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Research Article

Evaluation of Acute Phase Proteins, Some Cytokines and Hemostatic Parameters in Dogs with Sepsis [1]

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Abstract

The aim of this study was to evaluate the alterations in acute phase proteins, cytokines and hemostatic parameters in dogs with sepsis and to determine the importance of these parameters in diagnosis of the sepsis. Thirty dogs with sepsis and 9 healthy dogs were used in this study. Anorexia, depression, lethargy, hyperthermia, tachycardia, tachypnea, congestion in the mucosal membranes, prolonged capillary refill time, and leukocytosis or leucopenia were identified in the dogs with sepsis. The serum interleukin-1ß (IL-1ß), tumor necrosis factor α (TNF-α), interferon γ (INF-γ), C-reactive protein (CRP), serum amyloid A (SAA), prothrombin time (PT), activated partial thromboplastin time (aPTT), antithrombin III (AT III), fibrinogen, protein C (PC), and D-dimer levels were measured in all dogs. We found that the serum IL-1ß, TNF-α, INF-γ, CRP and SAA concentrations were significantly elevated in dogs with sepsis as compared with healthy controls. In addition, the plasma PT and APTT levels were notably prolonged, the plasma fibrinogen, D-dimers and protein C concentrations were significantly increased. However, the antithrombin III activity was significantly decreased in the dogs with sepsis. In conclusion, the results of this study indicate that the SAA, IL-1ß and TNF-α parameters play important roles in the inflammatory process in dogs with sepsis. The hemostatic abnormalities observed in dogs with sepsis may be due to the development of disseminated intravascular coagulation (DIC).

Keywords: *Dogs, Sepsis, Acute phase protein, Cytokines, Coagulation profile*

Sepsisli Köpeklerde Akut Faz Proteinler, Bazı Sitokinler ve Hemostatik Parametrelerin Değerlendirilmesi

Özet

Bu çalışmanın amacı; sepsisli köpeklerde akut faz proteinler, bazı sitokinler ve hemostatik sistem parametrelerin değişimlerini değerlendirerek, hastalığın tanısında bu parametrelerin önemini ortaya koymaktır. Bu çalışmanın materyalini 30 sepsisli ve 9 sağlıklı köpek oluşturdu. Sepsisli köpeklerde iştahsızlık, durgunluk, depresyon, vücut ısısında artış, mukoz membranlarda konjesyon, kapiller dolum zamanında uzama, taşikardi, takipnea, lökositozis veya lökopeni belirlendi. Bütün köpeklerin interlökin-1ß (IL-1ß), tümör nekroz faktör α (TNF α), interferon γ (INF-γ), C-reaktif protein (CRP), serum amiloid A (SAA) ve protrombin zamanı (PT), aktive edilmiş parsiyel tromboplastin zamanı (aPTT), antitrombin III (AT III), fibrinojen, protein C ve D-dimer seviyeleri ölçüldü. Sepsisli köpeklerde serum IL-1ß, TNF-α, INF-γ, CRP ve SAA düzeylerinde önemli artış belirlendi. Sepsisli köpeklerde plazma PT ve APTT sürelerinde önemli uzama, fibrinojen, D-dimer ve protein C düzeylerinde önemli artış, AT-III düzeyinde ise önemli azalma tespit edildi. Sonuç olarak sepsisli köpeklerde SAA, IL-1ß ve TNFα paramterelerinin yangısel olaylarda önemli rol aldığı belirlendi. Sepsisli köpeklerde tespit edilen hemostatik anormallikler dissemine intravaskuler koagulasyon (DİK) gelişimi ile ilgili olabilir.

Anahtar sözcükler: *Köpek, Sepsis, Akut faz proteinleri, Sitokinler, Koagulasyon profil*

INTRODUCTION

Sepsis is defined as a systemic inflammatory response against infection and characterized by fever, tachycardia, tachypnea and leukocytosis or leukopenia [1-3]. This disease has a complex pathophsiological state and is stil associated with a high degree of mortality. Sepsis is considered to be a common cause of morbidity and mortality in both veterinary medicine and human medicine. The incidance of sepsis in dogs was increased from 1 per 1.000 hospital

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cases in 1988 to 3.5 in 1998. In addition, the mortality rates of 33-50% have been described for dogs with sepsis [4-6]. Bacterial infections are the most common cause of sepsis in dogs and cats [7]. Patient with sepsis is more likely to develop multiple organ dysfunction syndrome, which carries a high mortality rate despite recent advances in critical care [4].

Acute phase proteins (APPs), cytokines and coagulation profiles might change in dogs with sepsis. Because sepsis is the clinical menifestation of body's response to an inciting stimulus which is severe enough to cause systemic release of circulating inflammatory mediators. The acute phase reaction, which occurs in sepsis, is stimulated by the release of cytokines such as IL-1ß, interleukin-6 (IL-6) and TNF-α from macrophage and monocytes at the site of inflammatory lesions or infections [8,9]. IL-1ß, IL-6, TNF-α and IFN, produced by inflammatory cells could induce local and systemic reactions [10]. It has been reported that serum IL-1ß, IL-6 and TNF-α levels are increased in sepsis. In addition, IL-6 might serve as a valuable marker for the determination of the severity of a systemic bacterial infection [6] and the measurement of serum IL-6 and TNF-α could be useful for evaluating septic patients [7].

Using acute-phase proteins for the assessment of healthy and sick animals has greatly increased in the last decade [11]. Acute-phase proteins are synthesized in the liver in response to release of proinflammatory cytokines in diseases such as bacterial and viral infections, immuno-mediated disease, neoplasia, tissue injury (trauma), necrosis and burns [12-14]. Clinical applications for APPs have been widely demostrated for prognostication as well as for detection of clinical disease and chronic iflammation [15]. Importantly, the APP assay has repeatedly demostrated its ability to enhance the diagnostic sensitivity for inflamatory processes [15]. Furthermore, CRP and SAA have been used for diagnosing the presence of infection in dogs. It is known that CRP is a major APP in dogs. Increased CRP and SAA concentrations have been detected in dogs with systemic inflammation [10,16,17]. It was reported that CRP and SAA levels were increased in dogs with pyometra [18].

The relationship between infection and coagulation is an area of intense investigation, with studies suggesting that hypercoagulability and subsequent microvascular trombosis contribute to multiple organ dysfonction during sepsis [2]. Dissemine intravascular coagulation (DIC), an acquired syndrome representing a hypercoagulable state, haemorrhagic symptoms and multiple organ failure, might occuring sepsis [2,19]. Protrombin time, aPPT, D-dimer and fibrinogen levels, AT III activity and trombosit count should be considered regarding to DIC [20]. It was reported that hemostatic disorder occurs in dogs with sepsis [2,21].

The aim of this study was to evaluate the importance of the acute phase proteins, cytokines and haemostatic parameters for diagnosis of sepsis.

MATERIAL and METHODS

Animals and Clinical Examination

Authorization to conduct this study has been taken from S.U. Faculty of Veterinary Medicine Animal Ethics Comittee (2011/061).

The materials of this study consist of thirty dogs with sepsis (experiment group) and 9 healthy dogs (control group) aged from 1 and 4 years, were brought into the Faculty of Veterinary Medicine, Internal Medicine Department. First, routin clinical examinations were performed for all dogs. In clinical examination, body temprature, heart rate, respiratory rate, capillary refill time, mucosa, and mental and consciousness states were evaluated. Total white blood cells and thrombocyte of dogs were counted. Dogs having sepsis criteria were included in the study.

Sepsis criteria;

Hyperthermia (>39°C) or Hypothermia (<35°C)

Tachycardia (heart rate >140 per minute)

Tachypnea (respiratory rate >20 breaths per minute)

Leukocytosis (>16.000/µL, or >3% bands) or leukopenia (<6000/µL) [2]. Bacteriologic culture for confirmation of infection was not performed.

Collection of Blood Samples

Blood samples were thereafter obtained through venipuncture of the cephalic or jugular vein then placed into a citrate tube (1 part 3.8% citrate: 9 part blood) and a serum tube (without anti-coagulant). Blood (with anti-coagulant and without anti-coagulant) was centrifuged for twenty minutes collection, after seperation of blood, plasma and serum samples, which would be used in the evaluation of coagulation profiles and acute phase proteins and cytokines, were kept in -80°C deep freeze until the measurement was completed.

Measurement of Leukocyte And Trombocyte

Leukocyte and trombocyte counts were measured by hemocell counter (Haematology analyser, MS4e, CFE 279, Melet Schlosing Laboratories, France).

Measurement of Serum Acute Phase Proteins

Canine C-reactive protein (Eastbiopharm, Cat. No.CK-E90977), canine serum amyloid A (Eastbiopharm, Cat. No.CK-E90978), canine protein C (TSZ ELISA Cat. No.CA 1033) and canine fibrinogen (Eastbiopharm, Cat. No.CK-E90979 concentrations were measured by ELISA method in Synergy HT multi-mode microplate reader (BioTek Inc. USA) device. Measurable sensitivity and test interval of CRP was 0.051 mg/L, and 0.1 mg/L - 30 mg/L,respectively. In addition, measurable sensitivity of SAA was 0.047 µg/mL and test interval of SAA level was 0.1 µg/mL and 40

µg/mL. For PC, measurable sensitivity was less than 0.15 ng/mL and test interval was 0.15 ng mL and 40 ng/mL. Lastly, measurable sensitivity of fibrinogen was 0.023 mg/mL and test interval of fibrinogen level was 0.05 mg/mL and 15 mg/mL.

Measurement of Serum Cytokines

Canine interleukin 1ß (Eastbiopharm, Cat. No. CK-E90800) canine tumor necrosis factor α (Eastbiopharm, Cat. No.CK-E90806) and canine interferon γ (Eastbiopharm, Cat. No.CK-E90877) levels were measured by ELISA method in Synergy HT multi-mode microplate reader (BioTek Inc. USA) device. Measurable sensitivity of IL-1ß was 0.1 pg/mL, and the test interval of IL-1ß level was 0.2 pg/mL and 60 pg/mL, measurable sensitivity of TNF-α is 0.01 ng/L and test interval of TNF-α level was 0,03 ng/L and 9 ng/L and measurable sensitivity of INF-γ is 2.35 ng/mL and the test interval of INF-γ level was 5 ng/L and 1.000 ng/L.

Measurement of Plasma Coagulatıon Profile

Protrombin time, APPT, AT III activity and D-dimer levels were measured by coagulometric method (Coagulometric method, Sysmex CA 1500 device, Siemens, A-7799, Germany).

Statistical Analysis

Two sample student test was used to determine the differences between groups. SPSS 19.0 for Windows was used to perform the test. P values <0.05 were considered statistically significant.

RESULTS

Clinical and Hematological Findings

Anorexia, depression, lethargy, hyperthermia, tachycardia, tachpnea, congession in mucosal membrans, prolonged capillary refill time were determined in dogs with sepsis. Leucocytosis in 28 of 30 dogs with sepsis was determined, but leucopenia in 2 of them was determined.

Acute Phase Proteins and Cytokines Findings

The concentrations of serum CRP (P<0.001), SAA (P<0.01), IL-1ß (P<0.001), TNF-α (P<0.001) and INF-γ (P<0.001) were significantly increased in dogs with sepsis as compared with healty dogs *(Table 1)*.

Coagulation Profile Findings

Global coagulation times (PT and APTT, P<0.01) and plasma fibrinogen (P<0.001), D-dimer (P<0.01) and PC (P<0.001) levels were dramatically increased in dogs with sepsis as compared with healthy dogs, but AT-III (P<0.05) activity was remarkably reduced. Haemotologically, the blood leukocyte count was found to be significantly elevated in dogs with sepsis as compared with healhty dogs,

Table 1. *Serum concentrations of IL-1ß, TNF-α, INF-γ, CRP and SAA of healthy dogs and dogs with sepsis (Mean±SE)*
Tablo 1. *Sepsisli ve sağlıklı köpeklerde serum IL-1ß, TNF-α, INF-γ, CRP ve SAA düzeyleri (Mean±SE)*

Parameters	Healthy Dogs (n=9)	Dogs with Sepsis (n= 30)	P Value
IL-1ß pg/mL	9.30±0.53	22.3±3.34	P<0.001
TNF-α ng/L	0.24±0.78	2.94±0.60	P<0.001
INF-γ ng/L	104±0.86	404±68.9	P<0.001
CRP mg/L	2.27±0.19	9.89±2.10	P<0.001
SAA µg/mL	2.30±0.18	10.8±2.43	P<0.01

IL-1ß: *interleukin-1ß;* ***TNF-α:*** *tumor necrosis factor α;* ***INF-γ:*** *interferon γ;* ***CRP:*** *C-reactive protein;* ***SAA:*** *serum amyloid A*

Table 2. *Plasma coagulation parameters of of healthy dogs and dogs with sepsis (Mean±SE)*
Tablo 2. *Sepsisli ve sağlıklı köpeklerde plazma koagulasyon parametreleri (Mean±SE)*

Parameters	Healthy Dogs (n=8)	Dogs with Sepsis (n=30)	P Value
PT (Sec)	6.32±0.17	7.80±0.14	P<0.001
APTT (Sec)	39.7±3.27	68.2±5.79	P<0.01
AT III (%)	78.1±2.90	54.9±2.92	P<0.05
Fibrinogen mg/mL	3.08±0.42	9.61±1.58	P<0.001
Protein-C ng/mL	0.63±0.10	2.37±0.45	P<0.001
D-dimer mg/dL	1.27±0.87	2.64±1.59	P<0.01
PLT x10^3 /mm^3	235±68.1	272±27.5	P>0.05
WBC x10^3 /mm^3	10.2±1.16	26.53±2.04	P<0.001

PT: *prothrombin time;* ***APTT:*** *activated partial thromboplastin time;* ***AT III:*** *antithrombin III;* ***PC:*** *protein C,* ***PLT:*** *platelet;* ***WBC:*** *white blood cell*

however no significant change was seen in thrombosit count *(Table 2)*.

DISCUSSION

Sepsis has a high mortality. Therefore, a rapid diagnosis would be important to get good prognosis. This study showed significant changes of acute phase proteins, cytokines and haemostatic para-metersin dogs with sepsis. Therefore, measurement of these parameters in dogs with sepsis could be a valuable approach to evaluate septic stages.

Cytokines play an important role in the development and the regulation of immune response, thus, cytokine profiles contribute to the effect of immunity level in diseases [22]. The release of proinflammatory cytokines such as IL-1ß, IL-6 and TNF-α by monocytes/macrophages and activeted T-lymphocytes is considered to be a key event in the development of sepsis [1]. Moreover, bacterial toxins lead to release of inflammation mediators from mononuclear phagocytes in sepsis. The measurement of acute phase proteins, cytokines and coagulation profiles could be useful for determining the stage of sepsis in patients. IL-1ß and TNF-α, important inflammatory cytokines, are implicated in a variety of disease in dogs [23-26]. It has been reported that IL-6 and TNF-α levels are significantly increased in sepsis, and these parameters for evaluating of sepsis might be useful marker [6,7,27]. Serum IL-1ß and TNF-α concentrations are usually increased in inflammatory disease of dogs [28,29]. Fransson et al.[30] reported that IL-6, TNF-α and CRP levels were increased in dogs with pyometra and dogs with systemic inflammatory response syndrome. In this study, serum IL-1ß, TNF-α and INF-γ concentrations were dramatically elevated in dogs with sepsis as compared with healthy dogs *(Table 1)*. The reason of this increase in IL-1ß, TNF-α and INF-γ levels could be explained by the initiation of releasing of inflammatoric mediators against bacterial toxins that cause sepsis. Therefore, selected cytokines including IL-1ß, TNF-α and INF-γ maight be useful in assessing the clinical severity of sepsis in dogs. These results are consistent with published studies [3,6,28,29].

Acute phase proteins are synthesized in the liver in response to release of proinflammatory cytokines in diseases such as bacterial and viral infections, immuno-mediated disease, neoplasia, tissue injury (trauma), necrosis and burns [4,13]. These noninvasive markers might be useful to determine the disease severity and more likely to response the treatment or prognosis, on a disease-spesific basis [11,13,31]. CRP and SAA are useful parameters to indicate the inflammation in human and animals [32]. In addition, increased CRP and SAA concentrations have been detected in dogs with systemic inflammation [10,16,17,30]. Furthermore, CRP and SAA were shown to be significantly increased in dogs with parvovirus enteritis [33], ehrlichiosis [23] and leptospirosis [34]. C-reactive protein and SAA concentrations have been shown to be dramatically elevated in dogs with pyometra [18]. In the present study, CRP and SAA concentrations were significantly increased in dogs with suspected sepsis as compared with healthy dogs. But, CRP level was normal range. The reason for higher concentrations of SAA in septic dogs may be related to inflammatory reactions and tissue damage. Similary, several studies have reported that CRP and SAA could be useful markers for diagnosis and prognosis in various disease [10,16,17,34,35]. However, CRP and SAA do not seem to be very spesific markers for the detection of bacterial infections because it might increase in a variety of diseases, not particularly in bacterial infections [10,16,17,35]. Besides, in this study, leukocytosis was found to be a common finding in dogs with sepsis. This observation agrees with the most studies [10,16]. In our study, SAA were detected to be specific markers of systemic inflammation in dogs with sepsis.

Disseminated intravascular coagulation a hemalotical syndrome, typically defined by the activation of intra-vascular coagulation resulting in excessive fibrin formation and consumption of caogulation factors [2,18,36] is a serious problem that threats lives of both people and animals. DIC might be developed due to septic caogulation, viremia, parazitic infection, severe tissue damage, toxication, intra-vascular hemolizis, autoantibody, hepatitis, pancreatitis and neoplasma [19,21,37-40]. Prolongations in PT and APTT, increase in FDP level, and decrease in AT-III activity and thrombosit count develop in dogs with DIC [41]. Prolongation in PT and APTT, increase in D-dimer, and decrease in AT-III level are detected in dogs with sepsis [2,42], systemic inflammatory syndrome [43] or septic peritonitis [44]. In the present study, prolonged PT and APTT values as well as increased levels of fibrinogen, D-dimer and PC concentrations revealed hemostatic alteration in response to sepsis in dogs. In the present study, the indicators of the activated coagulation of DIC including prolongation of both PT and APTT as well as a decrease in AT III activity and also an increase in the level of D-dimer which shows fibrinolitic activition were defined. The occurrence of significant changes in the hemostatic system was determined. D-dimer concentration was significantly increased in dogs with sepsis, indicating the precence of fibrinolysis. Increased activity of fibrinolytic system has been associated with DIC [2,42]. Our results were in line with previous studies [2,21,42-45].

Some authors [46-48] reported that the level of fibrinogen might increase in the first period of DIC due to an inflamatory response; and then this level might decrease because of fibrinolisis. In contrast, other researchers [19,45] reported a significant decrease in fibrinogen level. In this study, a significant increase in fibrinogen level in the dogs with sepsis was observed. This dramatic increase in fibrinogen level might be related to an elevation in acute phase proteins in spite of the presence of DIC in

dogs with sepsis. Therefore, our findings were in line with findings of earlier reporters [46-48].

It was reported that PC concentration was remarkably reduced in dogs with sepsis [4,49], and dogs with systemic inflammatory syndrome [43]. Fourrier et al.[50] noticed persistent decreases in PC activity in nonsurviving patients with sepsis and septic shock. However, de Laforcade et al.[2] determined that the PC and AT activities were decreased in first 24 h and then this activities were gradually increased in dogs with sepsis. In this study, the activity of PC was dramatically increased in dogs with sepsis as compare with healthy dogs, most probably due to time dependent effects of diseases. This result was not coincidet with the results of reported by Laforcade et al.[4] and Yan and Dhainaut [49], but this result was coincidet the results of reported by de Laforcade et al.[2].

In conclusion, results of tis study indicated that SAA, IL-1ß, TNF-α , and INF- γ concentrations were significantly changed in dogs with sepsis. Particularly, SAA, IL-1ß and TNF-α parameters seem to be reliable markers for systemic inflammation in sepsis. Besides hemostatic abnormalities in dogs with sepsis may be due to developing disseminated intravascular coagulation.

REFERENCES

1. de Boer JP, Wolbink GJ, Thijs LG, Baars JW, Wagstaff J, Hack E: Interplay of complement and cytokines in the pothohenesis of septic shock. *Immunopharmacol,* 24, 135-148, 1992. DOI: 10.1016/0162-3109(92)90019-9

2. de Laforcade AM, Freeman LMS, Shaw SP, Brooks MB, Rozanski EA, Rush JE: Hemostatic changes in dogs with naturally occuring sepsis. *Vet Intern Med,* 17, 674-679, 2003. DOI: 10.1111/j.1939-1676.2003.tb02499.x

3. Nemzek JA, Agrodnia MD, Hauptman G: Breed-specific pro-inflammatory cytokine production as a predisposing factor for susceptibility in the dogs. *Vet Emerg Crit Care,* 17, 368-372, 2007. DOI: 10.1111/j.1476-4431.2006.00215.x

4. de Laforcade AM, Rozanski EA, Freeman LM, Li W: Serial evalutaion of protein C and antitrombin in dogs with sepsis. *J Vet Intern Med,* 22, 26-30, 2008. DOI: 10.1111/j.1939-1676.2007.0021.x

5. Otto CM, Rieser TM, Brooks MB, Russell MW: Evidence of hyper-coagulability in dogs with parvoviral enteritis. *JAVMA,* 217, 1500-1509, 2000. DOI: 10.2460/javma.2000.217.1500

6. Rau S, Kohn B, Richter C, Fenske N, Kuchenhoff H, Hartmann K, Hartle S, Kaspers B, Hirschberger J: Plasma interleukin-6 response is predictive for severity and mortality in canine systemic inflammatory response syndrome and sepsis. *Vet Clin Pathol,* 36, 253-260, 2007. DOI: 10.1111/j.1939-165X.2007.tb00220.x

7. Song R, Kim J, Yu D, Park C, Park J: Kinetics of IL-6 and TNF-α changes in canine models of sepsis induced by endotoxin. *Vet Immunol Immunopathol,* 146, 143-149, 2012. DOI: 10.1016/j.vetimm.2012.02.008

8. Bochsler PN, Slauson DO: Inflammation and repair tissue. **In,** Mechanism of Disease. A Textbook of Comparetive General Pathology. Third ed., 140-245, Mosby, St. Louis, 2002.

9. Cary C: Acute phase proteins in animals. *Prog Mol Biol Transl Sci,* 105, 113-150, 2011. DOI: 10.1016/B978-0-12-394596-9.00005-6

10. Jain S, Gautam V, Naseem S: Acute-phase proteins: As daignostic tool. *J Pram Bioall Sci,* 1, 118-127, 2011. DOI: 10.4103/0975-7406.76489

11. Kjelgaard-Hansen M, Jacobsen S: Assay validation and diagnostic applications of major acute-phase proteins testing in companion animals. *Clin Lab Med,* 31, 51-70, 2011. DOI: 10.1016/j.cll.2010.10.002

12. Kocaturk M, Martinez S, Eralp O, Tvarijonaviciute A, Ceron J, Yilmaz Z: Prognostic value of serum acute-phase proteins in dogs with parvoviral enteritis. *J Small Anim Pract,* 51, 478-483, 2010. DOI: 10.1111/j.1748-5827.2010.00965.x

13. Murata H, Shimada N, Yoshioka M: Current research on acute phase proteins in veterinary diagnosis: An overview. *Vet J,* 168, 28-40, 2004. DOI: 10.1016/S1090-0233(03)00119-9

14. Turgut K: Veteriner Klinik Laboratuvar Teşhis. İkinci Baskı, Bahçıvanlar Basım Sanayi A.Ş. Konya, s.235-245, 2000.

15. Cary C: Biomarkers of inflammation in exotic pets. *J Exotic Pet Med,* 22, 245-250, 2013. DOI: 10.1053/j.jepm.2013.08.003

16. Gebhardt C, Hirschberger J, Rau S, Arndt G, Krainer K, Schweigert FJ, Brunnberg L, Kaspers B, Kohn B: Use of C-reaktive protein to predict outcome in dogs with systemic inflammatory response syndrome or sepsis. *Vet Emerg Crit Care,* 19, 450-458, 2009. DOI: 10.1111/j.1476-4431.2009.00462.x

17. Langhorn CMB, Goddard R, Andreasen A, Moldal E, Tvarijonaviciute A, Kirpensteijn J, Jakobsen S, Persson F, Kjelgaard-Hansen M: Comparison of serum amyloid A and C-reactive protein as diagnostic markers of systemic inflammation in dogs. *Can Vet J,* 55, 161-168, 2014.

18. Jitpean S, Holst BS, Höglund OV, Pettersson A, Olsson U, Strage E, Södersten F, Hagman R: Serum insulin-like factor-I, iron, C-reactive protein, and serum amyloid A for prediction of outcome in dogs with pyometra. *Theriogenelogy,* 30, 1-6, 2014. DOI: 10.1016/j.theriogenology.2014.02.014

19. Bruchim Y, Aroch I, Saragusty J, Waner T: Disseminated intravascular coagulation. *Compendium,* 3, 1-16, 2008. DOI: 10.1056/NEJM199908193410807

20. Caldin M, Furlanello T, Lubas G: Validation of an immunoturbidimetric D-dimer assay in canine citrated plasma. *Vet Clin Pathol,* 29, 51-54, 2000. DOI: 10.1111/j.1939-165X.2000.tb00398.x

21. Esmon CT: Inflammation and thrombosis. *J Thromb Haemost,* 1, 1343-1348, 2003. DOI: 10.1046/j.1538-7836.2003.00261.x

22. Ceremi A: Inflammatory cytokines. *Clin Immunol Immunolpathol,* 62, 3-10, 1999.

23. Alsemgeest SP, Van- Klooster GA, Van Miert AS, Hulskamp-Koch CK, Gruys E: Primary bovine hepatocytes in the study of cytokine induced acute-phase protein secretion *in vitro. Vet Immunol Immunopathol,* 53, 179-184, 1996. DOI: 10.1016/0165-2427(96)05602-4

24. Baggio V, Ott F, Fischer RW: Production of antibodies to canine IL-1ß and canine TNF to assess the role of proinflammatory cytokines. *Vet Immunol Immunopathol,* 107, 27-39, 2005.

25. Nakagawa-Tosa N, Morimatsu M, Kawasaki MN, Nakatsuji H, Syuto B, Saito M: Stimulation of haptoglobin synthesis by interleukin-6 and tumor necrosis factor, but not by interleukin-1, in bovine primary cultured hepatocytes. *J Vet Med Sci,* 57, 219-223, 1995. DOI: 10.1292/jvms.57.219

26. Ohtsuka H, Ohki K, Tanaka T, Tajima M, Yoshino T, Takahashi K: Circuling tumor necrosis factor and interleukin-1 after administration of lps in adult cows. *J Vet Med Sci,* 19, 227-229, 1997. DOI: 10.1292/jvms.59.927

27. Nemzek JA, Siddiqui J, Remick DG: Development and optimization of cytokine ELISAs using commercial antibody pairs. *J Immunol Methods,* 255, 149-157, 2001. DOI: 10.1016/S0022-1759(01)00419-7

28. Grone A, Aldinger S, Baumgartner W: Interleukin-1beta-6-12 and tumor necrosis factor-alpha experission in barin of dogs with canine distemper virus infection. *J Neuroimmunol,* 110, 20-30, 2000. DOI: 10.1016/S0165-5728(00)00332-5

29. Miyamota T, Fujinaga T, Yamashita K, Hagio M: Changes of serum cytokine activities and other parameters in dogs with experimentally induced endotoxic shock. *Jpn J Vet Res,* 44, 107-118, 1996. DOI: 10.14943/jjvr.44.2.107

30. Fransson BA, Lagerstedt AS, Bergstrom A, Hagman R, Park JS, Evans MA, Ragle CA: C-reaktive protein, tumor necrosis factor α and interleukin-6 in dogs with pyometra and SIRS. *Vet Emerg Crit Care,* 17, 373-

381, 2007. DOI: 10.1111/j.1476-4431.2006.00203.x

31. Ceron JJ, Eckersall PD, Martinez-Subiela S: Acute phase proteins in dogs and cats: Current knowledge and future perspectives. *Vet Clinical Pathol,* 34, 85-99, 2005. DOI: 10.1111/j.1939-165X.2005.tb00019.x

32. Eckersall PD, Bell R: Acute phase proteins: Biomarkers of infection and inflammation in veterinary medicine. *Vet J,* 185, 23-27, 2010. DOI: 10.1016/j.tvjl.2010.04.009

33. Yule TD, Roth MB, Dreier K, Johnson AF, Palmer-Densmore M, Simmons K, Fanton R: Canine parvovirus vaccine elicits protection from the inflammatory clinical consequences of the disease. *Vaccine,* 15, 720-729, 1997. DOI: 10.1016/S0264-410X(96)00232-0

34. Mastrorilli C, Dondi F, Agnoli C: Clinopathologic features and outcome predictors of leptospira interogans Australis serogroup infection in dogs: A retrospective study of 20 cases (2001-2004). *J Vet Intern Med,* 21, 3-10, 2007. DOI: 10.1892/0891-6640(2007)21[3:CFAOPO]2.0.CO;2

35. Mylonakis ME, Ceron JJ, Leontides L, Siarkou VI, Martinez S, Tvarijonaviciute A, Koutinas AF, Harrus S: Serum acute phase proteins as clinical phase indicators and outcome predicators in naturally occuring canine monocytic ehrlichiosis. *J Vet Intern Med,* 25, 811-817, 2011. DOI: 10.1111/j.1939-1676.2011.0728.x

36. Saba HI, Morelli GA: The pathogenesis and management of disseminated intravascular coagulation. *Clin Adv Hematol Oncol,* 4, 919-926, 2006.

37. Col R, Durgun Z: Sepsis, lökositler, sitokinler ve disseminant intravasküler koagulasyon. *Vet Bil Derg,* 23, 97-106, 2007.

38. Goddard A, Leisewitz AL: Canine parvovirus. *Vet Clin North Am: Small Anim Pract,* 40, 1041-1053, 2010. DOI: 10.1016/j.cvsm.2010.07.007

39. Levi M: Pathogenesis and treatment of DIC. *Thromb Res,* 1, 54-55, 2005.

40. Mischke R: Acute haemostatic changes in accidentally traumatised dogs. *Vet J,* 169, 60-64, 2005. DOI: 10.1016/j.tvjl.2004.01.008

41. Feldman BF, Madewell BR, O'Neill S: Disseminated intravascular coagulation: Antitrombin, plasminogen, and coagulation abnormalities in 41 dogs. *JAVMA,* 179, 151-154, 1981.

42. Yilmaz Z, Yalçın E, Ilçol Y: Septik şoklu köpeklerde koagulasyon profilinin değerlendirilmesi. *Turk J Vet Anim Sci,* 26, 171-176, 2002.

43. Bauer N, Moritz A: Coagulation response in dogs with and without systemic inflammatory response syndrome-Preliminary results. *Res Vet Sci,* 94, 123-121, 2013. DOI: 10.1016/j.rvsc.2012.07.029

44. Bentley AM, Mayhew PD, Culp WTN, Otto CM: Alterations in the hemostatic profiles of dogs with naturally occuring septic peritonitis. *Vet Emerg Crit Care,* 23, 14-22, 2013. DOI: 10.1111/vec.12013

45. Prins M, Schellens CJMM, van Leeuwen MW, Rothuizen J, Teske E: Coagulation disorders in dogs with hepatic disease. *Vet J,* 185, 163-168, 2010. DOI: 10.1016/j.tvjl.2009.05.009

46. Er C, Ok M: Levels of cardiac biomarkers and coagulation profiles in dogs with parvoviral enteritis. *Kafkas Univ Vet Fak Derg,* 21, 383-388, 2015. DOI: 10.9775/kvfd.2014.12575

47. Otto CM: Sepsis. **In,** Wingfield WE (Ed): The Veterinary ICU Book. 695-709, Jackson Hole, WY: Teton NewMedia, 2002.

48. Wada H: Disseminated intravascular coagulation. *Clin Chim Acta,* 344, 13-21, 2004. DOI: 10.1016/j.cccn.2004.02.015

49. Yan SB, Dhainaut JF: Activated protein C versus protein C in severe sepsis. *Crit Care Med,* 29 (Suppl. 7): S69-S74, 2001.

50. Fourrier F, Goudemand J, Hendrycx S, Caron C, Rime A, Marey A, Lesteval P: Septic shock, multiple organ failure, and disseminated intravascular coagulation. *Chest,* 101, 816-823, 1992. DOI: 10.1378/chest.101.3.816