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Can procalcitonin be used as a clinical biomarker during bacterial, viral and parasitic infections in dogs?

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Abstract

The aim of this study is to investigate the usability of serum procalcitonin level in dogs as a clinical biomarker for the distinction between bacterial, viral, and parasitic diseases. A total of 160 dogs were used. The animals were evaluated in four groups as control and those with bacterial, viral, and parasitic infections. Serum procalcitonin, tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interferon-gamma (IFN-γ) level, and total white blood cell (WBC), and differential leukocyte count were determined. Serum procalcitonin level was found to be higher in dogs with bacterial infection compared to the control group and dogs with viral disease ($P = 0.019$). It was observed that serum procalcitonin level in dogs with bacterial infection varies related to the disease agent and it was found higher in those with pyometra ($P = 0.009$). Serum procalcitonin level was higher in dogs which had parasitic infections but, the difference is not significant. IL-6 level was found higher in dogs with viral and parasitic diseases than those in the control ($P = 0.006$). A negative correlation was determined between procalcitonin and IFN-γ ($P = 0.001$). While a positive correlation was detected between procalcitonin and WBC count, a negative correlation was determined between procalcitonin and monocyte percentage. In conclusion, serum procalcitonin level can be used as a clinical biomarker in bacterial diseases and, perhaps, in some parasitic diseases in dogs. However, further studies should be conducted to determine threshold values that take the severity of infection, its prevalence, and clinical course into account.

Key Words: procalcitonin, cytokine, dogs, biomarker, infection

Introduction

Procalcitonin is a small peptide produced by parafollicular cells in the thyroid gland, weighing 13 kDa, and consisting of 116 amino acids. Its production is regulated by the calcitonin-1 (CALC-1) gene on the 11th chromosome[2,22]. It is produced as a prohormone of calcitonin in parafollicular cells in the thyroid gland. Its level in circulation is very low since it is converted to calcitonin and released into the circulation. It also begins to be produced by parenchymal cells during

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infections or in some pathological conditions. Since parenchymal tissues are quite common, it is secreted abundantly during inflammation[7]. The pancreas, liver, spleen, adrenal gland, lungs, kidneys, brain, medulla spinalis, testicles, stomach, small intestines, colon, abdominal fats, and white blood cells are the main sources of its extrathyroidal production[29]. Procalcitonin, which is normally produced in parafollicular cells in the thyroid gland, is reduced to peptides such as calcitonin, N-procalcitonin, or katacalcin by specific endopeptidases found in these cells. Since the enzyme in question cannot be found in parenchymal tissues, the prohormone produced is released into the circulation as procalcitonin without reduction[21]. Therefore, serum procalcitonin level increases significantly in a short time during infections[10].

During bacterial infection, released lipopolysaccharides, peptidoglycans, or other cellular fragments cause the release of cytokines such as interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α). These cytokines also stimulate the abundant production of procalcitonin by parenchymal tissues. In viral infections, a large amount of IFN-γ is produced[26]. Unlike cytokines released during bacterial infections, IFN-γ blocks the production of procalcitonin. In mixed infections with bacterial and viral agents, IFN-γ secreted by the viral agent blocks the pathway used by the bacterial agent and blocks the production of procalcitonin by parenchymal tissues. Linscheid et al.[21] proved that the production of procalcitonin in these cells is blocked when they added interferon-gamma (IFN-γ) to the differentiated adipocyte cell culture[21]. The stimulation of the production of procalcitonin during bacterial infections and its inhibition during viral infections have allowed this prohormone to be used as a clinical biomarker in people. In people exposed to bacterial infection or endotoxins, serum procalcitonin level is reported to increase rapidly in the early 3-6-hour period when clinical findings or white blood cell picture do not change[9]. Therefore, plasma procalcitonin level is also important in making the antimicrobial treatment decision quickly.

Calcitonin was discovered in tests conducted on dogs[8], and, although many experimental animals, especially rats, pigs, and baboons, have been used in the studies, there are few studies on procalcitonin in dogs[12,33]. In a study in which the mRNA level of procalcitonin was measured, it was detected that the mRNA production increased significantly in diseased dogs, but it was stated that serum mRNA level could not be used to differentiate those with infectious diseases and those without them[18]. In another study, it was determined that procalcitonin mRNA expression increased in the liver, lungs, and spleen taken from dogs who died as a result of systemic inflammatory response syndrome (SIRS)[10]. Interestingly, the serum procalcitonin level in dogs was measured only in two studies, as far as we could find. In one of them, it was reported that the serum procalcitonin level increased in dogs affected by *Babesia canis*, a blood parasite[4]. In the other study, it was reported that serum procalcitonin level increased when dogs were experimentally given endotoxin that is found in the cell membrane of gram negative bacteria[36].

Dogs brought to small animal clinics mostly consist of those with infection-related diseases. Similar clinical findings can also be observed in cases of infection caused by different agents. Furthermore, an infection-like clinical picture can be observed in non-infectious inflammatory conditions due to trauma, pancreatitis, or tumors. Therefore, it is sometimes difficult to distinguish bacterial infections from viral infections or non-infectious inflammatory diseases from infectious diseases[1]. Clinical findings and hematological parameters are needed for diagnosis. Procalcitonin has been widely used in humans for this reason, especially in recent years[29]. There are scarcely any studies published on dogs.

No study investigating whether procalcitonin could be used in dogs to distinguish bacterial infections from other inflammatory diseases was encountered. Moreover, no study examining the

Table 1. Breed, sex and age distribution of the 160 dogs included in the study.

Breed	Number of dogs	Sex			Age (year)*
		Male	Female	n	Mean ± SD
Mix Breed	38	23	15	22	4.43 ± 1.46
American Cocker Spaniel	18	7	11	13	7.23 ± 3.04
Golden Retriever	15	6	9	14	6.92 ± 3.23
Kangal Shepherd Dog	12	8	4	12	5.75 ± 2.21
German Shepherd Dog	10	8	2	7	6.75 ± 3.10
English Setter	7	2	5	5	6.40 ± 3.55
Rottweiller	7	4	3	5	6.33 ± 2.56
Labrador Retriever	5	3	2	3	7.00 ± 3.74
Cavalier King Charles Spaniel	5	1	4	5	6.60 ± 2.85
Welsh Springer Spaniel	4	1	3	4	6.50 ± 2.50
Miniature Pinscher	4	2	2	3	8.66 ± 1.69
Akbasch	4	3	1	3	6.33 ± 4.49
Beagle	3	2	1	3	7.0 ± 1.63
Dobermann	3	3		3	5.33 ± 3.68
Irısh Setter	3	1	2	3	9.66 ± 4.64
Bull Dog	3	3		1	9
Pekinese	2	1	1	2	4, 8
Siberian Husky	2	1	1	2	5, 6
Yorkshire Terrier	2		2	2	12, 2
Maltese	2		2	2	9, 7
Jack Russel Terrier	2	2		1	6
English Toy Terrier	2	2		1	4
Cairn Terrier	1		1	1	11
Daschhund	1	1		1	3
English Pointer	1	1		1	7
Pudelpointer	1		1	1	6
Schnauzer	1	1		1	6
Chow Chow	1	1		1	5
Chihuahua	1		1	1	8
Total	160	87	73	123	6.20 ± 3.03

* Data are expressed as mean ± standard deviation (if the number of dogs is less than three, mean ± standard deviation is not calculated and the real values are presented). Thirty-seven dogs (twenty five males, twelve females) of unknown age were not taken into account when performing age analysis. The "n" numbers indicate the number of dogs used in the age analysis.

relationship between procalcitonin and cytokines in dogs or other companion animals was found. In the present study, it was aimed to investigate the usability of serum procalcitonin level in dogs as a clinical biomarker for the distinction between bacterial, viral, and parasitic diseases. Moreover, it is aimed to examine the relationship between pro-inflammatory and anti-inflammatory cytokines and procalcitonin produced by extrathyroidal tissues.

Materials and Methods

Animals and groups: This study was approved by the local ethics committee of Istanbul

University (Approval number 2016/28). In current study, a total of 160 owned dogs, 87 male and 73 female, were used (Table 1). The owners of the dogs were informed, and they were asked for permission. The animals were evaluated in four seperate groups; control, bacterial, viral, and parasitic, each consisting of 40 dogs.

Forty healthy dogs over the age of one, who were brought to the university hospital for routine control or vaccination, were enrolled as control group. The decision was dependent upon on anamnesis, physical examination, complete blood count and blood serum biochemistry results.

A total of 40 dogs in accordance with at least two of the previously described SIRS criteria [16] were included in the bacterial infection group. These criteria are defined as fever or hypothermia, (> 40°C hyperthermia, or < 37°C hypothermia), leukocytosis (> 18,000 WBC/μL), tachycardia (HR >120 bpm) and tachypnea (RR >20 bpm). In addition to these common criteria, clinical findings or additionaly diagnostic methods were used to confirm the suspected disease. Briefly, in cases of bacterial enteritis, those with acute nausea, watery diarrhea, vomiting, and bloody diarrhea were considered in addition to their SIRS criteria. Furthermore, the treatment processes of these cases were monitored to see if they responded to antimicrobial therapy and supportive treatment. Bacterial pneumonia was diagnosed since the dogs were experiencing typical acute signs such as fever, dyspnea, tachypnea, lethargy, cough, nasal discharge. Clinical findings were also confirmed with thorax radiography. Pyometra was diagnosed related to criteria described previously[15] and the diagnosis was verified by postoperatively investigation of the uterus and ovaries. The diagnosis of pyoderma is based on clinical signs of superficial staphylococcal lesions consist of papules, pustules and epidermal collarettes, of deep skin infection with furuncles and draining tracts from the edematous skin. Bacterial urinary tract infections were detected based on the presence of lower urinary tract signs, such as hematuria, pyuria, bacteriuria, stranguria and bacterial culture and susceptibility results. In addition, dipstick, urine specific gravity and cytological examination of the sediment were performed in all cases to provide supporting evidence.

Forty dogs with distemper or parvovirus infection were selected to form the viral infection group. The diagnosis of canine viral diseases including canine parvovirus enteritis (CPE) and canine distemper virus (CDV) was based on positive speed test results and the clinical findings of the patient including diarrhea, bad odor lethargy, hyperthermia, ocular/nasal discharge .

Dogs positive for *Anaplasma* spp. or *Ehrlichia* spp. were selected to form the parasitic group. These blood parasites generate common blood diseases in dogs. A total of 102 dogs were screened depending on anemia, fever, leukopenia, thrombocytopenia and whether they had ticks on them. These two infections were clarified by quadruple test kits (Snap 4Dx Plus Test, IDEXX®) and PCR. PCR technique reveals that DNA of the agents in dog blood which causes the acute infections. Hence, 40 dogs that are blood positive with at least one or both of the two infections by PCR technique were included to this group.

The routine hematological tests of all dogs included in the study were performed in the laboratory of the university hospital. Moreover, a 3 ml blood sample without an anticoagulant was taken from each dog. The blood samples taken were centrifuged, their serum was separated and stored at -80 ºC for further analysis.

PCR analysis: Extractme DNA Blood Kit (EM05) (Blirt Company, Gdańsk, Poland) was used for DNA extraction of 102 dogs blood. PCR was performed using 16S8FE forward and B-GA1B reverse primers which targeted the 16S rRNA of both *Ehrlichia* spp. and *Anaplasma* spp. genus[32] PCR reactions were set up with 2x PCR TaqNova-Red- Master Mix with Taq Polymerase (RP85T) (Blirt Company, Gdańsk, Poland). The first round of the thermal cycling procedure was; an initial denaturation for 3 min at 94°C, followed by 45

Table 2. Diseases and case number that form bacterial, viral and parasitic infection groups

Diseases	Case number
Bacterial infections*	
Gastrointestinal tract infection	15
Pyometra	11
Bacterial pneumonia	7
Pyoderma	4
Urinary tract infections	3
Viral infections	
Parvovirus infection	23
Distemper	17
Parasitic infections	
Ehrlichiosis	12
Anaplasmosis	8
Ehrlichiosis + Anaplasmosis	20

* Cases in the bacterial infection category were selected from patients who responded positively to antibiotic therapy in addition to clinical and laboratory diagnosis.

cycles of 45 sec at 94°C, 45 sec at 57°C, 1 min at 72°C and a final 5 min at 72°C for extension. PCR products were electrophoresed through 1.5 % agarose gels containing ethidium bromide (10 mg/ml) and the expected DNA fragments which have 454-485 bp bands for *Ehrlichia* spp. and *Anaplasma* spp. genus visualised on the gel. As a result, 40 dogs positive with one or both of these blood parasites were included to the study.

Procalcitonin and cytokine analyses: Serum procalcitonin level and TNF-α, IL-1β, IL-6, and IFN-γ levels were determined by ELISA. In the detection of procalcitonin and cytokine levels, dog-specific commercial sets (Fine test, Fine Biotech, Wuhan, China) were used. Analyses were performed in accordance with the procedures of the kits.

Statistical analyses: The data of all groups were analyzed for normal distribution by the Shapiro-Wilk test. The data of the normally distributed parameters were analyzed by ANOVA. Tukey's HSD test was used for the post hoc analysis of these data. The non-parametric

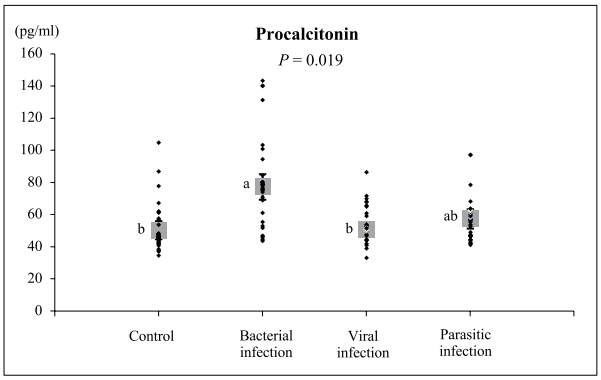

Fig. 1. Serum procalcitonin level in dogs with bacterial, viral, and parasitic disease (Erdal Matur). Each dot represents the value of a dog's serum procalcitonin level (n = 40). The large gray boxes represent mean values ± standard error. a and b = Means that have no letter in common are significantly different from each other (P<0.05).

Kruskal-Wallis test was performed for the non-normally distributed parameters. Mann-Whitney U test was used to compare serum procalcitonin levels in dogs regarding the ones with parvovirus enteritis to ones with distemper. Furthermore, Pearson's correlation analysis was conducted between procalcitonin and cytokines and between procalcitonin and white blood cell parameters. The correlation with the correlation coefficient between 0.7 and 1 was defined as strong, between 0.5 and 0.7 as moderate, and between 0.3 and 0.5 as weak correlation. The SPSS (SPSS for Windows, version 11.5.2.1.) packaged software was used for statistical analyses. Differences at the level of P<0.05 were considered significant. Those with a P-value between 0.05 and 0.1 were considered as a tendency.

Results

Data on serum procalcitonin level are presented in Fig. 1. The difference between the groups was detected to be significant (P = 0.019). It was determined that serum procalcitonin level was higher in dogs with bacterial infection compared to the control group and dogs with viral diseases. Although serum procalcitonin level was higher in dogs positive for blood parasites in

Table 3. The data on the fate of dogs in the bacterial group

Type of bacterial infections	Number of healed dogs	Number of dead dogs	Number of dogs whose fate is unknown*
Gastrointestinal tract infection (n=15)	8/15 (53.3 %)	3/15 (20 %)	4/15 (26.6 %)
Pyometra (n= 11)	7/11 (63.6 %)	2/11 (18.2 %)	2/11 (18.2 %)
Bacterial pneumonia (n= 7)	-	3 (42.8 %)	4 (58.2 %)
Pyoderma (n=4)**	4 (100 %)	-	-
Urinary tract infections (n=3)	3 (100 %)	-	-

* There not any informations about these dogs. Because they were not brought back to the university hospital. **Patients with pyoderma are generally healed, but 3 of them showed recurrence of skin infection.

Table 4. Changes in serum procalcitonin levels depending on the infection agent

	n	Mean ± SEM	*P* values
Bacterial infections			
Gastrointestinal tract infection	15	73.44 ± 1.63^{b}	
Pyometra	11	81.53 ± 2.03^{a}	0.009
Bacterial pneumonia	7	72.86 ± 2.88^{b}	
Viral infections			
Parvovirus infection	23	51.71 ± 0.43	0.655
Distemper	17	52.22 ± 0.95	
Parasitic infections			
Ehrlichiosis	12	53.52 ± 1.73	
Anaplasmosis	8	53.37 ± 0.84	0.406
Ehrlichiosis + Anaplasmosis	20	60.96 ± 6.31	

Data were presented as the mean and standard error of the means (SEM). Dogs with pyoderma and urinary tract infections were not compared with other groups because the number of cases was insufficient. a, b = Means with different superscripts within the same column are different.

comparison with the control group, the difference between them was not statistically significant. There was no difference between those with viral diseases and the control group.

Dogs evaluated in bacterial, viral and parasitic groups were classified related to disease agent. Accordingly, the bacterial group, consisted of dogs with bacterial enteritis (15/40 dogs), pyometra (11/40), bacterial pneumonia (7/40), pyoderma (4/40) and urinary tract infection (3/40) (Table 2). Data on the fate of dogs in the bacterial group are given in Table 3. Accordingly, it is seen that 22 of the dogs with bacterial infection recovered, 8 of them died, and no information was obtained about the fate of the remaining 10 dogs. In addition, it was determined that 2 of the 5 dogs with the highest procalcitonin level died, 1 recovered, and no information was obtained about the fate of 2 dogs.

Procalcitonin levels were compared statistically in gastroenteritis, pyometra and pneumonia cases where the number of cases was sufficient. The obtained results pointed out that the serum procalcitonin level was higher in dogs with pyometra than those with bacterial enteritis or pneumonia (P = 0.009) (Table 4). Dogs with distemper (17/40) and parvovirus infection (23/40) constituted the viral infection group. Regarding the Mann-Whitney U test, there was no significant difference in serum procalcitonin levels between dogs with distemper and parvovirus infection (P = 0.655) (Table 4). The parasitic infection group consisted of dogs positive for either *Ehrlichia* spp (12/40) or *Anaplasma* spp. (8/40) or for both

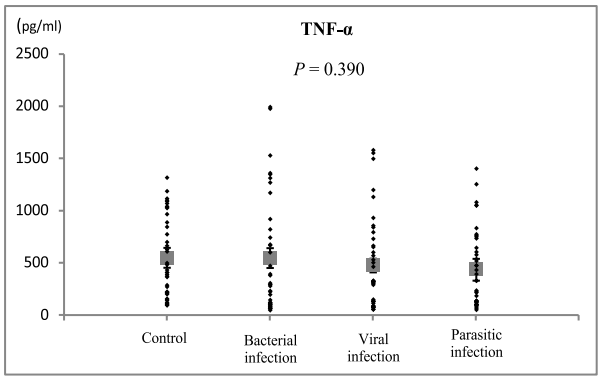

Fig. 2. Serum TNF-α level in dogs with bacterial, viral, and parasitic disease (Erdal Matur). Each dot represents the value of a dog's serum TNF-α level (n = 40). The large gray boxes represent mean values ± standard error.

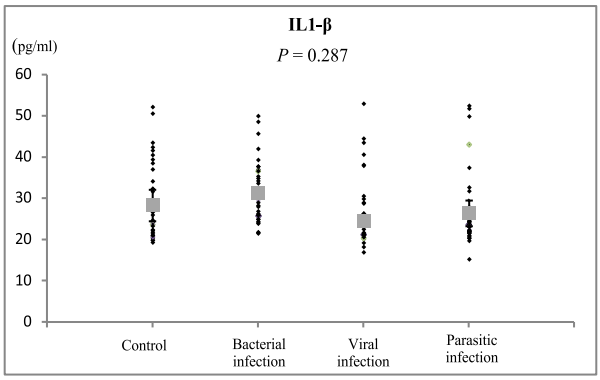

Fig. 3. Serum IL1-β level in dogs with bacterial, viral, and parasitic disease (Erdal Matur). Each dot represents the value of a dog's serum IL1-β level (n = 40). The large gray boxes represent mean values ± standard error.

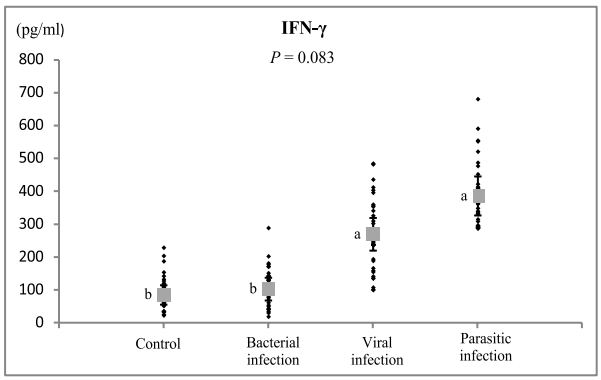

Fig. 4. Serum IFN-γ level in dogs with bacterial, viral, and parasitic disease (Erdal Matur). Each dot represents the value of a dog's serum IFN-γ level (n = 40). The large gray boxes represent mean values ± standard error. a and b = Means that have no letter in common are significantly different from each other (P<0.05).

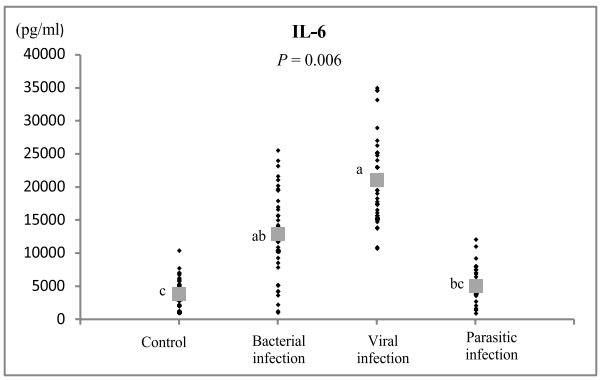

Fig. 5. Serum IL-6 level in dogs with bacterial, viral, and parasitic disease (Erdal Matur). Each dot represents the value of a dog's serum IL-6 level (n = 40). The large gray boxes represent mean values ± standard error. a, b and c = Means that have no letter in common are significantly different from each other (P<0.05).

(20/40). There were no significant differences between dogs positive with *Ehrlichia* spp., *and with Anaplasma* spp. or both (P = 0.406) (Table 4).

Serum TNF-α level is presented in Fig. 2, while IL-1β level is presented in Fig. 3. The obtained results demonstrate that there is no difference between the control and other groups in terms of serum TNF-α and IL-1β levels (P = 0.390 and P = 0.278, respectively). Serum IFN-γ level is presented in Fig. 4. The data obtained showed that the difference between the groups tends to be significant (P = 0.083). Serum IFN-γ level was found to be higher in dogs with viral and parasitic diseases compared to that in the control group and dogs with bacterial disease. Data on serum IL-6 level are presented in Fig. 5. The obtained data point out that the difference between the groups is significant. IL-6 level was found to be higher in dogs with viral and parasitic diseases compared to dogs in the control group. The difference between dogs with bacterial disease and the control group was insignificant.

Data on the correlation between serum procalcitonin level and cytokines are presented in Table 5. The correlation between procalcitonin and IFN-γ was found to be significant (P = 0.001).

Table 5. Correlations between cytokines and procalcitonin

Procalcitonin	TNF-α	IL-1β	IFN-γ	IL-6
Regression coefficient	0.019	-0.049	-0.347	-0.165
P-Value	0.434	0.336	0.001*	0.077

*There are negative correlation between serum procalcitonin level and IFN-γ.

Table 6. Correlations between procalcitonin and total leukocyte and leukocyte subtypes

Procalcitonin	WBC	Neutrophil	Lymphocyte	Eosinophil	Monocyte	Basophil
Regression coefficient	0.343	-0.063	0.125	-0.015	-0.291	0.010
P-Values	0.002*	0.576	0.269	0.896	0.009**	0.928

*There are positive correlation between serum procalcitonin level and WBC count. ** There are negative correlation between serum procalcitonin level and monocyte percentage.

Considering the calculated correlation coefficient, a weak negative correlation is observed between procalcitonin and IFN-γ. Data on the correlations between procalcitonin and total WBC count and leukocyte subtypes are presented in Table 6. The obtained data showed a low positive correlation between the total white blood cell count and procalcitonin. Furthermore, a low negative correlation was determined between the monocyte percentage and procalcitonin.

Discussion

The aim of the present study is to investigate whether serum procalcitonin level can be used as a clinical biomarker in bacterial, viral and parasitic infections in dogs. The results obtained indicate that serum procalcitonin level is significantly higher in dogs with bacterial infection than in healthy dogs and dogs with viral disease (Fig. 1). It also draws attention that procalcitonin level increases numerically, although not statistically significantly, in those with parasitic disease (Fig. 1). It is known that bacterial infections in humans significantly increase the production of procalcitonin[23]. However, the production of procalcitonin is closely related to the severity and prevalence of infection. Although it does not increase a lot in local infections, it is reported to increase up to thousands of times of normal in diffuse infections and sepsis[20]. Sepsis has recently been reported to increase the procalcitonin level in dogs[13]. This supports our finding indicating that the procalcitonin level increases in those with bacterial infection. In our study, it was observed that the procalcitonin level was high in dogs forming the bacterial group, although there was no sepsis. There are few studies related to the subject conducted on animals. It was reported that serum procalcitonin level increased within 0.5 hours in dogs experimentally administered with endotoxin, reached a maximum within 2 hours, and maintained this level for 4-48 hours[36]. In the mentioned experimental study, the recorded increase was observed to be 100% after the administration of endotoxin[36]. In our study, it was also calculated that the procalcitonin level in dogs with bacterial disease increased compared to those in the control group, but the increase rate was approximately 43%. This raises the question that why the procalcitonin level in dogs with bacterial infection does not increase at such a high level, as in humans or dogs experimentally administered with endotoxin. It is thought that this may have been caused by several different reasons. It is reported that the procalcitonin level reaches a peak soon after the infection starts, but it decreases by 50% 24-48 hours after reaching this peak[26]. It is also possible that dogs whose

disease was not at the initial stage were used in the current study. This may be one of the reasons why the procalcitonin level is not extremely high in those with bacterial infection. The other reason may be related to the fact that bacterial diseases are not diffuse or severe. As a matter of fact, upon examining the cases included in the study individually, we also observed that there were very significant increases (unpublished data). It is reported that the amount of procalcitonin varies in humans according to the level of bacterial infection[26]. For example, although serum procalcitonin level in humans increases above 1.0 ng/ml in case of bacteremia and above 2.0 ng/ml in septic infection, it is reported to remain at the level of 0.5-1.0 ng/ml in local infections[5]. The procalcitonin increase in bacterial infections is related to whether the agent is gram-positive or negative. Gram-negative ones cause more procalcitonin to be released[20]. Further studies need to be conducted on the subject and the level of procalcitonin has to be examined considering the severity of infection or its prevalence.

It was reported that plasma procalcitonin level increases in some parasitic diseases in humans[19]. For example, the procalcitonin level was also stated to increase significantly in visceral leishmaniasis[30]. Likewise, plasma procalcitonin level was reported to increase in individuals with systemic parasitic disease such as *P. falciparum malaria*[6]. and in people with *A. phagocytophilum* infection[34]. To our knowledge there is only one study on the subject carried out on dogs and it was reported that the serum procalcitonin level increased in dogs with *Babesia canis*[4]. In the present study, dogs diagnosed with *A. phagocytophilum* or *Ehrlichia canis* agents which are commonly observed in the veterinary practice constituted the parasitic disease group. It was observed that the plasma procalcitonin level in the mentioned dogs increased numerically in comparison with dogs in the control group. However, the difference between them was not statistically significant. Thus, it is impossible to say whether the serum procalcitonin value can be used as a clinical biomarker in dogs with blood parasites using the available data. More detailed studies on the subject need to be conducted.

In this study, each group consists of diseases caused by different infection agent. For example, the bacterial infection group consists of dogs with bacterial enteritis, pyometra, pneumonia, pyoderma and urinary tract infection. Therefore, whether there could be a difference in procalcitonin production related to the mentioned diseases was also statistically analyzed. Serum procalcitonin level was higher in dogs with pyometra than in dogs with bacterial enteritis or pneumonia. The fact that the level of serum procalcitonin in dogs with pyometra was higher than dogs with enteritis and pneumonia, indicated a possible relationship between the severity of the infection and procalcitonin production. Pyometra is one of the most serious conditions in veterinary medicine[14]. The fact that bacteria that colonized in the uterus of dogs with pyometra infection will threaten life by causing sepsis in a short time has already been proved[17]. We couldn't find any published data on the subject in dogs. However, the increase of the procalcitonin level due to the severity of the infection was considered a reasonable result. A number of methods can be used for the diagnosis of pyometra. On the other hand, in recent years, the interest in biomarkers that can be used to diagnose pyometra has been increasing[31]. In this context, our result suggested that procalcitonin can also be used as a biomarker in the early diagnosis of the pyometra.

In this study, it was observed that the procalcitonin level did not increase in those with viral disease. A similar result is observed in humans. It is reported that IFN-γ released in viral diseases and even viral-bacterial mixed infections blocks the pathway required for procalcitonin secretion[19]. Some inflammatory and anti-inflammatory cytokines were also examined to understand the mechanisms related to procalcitonin produced in non-thyroid tissues due to various infectious agents in dogs. In the current study, it was observed that the production

of IFN-γ tended to increase in dogs with viral and parasitic diseases. Furthermore, a negative correlation - although weak - was determined between IFN-γ gamma and procalcitonin (Table 5). The correlation in question means that the amount of procalcitonin will decrease as IFN-γ increases. The opposite is also true. As a matter of fact, the procalcitonin level is low and the INF-γ level is high in those with viral disease (Fig. 1 and Fig. 4 respectively). This has suggested that INF-γ has a negative effect on procalcitonin secretion, as in humans.

TNF and IL1-β are proinflammatory cytokines. Therefore, they are expected to increase during bacterial infections. However, the increase in these cytokines is not mentioned as much as the rise in procalcitonin. As a matter of fact, it has been reported that the increase in serum TNF-α and IL1-β levels after injection was less than twice in golden hamsters injected with *E. coli* and decreased to basal level in a short time[35]. In contrast, the same researchers state that the increase in procalciton is more than 100 times and lasts 24 hours[35]. Because of this feature, it is recommended to use procalcitonin as a clinical biomarker. In this study, the fact that serum TNF-α and IL1-B levels did not increase in the bacterial group is a result of insufficient bacterial stimulation or the period in which the infection peaked. In this study, the plasma IFN-γ level was found to be high in the parasitic group. Since a negative correlation was detected between procalcitonin and IFN-γ, the procalcitonin level was expected to be low in the parasitic group. However, a slight increase was observed. Thus, it is impossible to explain the fact that the production of procalcitonin does not decrease in the parasitic group by the IFN-γ mechanism. On the other hand, it is reported that mediators stimulating procalcitonin in some fungal or mixed infections exceed the blocking capacity of IFN-γ and cause a slight increase in procalcitonin[3]. In the present study, the fact that the production of procalcitonin does not decrease in the parasitic infection group in which the IFN-γ level is high can be explained by this mechanism.

It is reported that changes in the serum IL-6 level in humans are used as a biomarker in the early diagnosis of sepsis and monitoring of its prognosis[27]. It is stated that procalcitonin is widely used as a biomarker in septic cases[25]. In this study, the serum IL-6 level was observed to be high not only in dogs with bacterial disease but also in those with viral disease. However, a direct connection could not be established between procalcitonin production in extrathyroidal tissues because IL-6 level was high in both dogs with bacterial disease in which the procalcitonin level was high and in dogs with viral disease in which the procalcitonin level was low. Moreover, correlation analysis also confirms that there is no relationship between procalcitonin and IL-6.

The WBC count and percentage rates in the peripheral blood in humans and animals are parameters that are commonly used in the clinical diagnosis and monitoring of the prognosis. The current study investigated whether there was a correlation between the WBC count and leukocyte subtypes and procalcitonin. Accordingly, a positive correlation was observed between the WBC count and procalcitonin, while a negative correlation was observed with the monocyte percentage. The fact that the total white blood cell count increases, especially in bacterial infections, has already been known. It is stated that there is a correlation between procalcitonin and WBC in humans[24]. This study demonstrated that procalcitonin and WBC count increased together in bacterial infections in dogs, as well. It is stated that interleukin-10 (IL-10) suppresses monocytes in people with severe infection or sepsis[28]. It is also reported that IL-10 stimulates the production of procalcitonin at the same time[11]. In the current study, it was thought that the negative correlation between monocytes and procalcitonin might have been caused by IL-10. In this study, serum procalcitonin level was not monitored daily during the disease. It was measured only once; when the patients came to the clinic with an infection complaint. For this reason, this study

can be considered limited. More detailed studies and further research are needed to be conducted on the subject.

As a result, in this study, it can be said that procalcitonin can be used as a clinical biomarker in the differentiation of bacterial and viral diseases in dogs. Moreover, as a conclusion, it was stated that IFN-γ is effective in the procalcitonin mechanism in dogs, as in humans. However, it is obvious that further research needed to be carried out to determine threshold values to use in monitoring the severity or prognosis of the infection. Likewise, more detailed studies should be conducted , considering the fact that the procalcitonin level increases slightly in parasitic diseases.

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