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RESEARCH ARTICLE

Can Serum Amyloid A Levels be Used in the Diagnosis of SIRS in Cats with Pyometra?

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Abstract

Serum amyloid A (SAA) level increases in conditions such as infection, tissue damage and trauma and is not specific to a disease but provides information about the presence and severity of inflammation. As a life-threating conditions pyometra usually causes systemic inflammatory response syndrome (SIRS) and therefore may lead an increase in SAA levels. The present study was designed to determine SAA levels in cats with pyometra that developed SIRS, and to demonstrate the diagnostic value of SAA by comparing it with hematological and biochemical parameters as well as SIRS criteria. For this purpose, data were used from cats with open-cervix pyometra (OP, n=6) and closed-cervix pyometra (CP, n=6), which were identified as having developed SIRS and were admitted to hospital as well as from healthy cats brought in for routine neutering, which were identified to be in the diestrus phase of the sexual cycle (DE, n=6). Cats with pyometra had higher SAA levels and leukocytosis compared to cats in the DE group (P= 0.002 and P= 0.000, respectively). The highest SAA level was detected in the CP group (168.6 µg/ml) and this level is statistically significant compared to the other groups (P= 0.028). While there was no correlation between SAA levels and SIRS criteria, SAA levels were negatively correlated with both AST (P= 0.045, rs=-0.478) and GGT (P= 0.019, rs= -0.548). In the study, it was determined that sepsis and SIRS progressed with different symptoms in cats and SIRS criteria were less specific in cats. It was concluded that SAA levels may be an important marker in closed-cervix pyometra cases. We think that the study data are promising but further and comprehensive studies are needed considering the number of patients included in the study.

Key Words: Cat, pyometra, serum amyloid A, systemic inflammatory response syndrome

Serum amiloid A düzeyleri piyometralı kedilerde SİRS tanısında kullanılabilir mi?

Öz

Serum amiloid A (SAA) düzeyi enfeksiyon, doku hasarı ve travma gibi durumlarda artar ve bir hastalığa özgü olmamakla birlikte inflamasyonun varlığı ve şiddeti hakkında bilgi verir. Hayatı tehdit eden bir durum olan pyometra genellikle sistemik inflamatuar yanıt sendromuna (SIRS) neden olur ve bu nedenle SAA seviyelerinde artışa yol açabilir. Bu çalışma, SIRS gelişen piyometralı kedilerde SAA düzeylerini belirlemek ve SAA'nın tanısal değerini hematolojik ve biyokimyasal parametrelerin yanı sıra SIRS kriterleriyle karşılaştırarak göstermek amacıyla tasarlanmıştır. Bu amaçla SIRS geliştirdiği tespit edilen ve hastanemize getirilen açık serviks pyometralı (OP, n=6) ve kapalı serviks pyometralı (CP, n=6) kedilerin yanı sıra rutin kısırlaştırma için getirilen ve seksüel siklusun diöstrus döneminde olduğu tespit edilen sağlıklı kedilerden (DE, n=6) elde edilen veriler kullanılmıştır. Piyometralı kediler, DE grubundaki kedilere kıyasla daha yüksek SAA seviyelerine ve lökositoza sahipti (sırasıyla P= 0.002 ve P= 0.000). En yüksek SAA seviyesi CP grubunda tespit edilmiştir (168.6 µg/ml) ve bu seviye diğer gruplardakine göre istatistiksel olarak anlamlı bulunmuştur (P= 0.028). SAA düzeyleri ile SIRS kriterleri arasında korelasyon saptanmazken, SAA düzeyleri hem AST (P= 0.045, rs= -0.478) hem de GGT (P= 0.019 rs= -0.548) ile negatif korelasyon göstermiştir. Çalışmada sepsis ve SIRS'in kedilerde farklı semptomlarla ilerlediği, SIRS kriterlerinin kedilerde daha az spesifik olduğu tespit edilmiştir. Serum amilod A'nın CP olgularında önemli bir belirteç olabileceği sonucuna varılmıştır. Çalışma verilerinin umut verici olduğunu ancak çalışmaya dahil edilen hasta sayısı göz önünde bulundurulduğunda daha ileri ve kapsamlı çalışmalara ihtiyaç olduğunu düşünmekteyiz.

Anahtar Kelimeler: Kedi, piyometra, serum amiloid A, sistemik yangısal cevap sendromu

INTRODUCTION

Pyometra, which implies the acute or chronic suppurative infection of the uterus, occurs in the luteal period and is life-threatening in cats and dogs due to the general deterioration and systemic infection it causes (1). The repeated exposure of the reproductive system to hormonal stimulation has an important place in the etiology of the disease. Estrogen (E2) exposure during the follicular period prepares the uterus for the effect of progesterone (P4). Progesterone stimulates endometrial development and glandular secretion and suppresses myometrial contractions and uterine immunity. Eventually, this process prevents the uterus from eliminating bacterial contamination and creates favorable conditions for bacterial growth and the development of pyometra (2). This process may either occur as a result of repeated exposure to endogenous hormones or be triggered by exogenously administered E2 and P4 (3,4).

The clinical course of the disease varies, depending on the duration of its development, the presence of an irregular cycle, the effects of exogenous and endogenous hormones, the severity of liver and kidney dysfunction, the presence of bacterial infection, the patency of the cervix, and the patient's immune response (5,6). Usually, closed-cervix pyometra (CP) cases are more severe than open-cervix pyometra (OP) cases (7). However, the general clinical findings are similar in both types of pyometra. Symptoms such as purulent vaginal discharge (if the cervix is open), expansion of the uterine volume, polyuria, polydipsia, lethargy, anorexia, vomiting - which are actually not specific to pyometra - are observed (3).

Diagnosis can be made based on anamnesis, physical and gynecological examinations, laboratory findings, radiography, ultrasonography (USG), and the type of the genital discharge in cases, where the cervix is open (8). Recently, acute-phase proteins (APP) have also started to be evaluated in cats and dogs for diagnostic purposes (9,10).

Acute-phase proteins are produced by the liver against trauma, viral and bacterial infections, and inflammatory conditions. During the inflammatory process, species specific increases (positive APP) and decreases (negative APP) occur in some APP levels. For example, the APP, which increases most rapidly and at the highest level is the C-reactive protein (CRP) in the dog, and serum amyloid A (SAA) in the cat. This means that SAA is a positive acute-phase protein in the cat. Since SAA increases in various feline diseases, it can be used as a marker to determine the presence and severity of systemic inflammation and its prognosis in cats (11). Studies show that there is a correlation between APP production and the clinical signs of systemic inflammatory response (9,10).

Systemic inflammatory response syndrome (SIRS) is the body's clinical, hematological and immunological response to infectious and non-infectious factors. Toxins produced by bacteria involved in pyometra, after entering the systemic circulation, cause the development of sepsis and SIRS, which result in multiple organ failure and increased mortality rates (12). In veterinary medicine, clinical and laboratory findings adapted from human medicine, such as hyperthermia/hypothermia, tachycardia/bradycardia, tachypnea/bradypnea, leukocytosis/leukopenia and band neutrophils, are used as SIRS diagnostic criteria. These criteria show a significant correlation with the prognosis of the disease (13). Therefore, patients with sepsis or SIRS should be regularly assessed for these parameters (14). Furthermore, Yazlık et al. (15) suggested that the results of hematological and biochemical analyses could also be used as markers in dogs with pyometra that develop SIRS, especially in cases of CP.

Today, the diagnostic criteria established for dogs continue to be used for the diagnosis of non-species-specific diseases in cats. However, there are very important metabolic, enzymatic and endocrinological differences between cats and dogs. For example, as mentioned above, there are various species-specific APPs, and evaluating the CRP instead of SAA in cats would yield irrelevant results and mislead the physician (9). Furthermore, while pyometra occurs after the age of six in dogs not administered with any exogenous hormone, it may occur at any age in cats (1). Therefore, there is a need to detect the differences of cats. In this context, the present study was designed to determine SAA levels in cats with pyometra that developed SIRS, and to demonstrate the diagnostic value of SAA measurement by comparing it with hematological and biochemical parameters as well as SIRS criteria.

MATERIAL AND METHODS

In the present study, data were used from cats with open- and closed-cervix pyometra, which were identified as having developed SIRS and were admitted to our clinic, as well as from healthy cats brought in for routine neutering, which were identified to be in the diestrus phase of the sexual cycle.

Cats, which were admitted to the clinic with signs of vaginal discharge, anorexia, polyuria, polydipsia, and lethargy, diagnosed with pyometra by USG, vaginal cytology, and blood analysis, and determined to have developed SIRS, were included in the open-cervix pyometra group (OP, n=6). Cats showing the same clinical signs, except for vaginal discharge, were included in the closed-cervix pyometra group (CP, n=6). Cats, which were brought in for neutering, found to be healthy upon clinical examination, and identified to be in the diestrus phase of the sexual cycle based on gynecological examination and operational findings, were included in the diestrus group (DE, n=6).

In this study, animals with SIRS were identified according to the diagnostic criteria reported (16). Accordingly, the following reference intervals were used: <6, >20 $\times 10^9$/L for WBC,<37.2°C,>39.4°C for body temperature, >40 breaths/min for respiratory rate, and <140, >220 bpm for pulse. Cats, which met at least three of these four criteria, were considered to be SIRS-positive (16).

Animals with no history of exogenous hormone administration at any time, no treatment before being brought to our clinic, and no acute or chronic systemic and/or metabolic disorders other than pyometra, and without any organ dysfunction caused by pyometra were included in the study.

Routine procedures were performed on the cats. Accordingly, after anamnesis was performed, physical examinations were conducted, and the findings were recorded. The report of Thomovsky et al. (17) was used as a reference for these examinations, which were part of the routine evaluation.

Ultrasonographic examinations were performed transabdominally with an 5-7.5 MHz probe (Mindray VETUS 9,

Hasvet). During USG, a preliminary diagnosis of suspected pyometra was made by observing an anechoic content with a snowstorm appearance in the distended lumen, which expanded cranially and dorsally from the bladder. Cats diagnosed with pyometra were assigned to the OP group if they had vaginal discharge, the CP group if they had no vaginal discharge. Cats with no pathology in the uterus were assigned to the DE group. In all groups, uterine diameter measurements were performed with USG, as described by Gatel et al. (18), and the results were recorded.

The definitive classification of the animals into the study groups was based on the macroscopic and microbiological evaluation of the reproductive tissue after surgery. Accordingly, cats with suspected pyometra were definitively classified into the pyometra group if bacterial growth was found in the microbiological evaluation of the uterus. Cats with no pathology in the uterus and with corpus luteum identified in the ovary during the macroscopic evaluation were classified into the DE group.

For hemogram, serum biochemistry, and SAA measurement, blood samples were taken from the antebrachial cephalic vein into 10-ml serum tubes and EDTA-coated tubes before surgery (Mindray BC-60, Hasvet). In this study, only WBC (reference interval: 6-20 x10^9/L) were counted as the WBC count is included among the SIRS criteria. After the samples were centrifuged at 5000 rpm for 10 minutes, blood urea nitrogen (BUN; reference interval: 19-34 mg/dl), creatinine (CREA; reference interval: 0.8-2.1 mg/dl), alanine aminotransferase (ALT; reference interval: 28-109 U/L), alkaline phosphatase (ALP; reference interval: 11-49 U/L), aspartate amino transferase (AST; reference interval: 17-46 U/L) and gamma glutamyl transferase (GGT; reference interval: 0-2 U/L) levels were measured in the serum samples (Mindray BS-120Vet, Hasvet). The SAA levels were determined in the sera with the aid of an automatic veterinary hormone analysis and immunity testing device (Vcheck V200, Hasvet) using Feline SAA 3.0 test kits, which were 97% compatible with ELISA. Evaluations were performed according to the prospectus data, such that <5 µg/ml was accepted normal; 5-10 µg/ml raised a suspicion of systemic inflammation; and >10 µg/ml indicated the presence of systemic inflammation.

Statistical analysis

Statistical analyses were performed with the IBM SPSS 25.0 package program. The normality distribution of the study data was determined by the Shapiro-Wilk test. When the normal distribution of the data was verified, a one-way ANOVA test was used for the comparison of the groups, and when the data did not show a normal distribution, the Kruskal-Wallis and Mann-Whitney U tests were employed. The correlations between SAA and the other parameters were determined by Spearman's correlation coefficient.

RESULTS

No statistical differences were found between the mean ages and body weights of the study groups (Table 1). The clinical symptoms detected in the cats with pyometra and the severity of these symptoms are presented in (Table 2).

Table 1. Values of mean age and body weight of the groups

Group	Age (year; X±SE)	Body weight (kg; X±SE)
OP	2.8 ± 2.7	3.4 ± 0.2
CP	2.0 ± 1.3	3.3 ± 0.1
DE	1.3 ± 0.4	3.2 ± 0.1
P value	0.501	0.815

Data are presented as X ± SE.OP; Open-cervix pyometra, CP; Closed-cervix pyometra, DE; Diestrus.

Table 2. Clinical symptoms and its rates identified in cats with pyometra

Clinical Symptoms	OP (%; n)	OP (%; n)	Total (%; n)
Depression/Lethargy	0.0 (0/6)	100 (6/6)	50.0 (6/12)
Anorexia	66.7 (4/6)	100 (6/6)	83.3 (10/12)
Vomiting	0.0 (0/6)	16.7 (1/6)	8.3 (1/12)
Color change in mucous membranes	33.3 (2/6)	66.7 (4/6)	33.3 (6/12)
Prolongation of CRT	33.3 (2/6)	66.7 (4/6)	50.0 (6/12)
Hyperthermia	50.0 (3/6)	66.7 (4/6)	58.3 (7/12)
Dehydration	33.3 (2/6)	50.0 (3/6)	41.7 (5/12)
Abdominal pain	16.7 (1/6)	50.0 (3/6)	33.3 (4/12)
Polydipsia	0.0 (0/6)	50.0 (3/6)	25.0 (3/12)
Polyuria	0.0 (0/6)	50.0 (3/6)	25.0 (3/12)

Data were presented as % and "n" show the number of cats. OP; Open-cervix pyometra, CP; Closed-cervix pyometra, DE; Diestrus, CRT; Capillary refill time.

Data on the hematological and serum biochemical parameters, SAA levels and SIRS criteria are presented in (Table 3). The cats with pyometra had higher SAA levels and leukocytosis, when compared to cats in the DE group (P= 0.002 and P= 0.000, respectively). Also, bradycardia was more common than tachycardia in the cats with pyometra.

Table 3. Rates of hematological, serum biochemical and SAA findings of the groups

Parameters	Reference interval	OP (%; n)	Groups CP (%; n)	DE (%; n)	P value
High SAA levels	>5 µg[a]	50 (3/6)	100 (6/6)	0 (0/6)	0.002*
Leukocytosis	>20×10^9/L[b]	100 (6/6)	100 (6/6)	0 (0/6)	0.000**
Hyperthermia	>39.4°C[b]	50 (3/6)	66.7 (4/6)	0 (0/6)	0.207
Tachypnea	>40 bpm[b]	100 (6/6)	83.3 (5/6)	50 (3/6)	0.301
Bradycardia	<140 bpm[b]	66.7 (4/6)	83.3 (5/6)	0 (0/6)	0.04*
Tachycardia	>220 bpm[b]	0 (0/6)	16.7 (1/6)	0 (0/6)	-
Higher BUN levels	>34 mg/dl[c]	50 (3/6)	66.7 (4/6)	33.3 (2/6)	0.513
High GGT activity	> 2 U/L[c]	33.3 (2/6)	50 (3/6)	0 (0/6)	0.059
High ALT activity	> 109 U/L[c]	33.3 (2/6)	0 (0/6)	0 (0/6)	0.073
Low ALT activity	< 28 U/L[c]	0 (0/6)	33.3 (2/6)	0 (0/6)	-
High AST activity	> 48 U/L[c]	33.3 (2/6)	0 (0/6)	0 (0/6)	0.073
Low AST activity	< 17 U/L[c]	0 (0/6)	33.3 (2/6)	0 (0/6)	-
High ALP activity	> 49 U/L[c]	16.7 (1/6)	16.7 (1/6)	33.3 (2/6)	0.575
Low ALP activity	< 11 U/L[c]	16.7 (1/6)	0 (0/6)	0 (0/6)	-

Data were presented as % and "n" show the number of cats.OP; Open-cervix pyometra, CP; Closed-cervix pyometra, DE; Diestrus, SAA; Serum Amiloid A, BUN; Blood urea nitrogen, GGT; Gamma glutamyl transferase, ALT; Alanine aminotransferase, AST; aspartate amino transferase, ALP; alkaline phosphatase.[a] : Prospectus of Feline SAA 3.0 test kits, [b]: (16), [c]: Vet Cornell Refence Intervals, *; p<0.05, **p<0.001 .

The mean data for the SIRS criteria and SAA levels are presented in Table 4. Statistical significance was determined only between WBC, pulse and SAA levels among the parameters examined. The mean body temperature of the cats with CP was higher than the reference interval values, but this difference was statistically insignificant. The mean respiratory rates were above the reference values in all groups. Although the respiratory rates were higher in the pyometra groups than in the control group, no statistically significant difference was detected. The highest SAA level was determined in the CP group (168.6 µg/ml), and this level was statistically significant compared to the levels of both the OP and DE groups (P= 0.028). On the other hand, no significant difference was found between the SAA levels of the OP and DE groups (Table 4).

Table 4. Mean values obtained for SIRS criteria and SAA levels in the study

Parameters	Reference interval	Groups			P value
		OP	CP	DE	
WBC	<6 ; >20 x10^9/L	36.0 ± 5.3[a]	39.1 ± 6.4[a]	10.0 ± 1.4[b]	0.001**
Body temperature	<37.2; >39.4 °C	39.1 ± 0.2	39.4 ± 0.5	38.6 ± 0.2	0.216
Respiratory rates	>40 breaths/min	55.0 ± 3.9	58.7 ± 9.5	49.3 ± 9.4	0.714
Pulse	<140; >220 bpm	130.0 ± 7.7[a]	129.2 ± 18.5[a]	166.3 ± 8.3[b]	0.028*
SAA	0-5 µg/ml	18.2 ± 8.7[a]	168.6 ± 17.9[b]	5.0 ± 0.0[a]	0.028*

Data are presented as X ± SE. Different letters[(a, b)] in the same row indicates statistically significant. OP; Open-cervix pyometra, CP; Closed-cervix pyometra, DE; Diestrus, WBC; white blood cell, SAA; Serum Amiloid A, *; p<0.05, **p<0.001.

No statistically significant difference was detected in the serum biochemical parameters, except for AST (P= 0.040). Although the GGT results were higher than the reference interval values, no statistical significance was determined in the CP group (Table 5).

Table 5. Mean results of serum biochemical test

Parameters	Reference interval	Groups			P value
		OP	CP	DE	
BUN	19-34 mg/dl	38.7 ± 4.3	36.2 ± 5.2	31.1 ± 3.8	0.487
CREA	0.8 - 2.1 mg/dl	1.0 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.189
ALT	28 -109 U/L	85.7 ± 18.8	37.9 ± 9.5	67.5 ± 9.6	0.066
AST	17- 48 U/L	38.2 ± 5.1[a]	20.6 ± 5.7[b]	26.9 ± 0.9	0.040
ALP	11- 49 U/L	36.7 ± 9.5	30.9 ± 4.9	43.5 ± 3.6	0.414
GGT	0- 2 U/L	2.8 ± 1.4	3.2 ± 1.3	1.0 ± 0.0	0.353

Data are presented as X ± SE. Different letters[(a, b)] in the same row indicates statistically significant. OP; Open-cervix pyometra, CP; Closed-cervix pyometra, DE; Diestrus, BUN; Blood urea nitrogen, CREA; Creatinine, GGT; Gamma glutamyl transferase, ALT; Alanine aminotransferase, AST; aspartate amino transferase, ALP; alkaline phosphatase.

While the CP group had the largest uterine diameter, the DE group had the smallest diameter (1.6 ± 0.2 cm vs. 0.4 ± 0.1 cm). It was found that the uterine diameters were significantly larger in the CP group than in the OP (1.2 ± 0.3 cm) and DE (P= 0.023 and P= 0.001, respectively) groups, and were also higher in the OP group than in the DE group (P=0.01).

While no correlation was determined between the SAA levels and SIRS criteria (Table 6), the SAA levels were negatively correlated with both AST (P= 0.045, $r_{s=}$-0.478) and GGT (P= 0.019, $r_{s=}$ -0.548) (Table 7).

Table 6. Correlation between SAA level and SIRS criteria. r_s; Spearman correlation coefficient

		WBC	Body temperature	Respiratory rate	Pulse
SAA	P value	0.057	0.138	0.281	0.065
	rs	0.456	0.364	0.269	-0.444

SAA; Serum Amiloid A, WBC; White blood cells.

Table 7. Correlation between SAA level and serum biochemical results. r_s; Spearman correlation coefficient

		BUN	CREA	ALT	AST	ALP	GGT
SAA	P value	0.451	0.523	0.656	0.045	0.141	0.019
	rs	0.190	-0.161	0.113	-0.478	-0.361	-0.548

SAA; Serum Amiloid A, BUN; Blood urea nitrogen, CREA; Creatinine, GGT; Gamma glutamyl transferase, ALT; Alanine aminotransferase, AST; aspartate amino transferase, ALP; alkaline phosphatase.

DISCUSSION AND CONCLUSION

Despite being a life-threatening disease, the number of studies on the prevalence, characteristics and prognosis of pyometra in cats is much less than that in dogs, and therefore, pyometra in cats is assessed based on the data available for dogs (1). The incidence of pyometra in dogs is much higher than that in cats, such that it is >20% in ten-year-old dogs and 2% in 13-year-old cats. However, contrary to incidence, death from pyometra is higher in cats than in dogs (6% vs 4%), which means that pyometra is more fatal in cats than in dogs. Although clinical findings are similar to those observed in dogs, they are often nonspecific to cats, and therefore, pyometra is difficult to diagnose in cats (1,19). For that reason, it is necessary to determine the species-specific changes caused by pyometra in the cat, and this issue is open to study.

In the present study, none of the patients died and all survived after being treated. Therefore, an evaluation for the correlation between the SAA levels and survival rate or time could not be performed. On the other hand, the physical examination findings demonstrated that 50% to 55% of the cases presented with symptoms of depression/lethargy, prolongation of the CRT, loss of appetite, and color change in the mucous membranes, which can be observed in various diseases. It has been reported that the clinical findings of pyometra in cats are non-specific (1,19). However, the significant advantages offered by USG in the diagnosis of pyometra eliminated the disadvantage of the non-specific clinical findings.

As is the case with many bacterial infections, as pyometra progresses, it may lead to sepsis, endotoxic shock or SIRS, which can result in death (20). Although the definitions of sepsis and SIRS are different, they are closely related to each other: SIRS is found in cases with sepsis, but sepsis does not occur in every case with SIRS (21). The mortality rate of sepsis varies between 29% and 79% in cats (22-24). Therefore, diagnosing sepsis or severe sepsis in feline pyometra cases, based on clinical findings, is important for determining the extent of systemic disorder and managing the clinical course (25). However, although proven to be sensitive in both dogs (26) and cats (27), SIRS diagnostic criteria are not specific to these species and lack a consensus (25). This is because hyperthermia, tachycardia and tachypnea, which are among the SIRS criteria, have many causes other than systemic inflammation, such as anxiety and pain, especially in cats (28). As a matter of fact, in the present study, no statistically significant difference was determined between the body temperatures and respiratory rates of the groups. According to our results, these parameters increased only numerically in the pyometra groups.

In the hyperdynamic phase of sepsis, symptoms such as tachycardia, hyperthermia, and hyperemic mucosa develop due to peripheral vasodilation. As sepsis progresses, it evolves into the hypodynamic phase and presents with symptoms such as vasoconstriction, tachycardia, pale mucous membranes, prolonged CRT, and weakening of the pulse (25). However, in cats, this process progresses differently, and the hyperdynamic phase does not develop as it does in other species (25,29). In affected cats, although many of the classic clinical signs of sepsis in other animal species are observed, additional signs such as bradycardia, hypothermia, and abdominal pain also develop (22,24,25).

The results obtained for hyperthermia (58.3%; 7/12) and tachypnea (91.7%; 11/12) in the present study confirm with the changes observed during the hyperdynamic period of sepsis. These results agree with previous reports by DeClue et al. (25) and Brady et al. (30) indicating 42% and 59% of hypothermia, respectively. Brady et al. (25) also reported to have detected hyperthermia in 35% of cats with sepsis. These differences could be due to variances in the reference intervals used. In the study of Brady et al. (25), the mean body temperature was reported as 37.2 ± 2.5°C, and this value corresponds to the lower reference limit (<37.2 and >39.4°C) used in this study.

In addition, a total of 75% (9/12) of the cats were bradycardic and incompatible with the hyperdynamic phase (29). While similar data have been previously reported, indicating bradycardia in 66% of cats (25), the pathophysiology of this species-specific change in cats is unknown (30). Hypothermia may be a cause of bradycardia, but we cannot establish such a relationship since hypothermia did not occur in any of the animals in this study (31). On the other hand, much older studies have suggested that cats may not develop tachycardia in response to hypotension due to the simultaneous baroreceptor stimulation of the vagal and sympathetic fibers (25,32).

In cases of sepsis and SIRS, an increase occurs in inflammatory mediators such as pro-inflammatory cytokines, T cells and macrophages, and the activation of WBC during this increase plays a key role in the pathogenesis of SIRS (29). The most characteristic changes observed in the hemogram of cats and dogs with pyometra are a marked increase in WBC accompanied by an inflammatory leukogram and a regenerative left shift in WBC (1,13). Therefore, the leukocytosis observed in 100% of the pyometra cases in this study is an expected finding. The mean WBC values of the cats with pyometra in this study were higher than those reported in the studies of (25,30). These researchers reported mean WBC counts of $15x10^9$/L and $12.3x10^9$/L, respectively. However, they evaluated many different SIRS or septicemia cases such as pyothorax and septic peritonitis in their studies. Our results were obtained only from pyometra cases, and this could explain our higher results. On the other hand, it has been determined that WBC levels are higher in CP cases compared to OP cases in cats. It has been suggested that this is due to the drainage of the purulent content from the uterus through the open cervix, causing a milder level of septicemia compared to the closed cervix (7). According to these reports, there is a correlation between the uterine lumen being filled with pus and WBC levels, and this is expected to be more intense in CP in dogs (33,34). In our study, uterine diameter measurements how similarity to these data, but indicate no correlation. This may be because the uterine lumen of cats is smaller than that of dogs, although there are very large differences in size and uterine size between dog breeds, cases are detected and intervened faster in cats, and the cat's immune system has a different working mechanism than that of dogs. An example of this difference is the rapid increase in CRP levels in dogs and SAA levels in cats during the acute phase response (11).

There are differences between the evaluation of SIRS in cats and dogs. Since cats have their own pathophysiology, while two criteria are sufficient for the diagnosis of SIRS in dogs, three criteria are required in cats (29). Therefore, in this study, three of the four positive criteria were investigated. However, no specific findings were obtained, which seems to be in agreement with previous reports suggesting that SIRS criteria are not specific to cats and dogs (25-28). Besides, the fact that there was no correlation between SAA and body temperature, respiration rate and pulse in this study can be considered as proof of SIRS criteria not being specific to cats.

Acute-phase proteins are more sensitive markers than WBC in the early diagnosis of inflammation. During the inflammatory process, changes occur in both APP and WBC levels (35). Proteins that increase/decrease in the acute-phase response have been previously investigated in mares, cows and dogs with pyometra (36,37). It has been stated that SAA levels increase during inflammatory processes in many animal species, especially in reproductive tissues, and therefore can be used as a biomarker of inflammatory processes in reproductive tissues (11,38).

In the present study, it was determined that SAA levels had increased in the pyometra groups, but not in the control group, and these results are in agreement with previous reports (39,40). Similar results have been reported in previous studies in dogs (12,41), and it has been concluded that SAA could be used as a marker in dogs with pyometra (41). However, in the aforementioned studies, pyometra was evaluated without being classified under open- and closed-cervix cases. Given that clinical findings are more severe in CP (7), it is important to make the distinction between OP and CP cases. The statistical difference (P= 0.028) determined between the SAA levels of the two pyometra groups in this study confirm the importance of this distinction. Vilhena et al. (40) determined a mean SAA level of 63.6 µg/ml in their study, which included 96% of cats with OP. This level is much higher than the results we obtained in the OP group (18.2 µg/ml). The difference between the results of the two studies could be related to the number of animals used (6 vs 23) or the age of the animals. While this study included young animals, in the study of Vilhena et al. (40), most of the animals were old. It has been determined that the age-related increased incidence of subclinical disease may lead to increased SAA levels in older cats (42). Similar data were reported in dogs by Jitpean et al. (41). These researchers also determined that SAA levels were significantly higher in septic cases. On the other hand, in a pyometra study conducted without evaluating cervical patency, Yuki et al. (43) determined the mean SAA level as 154.8 µg/ml, which is similar to the mean SAA level of 168.6 µg/ml we determined in the CP group. Considering that all the cats with pyometra in this study were SIRS-positive, despite clinically exhibiting three out of the four positive criteria of SIRS, the difference between the SAA levels of the groups suggests that assessing SAA levels together with SIRS criteria would be beneficial in understanding the severity of the condition.

In human medicine, WBC and the CRP are important in detecting the presence of serious infection and determining the type of treatment required (44). On the other hand, although the use of APPs alongside WBC is beneficial for evaluating the inflammatory state in both humans and animals, APPs are reported to be more sensitive than leukocyte counts in detecting infection and inflammation (39,45). Our results agree with the aforementioned studies. In the present study, while no statistical difference was determined between the WBC levels of the pyometra groups, a difference was found between their SAA levels. We consider this to be an important finding in evaluating the clinical significance of OP or CP. Additionally, upon evaluating the relationship between SAA and WBC, although we thought there might be a tendency towards a positive correlation, it was determined that there was no correlation between these parameters (P= 0.057).

In cases of pyometra, sepsis or SIRS, the kidney and liver are the two most rapidly affected organs. There was no difference between the groups for the BUN and CREA values we measured in this study. The fact that these values were normal in the kidneys, which are the first organs affected by pyometra, indicates that kidney function changes had not started yet. The low rates of polyuria and polydipsia (25%) we detected confirm this data. This is because, in pyometra, the antigen-antibody complex affects the glomeruli and causes glomerulonephritis, the first clinical findings of which are polyuria and polydipsia (46,47). However, it has been reported that azotemia was detected in 77.8% of dogs with pyometra and high CREA levels were detected in a percentage of 42.2% (48). Jitpean et al. (41) reported that BUN and CREA levels in septic and nonseptic dogs with pyometra remained within the reference limits and there was no statistical difference between the two groups. DeClue et al. (30) showed that BUN levels increased in 26.3% and CREA levels increased in 10.5% of septic cats.

High AST and ALT activities may be detected in cats and dogs with sepsis (13,30). DeClue et al. (30) found an increase in ALT activity in 36.8% and ALP activity in 10.5% of cats with sepsis. Besides, they also reported that ALT activity was significantly increased in septic cats compared to healthy cats (30). The reason for this increase may be stress and damage to tissues such as the heart, liver and bone marrow due to sepsis (49). In addition, there are also reports suggesting that increased ALP activity in pyometra may result from intrahepatic cholestasis (50). However, as liver function parameters may not always increase in cases of sepsis or pyometra. In this study, GGT activity increased in 41.7% (5/12), AST and ALT activity increased in 33.3% (2/6), and ALP activity increased in 33.3% (2/6) of the cats in the pyometra groups. However, despite these increases and decreases, the mean values of the liver function parameters remained within the reference ranges. This may be due to liver enzyme activity in cats being faster than that of the dog. The half-lives of ALT, AST and ALP are 3.5 hours, 1.5 hours and 6 hours, respectively, in cats, and 60 hours, 12 hours and 66 hours, respectively, in dogs. The difference between these half-lives indicates that hepatic enzymes in cats will not increase as much as in dogs in cholestasis (51). In other words, ALT and AST levels, which increase in the serum following acute injury, will decrease very rapidly within hours after a significant increase due to differences in serum and the cellular localizations of the enzymes (52). Thus, we consider that the negative correlation detected in this study between SAA and AST or SAA and GGT has no clinical significance, as we are suspicious of the AST and GGT levels measured in the cats due to the half-lives of these enzymes.

When evaluating the results of the present study, we observed that there was 100% leukocytosis in all cases in the pyometra groups. Furthermore, tachypnea was detected at a level of 100% in the OP group and 83.3% in the CP group. Moreover, bradycardia was detected at a level of 66.7% in the OP group and 83.3% in the CP group, and finally, hyperthermia was determined at a level of 50% in the OP group and 66.7% in the CP group. Statistical significance was detected only for the WBC and pulse parameters, when compared to the control group. On the other hand, while SAA levels increased in only 50% of the animals in the OP group, they increased in all the cats in the CP group.

The serum biochemical test results demonstrated that the percentages of animals with increased/decreased levels of BUN, CREA, GGT, ALT, AST, and ALP did not differ significantly between the groups. The results of this study, we think that SAA levels could be used alongside SIRS criteria in cats, and can be use of as a marker especially in cases of CP, and even be evaluated before other SIRS criteria. We consider the study data to be promising but considering the number of patients included in the study, further and more comprehensive studies are needed.

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CONFLICT OF INTEREST

There is no conflict of interest to be declared by the authors.

ETHICAL STATEMENT

The procedures followed in the present study presented are part of routine veterinary practice and constitute a routine treatment/intervention to treat cats with pyometra. Therefore, since the presented study falls within the scope of "Clinical applications for diagnosis and treatment purposes", which is described in the first article of the "Regulation on the working procedures and principles of animal experiments ethics committees" said regulation, the HADYEK permission of the Local Ethics Committee for Animal Experiments (HADYEK) is was not required (Article 8, paragraph (k) of the "Regulation on the working procedures and principles of animal experiments ethics committees, February 15, 2014 / 28914).

AUTHOR CONTRIBUTIONS

Idea/Concept: SSA, AGA, Eİ
Design: SSA, MF, FB
Supervision/Consultancy: AGA, VF, FB, İİ
Data Collection and/or Processing: SSA, MF, AGA, Eİ
Analysis and/or Interpretation: AGA, VF, FB, İİ
Source Search: AGA, VF, FB, İİ, Eİ
Manuscript Writing: SSA, MF, AGA, VF, FB, İİ, Eİ
Critical Review: SSA, MF

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