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Cats

Evaluation of the Diversities in the Inflammatory Responses in Cats With Bacterial and Viral Infections

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ABSTRACT

Background: Understanding the nature of inflammatory responses in cats with bacterial and viral infections is essential for accurately managing the infection. This study aimed to investigate the diversities of inflammatory responses between bacterial and viral infections in cats to figure out their role in the pathophysiology of these infections.

Methods: Seventy-five owned cats were included in the study. The evaluations were performed based on three groups: healthy control, bacterial infection group (those with bronchopneumonia and gastrointestinal tract and urinary tract infections) and viral infection group (21 with feline coronavirus [FCoV], 3 with feline leukaemia virus [FeLV] and 1 with feline calicivirus), each containing 25 individuals. Total and differential leukocyte counts, C-reactive protein (CRP), transforming growth factor beta (TGF-β), interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and interleukin-10 (IL-10) concentrations were assessed in the blood samples collected from sick and healthy animals.

Results: No statistically significant difference was noted in serum TNF-α, IL-1β and IL-10 concentrations of the infected cats ($p = 0.996$, $p = 0.160$ and $p = 0.930$, respectively). Serum TGF-β concentration in the viral infection group was reduced compared to the healthy control ($p = 0.001$). In contrast, WBC count and IL-6 and CRP concentrations were increased in the cats with bronchopneumonia, gastrointestinal tract infections and urinary tract infections compared to the healthy control and viral infection groups ($p = 0.001$, $p = 0.001$ and $p = 0.001$, respectively).

Conclusion: This study revealed significant differences between bacterial and viral infections regarding the fashion of inflammatory responses in cats, and the relevant data will undoubtedly contribute to the management and control of feline infectious diseases, rendering the development of novel therapeutic strategies.

1 | Introduction

The diversity of the host's inflammatory responses to infectious agents has recently drawn growing attention since, as in their human counterparts, distinguishing a bacterial infection from a viral infection is quite troublesome in animal species. (Chalupa et al. 2011; De Nooijer et al. 2023). Cytokines are one of the most utilised biomolecules in monitoring these immune responses. Cytokines are small proteins recruited in intercellular communication and are secreted by several cells, including macrophages,

lymphocytes, endothelial cells and fibroblasts (Lacy and Stow 2011). They are the critical molecules for the defence cells' production, proliferation and activation (Lokau and Garbers 2020) and regulation of pro- and anti-inflammatory reactions induced during tissue injury or infections (Cicchese et al. 2018). They are concurrently involved in producing acute-phase proteins (APPs) during the inflammatory process (Burger and Dayer 2002), orchestrating the multicomponent immune responses (Watford et al. 2004).

Transforming growth factor beta (TGF-β) is one of the pleiotropic cytokines secreted mainly by leucocytes (Tsuda 2018). It plays a significant role in regulating inflammatory reactions and immune responses (Travis and Sheppard 2014) and, during inflammation, prompts fibroblasts and keratinocytes toward the inflammation zone, stimulating the synthesis of matrix proteins and promoting tissue regeneration (Frangogiannis 2020). However, persistent TGF-β activation is known to induce extracellular accumulation of matrix proteins, leading to fibrosis and organ dysfunction (Budi et al. 2021). Few studies have investigated TGF-β in feline medicine; these include an in vitro study that showed TGF-β production was reduced in herpes virus-infected feline tracheal epithelial cells (Lee et al. 2023) and research indicating that the placental gene expression of TGF-β was downregulated in response to feline immunodeficiency virus (FIV) infection (Meeker and Hudson 2017). Moreover, TGF-β production in bone marrow stromal cells was decreased during FeLV infection (Linenberger, Dow, and Abkowitz 1995). Limited data is available regarding the TGF-β production in feline bacterial infections. To our knowledge, whether or not the TGF-β response differs in bacterial infections involving the respiratory, digestive or urinary systems was yet to be investigated.

IL-6 is a pleiotropic cytokine that regulates inflammatory processes and initiates pro-inflammatory signals during the acute-phase response (Uciechowski and Dempke 2020), while also exhibiting anti-inflammatory effects in prolonged inflammation (Scheller et al. 2011). IL-6 plays a crucial role in immune responses by promoting neutrophil and monocyte chemotaxis during innate immunity (Choy and Rose-John 2017) and regulating B-cell differentiation and T-cell activity in adaptive immunity (Singh and Goyal 2013; Tanaka et al. 2014). However, limited data is available concerning the diversities in IL-6 response during bacterial and viral infections or the infections involving respiratory, digestive and urinary systems in cats.

Tumour necrosis factor alpha (TNF-α) is a pleiotropic cytokine mainly secreted by macrophages (Flynn et al. 1995). TNF-α was demonstrated to have elevated during bacterial (Parameswaran et al. 2010) and viral (Takano et al. 2007) infections in humans. Even though TNF-α production of macrophages was shown to have increased in cats infected with feline infectious peritonitis (FIP) virus (Takano et al. 2007), whether or not the TNF-α production differs in feline bacterial infections is yet to be investigated.

IL-1β is a pro-inflammatory cytokine secreted mainly by activated macrophages (Garlanda, Dinarello, and Mantovani 2013). IL-1β concentration was reported to have increased during bacterial infections in humans (Sahoo et al. 2011). On the other hand, whether there is a change in IL-1β production in viral and bacterial infections in felines has not been investigated sufficiently.

IL-10 is an anti-inflammatory cytokine mainly produced by lymphocytes and macrophages (Van der Poll et al. 1996). IL-10 expression was reported to have varied depending on the type of the bacteria and the course of the infection (Kang et al. 2020). IL-10 production was increased, directly correlated with the virus replication, in various tissues, mainly including cervical lymph nodes, in the FIV-infected cats (Dean and Pedersen 1998); on the contrary, IL-10 gene expression was reduced in kittens experimentally infected via the intranasal route (Gunn-Moore et al. 1998). Therefore, further studies concerning the status of IL-10 production are required to assess this cytokine's production in cats with bacterial or viral infections.

APPs are chemical mediators synthesised in the liver in response to infectious or non-infectious inflammation (Benson 1989). Like their human counterpart, APPs are also utilised in feline practice to detect and monitor inflammation, assess the efficacy of antimicrobial therapeutic approaches, and for the early diagnosis of remission or recurrence of the diseases (Rossi 2023). C-reactive protein (CRP) is one of the APPs utilised as the most prevailing biomarker in human medicine since its concentrations may elevate hundreds of folds during inflammation (Sproston et al. 2018). It is also recognised as the primary acute-phase reactant in dogs and other species (Schultz and Arnold 1990). On the other hand, CRP did not receive much attention in feline practice since inflammation-induced CRP production does not progress at high concentrations in cats, unlike the other species (Ceron, Eckersall, and Martínez-Subiela 2005); however, this does not necessarily rule out its value as a biomarker. Hence, a recent review indicated that CRP could be considered a minor acute-phase reactant in cats (Rossi 2023). The most challenging aspect of feline practice is the lack of particular clinical signs and prominent changes in the blood profile regarding leucocyte count or APP values during the infection, unlike in dogs (Cho et al. 2021). In such cases, a simultaneous assessment of multiple biomarkers is required for a differential diagnostic approach (Jain, Gautam, and Naseem 2011). Therefore, measuring the CRP concentrations, apart from the other APPs that were proved significant in cats, such as serum amyloid A (SAA) or alpha-1-acid glycoprotein, may serve as a diagnostic tool.

Accurately assessing the nature and severity of inflammatory responses in feline bacterial and viral infections is crucial for effectively managing these conditions. This study aims to explore the differences in immune responses between bacterial infections (including bronchopneumonia, gastrointestinal and urinary tract infections) and viral infections in cats to better understand their roles in the pathophysiological processes of these infections.

2 | Materials and Method

2.1 | Animals and Study Groups

This study was carried out with the permission of the Istanbul University Animal Experiments Local Ethics Committee (Approval no: 2018/25 118-481). Seventy-five owned cats referred to the internal medicine clinics of the university's animal hospital

TABLE 1 | Demographic data of the cats included in the study.

		Sex		Age (year)[a]
	n	**Male**	**Female**	**Mean ± SD**
Healthy control				
Non-pedigree cat	14	6	8	5.57 ± 4.2
Scottish fold	6	2	4	2.08 ± 0.8
British shorthair	1		1	9
Chinchilla	2		2	1–9
European shorthair	1	1		3
Sphynx	1	1		3
Bacterial infection				
Non-pedigree cat	20	11	9	3.94 ± 3.18
Scottish fold	2	1	1	3–4
British shorthair	1		1	5
Turkish angora	1		1	5
Persian	1	1		6
Viral infection				
Non-pedigree cat	20	7	13	2.2 ± 1.68
Scottish fold	2	2		1–1
Persian	2		2	1–2
Russian blue	1	1		3

[a]The animal's actual age was presented when the number of cats belonging to each breed was ≤ 2; when the number of individuals in each breed was ≥ 3, the mean age (mean) ± standard deviation (SD) are presented.

either with complaints or for routine follow-up were included in the study upon the owners' signed consent. The animals were divided into three groups: healthy control, bacterial infection group (those with bronchopneumonia and gastrointestinal tract and urinary tract infections) and viral infection group (21 with FCoV, 3 with FeLV and 1 with feline calicivirus), each containing 25 individuals. During the study, we did not interfere with the cats' housing, maintenance or treatment protocols. The patient demographics are shown in Table 1.

2.2 | Inclusion Criteria for the Cat Population

2.2.1 | Healthy Control

Twenty-five healthy cats over 1 year of age were included in the healthy control group. Blood samples from the healthy control group were collected in accordance with the Local Ethics Committee's guidelines. These cats were already undergoing routine clinical examinations or pre-surgical evaluations, typically blood sampling for health assessments and vaccination purposes. In addition, some cats were brought in for neutering procedures involving preoperative blood testing to ensure their suitability for anaesthesia and surgery. The blood samples used in this study were collected, ensuring no additional distress or risk to the animals. These cats were screened for patient history, physical inspections and whole blood and biochemical tests to rule out any pathological condition. Negativity for feline coronavirus (FCoV), FIV and FeLV was established by rapid antigen test kits (Antigen Rapid FcoV Ab and Antigen Rapid FeLV Ag/FIV Ab, Gentaur; Kampenhout, Belgium).

2.2.2 | Bacterial Infection Group

The cats with bacterial bronchopneumonia, enteritis and lower urinary tract infections were included in this group. Bacterial culture could not be performed since the owners did not consent to procedures such as bronchoscopy and endoscopy.

The diagnosis of bacterial bronchopneumonia was established based on general clinical inspection, radiography and leucocyte count. Purulent nasal discharge, dyspnoea of varying intensity, fever and leucocytosis supported the diagnosis. Rapid antigen kits ruled out a potential viral infection regarding FcoV, FeLV and FIV, and a potential calicivirus infection was eliminated by the quantitative RT-PCR method. The patients' response to antibacterial treatment was also monitored, and a positive response was considered affirmative for inclusion. Finally, a definitive diagnosis of bacterial bronchopneumonia was achieved in ten cats.

A preliminary clinical diagnosis of bacterial enteritis was confirmed by auxiliary diagnostic methods such as WBC count, radiography and ultrasonography. Clinical signs included diarrhoea, impaired general condition, lethargy and fever. Blood tests revealed leukocytosis. Initially, cats with a recent diet change were excluded from the study. Faecal examination, including the

Giardia test, was performed in all cats, and those positive for any parasitic infection were also excluded. Rapid antigen kits were applied to eliminate FcoV, FIV, FeLV and feline panleucopenia-associated enteritis. A positive response to antibacterial therapy was also considered as an inclusion parameter. Finally, nine patients who responded well to the therapy with subsided diarrhoea, fever, leukocytosis and improved general health conditions were elected.

A definitive diagnosis of lower bacterial urinary tract infection was established in six cats. Urine samples were collected via cystocentesis from the patients with strangury, pollakiuria, dysuria and haematuria for bacterial culture and antibiogram. Bacterial culturing revealed the bacterial pathogens in descending order, such as *Proteus* spp., methicillin-resistant *Staphylococcus* spp., *Enterococcus* spp., *Escherichia coli* and *Staphylococcus* spp.

2.2.3 | Viral Infection Group

The selection of cats with viral infections was based on a combination of clinical inspection, rapid tests (Anigen Rapid FcoV Ab and Anigen Rapid FeLV Ag/FIV Ab; Gentaur), and confirmatory diagnostics using quantitative RT-PCR. Cats included in this group were positive for at least one viral infection (21 for FcoV, 3 for FeLV and 1 for feline calicivirus), confirmed as follows: FIP diagnosis: A definitive diagnosis of FIP was established in two cats based on clinical signs and quantitative RT-PCR-confirmed FcoV positivity in effusion fluids, in accordance with the European Advisory Board on Cat Diseases (ABCD) guidelines. Non-effusive FcoV infections: 12 cats showed clinical signs consistent with non-effusive FcoV infection (uveitis, ataxia, vomiting, diarrhoea, dizziness, anorexia, fever and reduced albumin/globulin ratio < 0.4). These cases were confirmed using rapid tests and clinical criteria, with RT-PCR as necessary to support the diagnosis. FeLV and calicivirus infections: The diagnosis for FeLV and feline calicivirus infections was based on positive rapid tests, RT-PCR, and relevant clinical signs.

2.2.4 | Exclusion Criteria Used in the Selection of Cats for the Study

In this study, certain criteria were used to exclude cats from participation to ensure the accuracy and relevance of the results. Cats were excluded if they had previously received any form of treatment or were currently on medication, including antibiotics, corticosteroids or immunomodulators. In addition, cats with concurrent mycotic or parasitic infections were also excluded. Furthermore, cats with clinically evident or cytologically detected malignant or benign tumours were excluded from the study. Finally, cats suspected of having viral infections but with negative viral test results were also excluded.

2.2.5 | Blood Sampling

A blood sample of 3.5 mL was harvested from each cat involved in the study; 0.5 mL of blood was collected into EDTA tubes (MiniCollect; Kremsmünster, Austria) for assessing total and differential leucocyte counts, and the remaining blood samples were transferred into anticoagulant-free blood tubes (Vacutainer; Becton, Dickinson and Company) to obtain sera.

2.2.6 | Determining Total and Differential WBC Counts

The total WBC count and the differential leukocyte count were determined on a haemogram device using commercial kits (ProCyte Dx idexx; Hoofddorp, The Netherlands).

2.2.7 | Serum Cytokine and CRP Analyses

Serum CRP, TGF-β, IL-6, TNF-α, IL-1β and IL-10 concentrations were assessed by ELISA using feline-specific commercial kits (Abkine; Abbkine Scientific, GA, USA) according to the manufacturer's instructions. For each assay, 50 μL of serum samples were used, and the absorbance at 405 nm was measured using a microplate reader (RT6000; Rayto, Shenzhen, China).

2.3 | Statistical Analysis

The SPSS software (SPSS for Windows, version 11.5.2.1) was applied for the statistical analyses. The distribution of the data were initially analysed using the Shapiro–Wilk test. The normally distributed data were compared with the ANOVA, and the differences among groups were assessed using the Tukey HSD test. The Kruskal–Wallis test was applied for non-normally distributed data. The post hoc analysis of the parameters was performed using the Mann–Whitney *U* test. The independent sample Student *t*-test was utilised to determine the possible changes in TGF-β, IL-6 and CRP concentrations between surviving and non-surviving cats. The results are presented as means ± standard error of the mean for parametric variables and as medians with interquartile ranges for nonparametric variables. Significance was established at the $p < 0.05$ level. The statistical significance was established as $p = 0.05$.

3 | Results

Demographic data of the cats included in the study are presented in Table 1. A total of 75 cats were included in the study, of which 44 were female and 31 were male, with a mean age of 3.61 ± 2.04 years.

Figure 1 presents WBC counts in the peripheric blood. The WBC count was higher in cats with bronchopneumonia, gastrointestinal tract infections and urinary tract infections compared to both the healthy control and viral infection groups ($p = 0.001$). Regarding the leukocyte subtypes, neutrophil and monocyte counts were higher ($p = 0.045$ and $p = 0.002$), and, in contrast, eosinophil and basophil counts were lower than those of the healthy control ($p = 0.001$ and $p = 0.001$).

The data regarding serum CRP and serum cytokine concentrations are presented in Figure 2. Serum CRP concentration was significantly increased in cats with bacterial infections (bronchopneumonia, gastrointestinal tract infections and urinary

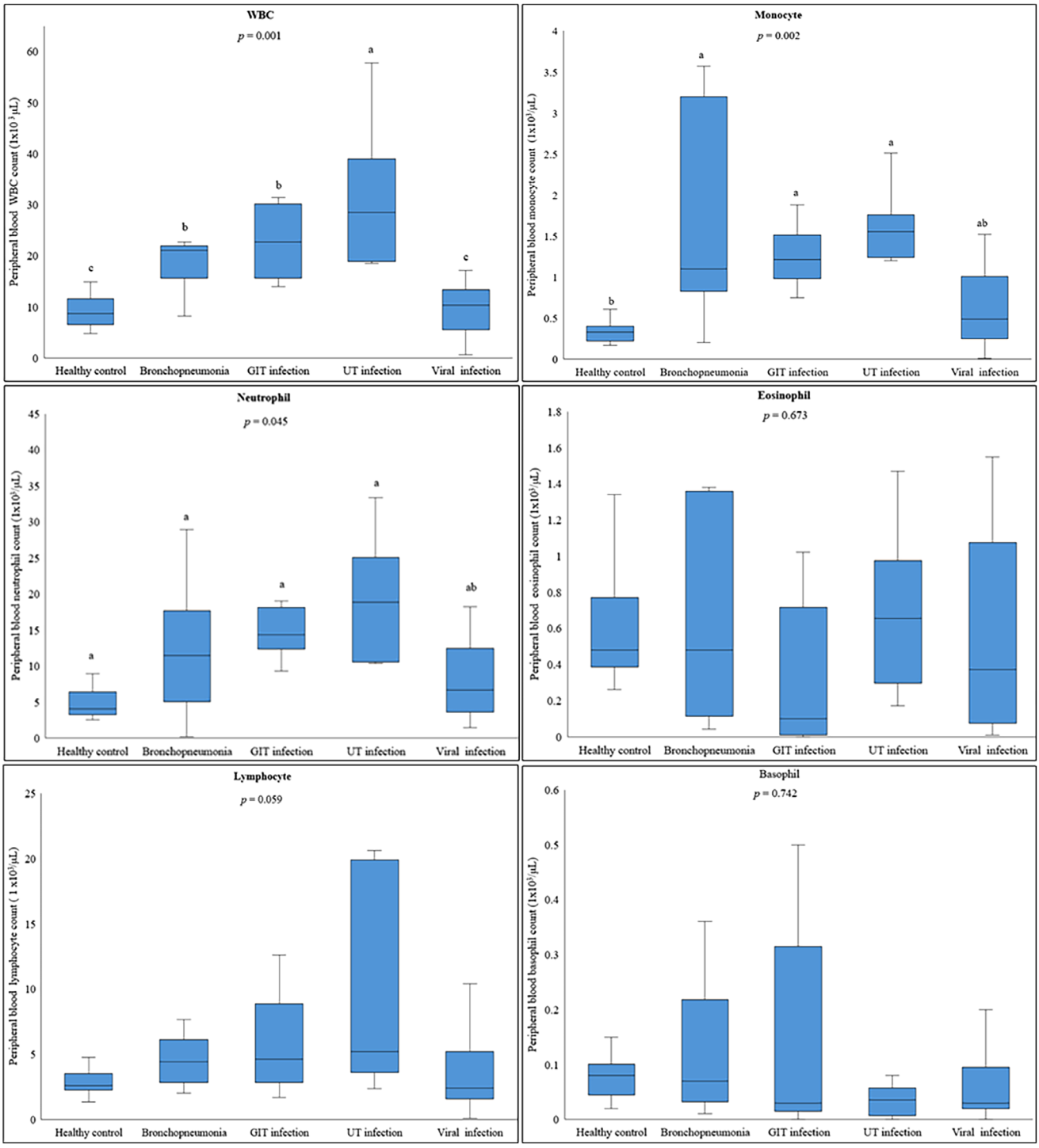

FIGURE 1 | Changes in leukocyte profile in cats with bacterial and viral infections. The central lines in the box plots represent the median, the edges of the boxes represent the interquartile range, and the whiskers represent the maximum and minimum values. a, b, c = The difference between means with different letters in the same graphic is significant. GIT, gastrointestinal tract; UT, urinary tract; WBC, white blood cells.

tract infections) than in healthy control and viral infection groups. The most prominent increase was noted in cats with bronchopneumonia, even higher than those with other bacterial infections (gastrointestinal tract infections and urinary tract infections) ($p = 0.001$).

The TGF-β concentration of the viral infection group was significantly decreased compared to the healthy control ($p = 0.001$). The difference in the relevant parameter between the bacterial infection group and the healthy control was statistically insignificant.

Serum IL-6 concentrations of the patients with bronchopneumonia, gastrointestinal tract infections and urinary tract infections were higher than those of healthy cats and the viral infection group ($p = 0.001$).

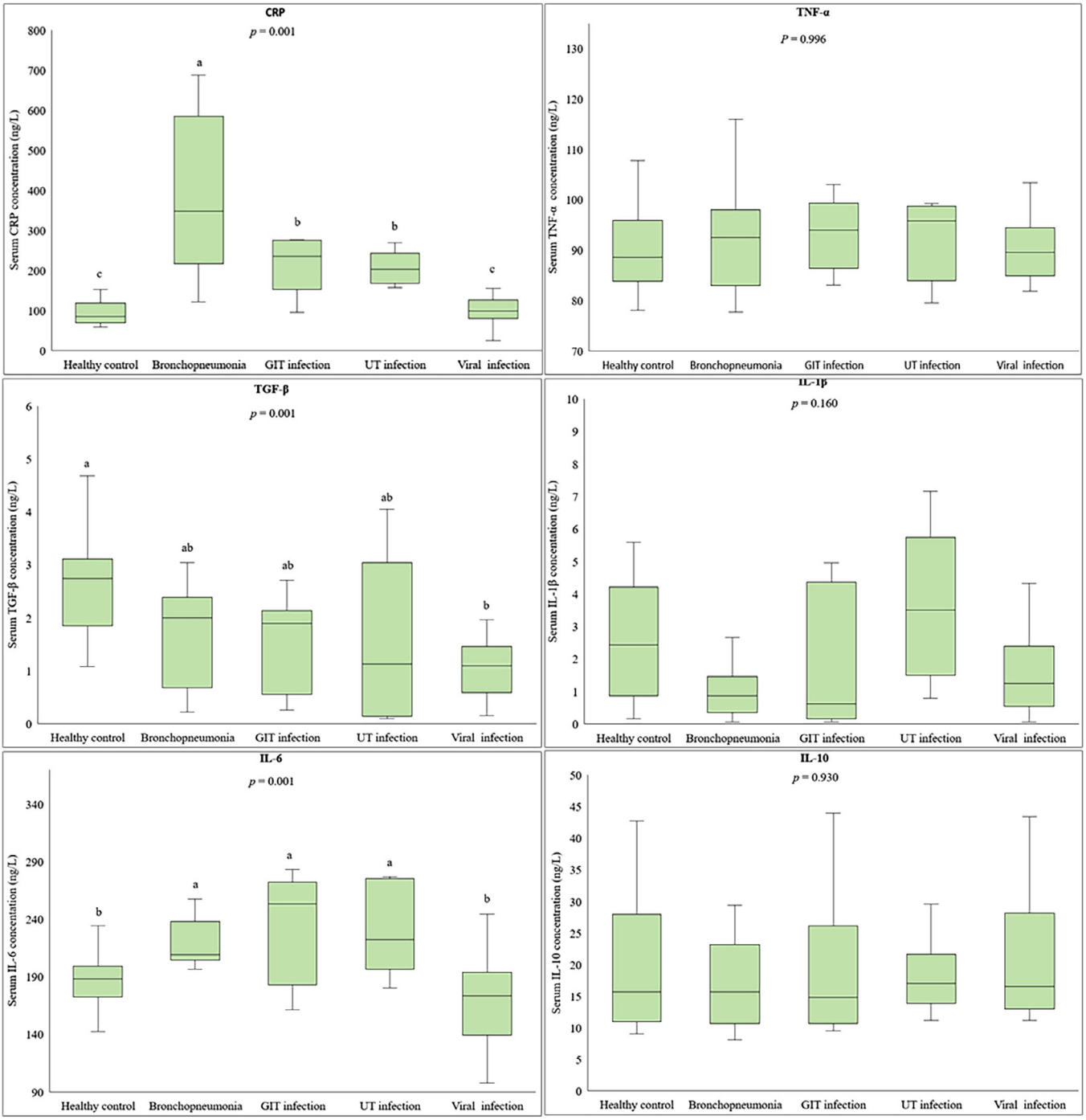

FIGURE 2 | The data regarding serum CRP and serum cytokine concentrations. The central lines in the box plots represent the median, the edges of the boxes represent the interquartile range, and the whiskers represent the maximum and minimum values. a, b, c = The difference between means with different letters in the same graphic is significant. CRP, C-reactive protein; GIT, gastrointestinal tract; IL-10, interleukin-10; IL-1β, interleukin-1 beta; IL-6, interleukin-6; TGF-β, transforming growth factor beta; TNF-α, tumour necrosis factor alpha; UT, urinary tract.

No statistically significant difference was noted between the healthy control and the other study groups regarding serum TNF-α, IL-1β and IL-10 production ($p = 0.996$, $p = 0.160$ and $p = 0.930$, respectively).

The data concerning serum CRP, IL-6 and TGF-β concentrations between survivors and non-survivors in cats are shown in Figures 3–5, respectively. In terms of serum CRP and TGF-β concentrations, no statistically significant difference was noted between survivors and non-survivors ($p = 0.165$ and $p = 0.392$, respectively). On the other hand, serum IL-6 concentrations of the patients who died were significantly higher than surviving cats ($p = 0.003$; Figure 4).

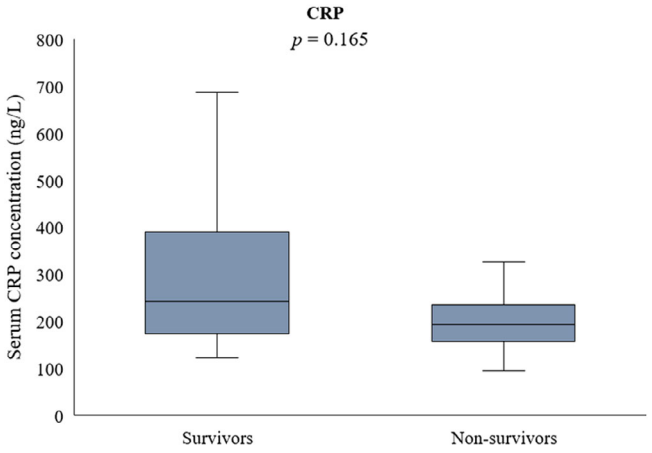

FIGURE 3 | Comparison of the serum CRP concentrations between surviving and non-surviving cats with bacterial infection. There was no significant difference in serum CRP concentrations between those who died and those who survived bacterial infection ($p = 0.165$). The central lines in the box plots represent the median, the edges of the boxes represent the interquartile range, and the whiskers represent the maximum and minimum values. Surviving cats $n = 14$, non-surviving cats $n = 7$. Since four cats in this group were lost to follow-up, no data was obtained regarding the death or survival status of these patients. Thus, the statistical analysis was performed on data from 21 cats. CRP, C-reactive protein.

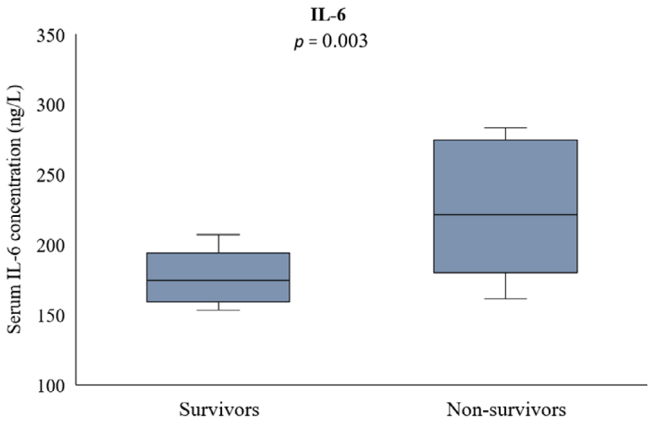

FIGURE 4 | Comparison of serum IL-6 concentrations between surviving and non-surviving cats with bacterial infection. The serum IL-6 concentration in non-surviving cats was higher than in survivors ($p = 0.003$). The central lines in the box plots represent the median, the edges of the boxes represent the interquartile range, and the whiskers represent the maximum and minimum values. Surviving cats $n = 14$, non-surviving cats $n = 7$. Since four cats in this group were lost to follow-up, no data was obtained regarding the death or survival status of these patients. Thus, the statistical analysis was performed on data from 21 cats. IL-6, interleukin-6.

4 | Discussion

The challenges of distinguishing bacterial infections from viral diseases have rendered assessing the host's inflammatory response to the infection essential in addition to the clinical parameters. (Ross et al. 2021). Herein, we evaluated the diversities in inflammatory responses in cats to multiple-system-associated

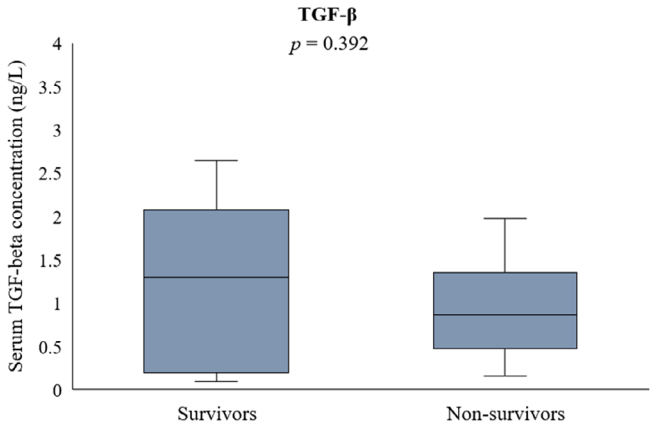

FIGURE 5 | Comparison of the serum TGF-β concentrations between surviving and non-surviving cats with viral infections. There was no significant difference in serum TGF-β concentrations between cats who died from viral infection and those who survived ($p = 0.392$). The central lines in the box plots represent the median, the edges of the boxes represent the interquartile range, and the whiskers represent the maximum and minimum values. Surviving cats $n = 9$, non-surviving cats $n = 12$. Since four cats in this group were lost to follow-up, no data was obtained regarding the death or survival status of these patients. Thus, the statistical analysis was performed on data from 21 cats. TGF-β, transforming growth factor beta.

bacterial infections and viral diseases. The data obtained revealed infection-type-wise significant differences in leukocyte counts and CRP, TGF-β and IL-6 productions.

Total WBC and differential neutrophil counts were increased in cats with bacterial infection (Figure 1), which was an anticipated finding since it is known that mediators secreted from various cells, debris released from injured tissues or bacterial degrading particles, such as lipopolysaccharides (LPS), stimulate the bone marrow's neutrophil production (Summers et al. 2010). Leukocyte count is the most accurate parameter for diagnosing a bacterial infection. However, bacterial infections manifest an elevation in WBC count in merely 30% of cats (Cho et al. 2021). Therefore, it is recommended that this parameter be evaluated simultaneously with other biomarkers.

CRP is used as a non-specific biomarker in monitoring the outcome of infectious diseases in humans (Vincent, Donadello, and Schmit 2011). However, it is less commonly applied in feline practice than SAA due to lower CRP elevation than SAA in cats during infections. However, the data revealed an approximately threefold increase in serum CRP concentrations in cats with bronchopneumonia compared to the healthy controls, while CRP concentrations were approximately doubled in cats with gastrointestinal tract and urinary tract infections. Considering the relevant data, CRP was to be useful in evaluating feline systemic infections in combination with other clinical parameters.

In the study, serum TGF-β concentration was significantly reduced in cats with viral infections. The replicating virus within the host cell is known to impact the cell's signalling pathways and protein production (Gaur, Munjal, and Lal 2011). Among the pathways it affects are the suppressor of mothers against decapentaplegic (SMAD) and other SMAD-associated signalling

pathways, which are effective in TGF-β production (Mirzaei et al. 2018). Despite the limited data, the FIP virus is known to upregulate SMAD4 (Drechsler et al. 2020), and the upregulated SMAD4 stimulates the LIM domain 7 (LMO7) gene expression, which inhibits TGF-β production by blocking the JUN and FOS signalling molecules (Hariyanto, Yo, and Wanandi 2021). Therefore, the reduced TGF-β concentration in cats with viral infection was associated with the adverse impacts of the viruses on the intracellular signalling pathways, including the SMAD pathways. A secondary cause might be linked with the impacts of viruses on the macrophages since macrophages transform TGF-β, which is secreted as a large, latent, inactive complex, into its active form. One of the targets of the viruses entering the host is the macrophages (Nunes, Shapiro, and Rifkin 1995), which might have been the cause of the restricted TGF-β production.

TGF-β plays a vital role in controlling inflammation, wound healing, tissue homeostasis and regulation of immune responses under pathophysiological conditions (Ong et al. 2021). The decreased TGF-β production in viral infections leads to increased inflammation, impaired tissue healing and inadequate immune response, likely adversely impacting the course of the infection (Deng et al. 2024). The high mortality in the viral infection group supported this assumption. Despite the duality of TGF-β activity, with both inflammatory and tissue injury-preventing properties (Saxena et al. 2008), inhibition of TGF-β production during viral infections generates a rapid-onset deterioration in clinical status (Li, Sanjabi, and Flavell 2006; Marie, Liggitt, and Rudensky 2006). Therefore, preventing the decline in TGF-β production in cats with viral infections may be the focus of a novel therapeutic strategy. However, utmost care should be taken while interpreting the data revealing the reduced TGF-β production in viral infections since most cats in the viral infection group had FIP. Thus, this observation may not apply to all types of viral infections. Hence, further studies are needed to evaluate TGF-β concentrations across various feline viral infections.

In the study, serum IL-6 concentration was higher in bacterial infections (bronchopneumonia, gastrointestinal tract infections and urinary tract infections) than in healthy control and viral infection groups. Similarly, IL-6 production was previously shown to have elevated in cats with sepsis (DeClue et al. 2011). In humans, IL-6 secretion was reported to have been significantly increased, especially during the early stages of bacterial infection (Panacek and Kaul 1999). Studies have shown that serum IL-6 concentrations are significantly higher in individuals with community-acquired bacterial infections than those with viral infections (Holub et al. 2013). Moreover, plasma IL-6 concentration was notably higher in patients with bacterial enterocolitis than in those with viral enterocolitis (Yeung et al. 2004). In the present study, a significant increase in serum IL-6 concentrations was observed in bacterial infections affecting the respiratory, urinary or gastrointestinal tracts. However, no significant differences were found among the specific types of bacterial infections. Therefore, serum IL-6 can be considered a valuable clinical parameter for evaluating bacterial infections in these systems more generally.

It has been reported that multiple mechanisms play a role in increasing IL-6 production during bacterial infections. One of these is the mechanism triggered in the host's immune system by small molecular motifs possessed by bacteria and known as pathogen-associated molecular patterns (Kumar, Kawai, and Akira 2011). These molecules released from bacteria bind to the host cell's toll-like surface receptors (TLRs) alongside other pattern recognition receptors and stimulate IL-6 production (Tanaka et al. 2014). Another mechanism involves triggering IL-6 production by binding LPS released from bacteria to the TLR4, particularly on the surface of monocytes, macrophages and granulocytes (Kim et al. 2023). Stimulation of TLR4 also activates signalling pathways such as nuclear factor kappa B, mitogen-activated protein kinase and NOD-like receptor protein 3, leading to further enhancement of IL-6 production (Ito et al. 2020). On the other hand, viruses exhibit lower secretion of inflammatory cytokines, such as IL-6, due to several reasons. First, viruses typically lack pathogenic molecules such as LPS that trigger robust immune responses, leading to lower concentrations of inflammatory cytokines (Lin et al. 2022). Second, some viruses may block the synthesis and maturation of cytokines or modulate cytokine signalling in the target cells, further contributing to reduced cytokine production (Alcami 2016). Moreover, viruses often adopt strategies that result in minimal damage to the host cell rather than destroying it (Sarikonda and von Herrath 2011). As a consequence, the weakened immune response triggered by viruses causes decreased IL-6 production. However, it should be noted that some viral infections may generate increased IL-6 production, as in the example of the cats infected with FeHV-1, in which the virus causes cellular necrosis in the trachea and lungs, inducing IL-6 production (Lawrence et al. 1995).

Serum IL-6 concentrations were higher in cats who died than in the survivors (Figure 4). No documented data concerning the IL-6 status during bacterial infections is available in cats or dogs. However, similar findings in humans have shown elevated IL-6 concentrations in patients admitted to intensive care units, where mortality rates were higher, suggesting IL-6 as a potential predictive biomarker for mortality (Liu et al. 2021). Based on our findings in cats, we recommend further studies to explore this potential biomarker in veterinary medicine.

The most important limitation of this study is that bacterial culture could not be performed in cats included in the bronchopneumonia and gastrointestinal tract infection groups. The main reason for this limitation was that patient owners did not consent to procedures such as endoscopy and bronchoscopy, which are generally performed under anaesthesia. One of the critical limitations of the study is that blood samples were collected from the cats only once. If blood samples could have been obtained at regular intervals throughout the study, it would have allowed for the detection of changes in the concentrations of cytokines or CRP, depending on the progression of the disease. The primary obstacle to obtaining blood samples at multiple time points was the preference of patient owners to continue treatment at other clinics. Another limitation of the study is that most cats included in the viral infection group had FIP infection. This observation may not apply to all types of viral infections.

5 | Conclusions

This study revealed significant differences between healthy cats and cats with bacterial infections, such as bronchopneumonia,

gastrointestinal and urinary system infections and viral infections regarding the inflammatory mechanisms. TGF-β production was significantly reduced in cats with viral infections compared to healthy individuals, pointing out the likelihood of the essential role of TGF-β in the pathophysiology of feline viral diseases. On the other hand, IL-6 production was increased in cats with bronchopneumonia and gastrointestinal and urinary system infections, highlighting the potential role of IL-6 in immune responses to these infections. Furthermore, the elevated CRP concentration in cats with bronchopneumonia indicated a more intense inflammatory response in the relevant group. The high serum IL-6 concentrations in the non-survivors suggested this cytokine's potential efficacy in assessing the severity of the infection. In conclusion, the diverse immune responses noted in the study offered valuable knowledge concerning the underlying pathophysiological mechanisms in feline bacterial and viral infections. Considering that few biomarkers are currently available in feline practice, we believe the data from this study will guide veterinary practitioners to an accurate diagnosis and treatment protocol and contribute to developing new strategies for managing and controlling feline infectious diseases.

Author Contributions

Songul Erhan: conceptualisation (lead), project administration (lead), writing–original draft preparation (lead), formal analysis (lead). **Bengu Bilgic**: resources (equal), investigation (equal). **Ezgi Ergen**: investigation (equal), formal analysis (equal), writing–review and editing (equal), visualisation (lead). **Mert Erek**: investigation (equal), formal analysis (equal), writing–review and editing (equal). **Elif Ergul Ekiz**: writing–review and editing (equal), methodology (equal). **Mukaddes Ozcan**: writing–review and editing (equal), methodology (equal). **Mehmet Erman Or**: resources (equal). **Banu Dokuzeylul**: resources (equal). **Erdal Matur**: conceptualisation (supporting), writing–original draft preparation (supporting), methodology (lead), funding acquisition (lead).

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Ethics Statement

This study was carried out with the permission of Istanbul University Animal Experiments Local Ethics Committee (Approval no: 2018/25 118-481). This work involved non-experimental animals (owned or unowned) and procedures that differed from established internationally recognised high standards ('best practice') of veterinary clinical care for the individual patient. The study, therefore, had ethical approval from an established committee, as stated in the manuscript.

Consent

Informed consent (either verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (either experimental or non-experimental animals) for the procedure(s) undertaken (either prospective or retrospective studies). For any animals or humans individually identifiable within this publication, informed consent (either verbal or written) for their use in the publication was obtained from the people involved.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer Review

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.70098.

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