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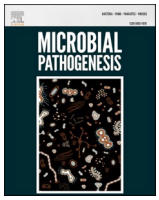



Changes in immuno-inflammatory and antioxidant biomarkers in serum and cerebrospinal fluid of dogs with distemper

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ABSTRACT

Canine distemper virus (CDV) causes a multisystemic disease with central nervous system involvement in dogs. Little is known about the role of immuno-inflammatory and redox-state biomarkers in the pathogenesis and diagnosis of naturally occurring CDV infection. Thus, the objectives of this study were: 1) to evaluate the potential differences in a profile of cytokines/chemokines, and inflammatory and redox-status biomarkers in serum between dogs with CDV-infection and healthy dogs, and 2) possible correlations between serum/blood and cerebrospinal fluid (CSF) of these biomarkers in dogs with CDV-infection.

Two groups of dogs were designed: 10 with CDV-infection, and 10 healthy. A total of 13 cytokines/chemokines, 3 inflammatory (C-reactive protein [CRP], haptoglobin [Hp], and butyrylcholinesterase [BChE]) and 3 antioxidants of redox status (cupric reducing antioxidant capacity [CUPRAC], Thiol, and ferric reducing ability of plasma [FRAP]) were analyzed in serum and CSF samples. Serum IL-7, IL-8, MCP-1, CRP, Hp, FRAP and Thiol levels were higher ($P < 0.05$) in dogs with CDV compared to controls. There were significant ($P < 0.05$) correlations only in IL-6 and MCP-1 between CSF and serum.

In conclusion, deregulated immune response, raised inflammation, and imbalances of redox homeostasis and antioxidant defense status may play role in the pathophysiological mechanism of neurological form of CDV-infection. The combination of clinical features and cytokine biomarkers (IL-7, IL-8 and MCP-1) might facilitate clinical diagnosis for neural involvement in dogs with CDV. Some cytokine/chemokine (IL-6 and MCP-1) in CSF are highly correlated with those of serum, indicating that serum samples could reflect the possible changes of these analytes in CSF.

1. Introduction

Canine distemper virus (CDV) causes a viral disease affecting a wide variety of mammals including dogs, foxes, wolves, ferrets, raccoons, and felines, as well as some primates [1,2]. CDV affects several systems including the gastrointestinal and respiratory tracts, and central nervous system (CNS) [3]. Common symptoms include fever, conjunctivitis, ocular/nasal discharge, coughing, vomiting, and diarrhea [4–6]. Serious neurological symptoms due to demyelinating temper of the virus may lead to blindness, paralysis, and convulsions followed by death of the animal [5].

Cytokine and chemokines are molecular mediators secreted by different cell types, and orchestrate immune-inflammatory response [7]. The effect of CDV infection on the production of some selected cytokines were investigated in an *in vitro* model, and results showed the differences in the cytokine expressions and viability of CDV among the cell cultures [8]. CDV induced interleukins (IL-1 and IL-6) and TNF-α expressions in the primary brain and macrophage-like cells. Also, cytokine induction was found to be correlated to the amount of CDV virions, and thus cytopathic effects were presumed to be due to a direct virus-mediated or cytokine-mediated process. These observations suggest that pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α, which might play an important role in CDV pathogenesis, are induced depend on the cell-specific manner [8]. Thus, their quantifications in biological samples such as serum and cerebrospinal fluid (CSF) are of importance to better understand the immune-inflammatory response, and to

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investigate whether the CNS involvement is present or not [9,10].

In the previous studies, cytokine mRNA expressions were investigated in experimental [11,12] and natural CDV-induced infections [13,14] or necropsied dogs [14]. In addition, there are two recently published studies on the association between neurodegeneration and cytokines/chemokines; one of them showed decreases in serum cytokines such as IL-1 and TNF-α in dogs with both gastrointestinal and neurological forms of CDV infection [15] and another reported the significantly elevated in serum levels of KC-Like/CXCL1 and MCP-1/CCL2 in CSF from CDV-infected dogs than those of healthy ones [16].

Acute phase proteins (APPs) are biomarkers of inflammation also known to cross the blood brain barrier (BBB) by saturable transport mechanism [17,18]. Thus, recent studies have focused on the role of these proteins in the mechanisms of the peripheral inflammation-induced neuroinflammation in humans [19,20]. Among APPs, CRP as a most widely used inflammatory marker is produced not only in the liver in response to proinflammatory cytokine IL-6 and TNF-α, but also in the brain due to demyelination [21]. In a previous study, haptoglobin (Hp), one of the major APPs, was reported to increase in patients with brain injury [22], and Alzheimer's disease [23]. Butyrylcholinesterase (BChE) is known as a negative APP, and associated with inflammation and oxidative stress in various diseases such as neurodegenerative disease in humans [24], and endocrine [25] and infectious diseases in dogs [26–28]. BChE plays a role in the neurotransmission by hydrolyzing the acetylcholine (ACh), and also reflects the intensity of cholinergic anti-inflammatory responses [29]. The relationship between APPs such as BChE activity, and neurodegenerative disorders have not been clearly determined [29].

Oxidative stress can lead to neural cell loss and eventually neurological dysfunctions [30]. The brain has high vulnerability to oxidative stress which is caused by a redox system imbalance [31]. This redox imbalance was characterized by the accumulation of reactive oxygen species (ROS) and consumption of the antioxidants was also reported during acute CDV infection in dogs [32]. Redox status and antioxidant capacity can be evaluated by the measurements of cupric reducing antioxidant capacity (CUPRAC), ferric reducing ability of plasma (FRAP) and thiol levels in biologicals samples such as serum [33,34] and saliva in dogs [35].

Taken together, little is known about the role of immuno-inflammatory and redox status biomarkers in the pathogenesis and diagnosis of the dogs with naturally occurring CDV infection. Thus, the objectives of this study were: 1) to evaluate if there could be differences in a profile of cytokines/chemokines, and biomarkers of inflammation and redox status in serum between dogs with CDV infection and healthy dogs, and 2) in the case of dogs with CDV infection, if there could be a correlation between serum/blood and CSF of these biomarkers. This could be useful to elucidate if any of the serum/blood analytes studied could reflect the changes that occur in CSF, knowing the fact that blood is much easier and minimal-invasive sample to obtain.

2. Material and methods

2.1. General variables and groups

A total of 10 un-vaccinated dogs diagnosed with CDV infection, and 10 healthy dogs (control group) were included in this study. Dogs with CDV were from different breeds (4 Anatolian shepherd dog, 3 mix-breed, 1 Golden retriever, 1 Siberian Husky, and 1 Cane Corso) and both sexes (5 males and 5 females), different ages (7.1 ± 1.7 months) and body weights (28.5 ± 9.4 kg). In control group, dogs were consisted of different breeds (3 Anatolian shepherd dog, 4 mix-breed, 2 Golden retrievers, and 1 Siberian Husky), both sexes (5 males and 5 females), different ages (6.9 ± 0.9 months) and body weights (26.3 ± 8.8 kg) (Table 1).

Table 1
Signalment and some clinical parameters (mean ± sd) in the dogs with canine distemper virus (CDV) and healthy dogs (controls).

Parameter	Healthy n = 10	Dogs with CDV n = 10	P value
Body weight (Kg)	26.3 ± 8.8	28.5 ± 9.4	NS
Age (months)	6.9 ± 0.9	7.1 ± 1.7	NS
Temperature (°C)	37.8 ± 0.6	39.9 ± 0.7	0.05
Pulsation (bpm)	122 ± 10	142 ± 12	NS
Respiration (rpm)	16 ± 6	24 ± 9	NS

Kg: Kilogram, M: male, F: female, bpm: beat per minute, rpm: respiration per minute.
NS: statistically not significant (P > 0.05) *P < 0.05.

2.2. Inclusion and exclusion criteria

CDV-infected dogs were selected from among the dogs which were referred by private vets or introduced by the owner to Bursa Uludag University Animal Hospital (Bursa – Turkey) for the diagnosis and treatments. Inclusion criteria were that the dogs had acute onset clinical signs (3–7 days) such as tremor, paraplegia, twitching of temporal and/or appendicular muscles along with the positive test results to CDV-antigen (CDV-Ag). Fever, innapetence/anorexia, and lethargy contributed to the neurological signs mentioned above in the dogs enrolled in this study. Both CSF and serum samples were collected on the same day that CDV-infected dogs presented to the clinic. However, the sampling days of CDV-infected dogs varied depending on the different days of presentation to the clinic and neurological signs of CDV (1 dog on day 4, 2 dogs on day 5, and 3 dogs on day 6). The days of reported neurological signs of CDV depended on the detailed patient history provided by the owners. Serum samples of control (healthy) dogs were sampled in the daily clinical routine on different days of presentation to the clinic. The presence of a concurrent disease such as external and/or internal parasitism, hepato-renal injury, anemia, and rickettsial infection was an exclusion criteria (Table 2).

Preliminary diagnosis of neurological CDV infection was confirmed by a snap chromatographic immunoassay diagnostic test kit showing the positive test results to CDV antigen in the conjunctival swap samples (Anigen® Rapid CDV Ag Test Kit, Bionote, Korea; sensitivity: 100 % *vs* RT-PCR, specificity: 98.5 % *vs* RT-PCR; detects all strains of CDV with no-cross reaction against CPV, CCV, ICH, and PI2). CDV-positive dogs were also checked for viral (canine parvovirus and coronavirus), protozoal (Giardia spp.) and parasitic antigens (D. immitis), and for

Table 2
Some hemato-biochemical parameters in the dogs with canine distemper virus (CDV) and healthy dogs (minimum – maximum).

Parameter	Healthy Dogs n = 10	Dogs with CDV n = 10	P value
WBC (x10^3/μL)	12.3 ± 3.5 (8.9–14.4)	3.6 ± 3.6 (0.3–12.0)	<0.001
RBC (x10^6/μL)	6.3 ± 0.8 (4.6–7.7)	5.8 ± 0.6 (4.5–5.9)	NS
HCT (%)	41.4 ± 5.2 (36.2–46.1)	40.4 ± 7.0 (31.1–48.3)	NS
PLT (x10^3/μL)	257 ± 61 (155–342)	309 ± 52 (251–363)	NS
Albumin (g/dL)	3.1 ± 0.7 (2.4–3.6)	2.9 ± 0.4 (2.5–3.2)	NS
ALP (U/L)	67.1 ± 26.4 (40.1–92.4)	80.7 ± 32.0 (50.1–110.3)	NS
ALT (U/L)	27.4 ± 6.5 (21.1–34.2)	44.3 ± 20.3 (24.5–67.8)	NS
BUN (mg/dL)	13.3 ± 4.4 (9.8–17.4)	16.8 ± 8.8 (10.1–26.2)	NS
Cr (mg/dL)	0.8 ± 0.4 (0.4–1.3)	0.9 ± 0.3 (0.5–1.4)	NS
TP (g/dL)	5.9 ± 0.9 (5.3–6.9)	6.2 ± 1.0 (5.0–7.2)	NS
Albumin (g/dL)	3.1 ± 0.7 (2.4–3.6)	2.9 ± 0.4 (2.6–3.3)	NS
Globulin (g/dL)	2.5 ± 0.4 (2.0–3.1)	3.7 ± 0.6 (3.1–4.2)	NS
Glucose (mg/dL)	122 ± 22 (101–145)	133 ± 35 (105–162)	NS
Na (mmol/L)	147 ± 5.2 (141–152)	148 ± 4.5 (140–153)	NS
K (mmol/L)	4.3 ± 0.3 (4.1–4.4)	4.7 ± 0.4 (4.2–5.2)	NS

WBC: White blood cell count. RBC: Red blood cell count. HCT: Hematocrit. PLT: Platelet count. ALP: Alkaline phosphatase. ALT: Alanine amino transferase. BUN: Blood urea nitrogen. Ca: Calcium. Cr: Creatinine. Na: Sodium. K: Potassium. TP: Total protein.

antibodies against vector-borne diseases (Anaplasma phagocytophilum, Anaplasma platys, Ehrlichia canis and Leishmania infantum) and protozoal infections (Toxoplasma gondii) with additional snap chromatographic immunoassay diagnostic test kits (Anigen® Rapid CPV/CCV/Giardia Ag Test Kit, Anigen Rapid CaniV-4, Anigen Leishmania Ab, Bionote, Korea; and FASTest® TOXOPLASMA; West Sussex - UK), as performed in our [36–38] and other previous studies [15,39]. The dogs which had negative test results for the diseases screened were enrolled in the present study.

Dogs that were found healthy based on the clinical and hematobiochemical examinations along with seronegative test results for the viral and parasitic agents mentioned above were used as controls. Additionally, all dogs enrolled in the study were not receiving any medications and vaccinations for 10 days at least before being admitted to the clinic.

2.3. Sampling

Routine diagnostic procedures such as anamnesis, physical examination, and laboratory analysis were applied to the dogs in this study. Blood samples were taken via venipuncture from cephalic veins into the vacutainer tubes with or without anticoagulant (EDTA) for hemogram (5-parts CBC differential with 22 parameters, VetScan® HM5, Abaxis, USA) and serum biochemistry analysis (Comprehensive profile, VetScan® VS2, Abaxis, USA), respectively.

Cerebrospinal fluid (CSF) samples were collected via the spatio atlanto-occipitalis after mild sedation (xylazine, Rompun Flacon, Bayer, Istanbul/Turkey) and local skin infiltration anesthesia (procaine 2 %, Pricain Flacon, Polifarma, Tekirdag/Turkey) for each of 10 dogs with CDV, as suggested [40]. EDTA anti-coagulated blood and serum specimens were collected from 10 clinically healthy dogs with the same ages admitted for a routine check-up procedure. After routine laboratory analysis, rest of the serum and CSF samples were frozen and stored at −80 °C until cytokines levels were analyzed.

2.4. Measurements

2.4.1. Hemato-biochemical analysis

Routine CBC analysis included leukogram with 5 part differentials, eritrogram, and thrombogram values, but only white blood cell (WBC), red blood cell (RBC) and platelet (PLT) counts were used in this study. Among serum biochemistry parameters analyzed, albumin, alkaline phosphatase (ALP), alanine amino transferase (ALT), blood urea nitrogen (BUN), creatinine (Cr), glucose (Glu), sodium (Na), potassium (K), total protein (TP) and globulin values were selected for this study.

2.4.2. Serum cytokine/chemokine measurements

The MILLIPLEX® Canine Cytokine Panel (Merck, Darmstadt, Germany), which including 13 canine specific plex kit (GM-CSF, IFN-γ, KC-Like, IP-10, IL-2, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, MCP-1, and TNF-α) with bead-based multiplex assays using the luminex technology were used for analyzing CSF and serum samples of dogs with CDV and serum samples of healthy dogs, according to the manufacturer assays protocol, as reported in our previous study [34].

2.4.3. Inflammatory and antioxidant markers

CRP and Hp levels which are known as major APP in dogs were measured in serum and CSF samples, as reported in our previous studies [26,27,34,37]. Briefly, CRP measurement was performed using an immunoturbidimetric assay (CRP OSR6147 Olympus Life and Material Science Europe GmbH, Hamburg, Germany). For Hp measurement, a commercially available method was used (Tridelta Ltd., Brey, Ireland). BChE activities were determined according to previously validated assays [27,41]. All analysis was performed using the Olympus AU600 (Olympus Diagnostica GmbH) analyzer. The concentrations of antioxidant markers of redox status including cupric reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP), and thiol were measured in the samples (serum and CSP), as reported in our previous study [34].

2.5. Statistics

Statistical analysis was performed by routine descriptive statistical procedures and software (SigmaPlot® Statistical and Graphing Software, California, USA). The changes of the analytes between the groups (healthy serum *vs* diseased serum) were assessed by the *student's t*-test. These data were expressed as mean ± sd. When Normality Test (Shapiro-Wilk) was failed for the evaluation of hemogram and biochemistry parameters, Mann-Whitney Rank Sum Test was performed, and their results were expressed as minimum and maximum. The Pearson correlation coefficient ***r*** was used for measuring a linear correlation between cytokines/chemokines levels and inflammatory and antioxidant markers of CSF and these same analytes in serum samples. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Clinical and hemato-biochemical findings

Of general variables, there were not statistical differences of ages and body weights between control dogs and dogs with CDV (Table 1). Among clinical findings, mean body temperature in CDV-infected dogs was higher ($P < 0.05$) than that of healthy controls (Table 1). Dogs with CDV had lower WBC due to neutropenia and lymphopenia with a normal Hct and RBC and PLT counts (Table 2). There were not any statistical differences in the biochemical parameters between the groups (Table 2).

3.2. Cytokine/chemokine profile

Serum and CSF cytokine results with statistically significant and non-significant changes between variables are given in Figs. 1 and 2, respectively. Mean IL-7 level was higher (68 ± 126 pg/dL; $P < 0.01$) in the serum samples than that of CSF (0.93 ± 0.67 pg/dL) from CDV-infected dogs and the serum samples from healthy dogs (7.0 ± 3.3 pg/dL, $P < 0.001$). Serum IL-8 levels were lower (2676 ± 3440 pg/dL; $P < 0.001$) than those of healthy controls (6864 ± 4001 pg/dL). MCP-1 chemokine increased significantly in serum samples from CDV-infected dogs (565 ± 538 pg/dL; $P < 0.001$) compared to controls (158 ± 52 pg/mL, Fig. 1). There were no statistically significances in serum levels of GM-CSF, IFN-γ, TNF-α, IL-2, IL-6, IL-10, IL-15, IL-18, and IP-10 between the dogs with CDV and controls (Fig. 2).

3.3. Inflammatory and antioxidant markers

The results of APPs (CRP, Hp, and BChE) and antioxidant biomarkers of redox status (FRAP, thiol, and CUPRAC) appear in Fig. 3. Serum CRP, Hp, FRAP and Thiol levels were higher ($P < 0.05$) in CDV dogs compared to those of healthy dogs, whereas serum BChE and CUPRAC levels did not show statistically significant changes in serum between the groups. CRP, Hp, FRAP, thiol, and CUPRAC levels were lower ($P < 0.05$–0.001) but BChE level was higher ($P < 0.001$) in CSF samples than those of serum samples in CDV-infected dogs.

3.4. Correlations

Among the parameters studied, there were statistically significant ($P < 0.05$) correlations only in IL-6 and MCP-1 between CSF and serum (Table 3). Serum levels of APPs and antioxidant markers did not show significant correlations with those of CSF samples in CDV-infected dogs (Table 4).

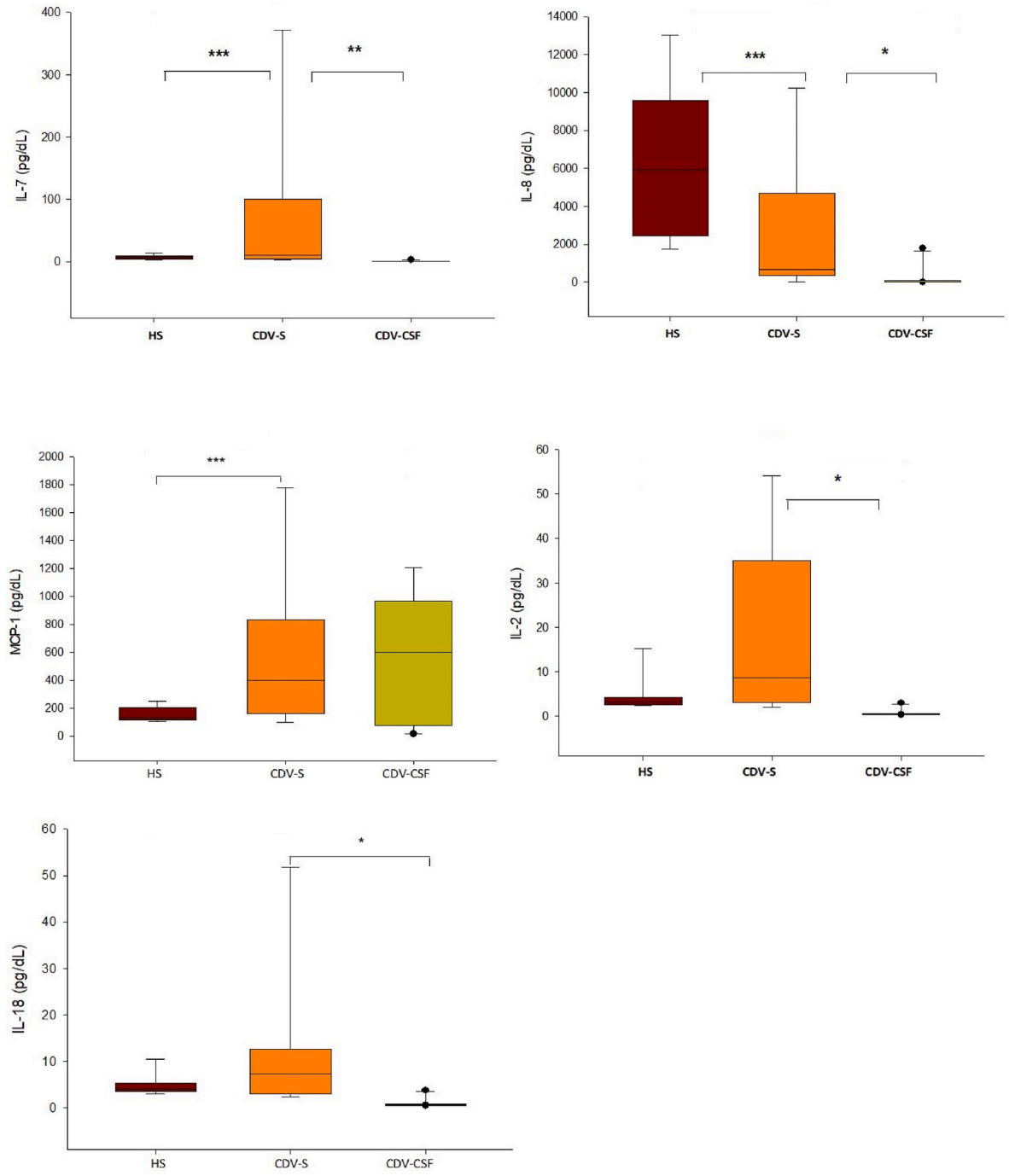

Fig. 1. Cytokine results were represented by the box-plot showing statistically significant changes between the serum samples (IL-7, IL-8, and MCP-1) in the dogs with neurological form of CDV infection (n = 10) and healthy control dogs (n = 10), and serum and CSF samples (IL-7, IL-8, IL-2, and IL-18) in diseased dogs. Note that CSF samples were collected only from diseased dogs. Healthy serum (HS), serum (CDV-S) and cerebrospinal fluid (CDV-CSF) from CDV- infected dogs. IL: Interleukin; MCP-1; Monocyte Chemotactic Protein-1
*P < 0.05, **P < 0.01, and ***P < 0.001 between two variables.

4. Discussion

Cytokines and chemokines are proteins coordinating the immune-inflammatory responses in the body, and their dysregulation plays a pivotal role in the development of neuroinflammation, neuro-degeneration, and demyelination both in the central and peripheral nervous systems [42]. CDV-induced demyelination is characterized clinically by the neurologic form of distemper in dogs. Some cytokines and their mRNA expressions were reported in the experimentally induced CDV infection [43], necropsied dogs [14], or naturally occurring cases [13,15,16]; however, how immune-inflammatory and antioxidant/redox status biomarkers in the CSF samples could be changed compared to those of serum, and which one of them would be of potential use as an additional tool for the diagnosis biomarker for the naturally occurring neurological CDV infection in dogs are not known yet.

In this study, observed clinical and hematological findings indicated the presence of systemic inflammatory response syndrome (SIRS) and immunosuppression, compatible with the early phase of the CDV infection. In this phase, when viremia is sufficiently high, CDV invades

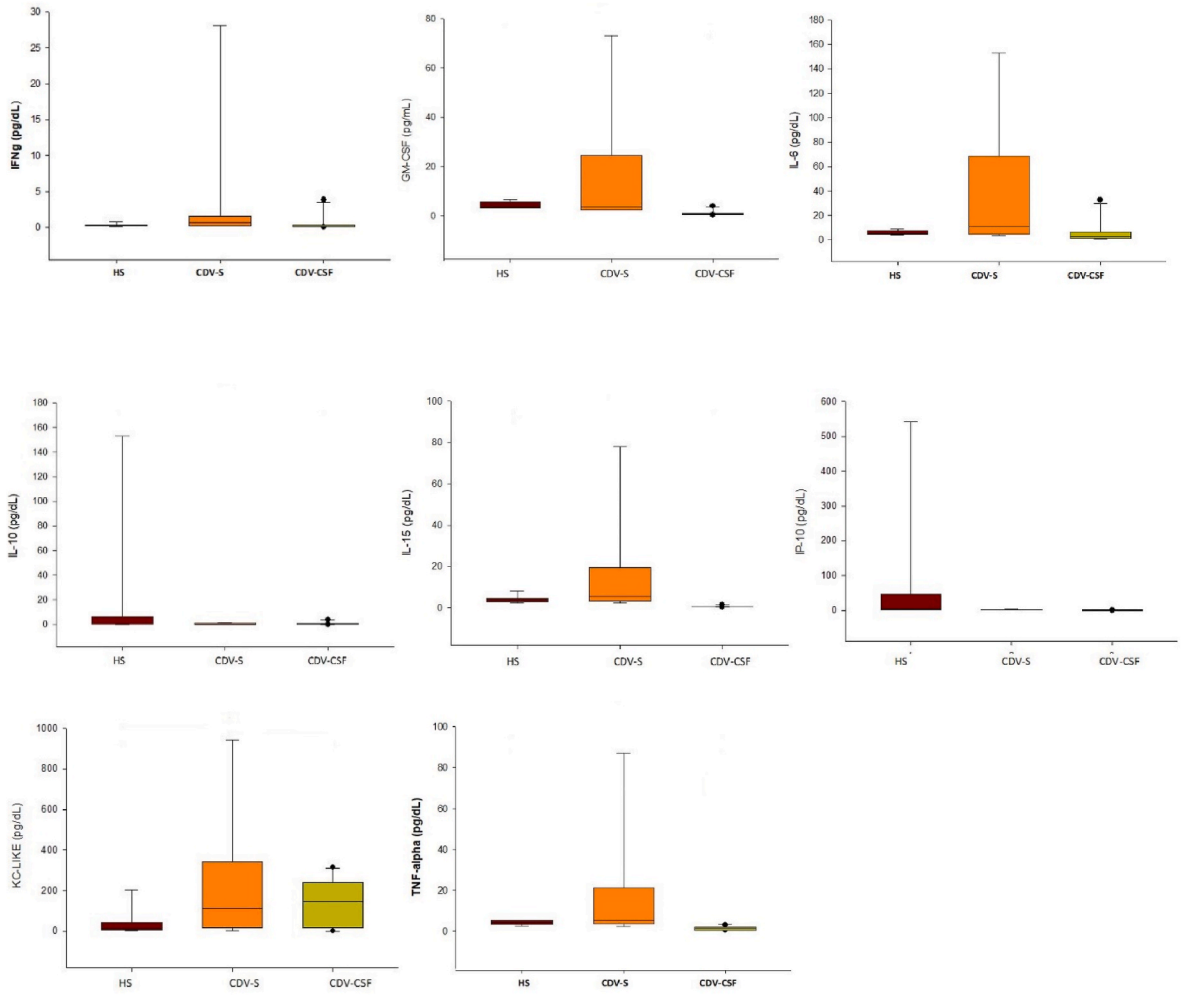

Fig. 2. Cytokine results were represented by the box-plot showing statistically not significant changes between the serum samples in the dogs with neurological form of CDV infection (n = 10) and healthy control dogs (n = 10), and serum and CSF samples in diseased dogs. Note that CSF samples were collected only from diseased dogs. Healthy serum (HS), serum (CDV-S) and cerebrospinal fluid (CDV-CSF) from canine distemper virus infected dogs.
GM-CSF: Granulocyte macrophage colony-stimulating factor; IL: Interleukin; IP-10: Interferon gamma inducible protein-10; KC-Like: Keratinocyte-derived Chemokine-Like; TNF-α: Tumor necrosis Factor alpha, and IFN-γ: Interferon gamma.

to the CNS [44], resulting in the neurological signs with or without gastrointestinal and respiratory signs of the illness. Dogs that are initially presented with neurological manifestation are also reported to show the systemic signs within a week or two [45]. However nervous signs may also occur without other signs [12].

From the cytokines analyzed in our study, only IL-7 (cytokine), IL-8 (chemokine/CXCL8) and MCP-1 (chemokine/CCL2) differed significantly between the serum samples, indicating their pathophysiological role and diagnostic potentials of neurological form of CDV infection. The increased IL-7 level in serum from CDV-infected dogs in our report agrees with the previous studies reporting elevated serum levels of IL-7 in dogs and humans with other inflammatory disease; atopic dermatitis [46] and multiple sclerosis [47], respectively. IL-7 is a cytokine of great importance for the development and survival of lymphocytes, and mediates its actions through binding to its specific receptor [48]. IL-7 is also known as a hematopoietic growth factor, secreted by stromal cells in the bone marrow, and examined as an immunotherapy agent for various diseases in animal and human studies [49,50]. Thus, observed increases in serum IL-7 levels in the present study may be related to the immunoregulatory response of the host to the viral agent, CDV. In this line, it would be interesting to explore if dogs that have more severe forms of CDV would have different values of the IL-7, and also its role as prognostic marker in this disease.

The decreased levels of serum IL-8 levels in dogs with distemper observed in this report may be resulted from overconsumption of IL-8 to prevent organ/tissue from oxidative stress, and to activate chemotaxis and phagocytosis [51]. This may also be explained by the fact that lack of detectable cytokine transcripts in whole blood samples was associated with a high antigen index and viremia, indicating that an overwhelming virus infection may suppress cytokine production, possibly due to paralysis of the immune system [13]. In contrast to our observation, serum IL-8 levels were higher in dogs with atopic dermatitis compared to healthy dogs [46], possibly reflecting differences in the physiopathology of these diseases.

In the two recent papers, cytokines/chemokines panels were studied in serum [15] or CSP sample [16] from dogs with CDV or ischemic stroke, respectively, whereas in the present study, these parameters were investigated in both samples from CDV-infected dogs. Although serum levels of INF-γ, IL-1β, IL-2, IL-6, and TNF-α were reported to decrease in the CDV-positive dogs in the previous study [15], they did not change at statistically significant levels in our study. This discrepancy may be due to the differences of the severity of the clinical cases and the measurement techniques between the studies. Other study investigating the changes in CSF cytokines by a multiplex immunoassay reported significantly elevations in CXCL1 and MCP-1 among 14 cytokines analyzed in dogs with ischemic stroke when compared to those of

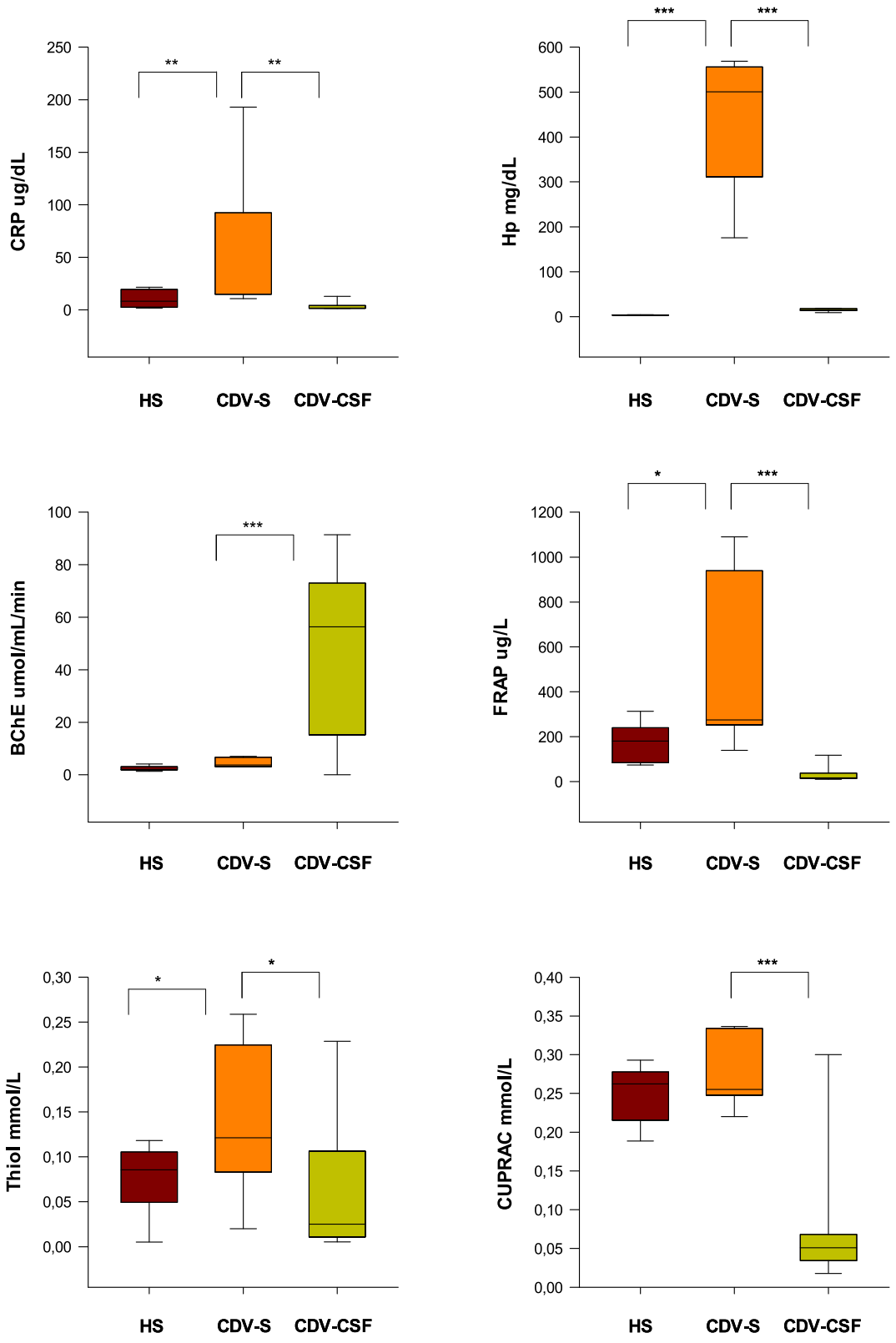

Fig. 3. The results of acute phase proteins (C-reactive protein [CRP], Haptoglobin [Hp] and Butyrylcholinesterase [BChE], and antioxidant markers (Thiol, Ferric Reducing Ability [FRAP], and Cupric Reducing Antioxidant Capacity [CUPRAC]) in the dogs with neurological form of CDV infection (n = 10) and healthy control dogs (n = 10). Note that CSF samples were collected only from diseased dogs. Healthy serum (HS), serum (CDV-S) and cerebrospinal fluid (CDV-CSF) from CDV-infected dogs. *P < 0.05, **P < 0.01, and ***P < 0.001 between two variables.

Table 3
Correlations in cytokines/chemokines between serum and cerebrospinal fluid (CSF) in dogs with CDV infection.

Serum *vs* CSF	*r*	P value
GM-CSF	−0.194	0.646
IL-2	−0.329	0.427
IL-6	0.712	0.047
IL-7	−0.080	0.849
IL-8	0.264	0.527
IL-10	−0.315	0.447
IL-15	−0.230	0.583
IL-18	−0.224	0.593
IP-10	0.131	0.757
KC-Like	0.039	0.925
MCP-1	0.783	0.021
TNF- α	0.581	0.131
IFN-γ	−0.169	0.689

Underlined results represent statistically significant correlation between the related parameters.
GM-CSF: Granulocyte macrophage colony-stimulating factor; IL: Interleukin; IP-10: Interferon gamma inducible protein-10; KC-Like: Keratinocyte-derived Chemokine-Like; MCP-1: Monocyte Chemotactic Protein-1; TNF-α: Tumor necrosis factor alpha, and IFN-γ: Interferon gamma.

Table 4
Correlations in acute phase proteins (APPs) and antioxidants/redox status markers between serum and cerebrospinal fluid (CSF) in dogs with CDV infection.

Serum *vs* CSF	*r*	P value
CRP	−0.366	0.333
Hp	0.151	0.698
BChE	0.359	0.342
FRAP	−0.128	0.742
Thiol	0.327	0.390
CUPRAC	−0.105	0.787

CRP: C reactive protein. Hp: Haptoglobin. BChE: Butyrylcholinesterase. FRAP: Ferric reducing ability of plasma. CUPRAC: Cupric reducing antioxidant capacity.

healthy dogs. In our study, CSF samples were not collected from healthy dogs, due to ethical reason. Thus, CSF MCP-1 level in dogs with CDV was compared to its serum levels from sick and healthy dogs, and observed increase in CSF was thought to be associated with the neurodegeneration/neuroinflammation [16], and related to disease severity and risk of death [52]. Since MCP-1 plays key roles in the clearance of axonal and myelin debris in neuroinflammatory conditions in dogs, this increase may also be considered as an adaptive physiological response to the demyelination [10]. MCP-1 is a chemokine known to recruit monocytes, T cells, and dendritic cells to sites of inflammation, and is involved in the neuroinflammatory processes that takes place in the various diseases of the CNS [53].

Our results showed the activation of systemic inflammation which was represented by increased serum levels of CRP and Hp. The higher cytokine and APPs levels in serum suggested that the neuroinflammation may be resulted in part from systemic inflammation. CRP and Hp could be transported from peripheral to cerebral circulation due to inflammation-induced BBB dysfunction, resulting the CNS injury [20]. Local production of both APPs in the brain may also contribute to the initiation or progression of the neuroinflammation in CDV-infected dogs [21–23].

In our report, there was enhanced anti-oxidant capacity which was represented by increased serum levels of redox status marker FRAP and anti-oxidant thiol in infected dogs. Oxidative stress and redox homeostasis play an important role in the pathophysiological mechanisms of neurodegenerative diseases by stimulating cytokines/chemokines production in the peripheral and cerebral circulations, and causes systemic and neuroinflammation [54]. The higher activity levels of serum systemic redox biomarkers (FRAP and thiol) measured in this study could have resulted from their overproductions to protected from oxidative stress and systemic inflammation in response to CDV infection. Our results would be in agreement with the previous study reporting the redox imbalance during acute CDV infection in dogs [32]. BChE is considered as a member of APPs and antioxidant system. Interestingly, in the present study, BChE was the only analyte that was found at higher concentrations in CSF samples than that of serum in infected dogs. This elevation in BChE in CSF may reflect a protective mechanism of inhibiting oxidative damage and neuroinflammation by the activation of cholinergic anti-inflammatory pathways [27–29]. Increased BChE may also be resulted from the degeneration of brain cells/tissue containing BChE activity [55]. Further studies are needed to investigate the relationship between BChE activity and neurodegenerative disorders, as suggested by Dong et al. [29].

Among the parameters studied, there were statistically significant correlations only in IL-6 and MCP-1. There were no significant correlations in APPs and antioxidants/redox markers, between CSF and serum in CDV-infected dogs, indicating that levels of CNS and peripheral inflammatory markers might be regulated independently of each other and that changes may not simply reflect the passive diffusion of circulating cytokines/chemokines into or out of the CNS [56]. The correlations of IL-6 and MCP-1 between serum and CSF showed that both analytes might play most important roles in BBB relation in CDV-infected dogs, as reported in humans with neuropsychiatric disorders [57].

There are some limitations of the study which are absence of 1) the necropsy and brain histopathology of the dogs with CDV, 2) long-term follow-up of the CDV-infected dogs and 2) CSF samples from healthy dogs, that could allow make comparisons to that of CDV positive dogs and see the outcomes. Long-term follow-up of all CDV-infected dogs could not be performed due to sampling in the daily clinical routine and different clinical preference of the owners, however, 3 dogs were euthanized 2–3 weeks follow-up in th clinic due to worsening clinical signs. Healthy CSF samples could not be collected for the ethical reasons. Another limitation of the study is that samples were not tested via PCR for CDV. However, as performed in the previous study [15], the diagnosis of CDV was confirmed according to the results of a snap chromatographic immunoassay diagnostic test kit with an acceptable sensitivity and specificity in this study. In addition, it could have been better if the groups with different etiology relating to CNS disorders were designed, and by this way, we could have speculated on whether observed changes in the parameters studied were due to only CDV infection or not. Interleukin changes in serum and CSF samples from the dogs with traumatic brain injury (TBI), tumor, and epilepsy were evaluated, and serum measurement of IL-1beta was found superior to that of CSF in dogs with TBI compared to epileptic dogs [58]. Pro- and anti-inflammatory cytokine expression and histopathological characteristics in canine brain with TBI was also reported [59]. These studies showed that serum and CSF cytokines might change regardless of the etiologic factors such as TBI, tumor, inflammation, and epilepsy in humans and dogs. New studies are needed to examine whether CDV infection can elicit different cytokine responses from other diseases affecting the CNS.

5. Conclusions

In conclusion, in dogs with the neurological form of distemper, there are increases in serum cytokines/chemokines (IL-7, IL-8 and MCP-1), and markers of inflammation (CRP and Hp), and redox status (FRAP and Thiol). This can reflect the activation of different physiopathological mechanisms associated with immuno-inflammation and redox homeostasis antioxidant defense status in the neurological form of distemper. The combination of clinical features and cytokine biomarkers might facilitate clinical diagnosis for neural involvement in dogs with CDV. In addition, the correlations that showed IL-6 and MCP-1 between serum

and CSF indicate that serum samples could reflect the possible changes of these analytes in CSF. Further studies are needed to clarify whether these analytes could serve as possible biomarkers in differentiating the neurological form of CDV-infection from among other diseases of the CNS involvement in clinical practice.

CRediT authorship contribution statement

Meriç Kocatürk: Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **A. Doğukan Öz:** Writing – original draft, Methodology, Data curation. **Alberto Muñoz:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Juan Diego Martinez:** Writing – review & editing, Writing – original draft, Software, Resources. **Jose Joaquin Ceron:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Data curation. **Zeki Yilmaz:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Funding acquisition, Formal analysis.

Institutional animal care and use committee approval

This study was performed in a daily routine clinical diagnostic approach, no ethical committee permission was needed. However, the owners were asked for permission via phone call for the rest of the freezed biological samples collected from their animals during their previous diagnostic procedures, and the samples allowed by the owners were used for this study.

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Declaration of competing interest

None of the other authors have any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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JJC and ZY conceived and supervised this study, MK, ADO, AM and JDM completed the main laboratory analysis, collected and analyzed data. MK, JJC and ZY wrote the first draft of the manuscript. All authors contributed to the critical revision of the manuscript and have read and approved the final version.

Data availability

Data will be made available on request.

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