Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2024.214

RESEARCH ARTICLE

Importance of Biomarkers and Cytokines in the Prognosis of Canine Parvovirus Infection

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ARTICLE HISTORY (24-148)

Received: March 08, 2024
Revised: July 05, 2024
Accepted: July 14, 2024
Published online: July 28, 2024

Key words:
Biomarker
Citrulline
CPV-2
Cytokine
Interferon

A B S T R A C T

Canine parvovirus (CPV) poses a significant threat to dogs globally, leading to both illness and death. Examining biomarkers may enhance early detection of the disease, gauge hospital stay duration, increase disease severity, and estimate patient prognosis. The purpose of this study was to examine the influence of diagnostic (hematological and biochemical) and prognostic biomarkers (Citrulline, serum amyloid A (SAA), thiobarbituric acid reactive substances (TBARS), glutathione (GSH), superoxide dismutase (SOD), interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-10, tumor necrosis factor-alpha (TNF-α), and interferon (IFN)) in CPV infection. Blood samples were collected from CPV-positive dogs (Experimental Group, n=20) and healthy dogs (Control Group, n=20) included in the study. Consequently of laboratory analyses, it was observed that citrulline, TNF-α, and SOD levels were significantly increased in CPV-positive animals compared to healthy animals, while IL-6 and SAA levels decreased. Also, leukocyte (WBC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), thrombocyte (THR), pH, chloride (Cl), lactate (Lac), glucose, sO_2, and HCO_3 levels were lower in CPV-positive dogs compared to the healthy ones ($P<0.05$). As a result, it was interpreted that the inflammatory and oxidative response changes can be measured with the investigated parameters and thus the animals can be in the recovery period despite the clinical symptoms. It was concluded that the measured biomarkers can provide important information in terms of the prognosis of CPV infection when measuring in different periods of the disease or in experimental infection model studies.

To Cite This Article: Dik I, Gulersoy E and Simsek A, 2024. Importance of biomarkers and cytokines in the prognosis of canine parvovirus infection. Pak Vet J, 44(3): 875-881. http://dx.doi.org/10.29261/pakvetj/2024.214

INTRODUCTION

Canine parvovirus (CPV) is a widespread viral disease that affects both domestic and wild carnivores, with a high mortality and morbidity rate. It manifests severe symptoms including persistent vomiting, hemorrhagic diarrhea, and leukopenia, with puppies being particularly susceptible to myocarditis (Dik and Şimşek, 2021; Dik *et al.,* 2022). CPV type-2 is a member of the *Parvoviridae* family, *Parvovirinae* subfamily, *Protoparvovirus* genus (Cotmore *et al.*, 2014). CPV demonstrates a strong affinity for rapidly proliferating cells such as lymphoid tissue cells, intestinal crypt epithelial cells, bone marrow precursor cells, and cardiac muscle cells (Goddard and Leisewitz, 2010). There are two types of canine parvovirus (CPV): CPV-1 and CPV-2. Three genetic variants of CPV-2 are further classified as CPV-2a, CPV-2b, and CPV-2c. Even with extensive immunization campaigns, CPV-2 still poses a serious risk to puppies (Polat *et al.*, 2019). Clinical indicators of CPV infection typically include lethargy, vomiting, loss of appetite and diarrhea, which can range from soft to mucoid and hemorrhagic. Although the gastrointestinal tract, bone marrow, lymphoid tissue, and myocardium are the main areas affected by CPV, a few studies have found results pertaining to the skin and neurological system (Ulas *et al.,* 2024).

In a broad sense, a 'biomarker' can be defined as an objectively measurable indicator of biological conditions, serving as markers of physiological or pathogenic processes. In the context of CPV infection, these biomarkers are instrumental in raising suspicion of the disease, assessing hospitalization duration, gauging disease severity, predicting patient prognosis, and guiding treatment decisions (Schoeman *et al.*, 2013).

Cytokines are low molecular weight proteins which are involved in many pathophysiological processes of the

body (Van Reeth and Nauwynck, 2000; Hatipoglu and Keskin, 2022). Cytokines have a regulatory role in the immune system and play a role in the pathogenesis of viral diseases, as in many areas. As a result of the humoral and cellular response that begins with the virus entering the cell, many cytokines are released. These cytokines are crucial for controlling the host immune response and preventing the spread of viruses (Velazquez-Salinas *et al.*, 2019). In various viral infections, it has been reported that the expression of cytokines such as interferon (IFN)-β, IFN-γ, interleukin (IL) -4 ,-1β, IL-8, IL-10, IL-6, and IL-12 is increased in various viral infections (Atmaca and Kul, 2012; Baron *et al.*, 2014). According to reports, CVP is an immune system suppressant (Qiu *et al.*, 2010) it has also been noted that dogs with a poor prognosis had higher levels of proinflammatory cytokines like tumor necrosis factor-alpha (TNF-α), with an emphasis on the fact that this increase in puppies was correlated with mortality (Schoeman *et al.*, 2013). In addition to cytokines, interferons are crucial in a variety of viral infections. Especially in some viral diseases, identification of endogenous interferon deficiency suggests that viral diseases can be treated with active interferon treatments (Ospel'nikova, 2013).

Nitrogen metabolites and reactive oxygen species (ROS) have complex functions in a number of illnesses. These metabolites function as a host defense mechanism in addition to influencing the growth of viruses in living cells. Furthermore, at low concentrations, ROS aid in mitogenic activation. Therefore, in the early phase of viral infections, respiratory burst is activated in the cells where the virus is located and in the phagocytic cells. With the entry of the virus into the cell, ROS production increases, and at the same time, buffer action starts, and the level of antioxidant enzymes such as superoxide increases. It has been reported that antioxidants, together with cytokines, could play a role in the treatment of viral diseases, and that there is a decrease in antioxidants due to viral replication (Dik *et al.*, 2016). It has been stated that in CPV infections, lipid peroxidation and oxidative stress in erythrocytes increase with anemia, and that there are changes in the antioxidant capacity (Schoeman *et al.*, 2013).

Citrulline is a non-essential amino acid that is mainly synthesized in the liver and intestine (Dik *et al.*, 2023). Citrulline is regarded as a possible biomarker in cases of intestinal disorders since it is produced by enterocytes of the mucosa of the small intestine (Crenn *et al.*, 2008; Gerou-Ferriani *et al.*, 2018). Citrulline is used as a quantitative biomarker to determine intestinal epithelial cell integrity and is not affected significantly by nutritional status or systemic inflammatory response. The citrulline level decreases in diseases with loss of enterocytes (Gerou-Ferriani *et al.*, 2018; Dik *et al.*, 2023). Although clinical indications such as hemorrhagic diarrhea and vomiting may lead to a provisional diagnosis of canine parvoviral enteritis, one of the most sensitive and specific methods for virus detection is a DNA-based PCR assay. Routine hematochemical measurements and specific biomarkers can also improve the accuracy of the diagnosis and offer useful clinical prognostic information (Alves *et al.*, 2020).

Hematological parameters and some prognostic biomarkers may be examined in order to determine the duration of hospital stay, assess the severity of the disease, and forecast the prognosis of the patient. Additionally, biomarkers can help with decision-making when clients are being discussed with treatment choices or euthanasia. The purpose of this study was to ascertain the changes in serum amyloid A (SAA), citrulline, oxidative state (TBARS), antioxidant enzymes (SOD and GSH), various hematological blood parameters, and several cytokines (INF-γ, TNF-α, IL-1β, IL-2, IL-4, IL-6 and IL-10).

MATERIALS AND METHODS

This study included a total of 40 owned, mix-breed unvaccinated dogs, aged between 0-12 months. All of them were brought to the Animal Hospital of Selcuk University's Faculty of Veterinary Medicine, either for diagnosis and treatment or for routine checkups and vaccinations. Of these, 20 dogs with gastroenteritis complaints such as bloody diarrhea and vomiting were diagnosed with CPV as a result of clinical, hematological and CPV antigen rapid diagnosis test (Asan Easy Test PARVO, Asan Pharma. CO. LTD., Gyeonggi-do Korea. For CPV subtypes (CPV2, 2a, 2b, 2c) sensitivity: 100%, specificity: 98.8% results, and consisted the Experimental Group (n:20). The same examination protocols and rapid diagnosis test kit applications were performed on 20 dogs. Healthy dogs with negative CPV antigen test results and determined to be healthy as a consequence of clinical and laboratory examinations were included in the Control Group (n:20). Anamnestic findings showed that the dogs in the Experimental Group did not receive any therapeutic intervention for the clinical symptoms and the admission time to the hospital was between 3-5 days.

Venous blood samples (5-6 mL) were collected with minimal stress from all dogs via cephalic vein venipuncture. As soon as the blood was collected, some of it was immediately moved into tubes containing K3EDTA for hemogram analysis, and some of it was placed into tubes containing heparin for blood gas analysis. The remaining samples were placed in tubes without anticoagulant for serum extraction and centrifuged (Hettich, Germany) at 2,500 rpm for 15 minutes. The serum samples were transferred to 1.5 mL sterile eppendorf tubes with a sterile pipette.

The selection criteria for the aforementioned biomarkers aimed to provide valuable insights into the pathophysiology of CPV infection, thereby assisting in diagnosis, prognosis, and treatment strategies. Specifically, serum amyloid A (SAA), interleukins (IL-1β, IL-2, IL-4, IL-6, IL-10), and tumor necrosis factor-alpha (TNF-α) were chosen as markers of systemic inflammation, reflecting the immune response. Thiobarbituric acid reactive substances (TBARS), glutathione (GSH), and superoxide dismutase (SOD) were selected as markers of oxidative damage during CPV infection. Additionally, interferon (IFN) was chosen to assess the antiviral response of dogs during CPV infection. Commercial ready-to-use ELISA kits were employed to assess Canine TNF-α (Cat. no: E0025Ca, BT LAB, China), Canine INF-γ (Cat. no: E0011Ca, BT LAB, China), Canine IL-1β (Cat. no: E0002Ca, BT LAB,

China), Canine IL-6 (Cat. no: E00041Ca, BT LAB, China), Canine IL-4 (Cat. no: E0003Ca, BT LAB, China), Canine IL-2 (Cat. no: E0201Ca, BT LAB, China), Canine IL-10 (Cat. no: E0006Ca, BT LAB, China), TBARS levels (TBARS Assay Kit, Cat. no: E0132Ca, BT LAB, China), GSH content (Cat. no: EA0021Ge, BT LAB, China), SOD activity (Item no: 706002, Cayman, USA), Canine Serum Amyloid A (Cat. no: E0125Ca, BT LAB, China), and Canine citrulline (Cat. no: E0231Ca, BT LAB, China). The assays were conducted using an ELISA reader (MWGt Lambda Scan 200, Bio-Tek Instruments, Winooski, VT, USA) following the manufacturer's instructions.

During hemogram analysis, leukocyte (WBC), erythrocyte (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hematocrit (Hct), hemoglobin (Hb), and thrombocyte (THR) an auto analyzer for hematology was used to measure the levels (MS4e CFE 279, Hematology Analyzer, Melet Schlosing Laboratories, France) within 5-15 minutes of sample collection. For blood gas analysis, heparinized blood samples were used and within the scope of blood gas analysis pH, partial pressure of carbondioxide (pCO_2), partial pressure of oxygen (pO_2), base excess (BE), oxygen saturation (sO_2), bicarbonate (HCO_3) and lactate levels were measured using an autoanalyzer (GEM Premier Plus 3000, 74351, Blood Gas/Electrolyte Analyzer, Model 5700, Instrumentation Laboratories, USA) within the first 5-15 min after the sampling.

A statistical study was carried out utilizing SPSS 25.00 (SPSS for Windows®). To determine the distribution of data, a one-sample Kolmogorov-Smirnov test was employed. Parametric data are presented as mean ± standard error of the mean (SEM) and analyzed using the T-test, while non-parametric data are presented as median (min-max) and analyzed using the Mann–Whitney U test. Statistical significance was set at $p < 0.05$.

Receiver operating characteristic (ROC) curve analysis was conducted to assess the diagnostic and/or prognostic performance of the biomarkers mentioned above. The diagnostic efficacy was evaluated based on parameters including area under the curve (AUC, >0.500), standard deviation (Std. error), diagnostic sensitivity, and specificity (>70%). According to Hosmer and Lemeshow (2000), an AUC <0.5 indicates no discrimination, 0.6–0.8 is considered acceptable, 0.8–0.9 excellent, and >0.9 outstanding.

RESULTS

Biomarker Analysis: In CPV-positive dogs, citrulline, TNF-α, and SOD levels were statistically higher, whereas IL-6 and Canine SAA levels were statistically lower compared to healthy dogs ($p<0.05$). Additionally, it was determined that there was no statistical difference in the levels of IFN-γ, IL-1β, IL-2, IL-4, IL-10, TBARS and GSH in CPV positive dogs compared to healthy dogs. Results of inflammatory (TNF-α, IL-1β, IL-2, IL-4, IL-6 and IL-10) parameters, IFN-γ, Canine SAA and Canine citrulline, and oxidative status and antioxidant capacity (TBARS, GSH with SOD) values by groups from serum samples are presented in Table 1.

Table 1: Changes in biomarkers in CPV positive dogs (mean±SEM).

Parameters	Control Group (n:20) Mean±SEM	Experimental Group (n:20) Mean±SEM
Citrulline (nmol/ml)	8.65 ± 0.057	8.89 ± 0.020*
INF-γ (ng/L)	161.38 ± 29.83	92.63 ± 24.83
IL-1β (pg/ml)	0.55 ± 0.07	0.43 ± 0.07
IL-2 (ng/L)	11.59 ± 0.41	11.38 ± 036
IL-4 (pg/ml)	33.03 ± 1.33	31.56 ± 1.05
IL-6 (ng/L)	0.19 ± 0.13	0.03 ± 0.03*
IL-10 (pg/ml)	61.48 ± 1.96	61.36 ± 2.10
SAA (µg/ml)	3.97 ± 0.84	1.77 ± 0.42*
TBARS (nmol/ml)	9.76 ± 0.68	11.11 ± 0.92
TNF-α (ng/L)	0.22 ± 0.01	0.34 ± 0.05*
SOD (U/ml)	0.40 ±0.30	0.46 ± 0.06*
GSH (ug/ml)	128.89 ± 10.30	94.36 ± 9.63

SAA: Serum amyloid A (SAA), TBARS: Thiobarbituric acid reactive substances, GSH: Glutathione (GSH), SOD: Superoxide dismutase (SOD), IL: Interleukin -1β, IL-2, IL-4, IL-6, IL-10, TNF-α: Tumor necrosis factor-alpha, IFN: Interferon (IFN), * represents $P < 0.05$.

Blood Gas Analysis: The blood gas analysis showed that, in the Experimental Group, pH, sO2, HCO_3, Cl, and glucose levels were lower than those of the Control Group. Conversely, lactate (Lac) levels were observed to be higher in the dogs of the Experimental Group compared to the Control Group dogs ($P<0.05$). Furthermore, no statistically significant difference in the values of pCO_2, pO_2, K, Na, and Ca could be found between the two groups. Blood gas analysis results were presented in Table 2.

Table 2: Blood gas findings of dogs with CPV and healthy dogs.

Parameters	Control Group (n:20) Median (min-max)	Experimental Group (n:20) Median (min-max)
pH	7.4 (7.37 - 7.45)	7.305 (7.14 - 7.40)*
pCO_2 (mmHg)	36.8 (32.12 – 39.78)	35.9 (22.7 – 45.3)
pO_2 (mmHg)	29.55 (25.9 – 32.45)	31.6 (23.5 – 44.7)
sO_2 (%)	68.7 (59.9 – 79.8)	42.6 (24.6 – 65.6)*
K (mmol/L)	4.05 (3.6 – 4.7)	3.85 (2.7 – 4.9)
Na (mmol/L)	148 (136 – 151)	148 (129 – 156)
Ca (mmol/L)	1.09 (0.83 – 1.21)	1.005 (0.56 – 1.21)
HCO_3 (mmol/L)	21.75 (20 – 23.9)	17.95 (13.2 – 23.4)*
Cl (mmol/L)	120.5 (115 – 126)	110.5 (86 – 118)*
Lac (mmol/L)	1 (0.6 – 1.3)	2.3 (1.6 – 4.7)*
Glu (mg/dL)	112.5 (98 – 130)	89 (45 – 115)*

pH: Power of hydrogen, pCO_2: Partial pressure of carbondioxide, pO_2: Partial pressure of oxygen, sO_2: Oxygen saturation, K: Potassium, Na: Sodium, Ca: Calcium, HCO_3: Bicarbonate, Cl: Chlorine, Lac: Lactate, Glu: Glucose, * represents $P < 0.05$.

Hemogram Analysis: The hemogram analysis revealed that dogs with CPV infection exhibited lower levels of WBC, MCH, MCHC, and THR compared to healthy dogs, while they demonstrated higher levels of MCV and Hct ($p<0.05$). It was found that the RBC and Hb values did not differ between the two groups. Hemogram analysis results were presented in Table 3.

Table 3: Complete blood count of dogs with CPV and healthy dogs

Parameter	Control Group (n:20) Median (min-max)	Experimental Group (n:20) Median (min-max)
WBC (m/mm^3)	14.78 (8.8 – 18.5)	5.965 (1.29 – 8.78)*
RBC (m/mm^3)	6.915 (5.45 – 7.86)	6.485 (5.27 – 9.29)
MCV	63.705 (56 – 712.42)	73.2 (61.4 – 91.3)*
MCH	23.485 (19.4 – 25.11)	20.7 (18.1 – 23)*
MCHC	33.12 (28.44 – 39.42)	27.8 (25.20 – 31.1)*
Hct (%)	45.28 (37.21 – 58.19)	53.6 (37.9 – 84.8)*
Hb (g/dL)	15.09 (13.45 – 19.7)	14.25(10.5 – 21.4)
THR (m/mm^3)	290 (254 – 348)	108.5 (48 – 228)*

WBC: Leukocyte, RBC: Erythrocyte, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, Hct: Hematocrit, Hb: Hemoglobin, THR: Thrombocyte, * represents $P < 0.05$

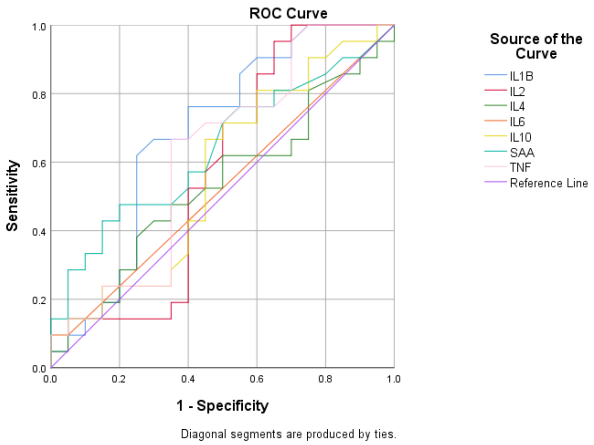

Fig. 1: ROC curves of inflammatory biomarkers

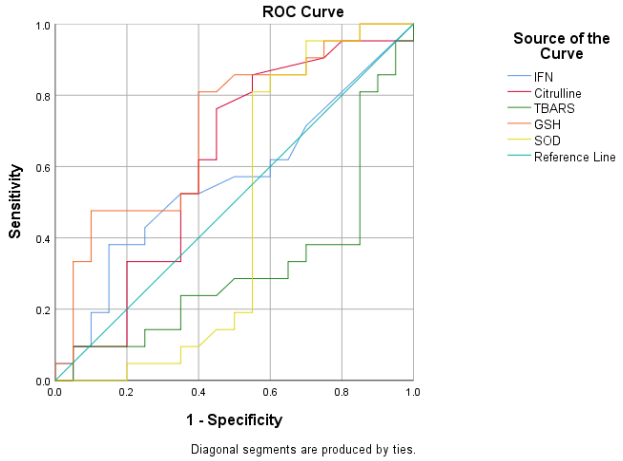

Fig. 2: ROC curves of prognostic and oxidative stress biomarkers.

Receiver Operating Characteristic (ROC) Curve Analysis: As a result of the ROC analysis, it was determined that all inflammatory biomarkers exhibited acceptable performance (0.6 < AUC < 0.8). Among these markers, TNF-α, IL-1β, IL-2 and IL-10 demonstrated high sensitivity (sensitivity > 70%) (Table 4).

In the ROC analysis of prognostic and oxidative stress markers, only citrulline and GSH demonstrated acceptable performance. Although citrulline, GSH, and SOD had high sensitivity (sensitivity > 70%), the overall performance of SOD was low (AUC < 0.5) (Table 5).

DISCUSSION

CPV leads enteritis and causes of morbidity and mortality, especially in young dogs (<6 months old). It is still life-threatening for puppies, in spite of the new strategies developed for the treatment and prevention of this infection (Mylonakis *et al.*, 2016). Some biomarkers are important for the diagnosis and prognosis of the disease (Schoeman *et al.*, 2013). Thus, it is believed that collectively evaluating specific biomarkers will provide an alternative approach to diagnosing, prognosing, and treating CPV infection.

Parvoviral infections can lead to damage to the intestinal mucosal barrier, potentially causing sepsis development and triggering systemic inflammatory response through TNF-α release (Ulas *et al.*, 2024). TNF-α, IL-1β, IL-6 cytokines and IFN-γ can cause local and systemic reactions (Nemzek *et al.*, 2007; Ok *et al.*, 2015).

Circulating citrulline is a protein produced by enterocytes of the small intestine. Therefore, it is considered as a plasma/serum biomarker for intestinal enterocyte function (Crenn *et al.*, 2008). Reduction in serum/plasma citrulline concentration has been reported to be due to the destructions occurring in enterocytes (Gerou-Ferriani *et al.*, 2018). It has been reported that in CPV enteritis, citrulline decreased significantly due to villous atrophy, but it is not a determinant biomarker of survival (Dossin *et al.*, 2011; Schoeman *et al.*, 2013). There are a limited number of studies on citrulline in CPV infection. It has been identified that in CPV infection, citrulline could not be produced because of the severe destruction of epithelial cells of the small intestine, especially in the crypts, and there was a decrease in serum/plasma citrulline levels compared to healthy animals (Dossin *et al.*, 2011). In the current investigation, the citrulline level in CPV positive animals increased statistically when compared with the healthy animals (Table 1). The increased levels of citrulline were seen as indicative of clinical improvement due to therapeutic interventions and high survival rates after the confirmation of the CPV diagnosis in diseased dogs. Additionally, their cytokine levels may have returned to normal levels since CPV-positive dogs are in the recovery phase. Citrulline is of great importance in repairing worn-out muscles and accelerating the recovery process (Rhim *et al.*, 2020). Decrease in citrulline levels is predicted in infections that cause destruction of intestinal enterocytes such as CPV (Dossin *et al.*, 2011). However, opposite results were observed in this study. This finding may be related to the admission time of the diseased dogs to the hospital and the dogs' being in the recovery period which is characterized by the repair of the destroyed enterocytes resulting in increased citrulline levels.

There is conflicting information about IL-6 levels in viral infections. It was reported that in some viral infections, IL-6 induced differentiation of immune system cells against pathogens, activated the immune system and inhibits virus replication (Kuo *et al.*, 2009), while in some viral infections, blocking IL-6 caused a decrease in viral load, an increase in IFN-γ and serine protease granzyme B production, and regulated the immune response (Wu *et al.*, 2015). However, it was reported that the increased IL-6 level stimulated anti-apoptotic genes and further exacerbated the infection by preventing the apoptosis of cells infected with the virus (Hou *et al.*, 2014). In another study, IL-6 knockout mice has weakened acute phase response compared with standard laboratory mice (Gomez *et al.*, 2006). In the presence of infection in dogs, SAA levels increased due to systemic inflammation (Ok *et al.*, 2015). However, on day 14 of the disease, it was seen that the SAA level in CPV positive dogs decreased to the levels as in healthy animals (Sahinduran *et al.*, 2016). In the present study, the IL-6 level decreased statistically in CPV positive animals (Table 1). Decreased IL-6 levels of dogs with CPV infection was interpreted that the immune system response in CPV positive animals suppressed the IL-6 level, increasing apoptosis and decreasing the viral load, thus reducing the level of SAA, which is an acute phase protein.

Table 4: ROC analysis of inflammatory biomarkers

Parameters	AUC	Std. Error	P value	Asymp. %95 CI		Cut-off	Sensitivity	Spesifity
				Lower Bound	Upper Bound			
IL-1β	0.685	0.086	0.043	0.515	0.854	0.25	85.7%	45%
IL-2	0.575	0.095	0.411	0.388	0.762	10.10	95.2%	35%
IL-4	0.533	0.092	0.715	0.354	0.713	29.39	66.7%	30%
IL-6	0.525	0.091	0.784	0.346	0.704	0.13	45%	50%
IL-10	0.567	0.092	0.465	0.386	0.747	57.12	81%	40%
SAA	0.638	0.087	0.130	0.467	0.809	1.72	57.1%	60%
TNF-α	0.621	0.091	0.183	0.444	0.799	0.17	81%	30%

Table 5: Prognostic and oxidative stress biomarkers

Parameters	AUC	Std. Error	P value	Asymp. %95 CI		Cut-off	Sensitivity	Spesifity
				Lower Bound	Upper Bound			
IFN	0.562	0.091	0.498	0.383	0.741	101.50	42.9%	75%
Citrulline	0.613	0.092	0.215	0.434	0.793	8.47	76.2%	55%
TBARS	0.318	0.087	0.046	0.148	0.488	9.12	38.1%	20%
GSH	0.708	0.082	0.022	0.547	0.869	93.30	81%	60%
SOD	0.454	0.102	0.611	0.254	0.653	0.28	85.7%	40%

In a previous study, Sahinduran *et al.* (2016) found that mice with CPV had considerably greater levels of IL-1, IL-6, and TNF-α. These levels started to decline on the third day after therapy began, and by the fourteenth day, they were even lower than those of healthy animals. It was also reported that IL-4 and IFN production was suppressed between days 7-21 of the disease (Liu *et al.*, 2017). In an in vitro study, it was reported that the nonstructural protein of CPV induced TNF-α and led to cell apoptosis (Sol *et al.*, 1999). In the current study, the TNF-α level was statistically high in CPV positive animals and IFN levels were partially decreased (Table 1). In the CPV-infected dogs of the present study, consistency of the increase in TNF-α level with the increase in IL-6 response might have contributed to the reduction of viral load by inducing apoptosis in cells, suggesting that most animals entered the recovery period, which is also consistent with the time of admission to the hospital of the diseased dogs.

According to reports, viral infections have the potential to damage cells, which raises the risk of oxidative stress (Hahn *et al.*, 2008). Oxidative stress markers such as MDA rise, while antioxidant enzyme activity (SOD, GPX, CAT) declines in virus-infected cells (Yavru *et al.*, 2015). Nevertheless, the treatment leads to a reduction in oxidative stress and cellular damage by alleviating inflammation and reducing virus replication (Chen *et al.*, 2015). A statistically significant increase in SOD levels and a non-statistical partial increase in TBARS levels were determined in the CPV-positive animals (Table 1). In the present study, in CPV infection, a non-statistical increase in TBARS level was seen due to oxidation and inflammation caused by virus replication ($p>0.05$). But, it is thought that the TBARS level started to decrease due to the time of admission to the hospital, which is between 3-5 days, (disease might be in the recovery phase) and the possibility of receiving antioxidant foods. This observation was supported by the higher levels of SOD and specific inflammatory markers, including TNF-α, SAA, and IL-6, in this investigation.

Some blood parameters are the most important for the evaluation of the health status and in the diagnosis of disease of animal. It is an important indicator for physiological and pathological changes in the organism (Terzungwe, 2018). Therefore, in veterinary medicine, hematological parameters are often evaluated for clinical status, nutritional balance, treatment follow-up, and disease diagnosis. Even though hematological parameters are not sufficient to make a definitive diagnosis of enteric disease, they help to create a differential diagnosis list and evaluate patient's response to treatment (Goddard and Leisewitz, 2010). In this context, whereas leukopenia with transient lymphopenia is a consistent finding observed in parvoviral enteritis cases, the absence of cytopenia has been reported as a 100% positive predictive value that could be used in evaluating survival in dogs with parvoviral enteritis for 24 hours after hospital admission (Goddard and Leisewitz, 2010; Terzungwe, 2018). In the current study, MCH, MCHC, WBC and THR levels were lower ($p<0.000$) while MCV and Hct values were greater ($p<0.017$ and $p<0.044$, respectively) in the Experimental Group compared to the Control Group. The statistical differences were not determined in RBC and Hb values between the groups ($p>0.05$) (Table 3). It was interpreted that the altered hematological parameters may be associated with cell hyperplasia seen in the bone marrow (Smith-Carr and Macintire, 1997). Furthermore, as the disease progresses, distinct hematological alterations could correspond with the manifestation of various cell-mediated immunological reactions (Terzungwe, 2018). Laboratory findings such as anemia, pancytopenia, thrombocytopenia/thrombocytosis, monocytosis and neutrophilic leukocytosis are commonly observed in dogs with enteritis associated with CPV (Prittie, 2004; Goddard and Leisewitz, 2010). Leukopenia, lymphopenia, and thrombocytopenia are seen more frequently in these findings (Hoskins, 1997). In the present study, MCHC and THR levels were lower in CPV positive dogs than the healthy ones. Higher MCV and Hct levels of the Experimental Group may depend on the combination of intestinal hemorrhage and dehydration (Macartney *et al.*, 1984). The hematological abnormalities found in this study were in line with results from earlier research on canines infected with canine CPV. However, absence of any statistical difference in RBC and Hb values was thought to be due to disease's period and dehydration and secondary polycythemia due to fluid lost from the gastrointestinal tract (Goddard and Leisewitz, 2010; Terzungwe, 2018).

One of the common findings in dogs with parvoviral enteritis is metabolic acidosis. In addition, it has also been reported that severe metabolic acidosis might be related to

poor clinical outcome and organ dysfunction (Toledo Maciel *et al.*, 2010). In the present study, metabolic acidosis (pH<7.32) (Hopper and Haskins, 2008) of the dogs in the Experimental Group is related to the loss of HCO_3 due to damage that was caused by the CPV intestinal villi (Goddard and Leisewitz, 2010). sO_2 is an important oxygenation parameter in animals with critical infections such as parvoviral enteritis. In addition, it has been reported that low levels of sO_2 together with pO_2 might be an important prognostic indicator of pulmonary oxygenation impairment in animals with sepsis and/or critical illness (Hückstädt *et al.*, 2016). As a result of blood gas and electrolyte analysis, pH, sO_2, HCO_3, Cl and glucose levels of the CPV positive dogs were found to be lower ($p<0.000$) and lactate levels were found higher ($p<0.000$) when compared to the healthy dogs (Table 2). In our study, when compared to the healthy Control Group, the low sO_2 levels of the Experimental Group might be related to poor oxygen perfusion and inadequate oxygenation due to circulatory disorders (Alves *et al.*, 2019).

Common electrolyte abnormalities seen in dogs with CPV related enteritis are hypokalemia, hyponatremia, hypomagnesemia, and hypochloremia. Together with these abnormalities, hypoglycemia or mild to moderate hyperglycemia has also been reported (Sykes, 2014). Lower Cl levels observed in CPV dogs that tested positive in this investigation as opposed to the healthy ones might be associated with vomiting and diarrhea accompanied by intestinal epithelial destruction (Mylonakis *et al.*, 2016). Low glucose levels together with the aforementioned electrolyte abnormalities might be related to severe malnutrition, stress-induced katecholamine activation, and depletion of glycogen stores, especially in puppies (Alves *et al.*, 2019). In the present study, Cl and glucose levels detected in the CPV positive dogs being within the reference ranges might be associated with the time of admission, disease period, nutritional status, and severity of vomiting and diarrhea (Goddard and Leisewitz, 2010). Studies in the field of human and veterinary medicine have stated that lactate measurement in body fluids such as plasma or serum is a prognostic indicator and a useful tool in triage and risk assessment (Rosenstein *et al.*, 2018). Lactate levels were found to be greater in CPV positive dogs than in healthy dogs in the current investigation. This finding was linked to dehydration, poor perfusion, and poor circulation as a result of the vomiting and diarrhea that are frequently observed in enteritis cases (Prittie, 2004; Mylonakis *et al.*, 2016).

A detailed examination of the present study's results reveals variance in proinflammatory cytokine levels and a decrease in IFN and SAA levels, suggesting that the affected animals brought to the hospital might be in the recovery period. High survival rates (0 deaths out of 20 diseased dogs) after the treatment may confirm these findings. Considering the fact that especially proinflammatory cytokines are activated in the first hours of infections, it is possible that these values tend to decrease and remain at normal levels in the later stages of infection. It has been observed that biomarker levels may reveal different results in the recovery period in CPV positive dogs, where age standardization cannot be achieved as in experimental studies and therefore the age range is wide. Especially since cytokines are produced locally in the organs selected by the infectious agent in the organism and pass to the plasma, they may not fully reflect the pathogenesis of the disease in terms of tissue response. This suggests that plasma cytokine concentrations may be a criterion for aiding diagnosis, but in some cases, it may be difficult to correlate them with disease severity or clinical status. For this reason, it is thought that especially clinician veterinarians can reveal the prognosis more clearly by considering some additional blood biomarker levels in their evaluations regarding the course of the disease. Consequently, in future studies with CPV, creating experimental models and performing periodic sampling will provide a more reliable evaluation of the results of blood parameters of sick animals that are obtained according to the course of clinical signs and clinical severity, and the effects of these parameters on the pathogenesis of the disease.

Ethical statement: The Veterinary Faculty Ethics Committee of Selcuk University in Konya, Turkey, approved the study procedure (Ethical approval number 2020/50 on 11.06.2020).

Acknowledgments: SUPABK provided assistance for this research (20401096).

Conflict of interest statement: There is no conflict of interest, according to the authors.

Authors contributions: I.D. Determination of the study's hypothesis, evaluation of data, literature review, and article writing. E.G. Collection of samples and conducting analyses. A.S. Analysis of data and evaluation of figures and tables. Additionally, all authors contributed to the writing and revision of the article.

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