therapeutic outcomes.

Methods

Cats

Blood samples from a total of 35 client-owned cats were included in this study, which was conducted between 2020 and 2022 at the Small Animal Clinic of the Veterinary faculty of the University of Ljubljana, Slovenia. The inclusion criteria consisted of a FIP diagnosis, confirmed by a veterinarian, following the AAFP diagnostic guidelines [5]. The diagnosis of FIP was based on relevant clinical signs with typical clinicopathological test results (haematology, biochemistry and cytologic examination of effusions) and the detection of FCoV in body effusions and/or affected organs (spleen, lymph node aspirates) with commercially available RT PCR detection of mutations in spike proteins (FIP virus RealPCR™, IDEXX laboratory).

The study was conducted as a retrospective study, utilizing clinical and laboratory data and archived blood samples collected for diagnostics and/or clinical

monitoring. A total of 32 cats were treated with a non-licensed antiviral drug containing the adenosine nucleoside analogue GS- 441524. At the time of the study, there were no licensed drugs containing GS- 441524 available in the European Union; therefore, the therapy was carried out as decribed in previously reported crowd-sourced GS- 441524-based therapies [7]. Cats that completed a 12-week observation period after the completion of therapy without experiencing a relapse were considered cured. Three out of 35 cats were not treated, but their results were included in the statistical pre-treatment group comparison with the control group.

The following clinical information was collected: weight before/after treatment, age, sex and neuter status, presenting clinical signs inclusive of effusive/non-effusive form, ocular and neurological involvement. In treated cats, clinical examinations and blood sample collections were performed at the time of diagnosis and 4, 8 and 12 weeks after the start of treatment. Blood samples were collected from all cats via venipuncture of the cephalic or jugular vein. Archived serum samples from 19 cats were stored at − 80°C for further analyses with the permission of the caregivers.

Haematology analysis and haematology-derived inflammatory markers

In all included animals, a complete blood count with a differential white blood cell count was performed using an ADVIA 120 automated haematology analyser with species-specific software (Siemens, Germany). From these results, the following haematology-derived inflamamtory markers were calculated from absolute counts of relevant parameters: NLR, LMR and PLR. Additionally SII was calculated as neutrophills x platelets/lymphocytes. Since reference values for these haematology-derived inflammatory markers are not available for cats, a control group of 28 age-matched healthy cats was included, for which the same indexes were calculated. In these cats, the analysis was performed as a part of a wellnes checkup, and results were used with the permission of the caregivers.

Biochemistry analysis

The biochemical parameters determined at each time point were selected at the discretion of the attending veterinarian, based on the clinical status of each animal. However, the minimal laboratory workup included the routine determination of serum urea, creatinine, total protein and albumin concentrations, and ALT activity. Serum protein electrophoresis and bilirubin mesurement were performed in 7 treated cats before treatment and 20 cats after treatment. All biochemical analyses were performed at a commercial laboratory (IDEXX Laboratories, Germany), and individual values were compared with the reference ranges reported by IDEXX Laboratories. Additionally, LDH activity was determined retrospectively in stored frozen serum samples. LDH activity was measured spectrophotometrically using an RX Daytona + automated biochemical analyser (Randox, Crumlin, United Kingdom) and a Randox lactate dehydrogenase L-lactate–pyruvate reagent kit in the Diagnostic Laboratory of the Small Animal Clinic.

Cytokine and APP response

Out of the 19 samples stored with caregivers' permission, samples from 8 cats were suitable for the determination of cytokine and/or APP response. The remaining cats had either missing samples at crucial time points (e.g. before or after therapy) due to the caregivers' lack of compliance, or the volume of the stored samples was insufficient for analysis.

An additional five blood samples from healthy cats, collected as part of a wellness check-up during routine examinations, served as a control group. Cats were considered healthy based on their history, physical examination and routine laboratory results (routine haematology and biochemistry parameters within reference ranges). Species-specific, commercially available quantitative sandwich ELISA tests were used to determine five selected serum parameters: IL- 6, TNF-α, IL- 1β, ferritin (Cat IL- 6 ELISA kit, Cat. No.: MBS284478; Cat TNF-α ELISA kit, Cat. No.: MBS1602676; Cat IL- 1β kit, Cat. No.: MBS9319075; Cat Ferritin ELISA kit, Cat. No.: MBS096404; all Mybiosource, USA), and haptoglobin (Feline and Canine Haptoglobin ELISA kit, Cat. No.: TE1033, Teco, Switzerland). The standards used to generate the calibration curve were prepared in six different concentrations for each kit separately according to the manufacturer's recommendations regarding the stated sensitivity for each parameter. All test samples, standards and quality controls were analysed in duplicate according to the manufacturer's instructions. Optical densities were measured at 450 nm using a Multiskan FC Microplate Photometer microplate reader (Thermo Fisher Scientific, USA), and the average absorbance of the two measurements of each sample was calculated. Sample concentrations were determined using the best-fit standard curve.

Statistical analysis

Results from the initial visit (pre-treatment) and final checkup (post-treatment, 12 weeks after the start of therapy) were statistically analysed for the study. The data were analysed using commercially available software (IBM SPSS 29, Chicago, Illinois, USA). The Shapiro–Wilk test was used to determine the data distribution. Based on the results, parametric or non-parametric tests were

used to compare the data. We performed the following comparisons:

(a) for haematology, cytokines and APPs, pre-treatment values of selected parameters (including results of cats that were not treated, where available) were compared with values of the control group. Post-treatment values of these parameters were compared with those of the control group and, for parameters where paired results for the same cat were available, pre- and post-treatment values were compared to determine the effect of the therapy. In addition, pre- and post-treatment data were also compared between cats with the effusive and non-effusive form of FIP.
(b) For biochemistry results, pre-treatment and post-treatment values were compared for those parameters where paired results for the same cat were available to determine the effect of the therapy.

Accordingly, an independent t-test was used in cases of normally distributed data, and the Mann–Whitney U-test was applied for non-normally distributed data to test for statistically significant differences in the measured parameters between FIP cats and the control group before and after therapy and between cats with the effusive and non-effusive form of FIP. Moreover, the Mann–Whitney test was used to test for statistically significant differences in weight between young and adult cats. For FIP cats, a paired sample t-test was used for normally distributed data, and a Wilcoxon signed-rank test was used for non-normally distributed data to compare parameters before and after therapy. A value of $p \leq 0.05$ was considered significant.

Results

Cats

Thirty-five cats diagnosed with FIP (14 females and 21 males) with a median age of 11 months (IQR: 6.0–24.0 months) and 28 healthy cats (10 females and 18 males) with a median age of 16.5 months (IQR: 10.0–23.8 months) were included in the study. Although the cats in the control group were 5.5 months (median) older, the age difference was not significant ($p = 0.065$). The study population comprised 16 domestic shorthair/longhair cats, 11 British shorthair/longhair cats, 4 Maine Coons and one each of the following breeds: Ragdoll, Bengal, Persian and Siberian.

In 23/35 cats (65.7%), the effusive form of FIP was diagnosed, while in 12/35 (34.2%), the non-effusive form was observed. Eight of the 35 cats (22.8%) exhibited neurological signs, primarily seizures, ataxia and incontinence. Six of eight cats with neurological signs had non-effusive disease and two had effusive form. Additionally, 10/35 (28.5%) cats had ocular involvement, primarily manifesting as clinical signs related to anterior uveitis, including blepharospasm, photophobia, conjunctivitis, myosis, positive flare and low intraocular pressure. Seven of these cats had non-effusive form of FIP and three had effusive form. There were six cats which had both neurological and ocular clinical signs and all of them had non-effusive disease.

Twenty-nine of the 32 patients (90.6%) were considered cured after a single course of 12-week therapy. In 2/32 patients (6.25%), both six months old at the time of the first diagnosis, relapse occurred after the completion of therapy. One cat (Ragdoll) relapsed within 80 days, and another (Maine Coon) within 30 days. However, both were treated again with an increased dosage (approximately 50% higher as the initial dose) of the same medicine and successfully completed a second 12-week observation period without relapse. The results obtained in both treatment cycles were used independently in the study. One cat (3.1%) in severe clinical condition died three days after the start of therapy.

Complete data regarding weight (before and after therapy) were available for 29 cats. Before treatment, the treated cats had significantly ($p = 0.010$) lower weight compared to the age-matched control group. After treatment, the treated cats showed a significant increase in weight ($p < 0.001$) (Table 1), which was no longer significantly lower compared to the control group ($p = 0.192$). The increase in weight was significant and independent of their age (young cats under one year ($p < 0.001$) vs. adult cats over one year ($p = 0.001$)).

Table 1 Weight in treated and control cats before and after treatment

Weight [kg]	Weight before treatment [kg] Median; IQR	Weight after treatment [kg] Median; IQR	*p* value
All FIP cats; *n* = 29	2.85; 2.38–4.00	4.50; 3.80–5.03	**< 0.001**
FIP cats < 1 year old; *n* = 16	2.55; 1.83–3.05	4.18; 3.19–4.65	**< 0.001**
FIP cats > 1 year old; *n* = 13	3.50; 3.01–4.35	4.55; 4.00–5.38	**0.001**
Control group *n* = 22	3.90; 3.53–4.62*		

* Significant difference in weight in control group cats ($p = 0.010$) compared to the group of all FIP cats before treatment; $p < 0.05$ (bold), significant difference before vs after treatment; IQR, 25 th – 75 th percentile; n = number of data included in the statistical analysis

Haematological parameters

Pre-treatment haematological parameters indicated moderate leucocytosis with absolute and relative neutrophilia and lymphopenia and moderate anaemia (Table 2). All haematological parameters, with the exception of platelet and monocyte concentrations, were significantly different compared to those of the control group of healthy cats (Table 2). Post-treatment values of these haematological parameters normalised, showing no significant difference in comparison with those of the control group. Platelet concentration was the only parameter that was significantly higher compared to the control group after treatment ($p = 0.002$). Similarly, all pre-treatment haemogram-derived inflammatory markers significantly differed from those in healthy cats, with NLR ($p < 0.001$), PLR ($p = 0.007$) and SII ($p < 0.001$) having higher values, and LMR ($p = 0.014$) having a lower value compared to the control group (Table 2). Post-treatment, only LMR remained significantly different compared to healthy cats ($p = 0.001$).

Statistical analysis of the effects of treatment on haematological parameters was not possible for all included cats due to missing data (Table 3). However, all selected haematological and haemogram-derived inflammatory markers were significantly different post-treatment compared to pre-treatment values (Table 3). Anaemia improved markedly, as all erythrocyte-dependent parameters (RBC, HCT, HGB) increased significantly after treatment (all $p < 0.001$). The most profound change in the white blood cell differential count was a significant decrease in neutrophils and a significant increase in lymphocytes (both $p < 0.001$). However, we observed a less pronounced but still significant decrease in total white blood cell count ($p = 0.047$). Treatment also had a notable impact on haemogram-derived inflammatory markers, as the comparison of pre- and post-treatment results showed significant differences in all these parameters (Table 3).

An additional comparison of both pre- and post-treatment haematological parameters between cats with effusive and those with non-effusive FIP was performed.

Table 2 Haematological and haemogram-derived inflammatory markers in healthy cats and FIP cats before and after treatment

Parameter	HEALTHY cats	FIP cats			
		Before treatment	p^a	After treatment	p^b
WBC ($\times 10^9$/L)	n = 28 8.80; 7.65–12.68	n = 36 14.57; 9.07–20.76	**0.006**	n = 30 10.60; 8.93–12.10	0.225
RBC[c] ($\times 10^{12}$/L)	n = 28 9.38 ± 1.56	n = 36 7.15 ± 1.90	**< 0.001**	n = 30 9.94 ± 1.16	0.065
HCT[c] (L/L)	n = 28 0.413 ± 0.059	n = 35 0.279 ± 0.067	**< 0.001**	n = 30 0.394 ± 0.051	0.131
HGB[c] (g/L)	n = 28 127.6 ± 16.1	n = 36 91.9 ± 22.3	**< 0.001**	n = 30 131.2 ± 14.0	0.362
NEUT ($\times 10^9$/L)	n = 28 4.58; 2.74–7.23	n = 36 12.03; 8.26–18.17	**< 0.001**	n = 30 4.83; 4.17–6.05	0.560
LYMPH ($\times 10^9$/L)	n = 28 3.24; 2.68–4.26	n = 36 1.55; 1.05–2.74	**< 0.001**	n = 30 3.98; 2.91–6.09	0.102
MONO ($\times 10^9$/L)	n = 28 0.290; 0.190–0.608	n = 36 0.390; 0.150–0.680	0.839	n = 30 0.210; 0.130–0.308	0.072
PLT ($\times 10^9$/L)	n = 28 263; 221–333	n = 27 258; 168–346	0.613	n = 29 407; 287–435	**0.002**
NLR	n = 28 1.47; 0.82–2.45	n = 36 7.43; 3.95–11.13	**< 0.001**	n = 30 1.16; 0.78–1.58	0.234
LMR	n = 28 10.50; 6.41–15.95	n = 34 6.01; 1.71–12.70	**0.014**	n = 29 18.50; 14.09–26.93	**0.001**
PLR	n = 28 74.9; 57.8–128.6	n = 27 156.3; 94.2–238.5	**0.007**	n = 29 88.0; 62.1–126.9	0.737
SII ($\times 10^9$/L)	n = 28 444.5; 185.4–652.4	n = 27 2265.7; 869.3–3158.4	**< 0.001**	n = 29 431.5; 264.2–722.6	0.503

IQR (25 th to 75 th percentile); *WBC* white blood cell count; *RBC* red blood cell count; *HGB* haemoglobin concentration; *HCT* haematocrit; *NEUT* neutrophil concentration; *LYMPH* lymphocyte concentration; *MONO* monocyte concentration; *PLT* platelet concentration; *NLR* neutrophil-to-lymphocyte ratio; *LMR* lymphocyte-to-monocyte ratio; *PLR* platelet-to-lymphocyte ratio; *SII* systemic immune-inflammatory index; *n* number of data included in the statistical analysis

[a] control vs FIP before treatment, significant difference, $p < 0.05$ (bold)

[b] control vs FIP after treatment, significant difference, $p < 0.05$ (bold)

[c] values reported as mean ± standard deviation, other parameters are reported as median

Table 3 Haematological parameters and haemogram-derived inflammatory markers before and after treatment of FIP

Haematological parameters	Before treatment	After treatment	*p*
WBC ($\times 10^9$/L); n = 30	11.50; 8.43–18.60	10.60; 8.93–12.1	**0.047**
RBC[a] ($\times 10^{12}$/L); n = 30	7.11 ± 1.57	9.94 ± 1.16	**< 0.001**
HCT[a] (L/L); n = 29	0.276 ± 0.059	0.394 ± 0.051	**< 0.001**
HGB[a] (g/L); n = 30	91.1 ± 20.4	131.2 ± 14.0	**< 0.001**
NEUT ($\times 10^9$/L); n = 30	10.94; 5.81–17.17	4.83; 4.17–6.05	**< 0.001**
LYMPH ($\times 10^9$/L); n = 30	1.48; 1.01–2.48	3.98; 2.91–6.09	**< 0.001**
MONO ($\times 10^9$/L); n = 30	0.390; 0.143–0.643	0.210; 0.130–0.308	**0.016**
PLT ($\times 10^9$/L); n = 23	248; 163–346	400; 283–432	**0.006**
NLR; n = 30	7.43; 4.03–10.28	1.16; 0.78–1.58	**< 0.001**
LMR; n = 27	6.33; 1.75–12.25	18.50; 14.19–26.35	**< 0.001**
PLR; n = 23	144.2; 92.1–246.3	91.7; 67.4–120.5	**0.013**
SII ($\times 10^9$/L); n = 23	2160.4; 869.3–3158.4	431.5; 247.5–749.0	**< 0.001**

WBC white blood cell count; *RBC* red blood cell count; *HGB* haemoglobin concentration; *HCT* haematocrit; *NEUT* neutrophil concentration; *LYMPH* lymphocyte concentration; *MONO* monocyte concentration; *PLT* platelet concentration; *NLR* neutrophil-to-lymphocyte ratio; *LMR* lymphocyte-to-monocyte ratio; *PLR* platelet-to-lymphocyte ratio; *SII* systemic immune-inflammatory index; *n* number of data included in the statistical analysis

[a] values reported as mean ± standard deviation, other parameters are reported as median and IQR (25 th to 75 th percentile); $p < 0.05$ (bold), significant difference before vs after treatment

Statistical analysis revealed significantly higher NLR ($p = 0.044$) pre-treatment values in effusive FIP (median: 8.90; IQR: 4.63–14.55) compared to non-effusive FIP (median: 4.98; IQR: 2.37–8.39). Similarly, pre-treatment SII values were significantly ($p = 0.045$) higher in effusive FIP (median: 2719.4 $\times 10^9$/L; IQR: 1190.0 $\times 10^9$/L – 3971.5 $\times 10^9$/L) than in non-effusive FIP (median: 1056.5 $\times 10^9$/L; IQR: 701.5 $\times 10^9$/L – 1804.9 $\times 10^9$/L). Conversely, we observed a much lower, though non-significant ($p = 0.052$), pre-treatment lymphocyte concentration in effusive FIP (median: 1.23 $\times 10^9$/L; IQR: 1.02 $\times 10^9$/L – 2.20 $\times 10^9$/L) than in non-effusive FIP (median: 2.40 $\times 10^9$/L; IQR: 1.31 $\times 10^9$/L – 4.69 $\times 10^9$/L). All other haematological parameters and haemogram-derived inflammatory markers did not differ significantly between these two clinical presentations either before or after treatment.

Biochemical parameters

Biochemistry profiles of treated cats, which were performed as a part of the diagnostics of the disease, revealed hyperproteinaemia with hyperglobulinemia, hypoalbuminemia, decreased albumin/globulin ratio and hyperbilirubinemia (Table 4).

Post-treatment biochemical parameters were not available for all patients; therefore, the comparison of pre- and post-treatment parameters in treated cats was based on a smaller subset (Table 4). Therapy profoundly affected the majority of biochemical parameters, which returned toward reference values: there was a significant decrease in the concentrations of total proteins, bilirubin and absolute and relative globulins (all $p < 0.001$). Albumin levels, A/G ratio and alanine aminotransferase (ALT) activity significantly increased (all $p < 0.001$). Electrophoresis of serum proteins showed a significant decrease only in the concentrations of alpha- 2 ($p = 0.018$) and gamma globulins ($p = 0.028$); changes in other fractions did not reach statistical significance.

Cytokine and APP response

TNF-α concentration (Fig. 1A) significantly increased after therapy from a median concentration of 77.4 pg/mL (IQR: 46.5–103.5 pg/mL) to 114.2 pg/ml (IQR: 111.0–158.4 pg/mL) ($p = 0.028$). Additionally, its median concentration after therapy was significantly higher compared to the control group (99.0 pg/mL, IQR: 78.7–99.2 pg/mL; $p = 0.042$). IL- 1ß concentration (Fig. 1B) slightly decreased after therapy, from its median concentration of 20.3 pg/mL (IQR: 13.9–26.3 pg/mL) to 19.7 pg/mL (IQR: 15.4–26.3 pg/mL). IL- 6 concentration (Fig. 1C) decreased from a median concentration of 61.1 pg/mL (IQR: 36.6–286.0 pg/mL) to 53.4 pg/mL (IQR: 11.2–90.0 pg/mL). However, the changes in these two variables did not reach statistical significance. Furthermore, there were no significant differences in IL- 1ß and IL- 6 concentrations either before or after therapy compared to the control group.

Of the acute-phase proteins, the haptoglobin concentration (Fig. 2A) showed significant changes after treatment, significantly decreasing ($p = 0.018$) from 9.91 g/L (IQR: 6.41. – 10.53 g/L) to 4.77 g/L (IQR: 3.56–5.47). Compared to the control group, the haptoglobin concentration was significantly higher both before ($p = 0.004$) and after therapy ($p = 0.042$). However, the post-treatment concentration almost reached that of the

Table 4 Selected biochemical parameters before and after treatment of FIP and their reference values

Biochemical parameters	Reference values*	Before treatment	After treatment	p
TP[a] (g/L); n = 29	59–87	84.0 ± 15.6	72.9 ± 6.2	**< 0.001**
ALB[a] (g/L); n = 29	31–47	23.6 ± 6.3	36.7 ± 3,6	**< 0.001**
ALB[a] (%); n = 29	44.5–62.3	28.7 ± 8.9	50.6 ± 6.2	**< 0.001**
GLOB[a] (g/L); n = 29	28.0–51.0	60.1 ± 16.2	36.3 ± 7.0	**< 0.001**
GLOB[a] (%); n = 29	5.9–23	70.9 ± 9.6	49.4 ± 6.4	**< 0.001**
A/G[a]; n = 29	> 0.57	0.433 ± 0.224	1.058 ± 0.265	**< 0.001**
Alpha- 1 globulins (g/L); n = 7	1.6–5.2	1.8; 1.6–2.1	2.3; 2.0–2.5	0.176
Alpha- 2 globulins (g/L); n = 7	4.0–12.8	8.4; 6.1–11.1	5.5; 4.6–7.4	**0.018**
Beta- 1 globulins (g/L); n = 7	1.9–7.4	4.5; 3.8–5.1	5.2; 5.0–5.5	0.150
Beta- 2 globulins (g/L); n = 7	2.0–8.5	7.0; 5.8–8.1	6.1; 5.0–6.7	0.128
Gamma-globulins (g/L); n = 7	3.6–16.9	32.9; 19.0–47.6	12.7; 12.3–19.4	**0.028**
ALT(U/L); n = 28	27–110	39.0; 31.3–50.8	67.5; 50.0–80.0	**< 0.001**
Bilirubin (µmol/L); n = 25	0–6.8	8.00; 4.05–23.40	3.20; 2.60–3.80	**< 0.001**
LDH (U/L); n = 9	21–217	119.0; 80.5–152.5	110.0; 75.5–172.5	0.594

TP total protein concentration; *ALB* albumin; *GLOB* globulins; *A/G* albumin-to-globulin ratio; *ALT* alanine aminotransferase; *LDH* lactate dehydrogenase; *n* number of data included in the statistical analysis

* IDEXX laboratories; [a] values reported as mean ± standard deviation, other parameters are reported as median and IQR (25 th to 75 th percentile); $p < 0.05$ (bold), significant difference before vs after treatment

control group (median: 3.21; IQR: 2.55–3.50 g/L), with the p-value at the threshold of statistical significance.

Pre-treatment and post-treatment ferritin concentrations (Fig. 2B) of 10.76 ng/mL (IQR: 8.05–12.30 ng/mL) and 10.52 ng/mL (IQR: 10.00–12.83 ng/mL), respectively, were lower compared to those of the control group (median: 12.39 ng/mL; IQR: 10.54–13.51 ng/mL); however, these differences did not reach statistical significance in any of the comparisons.

Discussion

Our study demonstrated that clinically successful treatment of FIP in cats results in the normalisation of the majority of haematological and biochemical parameters presented at the time of diagnosis, including haemogram-derived inflammatory markers. This includes parameters that were severely altered pre-treatment. Furthermore, we observed a significant decrease in haptoglobin and an increase of TNF-α following clinical remission.

Specific antiviral treatment with GS- 441524-based therapy has been demonstrated to be highly successful in treating FIP in cats. Large-scale studies report remission rates between 80 and 94%, with some studies even reaching a 100% success rate [6–15, 17, 19, 20]. A relapse rate of approximately 10% has been reported in a large-scale study, which aligns with our findings [19], where relapse was observed in 2 cats out of a surviving 31. Both of these cats were successfully treated with a repeated course of therapy. Notably, one of the two cats that relapsed in our study was a Ragdoll. Green et al. (2023) reported a poorer response to therapy in Ragdoll cats, noting a delayed response to treatment, with 3/5 Ragdoll cats being euthanized [20]. On the other hand, study by Goto et al. [17] reported that one out of three Ragdoll cats, included in the study, died. Furthermore, they didn't observe any significant differences in short-term mortality between pedigree and non-pedigree cats. While the number of Ragdolls in these studies is too low to draw definitive conclusions, further research should consider investigating breed-specific responses to treatment, particularly since pure-bred cats are at higher risk of developing FIP [5].

An important outcome in treated cats was the significant increase in their body weight [21, 22], which was also confirmed in our study. Notably, the weight gain was not associated with the accumulation of ascitic fluid, as ascites resolved within the first month of therapy in all cats enrolled in the study. FIP commonly affects younger

(See figure on next page.)

Fig. 1 Tumour necrosis factor-alpha (TNF-α) (**A**), interleukin- 1 ß (IL- 1ß) (**B**) and interleukin- 6 (IL- 6) (**C**) concentrations before and after FIP treatment and in the control group of healthy cats. The Mann–Whitney U-test was used to test for statistically significant differences in TNF-α (n = 7), IL- 1β (n = 5) and IL- 6 (n = 7) concentrations between FIP cats and the control group (n = 5) before and after therapy. A Wilcoxon signed-rank test was used to compare TNF-α (n = 7), IL- 1β (n = 5) and IL- 6 (n = 7) concentrations before and after therapy. A value of $p \leq 0.05$ was considered significant

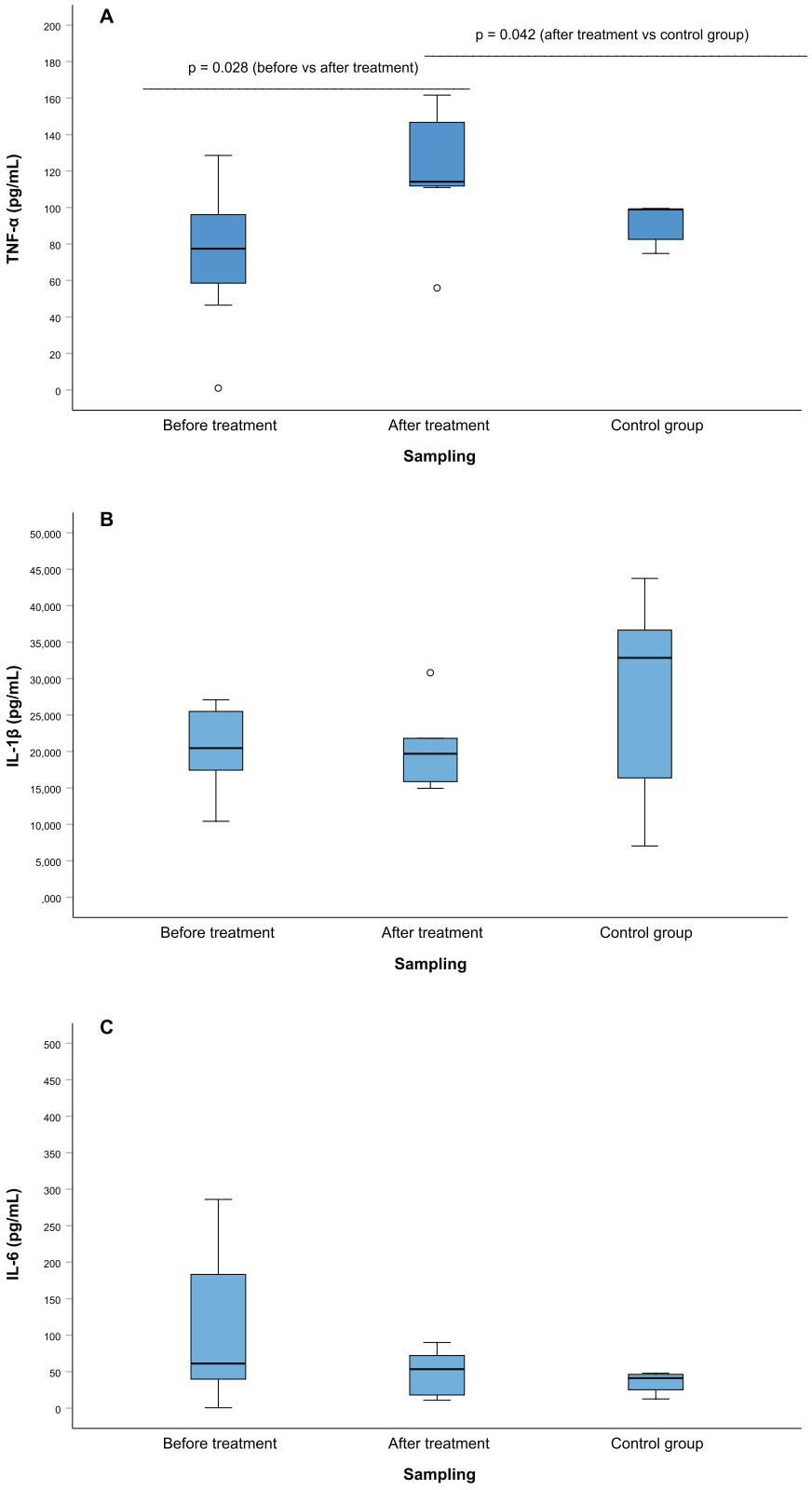

Fig. 1 (See legend on previous page.)

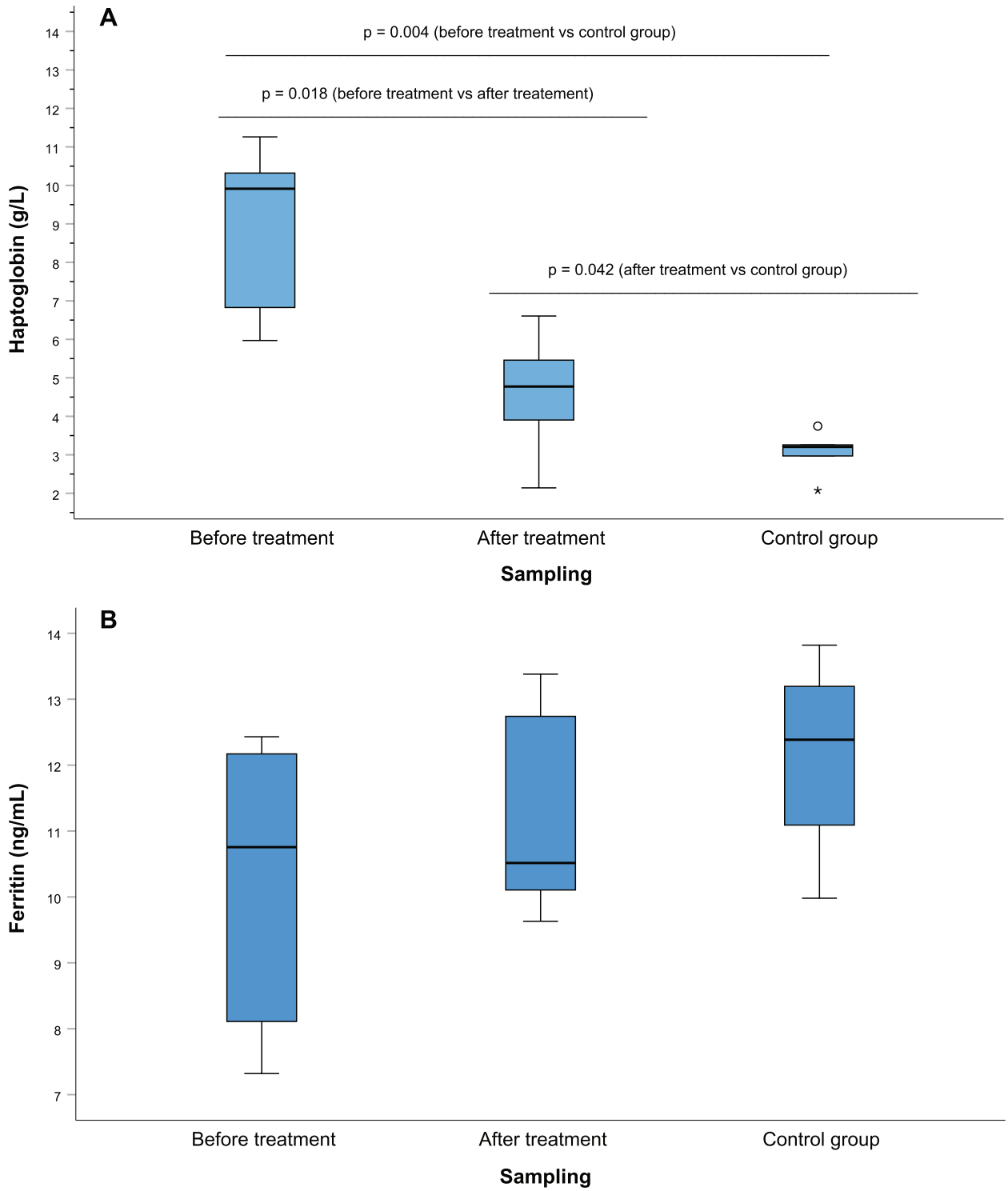

Fig. 2 Haptoglobin (**A**) and ferritin (**B**) concentrations before and after FIP treatment and in the control group of healthy cats. The Mann–Whitney U-test was used to test for statistically significant differences in haptoglobin (n = 7) and ferritin (n = 8) concentrations between FIP cats and the control group (n = 5) before and after therapy. A Wilcoxon signed-rank test was used to compare haptoglobin (n = 7) and ferritin (n = 8) concentrations before and after therapy. A value of $p \leq 0.05$ was considered significant

or adolescent cats, and thus it was important to discern whether growth could be influencing the observed weight gain. To address this, we divided cats into two subcategories based on their age (young cats under 1 year and adult cats over 1 year). Weight gain was significant in both age groups. Therefore, regular weighing should be recommended for all cats during FIP treatment, serving as a simple, non-invasive monitoring tool and for the necessary adjustment of drug dosages.

The focus of our study was the evaluation of selected clinicopathologic parameters, with an emphasis on inflammatory markers, to ascertain if they correlate with clinical improvement. Routine laboratory findings of cats at the time of diagnosis displayed typical haematological and biochemical alterations associated with FIP, as previously described [12, 15, 18–20, 23]. In summary, these included lymphopenia with neutrophilia and non-regenerative anaemia for haematological parameters, and hyperproteinaemia, hyperglobulinemia, hypoalbuminemia, low A/G ratio and pronounced hyperbilirubinemia for biochemical parameters. An important finding was that treatment had a profound impact on these parameters. We succeeded in demonstrating a significant change in almost all clinicopathological parameters compared to pre-treatment values. Furthermore, the post-treatment values were either within reference ranges (IDEXX laboratories) or did not differ significantly from the healthy control group. A similar observation was noted in other studies describing different approaches to the treatment of FIP, with either parenteral or peroral application of GS- 441524 or remdesivir [12, 13, 15, 18–21]. However, in contrast to our study, the majority of previous studies relied only on descriptive statistics of clinicopathological changes during treatment, without using statistical methods to compare measured parameters, or they compared a much smaller set of laboratory parameters.

Haemogram-derived inflammatory markers, such as NLR, LMR, PLR and SII, have already been identified as prognostic and/or diagnostic markers in various neoplastic and inflammatory diseases in cats [24–27], yet their role in FIP has not been previously studied. Our study demonstrated that cats with FIP have significantly higher NLR, PLR and SII levels and significantly lower LMR levels compared to healthy cats. This is similar to findings in human patients with acute COVID- 19 [28], where the severity of clinical signs and treatment outcomes are associated with the values and dynamics of haemogram-derived inflammatory markers, particularly NLR [28–30]. The post-treatment values of these inflammatory markers aligned with those of the healthy control group, with the exception of LMR, which significantly increased beyond the healthy group's values. This increase can be attributed to a significant increase in the absolute lymphocyte count, which influences the ratio. Similar normalisation of these inflammatory parameters has been observed in patients with severe COVID- 19 who successfully recovered [29]. Another notable finding in our study was that cats with effusive FIP had significantly higher NLR and SII levels compared to cats with non-effusive FIP. The difference in lymphocyte count approached the threshold of statistical significance ($p = 0.052$), with lower concentrations observed in effusive cases. If left untreated, effusive FIP is reported to have a worse prognosis compared to the non-effusive form of the disease, with an MST of 21.3 days and 38.4 days, respectively [31]. Typically, non-effusive FIP has a more chronic and less severe clinical presentation, and APP concentrations are usually lower compared to the effusive form [8, 18], which is also likely reflected in haemogram-derived inflammatory markers.

Recently, Donato et al. studied the relationship between NLR, MLR (an inverse value of the parameter we included, LMR) and PLR ratios and other markers of inflammation in cats [32]. The highest values of these markers were found in cats with hypoalbuminemia, hyperglobulinemia and increased SAA – conditions typically present in FIP patients – but also with a normal albumin-to-globulin ratio, and the presence of left shift response. In the group of cats without any indications of inflammation, the maximum values of NLR, MLR and PLR were 11.25, 0.42 and 528.3, respectively, which the authors propose as possible cut-off values for "cats with no inflammation". In our study, the vast majority of cats with FIP had values well below these proposed cut-off values but were still significantly higher compared to our healthy control group. These findings align more closely with results obtained from 76 healthy cats (median NLR of 1.9) and cats with systemic inflammatory response syndrome and sepsis (median NLR of approximately 9) [33]. Given that FIP is a disease related to a pronounced systemic inflammatory response, which is also reflected in elevated levels of other inflammatory markers (e.g. SAA, AGP, etc.), it is reasonable to expect an increase in haemogram-derived inflammatory markers. The discrepancy between our results and those of Donato could stem from differences in sample size, the age of the cats included, methodology (analyser), or, most likely, the different diagnoses of the included cats. In Donato's study, none of the cats had FIP, and the group of "cats with no inflammation" consisted of cats without leucocyte alterations suggestive of inflammation, which does not necessarily equate to healthy cats. Therefore, it is plausible that the cut-off values proposed by Donato might be too high or at least warrant further investigation. Nevertheless, these calculated markers could prove beneficial in clinical

use, as they are easily measurable, inexpensive and accessible parameters.

Previous publications have reported that approximately 30–40% of cats with FIP exhibit an increase in ALT activity at the time of diagnosis, which can persist until the completion of therapy [9, 20, 21]. In our study, only 3 out of 35 cats with FIP (8%) exhibited a minor increase in ALT activity – the highest value was 178 U/L, which is approximately 50% above the upper reference limit. For all these cats, the values normalised after treatment. However, we observed that four cats (11%) with ALT values within reference limits pre-treatment developed an increase in ALT activity after treatment. Three of them had just minor increases (between 119 and 168 U/L, with a reference range of 27 to 110 U/L), and the fourth, a Ragdoll cat with a relapse, exhibited a threefold increase over the upper reference range. This particular cat also experienced an increase in the activities of alkaline phosphatase (two-fold increase) and aspartate transaminase (20% over the higher reference limit) following the second cycle of treatment, which persisted for at least 6 months. However, the cat remains asymptomatic and in remission two years later. The measurement of plasma levels of the therapeutic drug and a liver biopsy would be of particular interest in this cat to identify any problems with the metabolism of GS- 441524 and the mechanism of its possible hepatotoxic effect. There have been reports of minor and reversible increases in ALT in human patients with COVID- 19 following treatment with remdesivir, without any other signs of liver damage [34]. In feline patients, there have been sporadic observations of increased ALT during treatment of FIP [9, 19, 20], and they were more pronounced when treatment included GS, compared to monotherapy with remdesivir [19]. However, the precise cause of possible hepatopathy associated with administration of remdesivir and GS- 441524 is still not fully understood [20]. It should also be noted that all cats in our study were receiving an unauthorised treatment, so it is possible that this is the cause of the increase in ALT activity. As more and more authorised drugs become available, it will become clearer in the future whether the hepatotoxic effect is intrinsic to the therapeutic agent or caused by possible unidentified ingredients in unauthorised products.

Several reports from human medicine have demonstrated that increased LDH activity indicates a negative clinical prognosis and higher mortality in human patients with COVID- 19 [35, 36]. In cats, it is proposed as a prognostic factor in various diseases, such as acute kidney injury, malignant diseases [37–39] and most recently FIP [17]. Goto et al. (2025) showed that plasma LDH activity $\geq$323 U/L is associated with short-term mortality in treated cats with FIP [17]. Our study's statistical analysis of paired LDH activities in cats before and after therapy did not reveal significant changes. However, LDH activity above the reference range of our analyser (217 U/L) was detected in three samples prior to treatment: in both patients that relapsed (375 U/L in the Ragdoll and 440 U/L in the Maine Coon) and in the only cat that died within a few days of starting treatment (1080 U/L). This extremely high LDH activity in the non-surviving cat supports the findings of Goto et al. [17]. However, in our study, the two cats with relapse had LDH activities well above the suggested threshold for short-term mortality [17], but survived long-term (over 2 years) after the second treatment. This is also in contrast to the LDH values reported by Goto et al. in two presumed relapses, both of which had LDH activities below 201 U/L. Although different chemistry principles were used for LDH measurements in Goto's study compared to our study (dry vs. wet chemistry), the upper reference limits established for both analysers are similar (up to 187 U/L vs. up to 217 U/L), so the absolute values of these results are generally comparable.

Both studies shed light on LDH activity as a promising prognostic or predictive factor in cats with FIP. Therefore, future attempts should be made to investigate this enzyme in more detail as a marker of cell damage. At this stage, it is not possible to determine whether the increase in LDH activity in these patients is a consequence of specific tissue/organ damage or an indicator of a general inflammatory response. In our case, the cats had no particular clinical or laboratory features compared to other cats that would allow speculation as to the cause of the increased LDH activity. The only common feature of all three cats was the effusive form of the disease, which is usually considered to be more acute with a more pronounced systemic inflammatory response. The majority of non-responder cats in the Goto et al. study (12/13 cats) also had the effusive form of the disease. This fact may support the theory that LDH elevation is more indicative of generalised inflammation. However, simultaneous determination of other tissue enzymes, including creatine kinase, cardiac troponin I, aspartate aminotransferase, gamma-glutamyl transferase and ALT, may help to identify the exact pathophysiology of the elevated LDH in these patients. Another interesting approach could be the determination of different LDH isoenzymes in plasma/serum or in effusions of treated cats [40–42]. However, measurement in effusion would not be feasible for monitoring of therapy, as effusions usually resolve in less than 6 weeks (sometimes as quickly as 1 week) with successful treatment [19–21], which was also observed in our study. Therefore, any long-term serial monitoring is not possible. Furthermore, fluid is not available in the non-effusive form of the disease.

In a small number of included cats with sufficient archived serum samples, the pre- and post-treatment concentrations of selected APPs and proinflammatory cytokines were determined. In FIP, concentrations of haptoglobin, SAA and AGP are known to increase [18, 43–45], with the latter two aiding in diagnosis [3, 18]; however, increased concentrations are not pathognomonic for the disease. We decided to include haptoglobin, which has not been extensively studied in cats with FIP, and ferritin, which has previously been demonstrated to have a prognostic value in people with COVID- 19 [16, 46]. Haptoglobin was significantly higher compared to the healthy control and showed significant decline post-treatment, aligning with control group levels. Its normalisation after treatment is a novel finding, making it another candidate for treatment monitoring, along with previously described AGP and SAA, which exhibit similar dynamics [8, 18, 19].

To evaluate the changes in pro-inflammatory cytokines in treated cats, we measured the concentration of the three main feline proinflammatory cytokines TNF-α, IL-6 and IL-1β [47]. These are also among the most important prognostic factors in COVID-19 [48]. In cats with coronaviral infection these three cytokines have been studied by Safi et al. (2017) [1] using different methodologies, including real-time PCR and a multiplex bead-based immunoassay. They found that IL- 1β and IL- 6 could not be detected in the serum of the majority of cats with FIP but were present in their abdominal effusions. In contrast, TNF-α production was detected in both FCoV-positive and negative groups. Our study demonstrated a significant increase of TNF-α concentration after therapy. TNF-α is a major contributor to the inflammatory response and pathogenesis of FIP, causing apoptosis of lymphocytes and subsequently lymphopenia [18, 49]. It would be reasonable to expect that TNF-α would be elevated before therapy and normalise after treatment; however, our study showed the opposite. Similar outcome has been previously reported in people with severe COVID- 19 treated with antiviral therapy, such as favipiravir or lopinavir/ritonavir [50]. The concentration of TNF-α at the time of diagnosis did not differ compared to the control group but significantly increased after therapy, especially in patients requiring intensive care. The authors suggested that might be due to the inability of the anti-viral therapy to suppress the systemic inflammatory response, despite clinical improvement and improvement in other inflammatory markers associated with COVID-19 infection.

On the other hand, the concentrations of IL- 1β and IL-6 decreased during therapy, although this change was not significant. These two proinflammatory cytokines play a crucial role in both acute and chronic inflammation, for example in the recruitment of leukocytes, in changes in vascular permeability and in the production of APPs [51]. They are also a driving force behind platelet activation, which is another important aspect of the systemic inflammatory response [52]. In our patients, the platelet concentration increased significantly during therapy, although the values were within the reference range both before and after treatment. There are several possible causes for the increase in platelet count after treatment, e.g. reduction of the inflammatory response leading to resolution of immune-mediated thrombocytopenia and restoration of bone marrow function. As both median values remained within the reference ranges, the quantitative measurement of platelet count is probably less useful for monitoring response to treatment. Other methods that could be used to assess platelet activation are specific haematological markers (e.g. mean platelet volume, plateletcrit or assessment of platelet morphology on blood smears) or functional platelet activation tests (e.g. platelet aggregometry or flow cytometry for expression of P-selectin, CD62P and platelet-leukocyte aggregates) [52, 53]. As far as we know, platelet activation in cats with FIP has not been specifically studied, so it is not possible to speculate on the specificities in this patient population. Based on the pathophysiology, it is possible that there is a marked difference between the wet and dry forms of the disease. Furthermore, the degree and speed of normalisation most likely depends on the severity of the disease, which can vary greatly in cats with FIP. However, this could be another aspect that future studies should focus on to evaluate another possible venue of treatment monitoring.

It must be emphasised that the number of samples in the part of our study dealing with the dynamics of cytokines and APPs was very small and that the concentrations were measured using the ELISA method. Although the ELISA method is accurate and can be useful for clinical laboratories, it has drawbacks that need to be considered when interpreting these results. These include limitations in sensitivity and specificity for selected biomarkers, possible cross-reactivity or interference with other substances present in complex samples (sample matrix effect) and batch-to-batch variations. The use of species-specific commercial kits is essential to ensure discrimination between closely related molecules. All sandwich ELISA measurements reported in this study were performed by the same operator in the same laboratory on the same plate in duplicate per biomarker measured to minimise assay-related variability. Ideally, the same parameters should be analysed with kits from different manufacturers, but few species-specific commercial kits are available in veterinary medicine. Nevertheless, validation of the results on a larger sample

is necessary and the inclusion of alternative validation methods, such as Western blot, magnetic bead-based multiplex ELISA system, would improve the reliability of our results. Although the statistical analysis showed statistically significant differences in haptoglobin and TNF-α concentrations, the small sample size could influence these results. Unfortunately, due to the retrospective nature of the study, only such a small number of samples were available. However, we think that these results may be a useful starting point for future studies evaluating the inflammatory response in cats with FIP.

The findings of this study must be viewed in light of several limitations. The main limitation of this study is the nature of the treatment. Since GS- 4441524 is currently produced without a licence and uncontrolled, it is not possible to ascertain the exact quantity of the active ingredient received by the cats. However, two research groups have recently reported the results of quality assessments of a wide range of products (30 vials from 17 brands by Mulligan and Browning and 127 different products by Kent et al.) [54, 55]. All parenteral products marketed as GS- 44125 contained this active ingredient at the advertised or higher concentrations, with peroral products showing more variable concentrations. Although procuring licensed GS- 441524 is currently not possible in the majority of countries worldwide, the patients appear to be treated with the advertised drug, as evidenced by the high survival rate of treated cats. However, it should be emphasised that the uncontrolled manufacture of this drug may affect the consistency and accuracy of dosing, leading to variations in treatment protocols that could affect laboratory results. Another limitation is the unavailability of all studied parameters for every treated cat before and after therapy, due to the retrospective nature of the study and issues with client compliance. This is particularly true for the small number of samples in which cytokines and APPs were determined using the ELISA method, which has its own set of limitations. Furthermore, AGP and SAA, as the two routinely available APPs were not included in the measurements due to limited amount of available serum.

Conclusions

In conclusion, we demonstrated that successful treatment of FIP results in significant changes in the majority of clinicopathological parameters in treated patients. For the first time, we demonstrated that haemogram-derived inflammatory markers follow a similar pattern and significantly differ between effusive and non-effusive FIP. They could serve as simple, cost-effective and readily available parameters for diagnostic and monitoring purposes, similar to the currently more commonly used but more expensive or less available APPs. Haptoglobin normalised with treatment of FIP, and TNF-α increased after therapy. We suggest that LDH activity may be a new prognostic factor for cats with FIP. Our results encourage further studies on LDH activity, cytokines and APP responses to treatment of FIP in a larger group of cats and to investigate other aspects of systemic inflammation, such as platelet activation.

Abbreviations

A/G	Albumin to globulin ratio
AGP	Alpha- 1-acid glycoprotein
ALT	Alanine aminotransferase
APPs	Acute-phase proteins
FCoV	Feline coronavirus
FIP	Feline infectious peritonitis
IL- 1ß	Interleukin- 1ß
IL- 6	Interleukin- 6
LDH	Lactate dehydrogenase
LMR	Lymphocyte-to-monocyte ratio
NLR	Neutrophil-to-lymphocyte ratio
PLR	Platelet-to-lymphocyte ratio
SAA	Serum amyloid A
SII	Systemic inflammatory index
TNF-α	Tumour necrosis factor-α

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Authors' contributions

Study concept/study design: D.P., N.T., A.N.S.; data acquisition: D.P., L.T., M.Š., M.K.Ž., A.N.S., S.K.; statistical analysis: A.N.S.; data analysis/interpretation: all authors; manuscript drafting: L.T., M.Š., D.P.; manuscript revision for important intellectual content: all authors; approval of the final submitted manuscript: all authors.

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Data availability

All data are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

The authors confirm that the study was carried out in compliance with the ARRIVE guidelines. All caregivers provided informed consent for use of archived samples for the study. The Committee for Animal Welfare of Veterinary Faculty, University of Ljubljana, Slovenia considered that this type of project does not fall under the legislation for the protection of animals used for scientific purposes since all the procedures employed were performed as part of a routine clinical veterinary examination (decision No. 033–5/2025–6).

Consent for publication

Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Safi N, Haghani A, Ng SW, Selvarajah GT, Mustaffa-Kamal F, Omar AR. Expression profiles of immune mediators in feline Coronavirus-infected cells and clinical samples of feline Coronavirus-positive cats. BMC Vet Res. 2017. https://doi.org/10.1186/s12917-017-1019-2.
2. Addie D, Belák S, Boucraut-Baralon C, Egberink H, Frymus T, Gruffydd-Jones T, et al. Feline infectious peritonitis. ABCD guidelines on prevention and management. J Feline Med Surg. 2009; https://doi.org/10.1016/j.jfms.2009.05.008.
3. Tasker S, Addie DD, Egberink H, Hofmann-Lehmann R, Hosie MJ, Truyen U, et al. Feline infectious peritonitis: european advisory board on cat diseases guidelines. Viruses. 2023. https://doi.org/10.3390/v15091847.
4. Sykes JE. Feline Coronavirus Infection. In: Sykes JE, editor. Canine and Feline Infectious Diseases. Philadelphia:Saunders; 2014. p.195–208. https://doi.org/10.1016/B978-1-4377-0795-3.00020-X.
5. Thayer V, Gogolski S, Felten S, Hartmann K, Kennedy M, Olah GA. 2022 AAFP/EveryCat feline infectious peritonitis diagnosis guidelines. J Feline Med Surg. 2022; https://doi.org/10.1177/1098612X221118761.
6. Pedersen NC, Perron M, Bannasch M, Montgomery E, Murakami E, Liepnieks M, et al. Efficacy and safety of the nucleoside analog GS-441524 for treatment of cats with naturally occurring feline infectious peritonitis. J Feline Med Surg. 2019. https://doi.org/10.1177/1098612X19825701.
7. Jones S, Novicoff W, Nadeau J, Evans S. Unlicensed GS-441524-Like antiviral therapy can be effective for at-home treatment of feline infectious peritonitis. Animals. 2021. https://doi.org/10.3390/ani11082257.
8. Katayama M, Uemura Y. Prognostic prediction for therapeutic effects of mutian on 324 client-owned cats with feline infectious peritonitis based on clinical laboratory indicators and physical signs. Vet Sci. 2023. https://doi.org/10.3390/vetsci10020136.
9. Krentz D, Zenger K, Alberer M, Feltern S, Bergmann M, Dorsch R, et al. Curing cats with feline infectious peritonitis with an oral multi-component drug containing GC-441524. Viruses. 2021. https://doi.org/10.3390/v13112228.
10. Roy M, Jacque N, Novicoff W, Li E, Negash R, Evans SJM. Unlicensed molnupiravir is an effective rescue treatment following failure of unlicensed GS-441524-like therapy for cats with suspected feline infectious peritonitis. Pathogens. 2022. https://doi.org/10.3390/pathogens11101209.
11. Izes AM, Yu J, Norris JM, Govendir M. Current status on treatment options for feline infectious peritonitis and SARS-CoV-2 positive cats. Vet Q. 2020. https://doi.org/10.1080/01652176.2020.1845917.
12. Zwicklbauer K, Krentz D, Bergmann M, Felten S, Dorsch R, Fischer A, et al. Long-term follow-up of cats in complete remission after treatment of feline infectious peritonitis with oral GS-441524. J Feline Med Surg. 2023. https://doi.org/10.1177/1098612X231183250.
13. Krentz D, Zwicklbauer K, Felten S, Bergmann M, Dorsch R, Hofmann-Lehmann R, et al. Clinical follow-up and postmortem findings in a cat that was cured of feline infectious peritonitis with an oral antiviral drug containing GS-441524. Viruses. 2022. https://doi.org/10.3390/v14092040.
14. Meli ML, Spiri AM, Zwicklbauer K, Krentz D, Felten S, Bergmann M, et al. Fecal feline coronavirus RNA shedding and spike gene mutations in cats with feline infectious peritonitis treated with GS-441524. Viruses. 2022. https://doi.org/10.3390/v14051069.
15. Yin Y, Li T, Wang C, Liu X, Ouyang H, Ji W, et al. A retrospective study of clinical and laboratory features and treatment on cats highly suspected of feline infectious peritonitis in Wuhan. China Sci Rep. 2021. https://doi.org/10.1038/s41598-021-84754-0.
16. Gallo Marin B, Aghagoli G, Lavine K, Yang L, Siff EJ, Chiang SS, et al. Predictors of COVID-19 severity: A literature review. Rev Med Virol. 2021. https://doi.org/10.1002/rmv.2146.
17. Goto S, Kamiyoshi T, Iwasaki R. Predictive factors associated with short-term mortality in cats with feline infectious peritonitis treated with remdesivir or GS-441524 or both. J Vet Intern Med. 2025. https://doi.org/10.1111/jvim.17249.
18. Addie DD, Silveira C, Aston C, Brauckmann P, Covell-Ritchie J, Felstead C, et al. Alpha-1 acid glycoprotein reduction differentiated recovery from remission in a small cohort of cats treated for feline infectious peritonitis. Viruses. 2022. https://doi.org/10.3390/v14040744.
19. Taylor SS, Coggins S, Barker EN, Gunn-Moore D, Jeevaratnam K, Norris JM, et al. Retrospective study and outcome of 307 cats with feline infectious peritonitis treated with legally sourced veterinary compounded preparations of remdesivir and GS-441524 (2020–2022). J Fel Med Surg. 2023. https://doi.org/10.1177/1098612X231194460.
20. Green J, Syme H, Tayler S. Thirty-two cats with effusive or non-effusive feline infectious peritonitis treated with a combination of remdesivir and GS-441524. J Vet Intern Med. 2023. https://doi.org/10.1111/jvim.16804.
21. Coggins SJ, Norris JM, Malik R, Govendir M, Hall EJ, Kimble B, et al. Outcomes of treatment of cats with feline infectious peritonitis using parenterally administered remdesivir, with or without transition to orally administered GS-441524. J Vet Intern Med. 2023. https://doi.org/10.1111/jvim.16803.
22. Cosaro E, Pires J, Castillo D, Murphy BG, Reagan KL. Efficacy of oral remdesivir compared to GS-441524 for treatment of cats with naturally occurring effusive feline infectious peritonitis: a blinded, non-inferiority study. Viruses. 2023. https://doi.org/10.3390/v15081680.
23. Riemer F, Kuehner KA, Ritz S, Sauter-Louis C, Hartmann K. Clinical and laboratory features of cats with feline infectious peritonitis – a retrospective study of 231 confirmed cases (2000–2010). J Feline Med Surg. 2016. https://doi.org/10.1177/1098612X15586209.
24. Petrucci GN, Lobo L, Queiroga F, Martins J, Prada J, Pires I, et al. Neutrophil-to-lymphocyte ratio is an independent prognostic marker for feline mammary carcinomas. Vet Comp Oncol. 2021. https://doi.org/10.1111/vco.12686.
25. Chiti LE, Martano M, Ferrari R, Boracchi P, Giordano A, Grieco V, et al. Evaluation of leukocyte counts and neutrophil-to-lymphocyte ratio as predictors of local recurrence of feline injection site sarcoma after curative intent surgery. Vet Comp Oncol. 2020. https://doi.org/10.1111/vco.12534.
26. Neumann S. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in dogs and cats with acute pancreatitis. Vet Clin Pathol. 2021. https://doi.org/10.1111/vcp.12979.
27. Tagawa M, Shimbo G, Miyahara K. Prognostic role of lymphocyte to monocyte ratio in feline high-grade lymphomas. Can Vet J. 2021;62:1095–103.
28. Kosidło JW, Wolszczak-Biedrzycka B, Matowicka-Karna J, Dymicka-Piekarska V, Dorf J. Clinical significance and diagnostic utility of NLR, LMR, PLR and SII in the course of COVID-19: A Literature Review. J Inflamm Res. 2023. https://doi.org/10.2147/JIR.S395331.
29. Liu A, Hammond R, Donnelly PD, Kaski JC, Coates ARM. Effective prognostic and clinical risk stratification in COVID-19 using multimodality biomarkers. J Intern Med. 2023. https://doi.org/10.1111/joim.13646.
30. Zahorec R. Neutrophil-to-lymphocyte ratio, past, present and future perspectives. Bratisl Lek Listy. 2021. https://doi.org/10.4149/BLL_2021_078.
31. Tsai H-Y, Chueh L-L, Lin C-N, Su B-L. Clinicopathological findings and disease staging of feline infectious peritonitis: 51 cases from 2003 to 2009 in Taiwan. J Fel Med Surg. 2011; https://doi.org/10.1016/j.jfms.2010.09.014.
32. Donato G, Pennisi MG, Persichetti MF, Archer J, Masucci M. A Retrospective comparative evaluation of selected blood cell ratios, acute phase proteins, and leukocyte changes suggestive of inflammation in cats. Animals. 2023. https://doi.org/10.3390/ani13162579.
33. Gori E, Pierini A, Lippi I, Lubas G, Marchetti V. Leukocytes ratios in feline systemic inflammatory response syndrome and sepsis: a retrospective analysis of 209 cases. Animals. 2021. https://doi.org/10.3390/ani11061644.
34. Casalini G, Giacomelli A, Antinori S. Liver tests abnormalities with licensed antiviral drugs for COVID-19: a narrative review. Expert Opin Drug Saf. 2022. https://doi.org/10.1080/14740338.2022.2160446.
35. Fialek B, Pruc M, Smereka J, Jas R, Rahnama-Hezavah M, Denegri A, et al. Diagnostic value of lactate dehydrogenase in COVID-19: A systematic review and meta-analysis. Cardiol J. 2022. https://doi.org/10.5603/CJ.a2022.0056.
36. Huang Y, Guo L, Chen J, Wu M, Zhang C, Liu Z, et al. Serum lactate dehydrogenase level as a prognostic factor for COVID-19: a retrospective study based on a large sample size. Front Med (Lausanne). 2022. https://doi.org/10.3389/fmed.2021.671667.

37. Terragni R, Morselli-Labate AM, Vignoli M, Bottero E, Brunetti B, Saunders JH. Is serum total LDH evaluation able to differentiate between alimentary lymphoma and inflammatory bowel disease in a real world clinical setting? PLoS ONE. 2016. https://doi.org/10.1371/journal.pone.0151641.
38. Naito E, Shimada R, Yuki M. Diagnostic utility of measuring lactate dehydrogenase levels and its isoenzyme activities for the evaluation of malignancy in feline pleural effusion and ascitic fluid. Open Vet J. 2022. https://doi.org/10.5455/OVJ.2022.v12.i5.19.
39. Lee YJ, Chan JP, Hsu WL, Lin KW, Chang CC. Prognostic factors and a prognostic index for cats with acute kidney injury. J Vet Intern Med. 2012. https://doi.org/10.1111/j.1939-1676.2012.00920.x.
40. Romanelli P, Paltrinieri S, Bonfanti U, Castaman MG, Monza E, Bertazzolo W. Utility of the ratio between lactate dehydrogenase (LDH) activity and total nucleated cell counts in effusions (LDH/TNCC Ratio) for the diagnosis of feline infectious peritonitis (FIP). Animals. 2022. https://doi.org/10.3390/ani12172262.
41. Zoia A, Slater LA, Heller J, Connolly DJ, Church DB. A new approach to pleural effusion in cats: markers for distinguishing transudates from exudates. J Feline Med Surg. 2009. https://doi.org/10.1016/j.jfms.2009.04.005.
42. Mon Hla H, Hunprasit V, Siripoonsup J, Rungsipipat A, Radtanakatikanon A. Diagnostic utility of LDH measurement for determining the etiology of modified transudate pleural effusion in cats. Front Vet Sci. 2022. https://doi.org/10.3389/fvets.2022.1044192.
43. Paltrinieri S. The feline acute phase reaction. Vet J. 2008. https://doi.org/10.1016/j.tvjl.2007.06.005.
44. Giordano A, Spagnolo V, Colombo A, Paltrinieri S. Changes in some acute phase protein and immunoglobulin concentrations in cats affected by feline infectious peritonitis or exposed to feline coronavirus infection. Vet J. 2004. https://doi.org/10.1016/s1090-0233(03)00055-8.
45. Hazuchova K, Held S, Neiger R. Usefulness of acute phase proteins in differentiating between feline infectious peritonitis and other diseases in cats with body cavity effusions. J Feline Med Surg. 2017. https://doi.org/10.1177/1098612X16658925.
46. Para O, Caruso L, Pestelli G, Tangianu F, Carrara D, Maddaluni L, et al. Ferritin as prognostic marker in COVID-19: the FerVid study. Postgrad Med. 2022. https://doi.org/10.1080/00325481.2021.1990091.
47. Eckersall PD, Bell R. Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. Vet J. 2010. https://doi.org/10.1016/j.tvjl.2010.04.009.
48. Del Valle DM, Kim-Schulze S, Huang HH, Beckmann ND, Nirenberg S, Wang B, et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat Med. 2020. https://doi.org/10.1038/s41591-020-1051-9.
49. Takano T, Hohdatsu T, Hashida Y, Kaneko Y, Tanabe M, Koyama H. A "possible" involvement of TNF-alpha in apoptosis induction in peripheral blood lymphocytes of cats with feline infectious peritonitis. Vet Microbiol. 2007. https://doi.org/10.1016/j.vetmic.2006.08.033.
50. Mortaz E, Bassir A, Roofchayee ND, Dezfuli NK, Jamaati H, Tabarsi P, et al. Serum cytokine levels of COVID-19 patients after 7 days of treatment with Favipiravir or Kaletra. Int Immunopharmacol. 2021. https://doi.org/10.1016/j.intimp.2021.107407.
51. Bester J, Pretorius E. Effects of IL-1β, IL-6 and IL-8 on erythrocytes, platelets and clot viscoelasticity. Sci Rep. 2016. https://doi.org/10.1038/srep32188.
52. Wiinberg B, Jessen LR, Tarnow I, Kristensen AT. Diagnosis and treatment of platelet hyperactivity in relation to thrombosis in dogs and cats. J Vet Emerg Crit Care (San Antonio). 2012. https://doi.org/10.1111/j.1476-4431.2011.00708.x.
53. Tablin F, Schumacher T, Pombo M, Marion CT, Huang K, Norris JW, Jandrey KE, Kittleson MD. Platelet activation in cats with hypertrophic cardiomyopathy. J Vet Intern Med. 2014. https://doi.org/10.1111/jvim.12325.
54. Mulligan AJ, Browning ME. Quality assessment and characterization of unregulated antiviral drugs for feline infectious peritonitis: implications for treatment, safety, and efficacy. Am J Vet Res. 2024. https://doi.org/10.2460/ajvr.23.10.0221.
55. Kent AM, Guan S, Jacque N, Novicoff W, Evans SJM. Unlicensed antiviral products used for the at-home treatment of feline infectious peritonitis contain GS-441524 at significantly different amounts than advertised. J Am Vet Med Assoc. 2024. https://doi.org/10.2460/javma.23.08.0466.

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