EVALUATION OF SELECTED INFLAMMATORY MARKERS IN CATS WITH FELINE INFECTIOUS PERITONITIS BEFORE AND AFTER THERAPY

1. Study design

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. 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.

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.

At each followup the following information was collected: clinical information (for details see 6.1.), hematology analysis and haematology-derived inflammatory markers (for details see 6.2.), biochemistry analysis (for details see 6.3.) and cytokine and acute phase proteins measurements in 8 cats (for details see 6.4.).

Since reference values for 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 blood analysis was performed as a part of a wellnes checkup, and results were used with the permission of the caregivers.

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2. Sample size

Laboratory results of a total of 35 client-owned cats with FIP 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. Additional laboratory results of 28 age-matched healthy cats was included as a control group.

Archived samples from 8 cats from the study group were suitable for the determination of cytokine and/or acute phase proteins response.

3. Inclusion and exclusion criteria

The inclusion criteria for the study population consisted of a FIP diagnosis, confirmed by a veterinarian, following the AAFP diagnostic guidelines. 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).

Haematology results of 28 age matched cats served as a control group for haematology-derived inflammatory markers. Cats were considered healthy based on their history, physical examination and routine laboratory results (routine haematology and biochemistry parameters within reference ranges).

4. Randomisation

There was no randomisation of participant patients in the study.

5. Blinding

Blinding was not considered necessary in the study.

6. Outcome measures

6.1. Clinical information:

- weight before/after treatment,
- age,
- sex and neuter status,
- presenting clinical signs inclusive of effusive/non-effusive form, ocular and neurological involvement.

6.2. Haematology analysis and haematology-derived inflammatory markers

- 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).

- NLR (ratio of neutrophils to lymphocytes)

- LMR (ratio of lymphocytes to monocytes)

- PLR (ratio of platelets to lymphocytes)

- SII (systemic immune-inflammatory index) was calculated as neutrophills x platelets/lymphocytes.

6.3. Biochemistry analysis

- 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.

- Lactate dehydrogenase (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.

6.4. Cytokine and acute phase proteins response

Species-specific, commercially available quantitative sandwich ELISA tests were used to determine five selected serum parameters:

- Interleukin-6 (IL-6),
- Tumor necrosis factor -α (TNF-α),
- Interleukin-1β (IL-1β),
- ferritin
- haptoglobin

7. Statistical methods

Results from the initial visit (pre-treatment) and final checkup (post-treatment, 12 weeks after the start of therapy) were statistically analyzed. 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.

8. Experimental animals

The study was conducted as a study of laboratory results gained during health checkup of clinical patients and their archived blood samples. Experimental animals were not used. All diagnostic/therapeutic/monitoring procedures were performed according to the clinical guidelines for such patients.

9. Experimental procedures

There were no experimental procedures performed in the study. All cats were clinical patients, which were managed according to the standards of treatment.

10. Results

Pre-treatment haematological parameters in 32 treated cats showed moderate leukocytosis, neutrophilia, lymphopenia and anaemia, which normalized post-treatment. Pre-treatment values of all haemogram-derived inflammatory markers 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 normalized 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 TNF-α ($p = 0.028$) before and after therapy. Therapy didn't elicit any statistically significant changes in concentrations of ferritine, IL-1β and IL-6.