Investigation of some biochemical parameters, hematological changes, neopterin and procalcitonin levels in dogs with Canine distemper virus

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ABSTRACT: Canine distemper virus (CDV) infection is a highly important infectious disease impacting both domestic dogs and wild carnivores. This virus spreads rapidly and is prevalent among carnivorous species, with high transmission and mortality rates. Clinically, it can cause respiratory, gastrointestinal, dermatological, and neurological symptoms. Dogs infected with CDV that lack colostrum intake, are unvaccinated, or have a weakened immune system tend to experience a more severe progression of the disease. This study aimed to investigate serum neopterin and procalcitonin levels as diagnostic indicators in CDV infected dogs. The study included a sample of 35 dogs diagnosed with distemper and 15 healthy dogs of varying breeds and genders, all aged between 1.5 and 6 months. The diagnosis was confirmed via rapid antigen tests and PCR analysis. Subsequently, hematological and biochemical analyses were conducted. Blood cell counts, such as white blood cells (WBC), lymphocytes (LYM), granulocytes (Gra), red blood cells (RBC), hematocrit (HCT), thrombocytes (THR), Gra%, and hemoglobin (Hb), showed a statistically significant difference (P<0.001). Similarly, the monocyte values in sick dogs were significantly different from those in the control (P<0.01). The biochemical parameters urea, alkaline phosphatase (ALP), and amylase (AMY) of sick dogs also showed statistically significant differences (P<0.001) compared to control. Furthermore, serum GGT and glucose in sick dogs were significantly different from those in control (P<0.01). The serum neopterin concentration in sick dogs was 4.94 ± 0.31 nmol/L, while it was 3.02 ± 0.33 nmol/L in the control, indicating a statistically significant difference (P<0.001). Similarly, the serum procalcitonin concentration in sick dogs was 65.11 ± 3.12 ng/L, compared to 44.05 ± 3.20 ng/L in the control, also showing a significant difference (P<0.001). In conclusion, given that distemper is a multisystemic disease, significant changes in serumbiochemical and hematological parameters were observed. Additionally, neopterin and procalcitonin biomarkers may play a valuable diagnostic role in distemper.

Keyword: Dog; Neopterin; Procalcitonin; Distemper.

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INTRODUCTION

Canine distemper (CD), also known simply as distemper, is one of the most significant diseases affecting domestic dogs and wild carnivores (Beineke et al., 2009). This viral infection, characterized by high infection and mortality rates, is commonly observed in both domestic and wild carnivorous species (Appel and Summers, 1999). Among viral infections in dogs, rabies has the highest mortality rate, followed by distemper (Deem et al., 2000).

The canine distemper virus shares an antigenic relationship with the rinderpest virus in ruminants and the measles virus in humans. It belongs to the family Paramyxoviridae, within the order Mononegavirales (Barrett, 1999; Beineke et al., 2009). Clinically, it primarily affects the respiratory, gastrointestinal, dermatological, and neurological systems of its hosts. The immunosuppressive nature of the virus predisposes infected animals to secondary infections caused by both pathogenic and non-pathogenic agents (Greene and Vandevelde, 2012). Transmission occurs through all secretions and excretions of infected animals (Greene and Vandevelde, 2012).

Infection with the canine distemper virus results in a multisystemic disease, with the duration and clinical presentation varying based on factors such as the virus strain, the immune status of the dog, age, stress, and the presence of secondary infections (Sherding, 1994; Appel and Summers, 1995; Lan et al., 2005; Jensen et al., 2009; Sykes, 2014). Early clinical signs include anorexia, depression, and fatigue, followed by conjunctivitis and serous or mucopurulent ocular and nasal discharge (Lan et al., 2005; Sherding, 1994; Appel and Summers, 1999). In cases where the immune response is delayed or inadequate, the virus may persist in the body, leading to chronic signs in the uvea, lymphoid organs, footpads, and central nervous system. These patients often develop secondary infections due to opportunistic pathogens (Sykes, 2014).

In veterinary medicine, the most commonly used methods for Procalcitonin (PCT) measurement are generally Enzyme-Linked Immunosorbent Assay (ELISA), Chemiluminescent Immunoassay (CLIA), and Immunofluorescence Assay (IFA) (Meindertsma & Tvedten, 2019). On the other hand, High-Performance Liquid Chromatography (HPLC) and immunological assays are widely preferred for Npt measurement (Schroecksnadel et al., 2006). Among these methods, ELISA, which is based on antigen-antibody interactions, is the most frequently utilized technique and is extensively applied in veterinary medicine (Kutzler, 2015).

Neopterin (Npt) is a marker indicative of macrophage activation, particularly elevated in viral infections, and plays a crucial role in assessing the cellular immune response (Murr et al., 2002) PCT, on the other hand, is an inflammatory biomarker that can be used to differentiate between sepsis and systemic infections (Matur et al., 2021) The combined evaluation of these biomarkers in CDV infection may facilitate diagnosis and aid in determining appropriate supportive treatment strategies.

Procalcitonin, synthesized by the C cells of the thyroid gland, serves as a precursor of the hormone calcitonin and is present in low concentrations under normal conditions. During inflammation, PCT is released via two mechanisms: direct stimulation by microbial toxins and cytokine-mediated activation of a host response. Additionally, PCT is produced by neuroendocrine cells in the lungs, intestines, liver, and pancreas during inflammation (Maruna et al., 2000).

Monitoring immunological changes in patients is essential for identifying the underlying causes of diseases and determining effective treatment strategies. One key biomarker in this context is Npt, a molecule derived from guanosine triphosphate (GTP) and activated by interferon-gamma (IFN-γ) in T-lymphocytes upon macrophage stimulation (Şimşek, 2019). Neopterin plays a role in oxidative stress and nitric oxide synthesis and is strongly associated with endothelial damage and septic complications (Ruokonen et al., 2002). Veterinary research has identified changes in Npt levels in bacterial infections, sepsis, and babesia-like diseases, making it a valuable biomarker for diagnosing and treating infections in animals (Mrljak et al., 2004).

Neopterin and PCT are key biomarkers used in diagnosing infections and evaluating the immune response (Basbug & Aydogdu, 2020; El-Deeb et al., 2021). Neopterin is typically elevated in viral infections, suggesting a robust cellular response by the immune system (Akyüz & Gökce, 2021; Ercan & Başbuğ, 2016). While PCT is generally recognized as a marker for bacterial infections, it has been observed to rise in severe viral infections due to cytokine storms and systemic inflammatory response syndrome (SIRS). (Gautam et al., 2020). The combined evaluation of these two biomarkers enhances clinical management by offering more precise insights into the severity of viral infections

and potential bacterial superinfections (Akyüz & Gökce, 2021).

This study aims to comprehensively investigate the dynamic changes of neopterin (Npt) and procalcitonin (PCT) biomarkers during canine distemper infection. By elucidating the roles and diagnostic value of these biomarkers throughout the course of the disease, the research seeks to contribute to the development of more precise and effective diagnostic approaches for distemper.

MATERIALS AND METHODS
This study was conducted with the approval of the Kafkas University Animal Experiments Local Ethics Committee (decision dated 24.12.2020, number KAÜ-HADYEK/2020-173) to ensure compliance with ethical standards. Additionally, blood samples were obtained following the recommended "standard sample collection procedure," ensuring that animals were neither stressed nor harmed in any way.

Animal Material
The animal material for this study consisted of 35 dogs with distemper (DD) of various breeds and sexes, aged 1.5–6 months, as well as 15 healthy dogs (HD) of similar breeds, sexes, and age range. In the group infected with CDV, there were 17 males and 18 females. Two were Kangals, two were Golden Retrievers, and thirty-one were mixed breeds. The 15 dogs in the control group were mixed-breed, consisting of 7 males and 8 females. All dogs were presented to the Department of Internal Medicine Clinics at the Faculty of Veterinary Medicine, Kafkas University. Dogs in the DD tested positive for distemper using a Distemper Ag rapid test kit and RT-PCR method, exhibiting signs of systemic illness. The HD group included dogs brought to the clinic for vaccination or general examination, who showed no health issues during clinical examination and tested negative with the CDV Ag rapid test kit.

Clinical Examination
During the clinical examinations of the DD and HD groups, various parameters, including body temperature, respiratory and pulse rates, conjunctival status, breed, sex, and age, were recorded.

The HD group underwent comprehensive general examinations, encompassing body temperature, respiratory rate, heart rate, lymph node palpation, and lung and heart auscultation, which confirmed normal respiratory and cardiac sounds. The evaluations also included assessments of mucous membranes, capillary refill time (CRT), skin and coat condition, abdominal palpation, and musculoskeletal system function, none of which revealed any pathological abnormalities. To further confirm their healthy status, laboratory analyses—comprising hemogram, biochemical profiling, urinalysis, and fecal examination—were performed, and all results indicated the absence of underlying health problems.

Dogs in the DD group that exhibited clinical signs such as tachypnea, abdominal respiration, cough, nasal and/or ocular discharge, diarrhea, vomiting, high fever, anorexia, retinal hyperreflexia, unconscious chewing movements, tremors, tics, seizures, involuntary circling, barking, and other unconscious movements were included in the study.

Blood Sample Collection
Blood samples were collected once from DD based on clinical and laboratory findings. Blood was drawn into vacuum gel serum tubes (BD Vacutainer®, BD, UK) and vacuum EDTA blood tubes (BD Vacutainer®, BD, UK) using a holder and compatible sterile needle tip (Vacuette®, Greiner Bio-One GmbH, Austria). To ensure accuracy and prevent variability, the collection process was standardized,. The cephalic or saphenous vein was generally preferred for sampling. Blood samples collected in vacuum tubes without anticoagulant were centrifuged at 3000 rpm for 10 minutes at +4 °C in a refrigerated centrifuge (Hettich Rotina 380R®, Hettich, Germany) to separate the serum. The centrifuge was regularly calibrated, and quality control checks were performed to ensure consistent performance. Samples collected in EDTA tubes were subjected to complete blood count (CBC) analysis using automated hematology analyzers, which were maintained following manufacturer-recommended calibration protocols to minimize technical variability. Biochemical parameters were measured from the obtained serum samples, with quality control procedures implemented to ensure the reliability of the measurements. Serum samples for Npt and PCT measurements were stored at -20°C until analysis, following strict protocols to prevent degradation of analytes.

Biochemical and Complete Blood Count Analyzes
Biochemical analyses were conducted in the laboratory of the Department of Internal Medicine, Faculty of Veterinary Medicine, Kafkas University, using a fully automatic biochemistry analyzer (Mindray BS120®, Mindray Medical Technology, Türkiye) in

accordance with the manufacturer's instructions. The following parameters were measured: ALT (IU/L), AST (IU/L), GGT (IU/L), ALP (IU/L), amylase (IU/L), glucose (mg/dL), creatinine (mg/dL), urea (mg/dL), total protein (g/dL), and albumin (g/dL).

For CBC, blood samples were collected in EDTA tubes, and the following values were recorded using a VG-MS4e® analyzer (Melet Schloesing, France): total leukocyte count (WBC x10^3/µL), lymphocyte percentage (LYM %), monocyte percentage (Mon %), granulocyte percentage (Gra %), lymphocyte count (LYM), monocyte count (Mon x10^3/µL), granulocyte count (Gra x10^3/µL), erythrocyte count (RBC x10^6/µL), mean erythrocyte volume (MCV fL), hematocrit percentage (HCT %), hemoglobin concentration (HGB g/dL), and platelet count (THR x10^3/µL).

Virological Analysis

Immunochromatographic Method

An immunochromatographic-based test kit (Canine Distemper Virus Antigen Test®, Cat no: 022321, Asan Pharm CO. LTD, Korea) was used to detect the presence of CDV antigen in the dogs included in the study. The test was performed and interpreted according to the manufacturer's instructions. As described in the instructions, an eye and nose swab was immersed in a tube containing 300 µL of diluent, and the sample was stirred in the diluent for 20 seconds. Four drops of the resulting mixture were then added to the sampling area of the test kit. After 15–20 minutes, the results were interpreted. Samples showing a double line in both the control and test sections were considered positive, while those with a single line in the control section were considered negative. Whole blood samples with EDTA from dogs identified as positive by the immunochromatographic method were subsequently subjected to the RT-PCR test.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Blood samples collected in EDTA tubes were centrifuged at 2000 rpm for 5 minutes. The leukocyte layer was removed with a Pasteur pipette, reconstituted with 2 mL Phosphate Buffer Saline (PBS), and placed in stock tubes. For molecular analysis, samples were stored at -20°C until testing. Prior to molecular testing, viral nucleic acid was extracted from the samples using the phenol-chloroform method (Sambrook and Russell, 2006). Since CDV nucleic acid is RNA, complementary DNA (cDNA) was synthesized from the viral RNA by reverse transcription. A pair of primers targeting the conserved nucleoprotein region for PCR amplification was selected (Frisk et al., 1999), producing an amplicon of approximately 287 base pairs. For reverse transcription and DNA amplification in a single reaction, a 2x One-step RT-PCR master mix (Hibrigen) kit was used according to the manufacturer's instructions. Thermal cycler conditions were as follows: 55°C for 1 hour, 94°C for 4 minutes for initial denaturation, followed by 35 cycles of 94°C for 1 minute, 59°C for 2 minutes, and 72°C for 1 minute. Amplification concluded with a final extension step at 72°C for 10 minutes. A 100 bp standard ladder was used for evaluation, and amplified DNA products were stained with ethidium bromide in 1% agarose gel and electrophoresed at 100 V. The gel was examined under ultraviolet (UV) light, and products with bands of the appropriate size were identified as positive **(Figure 1)**.

Neopterin and Procalcitonin Analyzes

Serum Npt and PCT concentrations were measured using commercial canine-specific ELISA kits (Canine Neopterin ELISA kit®, Canine Procalcitonin ELISA kit®, BT Lab, China). The ELISA tests were performed according to the manufacturer's instructions, and optical densities were measured on an ELISA reader at a wavelength of 450 nm. Npt and
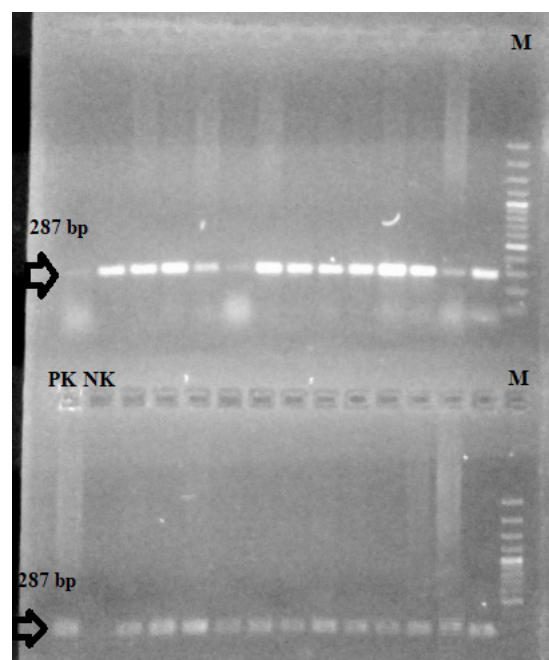

Figure 1. Rt-PCR result of a dog in the study (PK: Positive Control, NK: Negative Control, M: Ladder 287bp: 287 Base Pairs)

PCT were determined colorimetrically through regression analysis, and their values were recorded.

Statistical Analysis

All statistical analyses were performed using SPSS® software (Version 26.0, Chicago, IL, USA). All parameter values in the study were presented as mean ± standard error of mean (SEM). Since the groups (DD and HD) were normally distributed according to the Shapiro-Wilk test, they were compared using an independent sample t-test.Receiver Operating Characteristic (ROC) analysis was used to evaluate the diagnostic performance of Npt and PCT in distemper infection. The Area Under the Curve (AUC) values were found to be statistically significant, and values greater than 0.7 were considered diagnostically meaningful. Optimal cut-off values were determined using the Youden Index (J = Sensitivity + Specificity - 1). Differences between the groups in terms of the examined parameters were considered statistically significant at the P<0.05 level.

RESULTS

Clinical Findings

Tachypnea, abdominal respiration, cough, nasal and ocular discharge, diarrhea, vomiting, high fever, anorexia, retinal hyperreflexia, "chewing gum" fits, tremors, tics, seizures, involuntary circling, barking, and unconscious movements were among the clinical findings observed in the dogs included in the study. The rectal body temperature, respiratory rate, and pulse rate of both DD and HD are presented in Table 1, and it was determined that these values were not statistically different (P > 0.05).

Hematologic Findings

The WBC, LYM %, Mon %, Gra %, LYM, Mon, Gra, RBC, MCV, HCT %, Hb, and THR values of the dogs in the patient and control groups are presented in Table 2. Among the hematologic parameters, WBC, LYM%, LYM, Gra, RBC, HCT %, THR, Gra %, and Hb showed statistically significant differences in the patient group compared to the control group

Table 1. Comparison of clinical parameters between groups

Parameters	DD (n= 35)	HD (n= 15)	P value
	Mean ± Standard error of mean		
Rectal Body Temperature (°C)	38.80 ± 0.28	38.86 ± 0.15	> 0.05
Respirations/minute	58.45 ± 5.33	44.93 ± 2,36	> 0.05
Pulse/minute	136.91 ± 7.69	125.60 ± 3.72	> 0.05

HD: Dogs with distemper, HD: Healthy dogs

Table 2. Mean and standard error of mean ofhematologic parameters in groups

Parameters	DD (n= 35)	HD (n= 15)	P value
	Mean ± Standard error of mean		
WBC (x10^3/µL)	5.12 ± 0.57	14.81 ± 1.46	<0.001
LYM (%)	14.98 ± 1.73	29.21 ± 3.36	<0.001
Mon (%)	4.25 ± 0.37	4.46 ± 0.27	>0.05
Gra (%)	54.52 ± 3.58	68.64 ± 1.64	<0.001
LYM (x10^3/µL)	2.26 ± 0.24	5.31 ± 0.71	<0.001
Mon (x10^3/µL)	0.20 ± 0.14	0.74 ± 0.10	<0.01
Gra. (x10^3/µL)	3.2 ± 0.33	10.37 ± 1.3	<0,001
RBC (x10^6/µL)	6.06 ± 0.23	9.32 ± 0.20	<0.001
MCV (fL)	69.96 ± 0.89	68.86 ± 0.47	>0.05
HCT (%)	41.72 ± 2.09	65.00 ± 0.99	<0.001
HGB (g/dL)	12.73 ± 0.58	15.03 ± 0.25	<0.001
THR (x10^3/µL)	142.86 ± 11.40	304.25 ± 40.16	<0.001

HD: Dogs with distemper, HD: Healthy dogs

(P<0.001). Similarly, Mon showed a significant difference (P<0.01). However, Mon % and MCV were not statistically different (P>0.05).

Biochemical Findings

The ALT, AST, GGT, ALP, amylase, glucose, creatinine, urea, total protein, and albumin values in the DD and HD are presented in Table 4. Significant differences were observed between the DD and HD for the urea (P<0.001), ALP (P<0.001), amylase (P<0.001), GGT (P<0.01), and glucose (P<0.01). However, no statistically significant differences were found for ALT, AST, creatinine, total protein, and albumin (P>0.05).

Neopterin and Procalcitonin Values

The Npt and PCT concentrations were significantly higher in the DD than in the HD (P<0.001, Table 3). It has been determined that neopterin concentrations are influenced by the CDV. Neopterin concentrations were measured as 4.94 nmol/L in the DD group and 3.02 nmol/L in the HD group, with the difference between these groups found to be statistically significant (P < 0.001). Similarly, it was observed that PCT concentrations are also affected by the CDV. Procalcitonin concentrations were identified as 65.11 ng/L in the DD group and 44.05 ng/L in the HD group, and this difference was likewise found to be statistically significant (P < 0.001). The descriptive statistics of Npt and PCT in the DD and HD are presented in Table 3. In our study, ROC analysis was applied to evaluate the diagnostic value of Npt in dogs with distemper. According to the ROC analysis, the discriminatory power of Npt was calculated as AUC = 0.812 (P = 0.001, 95% CI: 0.679–0.946). In

terms of diagnostic performance, the optimal cut-off value for Npt was determined as 3.31, with a sensitivity of 80% and a specificity of 73.3% at this threshold. These findings are presented in Figure 2. In our study, according to the ROC analysis, the diagnostic power of PCT was calculated as AUC = 0.817 (P < 0.001, 95% CI: 0.698–0.936). In terms of diagnostic performance, the optimal cut-off value for PCT was determined as 58.67, with a sensitivity of 62.9% and a specificity of 93.3% at this threshold. These findings are presented in Figure 3.
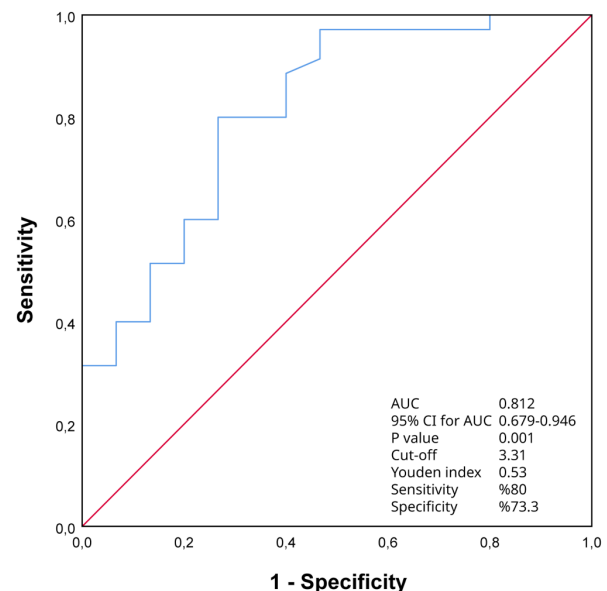

AUC	0.812
95% CI for AUC	0.679-0.946
P value	0.001
Cut-off	3.31
Youden index	0.53
Sensitivity	%80
Specificity	%73.3

Figure 2. Receiver Operating Characteristicanalysis for Neoperin (AUC: Area Under the Curve, CI: Confidence Interval)

Table 3. Changes ofneopterin and procalcitonin in groups

Parameters		DD (n= 35)	HD (n= 15)	P value
Neopterin (nmol/L)	Mean	4.94	3.02	
	SEM	0.31	0.33	
	Min	1.92	0.81	<0.001
	Max	8.86	5.82	
	Median	4.39	2.79	
Procalcitonin (ng/L)	Mean	65.11	44.05	
	SEM	3.12	3.20	
	Min	24.86	25.69	<0.001
	Max	98.76	61.44	
	Median	61.08	44.05	

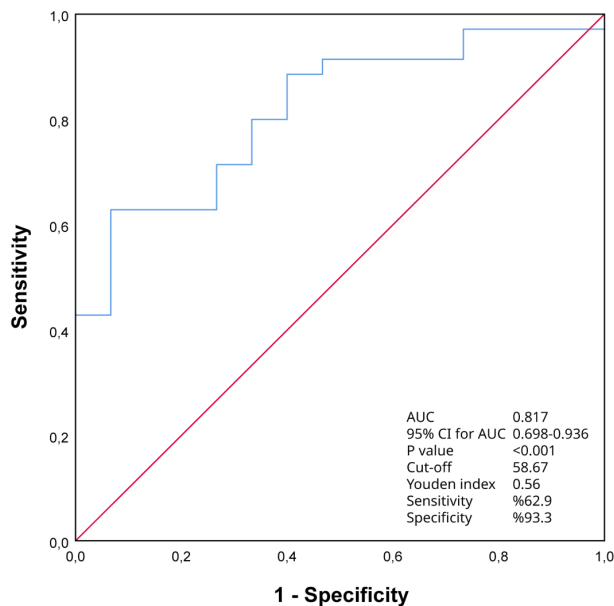HD: Dogs with distemper, HD: Healthy dogs, SEM: Standard error of mean

Figure 3. Receiver Operating Characteristic analysis for Procalcitonin (AUC: Area Under the Curve, CI: Confidence Interval)

DISCUSSION

Hypothermia or hyperthermia may occur in canine CDV infection, and body temperature may remain unchanged in the chronic form of the disease (Aiello et al., 2016). Yagci and Gunes (2017) found no statistical difference in body temperature between CDV-infected and healthy dogs. In our study, consistent with these findings, no statistical difference was observed in body temperature between DD and the HD. Some of the dogs in our study exhibited hypothermia, some hyperthermia, and others had body temperatures within the reference range. We believe that these variations in body temperature could be attributed to individual differences, causing the overall average to fall within the reference range.

Distemper virus infection is known to affect multiple systems, including the respiratory system, gastrointestinal system, genital system, CNS surface epithelium, kidneys (Appel and Summers, 1995; Rima and Duprex, 2006), skin (Appel and Summers, 1999), and even liver Kupffer cells (Maclachlan and Dubovi, 2010), leading to a generalized infection (Ettinger and Feldman, 2010). In respiratory system involvement, Bordetella bronchiseptica and other secondary bacteria are reported to act as co-pathogens (Sykes, 2014). Increased respiratory rates have been observed in dogs with bacterial, viral, or mixed pneumonia (Dear, 2020). In our study, no statistical difference was found in respiratory rates between DD and the HD. It is possible that the dogs in this study presented with varying forms of CDV infection, resulting in a range of respiratory rates that, when averaged, fell within normal limits.

A study reported an increase in pulse rate in newborn dogs with CDV infection (Saaed and Al-Obaidi, 2021). In the present study, however, no statistical difference was observed in heart rate between DD and the HD. We believe that the average pulse rate remained within normal limits in our study due to either the absence of viremia in the CDV infection cases or individual variations among the dogs.

The clinical variability of distemper infection and the presence or absence of viremia may contribute to individual differences in vital parameters among affected dogs. As a result, no statistically significant differences were identified in body temperature, heart rate, or respiratory rate between the HD and DD groups. In contrast, hematological and biochemical parameters, as well as Npt and PCT levels, exhibited more pronounced changes, likely reflecting the virus-induced tissue damage. These findings, particularly highlighting systemic inflammation and immune response mechanisms, offer valuable insights into the pathophysiology of distemper infection.

Canine distemper virus settles in the bone marrow and causes erythroid hypoplasia, leading to significant changes in hematologic parameters and a decrease in all leukocyte types, including neutrophils, eosinophils, basophils, and monocytes (Amude et al., 2007; Ezeibe and Udegbunam, 2008). In this study, WBC were statistically lower in DD compared to the HD, which aligns with the disease's progression, as the virus often affects the bone marrow. Okada et al. (2000) found that the reduction in total leukocytes and lymphocytes was associated with CDV-induced apoptosis in these cells. Most granulocytes are neutrophils, with a smaller proportion consisting of eosinophils and basophils. Neutrophils are the body's primary defense cells, engaging first in the immune response against pathogens (Brown and Rogers, 2001). Williams and Barker (2001) reported that the spread of CDV leads to the loss of T and B lymphocytes. In our study, we observed low white blood cell counts, granulocytopenia, lymphopenia, and monocytopenia. We believe this may be due to the virus settling in the bone marrow and lymph nodes, significantly weakening the immune system.

Saaed and Al-Obaidi (2021) noted that the per-

sistence of CDV in the bone marrow can lead to bone marrow depletion or suppression, thereby reducing erythrocyte production by affecting hematopoietic precursors. Additionally, the production of inflammatory mediators that inhibit erythropoiesis and shorten RBC lifespan are considered other possible causes for the reduction in erythrocyte count in dogs infected with CDV (Ezeibe and Udegbunam, 2008; Buragohain et al., 2017). In line with these findings, we believe that the release of interleukin-6 in infected dogs may result in a decrease in hemoglobin due to reduced iron utilization. The presence of CDV in the bone marrow likely slows down erythrocyte production by impacting hematopoietic precursors, contributing to the observed drop in erythrocyte count. This is further supported by the decrease in HCT, a parameter indicative of anemia. However, in our study, no statistical difference was observed between the MCV groups.

A primary cause of thrombocytopenia is the destruction or loss of bone marrow megakaryocytes due to viral antibody complexes adhering to platelet walls. Additionally, thrombocytopenia or reduced platelet production caused by viral effects on megakaryocytes has been observed in dogs with CDV (Axthelm and Krakowka, 1987). Studies have also reported that thrombocytopenia can occur in dogs following vaccination with attenuated live CDV (Axthelm and Krakowka, 1987; Weiss et al., 2000). Consistent with these findings, we believe that the decrease in platelet count observed in our study may result from fragmentation or a reduction in bone marrow megakaryocytes.

Serum ALP increase 2-6 fold in puppies six months of age and younger due to the predominance of bone ALP isoenzyme, which decreases as dogs grow and their epiphyseal plates close (Turgut, 2000). Buragohain et al. (2017) also reported elevated ALP in the blood of dogs with CDV, attributing this to the presence of ALP in the villus tips of enterocytes and villus damage in gastrointestinal disorders. These findings align with our study results. We believe that the increase in ALP observed in our study may be due to the inclusion of dogs younger than six months and severe degeneration of enterocytes resulting from viral presence in the gastrointestinal tract.

Increased serum ALP and GGT have been associated with intestinal mucosal and skeletal diseases (Buragohain et al., 2017; Gulersoy et al., 2022). Elevated GGT may also result from damage to the biliary system (Turgut, 2000). In our study, we believe that the increase in GGT could indicate villous damage due to diarrhea caused by CDV or damage to the biliary system, consistent with findings in the literature.

Low glucose concentration can occur in dogs with viral infections, including Canine Parvovirus (CPV) and CDV, which cause gastroenteritis due to villous damage and diarrhea (Prittie, 2004; Gulersoy et al., 2022). Severe bacterial infections may also lead to hypoglycemia associated with sepsis. Additionally, some viral infections with a non-fatal high viral titer have been reported to cause relative hypoglycemia (Castro et al., 2013; Gulersoy et al., 2022). Hypoglycemia has also been observed in patients with anorexia, enteropathy, and severe malnutrition (Turgut, 2000). In our study, glucose decreased in some CDV-infected dogs due to anorexia, severe diarrhea, and enteropathy.

In dogs, pancreatitis, obstruction of the pancreatic duct, pancreatic tumors, and abscess formation can lead to increased amylase activity (Turgut, 2000). CDV has been reported to form inclusion bodies in the pancreatic duct, pancreatic exocrine cells, and cells of the biliary system (Pardo et al., 2005; Maxie, 2015). Based on these findings, we believe that the increase in amylase activity observed in our study may be due to CDV forming inclusion bodies in pancreatic and other related tissues.

Biomarkers are key molecules that play a crucial role in assessing diseases by indicating fluctuations in physiological and pathological states. Both human and veterinary medicine emphasize the importance of reliable biomarkers for evaluating the prognosis and diagnosis of diseases (De Loor et al., 2013; Köse and Maden, 2013).

Neopterin is considered a biochemical marker of cell-mediated immunity, released in response to IFN-γ signals from monocytes, macrophages, and T lymphocytes. It is a byproduct of pteridine metabolism, which occurs during infectious diseases, oxidative stress, and immune activation (Berdowska and Zwirska-Korczala, 2001; Miao et al., 2018; Unuvar and Aslanhan, 2019; Akyuz and Gokce, 2021; Akyüz et al., 2022; Batı et al., 2023). Npt release reportedly begins three days before T cell proliferation peaks and increases about a week before specific antibodies appear, making it a reliable early indicator of inflammation and cell-mediated immunity (Cesur et al., 2014; Basbug et al., 2020). Elevated Npt have been observed in body fluids of individuals with

bacterial and viral infections (Akyuz et al., 2022; Murr et al., 2014), autoimmune diseases, and various stages of cancer (Basbug et al., 2020). Previous studies have demonstrated that Npt levels in body fluids increase in various conditions, including bacterial and viral infections, autoimmune diseases, and malignant tumors (Yildirim et al., 2008; Akyuz et al., 2022). It has been reported that Npt levels rise in response to Gram-negative bacterial infections, making it a significant biomarker for diagnostic purposes (Kozłowska-Murawska & Obuchowicz, 2008). Additionally, serum Npt levels have been found to be significantly higher in dogs with systemic inflammatory response syndrome compared to healthy dogs (Başbug et al., 2020). Our study suggests that CDV is a lymphotropic virus frequently associated with Gram-negative bacterial infections; thus, the increase in Npt levels may be attributed both to the direct effects of CDV and to the activation of monocytes and macrophages. The observation of increased Npt levels in the neurological, respiratory, and gastrointestinal forms of the virus, as well as in mixed forms, provides significant insights into the immunological dynamics of CDV infections. Elevated Npt levels have also been reported in immunological pathologies such as Alzheimer's disease (Leblhuber et al., 1999), inflammatory bowel diseases (Ertuğrul et al., 2007), and pulmonary tuberculosis (Soedarsono& Dolli 2019) in humans. The common feature of these conditions is the enhancement of the cellular immune response due to macrophage and monocyte activation (Başbug et al., 2020; Kozłowska-Murawska & Obuchowicz, 2008). Similarly, in CDV infections, secondary Gram-negative bacterial infections are frequently observed as a consequence of immune suppression. This indicates that the effects of CDV are not limited to viral infection alone but also contribute to immune system weakening, thereby increasing the susceptibility to other pathogens. The findings obtained reveal that CDV pathogenesis triggers a multifaceted immune response, which serves as a critical determinant of clinical progression. In this study, the potential role of Npt in the diagnosis of CDV was evaluated. According to the ROC analysis results, Npt demonstrated good diagnostic power (AUC = 0.812), suggesting its reliability as a biomarker for distinguishing CDV. With a sensitivity of 80%, Npt effectively identifies a significant proportion of affected individuals, although its specificity of 73.3% indicates a possibility of false positives among healthy individuals. Npt has been evaluated not only in relation to different forms of CDV but also in connection with the presence of secondary infections. Previous studies have reported that Npt is a biomarker reflecting immune system activation during viral infections, with levels increasing particularly in conditions associated with heightened cellular immune responses (Unuvar and Aslanhan, 2019; Akyuz and Gokce, 2021). In viral diseases such as CDV, which induce systemic inflammation, Npt is considered to play a crucial role in assessing disease severity. The results of our study further support the potential use of Npt as a valuable biomarker for the diagnosis of dogs with distemper.

Procalcitonin is produced as a prohormone of calcitonin in the parafollicular cells of the thyroid gland. Since it is converted into calcitonin and released into circulation, its circulating level remains very low under normal conditions. However, during infections or certain pathological conditions, parenchymal cells start producing PCT. This biomarker is abundantly secreted in response to inflammation due to its high prevalence in parenchymal tissues (Christ-Crain and Müller, 2007). Major sources of extrathyroidal PCT production include the pancreas, liver, spleen, adrenal glands, lungs, kidneys, brain, spinal cord, testes, stomach, small intestine, colon, abdominal fat, and WBC (Matur et al., 2021). PCT is released widely in response to microbial toxins and specific proinflammatory mediators, with concentrations rising rapidly following exposure to an infectious stimulus (Wacker et al., 2013; Batı, 2023). PCT have been shown to increase significantly in dogs with sepsis (Troia et al., 2018). Elevated levels have also been observed in dogs infected with *Babesia canis*, as well as in those with sepsis and severe bacterial infections (Brkljacic et al., 2014; Troia et al., 2018).

In contrast to the findings by Matur et al. (2021), which reported no statistically significant PCT elevation in dogs with viral infections, our study found that serum PCT in the DD were higher than the HD. Even there is a study in current literature (Kubesy et al., 2019) that reported an insignificant PCT increase in dogs with canine parvovirus infection same as our findings, we attribute this increase to the presence of sepsis in some of the study dogs and the systemic spread of CDV to various tissues, including the respiratory, gastrointestinal, and CNS. Clinical signs related to these systems were observed in the DD, along with frequent secondary bacterial infections, which may have contributed to elevated PCT. Additionally, the severe inflammation in these dogs

likely played a role in the increase of PCT. This observation suggests that PCT serves as an important biomarker for assessing CDV. In this study, the potential role of PCT in the diagnosis of CDV was evaluated, and according to the ROC analysis results, this biomarker was shown to have good diagnostic power (AUC = 0.817). This value suggests that PCT could be a reliable biomarker for diagnosing CDV. In terms of specificity, PCT's specificity of 93.3% indicates a high accuracy in correctly classifying healthy individuals as negative. The findings of this study support the use of PCT as a marker of immune response in CDV. However, the relatively low sensitivity of PCT (62.9%) suggests that its diagnostic accuracy may be limited, particularly in the early stages of the disease.

Our study has some limitations. First, this study should be carried out with a larger number of samples. Second, more relevant parameters should have been examined as well as Npt and PCT to better interpret the results and better reflect the clinical relevance. Third, we should have been ruled out the secondary bacterial infections to eliminate the non-viral infections for evaluating elevated PCT level. Then, age-related changes in biochemical parameters should have been evaluated. These limitations will be considered to get better and accuracy results for further studies.

CONCLUSION

In our study, we found that Npt and PCT biomarkers are valuable tools for diagnosing DD, as they act as positive acute phase reactants. Npt appears to have a significant diagnostic potential in dogs with distemper, while PCT may serve as a reliable biomarker that reduces the false-positive rate.These two biomarkers, which are widely used in human medicine but less frequently in veterinary medicine, show promise as indicators for various virus-induced diseases, particularly in dogs. We believe that they can illuminate future research on biomarkers in veterinary medicine

and provide valuable contributions to the existing literature. Future studies aim to conduct molecular investigations into oxidative stress mechanisms by analyzing parameters such as superoxide dismutase (SOD), catalase (CAT), lipid peroxidase (LPO), and malondialdehyde (MDA). Additionally, research will focus on the PI3K, MAPK, and JAK/STAT cellular signaling pathways, which play a crucial role in the inflammatory process. Furthermore, considering that chronic diseases may exhibit distinct biomarker patterns, future research should also explore the potential role of Npt and PCT in chronic conditions and other viral infections. Expanding the scope to include other viral diseases affecting companion animals will provide a broader perspective on the diagnostic and prognostic value of these biomarkers. This approach will enhance our understanding of their applicability across different disease processes, ultimately contributing to the development of more effective diagnostic and therapeutic strategies in veterinary medicine.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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