RESEARCH ARTICLE

THE USE OF hG-CSF IN CANINE PARVOVIRAL ENTERITIS: ITS EFFECT ON CLINICAL AND LABORATORY VARIABLES

Erdem Gülersoy[1*], Canberk Balıkçı[1], Esma Kısmet[1], Adem Şahan[1], İsmail Günal[1]

[1] Harran University, Veterinary Faculty, Department of Internal Medicine, Şanlıurfa, 63200, Türkiye

***Corresponding author:**
Assistant Professor Erdem Gülersoy

Address: Harran University, Veterinary Faculty, Department of Internal Medicine Şanlıurfa, Türkiye

Phone: +905333344042
ORCID: 0000-0001-8511-0150
E-mail:egulersoy@harran.edu.tr

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ABSTRACT

Canine parvovirus is one of the most important pathogenic viruses, and canine parvoviral enteritis (CPE) is a highly contagious and often fatal disease of canines. The presence of neutropenia has been considered a hallmark of CPE since it is a negative prognostic indicator. Granulocyte colony stimulating factors (G-CSF) are essential molecules that control blood cell differentiation, proliferation and survival. Thus, the aim of this study is to investigate the efficacy of filgrastim, a human G-CSF, on clinical and laboratory variables compared to the standard treatment regimen of CPE. Of the 40 dogs with CPE, each treatment group within study had 20 dogs randomly assigned. In addition to standard treatment protocols, dogs in the Filgrastim group received hG-CSF subcutaneously once a day at a dose of 10 µg/kg for 3 days. Physical and laboratory examinations were performed at study admission and for 3 days during the hospitalization period. The most prominent finding was a higher granulocyte count of the Filgrastim group on the 1st and 3rd days than that of the Standard group (p=0.024 and p=0.05, respectively). Although the use of hG-CSF at the first admission prevents the decrease in the granulocyte count and positively affects the prognosis, it should be kept in mind that the severity of clinical and laboratory findings at the first admission and at the initiation of the treatment is important in determining the prognosis and clinical outcome of CPE. As a result, it was concluded that the use of hG-CSF may have therapeutic significance in cases of CPE.

Keywords: Blood cell count, dog, prognosis, treatment

INTRODUCTION

Canine parvoviral enteritis (CPE) is one of the most common and virulent enteric diseases of the canine population. CPE causes high rate of morbidity and mortality in dogs, especially in young puppies (Mylonakis et al., 2016; Mazzaferro, 2020). Canine parvovirus (CPV) infection occurs via oronasal exposure in unvaccinated or inadequately immunized dogs by ingestion of CPV shed in the vomit or feces of affected animals. After the viral replication in oropharyngeal and mesenteric lymph nodes and subsequently in thymus, infected animals become viremic within 1 to 5 days of exposure (Mazzaferro, 2020). Then CPV targets rapidly dividing cells of the bone marrow, intestinal epithelial crypts, cardiac myocytes, and occasionally lung, spleen, liver, and kidneys (Strom et al., 2015; Ford et al., 2017). Following exposure and an incubation period that may vary from 4 to 14 days, virus shedding begins mostly in a few days before the onset of clinical symptoms such as hemorrhagic diarrhea and vomiting. Next, the intestinal mucosa becomes grossly denuded as enterocyte turnover is disrupted, resulting in blunting and atrophy of the intestinal villi, which causes nutrient malabsorption and enteric bacterial translocation, along with the clinical signs, including vomiting and diarrhea (Mazzaferro, 2020). Subsequently, thymic cortex collapses and is destroyed as a result of viral infection. Together with destruction of leukocyte precursors in the bone marrow, this situation results in prominent leukopenia in infected animals (Decaro et al., 2005; Mazzaferro, 2020).

Canine parvoviral enteritis has an approximately 91% mortality rate among dogs when left untreated (Prittie, 2004). Clinical outcome, prognosis and survival often depend on the severity of clinical and laboratory findings at the time of initiation of treatment (Goddard et al., 2008; Kalli et al., 2010). Mortality can be decreased to 5–20% from 91%, and survival chances may reach to 80–95% with an appropriate treatment regimen (Kalli et al., 2010; Mylonakis et al., 2016). Among the abnormal laboratory findings, leukopenia is an important contributor to impaired immune function, and morbidity and mortality are mostly associated with bacteremia in cases of CPE (Decaro et al., 2005; Mylonakis et al., 2016; Gulersoy et al., 2022). Granulocyte colony-stimulating factors (G-CSFs) are a class of hematopoietic regulatory glycoproteins that promote the proliferation, differentiation, and activation of neutrophils in the bone marrow (Armenise et al., 2019). Increases in endogenous concentrations of canine granulocyte colony-stimulating factor (cG-CSF) have been reported to improve neutrophil counts in puppies with experimental CPE (Kraft and Kuffer, 1995; Armenise et al., 2019). Also, the use of human G-CSF (hG-CSF) was reported to promote bone marrow stimulation and release of neutrophils (Duffy et al., 2010). Although one study did show that the use of recombinant hG-CSF improved neutrophil counts in a small population of puppies infected with parvovirus (Kraft and Kuffer, 1995), other studies showed no improvement in neutrophil count, length of hospitalization, or survival (Mischke et al., 2001; Duffy et al., 2010). However, studies demonstrated that cG-CSF at a dose of 5µg/kg once daily is effective at statistically increasing neutrophil, monocyte, lymphocyte and total leukocyte counts (Duffy et al., 2010; Armenise et al., 2019). The reasons why the expected clinical and laboratory improvements were not observed are as follows: depletion of the storage pool and of more mature progenitor cells in the bone marrow, lack of C-GSF receptors secondary to granulopoietic progenitor cell depletion, and the latency before the effects of the

C-GSFs become measurable (Hammond et al., 1991; Mischke et al., 2001).

Treatment of CPE primarily is supportive and symptomatic until clinical signs such as vomiting and hemorrhagic diarrhea resolve (Gerlach et al., 2020). In general, improvement in clinical symptoms often corresponds with rebound in leukocyte count (Prittie, 2004). Although hG-CSF is known to increase hematopoietic stem cell and neutrophil counts and modulate the immune system (Johannesen et al., 2018), its effectiveness in dogs with CPE is controversial, and the use of recombinant cG-CSF may not necessarily improve survival (Mischke et al., 2001; Mazzaferro, 2020). Therefore, the aim of this randomized controlled study is to investigate the efficacy of filgrastim, an hG-CSF, on clinical and laboratory variables compared to the standard treatment regimen in dogs with CPE.

MATERIAL AND METHODS

Ethics committee approval

The protocol used was approved by Harran University Institutional Animal Use and Care Committee before study initiation (session and permit no: 2022-010/01-09), and informed owner consent was obtained.

Animals

The animal material of the present study was consisted of a total of 40 dogs with CPE, all were admitted to the animal hospital of Harran University Veterinary Faculty for diagnostic/ treatment purposes, each treatment group within study had 20 dogs randomly assigned (Filgrastim group n: 20, Standard group n: 20). Dogs were considered eligible for study inclusion if they had never been vaccinated against CPV, were demonstrating clinical signs consistent with CPV such as anorexia, lethargy, vomiting, hemorrhagic diarrhea or some combination of these symptoms, tested positive for CPV using an ELISA-based parvovirus antigen test kit (SNAP Parvo Test, Idexx, Westbrook, Maine), and had not received any treatment for their condition. All levels of clinical severity relating to CPV infection were considered eligible for study inclusion. Dogs were excluded from the study if they had any comorbidities such as intussusception, parasitic infestation and concurrent infection upon hospital presentation that could affect the prognosis or outcome, had received prior treatment or displayed a temperament such as aggression that would affect study participation.

Baseline anamnestic data obtained from each dog included breed, sex, age, duration of clinical symptoms before hospital administration, related medical history, baseline vital signs and physical examination findings, including pulse rate, rectal temperature (°C), respiratory rate, gingival capillary refill time (seconds), and body weight (kg). The study population consisted of 16 intact males and 24 intact females, all were assigned randomly. In order not to affect the results, breeds such as Rottweiler, Doberman and German Shepherd, which are known to be susceptible to viral diseases such as CPE (Mylonakis et al., 2016), were not included in the study, and most of the dogs were cross breeds.

Treatment protocol

All the dogs from both study groups had intravenous (IV) catheters placed (*vena cephalica*) and received goal-directed fluid therapy at the first admission to the hospital using an isotonic crystalloid (Polifleks, 0.9 % İzotonik Sodyum Klorür IV İnfüzyon İçin, Polifarma, İstanbul, Türkiye), with dextrose (Polifleks, 5% Dekstroz, Polifarma, İstanbul, Türkiye) supplementation,

as indicated by the initial venous blood gas and electrolyte results. After fluid therapy, all the dogs had an IV catheter maintained throughout the hospitalization period for delivery of isotonic crystalloid fluids (120 mL/kg/d IV) with potassium chloride (Polifleks, İzolen, İstanbul, Türkiye) supplementation, maropitant citrate (1 mg/kg IV q24h; Cerenia, Zoetis, Michigan), ceftriaxone (25 mg/kg IV q12h; Unacefin, Yavuz İlaç, İstanbul, Türkiye), and dextrose supplementation, as indicated. Ondansetron (0.5 mg/kg IV or SC; West-Ward, New Jersey) was given to any dog with vomiting that was refractory to maropitant. In addition to this standard treatment protocol, hG-CSF (Filgrastim, Neupogen, Amgen İlaç, İstanbul, Türkiye) was injected subcutaneously once a day at a dose of 10 µg/kg (Mischke et al., 2001) for 3 days to the dogs of the Filgrastim group. Additional medications could be provided during the hospitalization period depending on the condition of the diseased dogs and were recorded in the medical record. The treatment protocol is summarized in Table 1.

Table 1 The treatment protocol of the study

Fluid therapy‡	Antibiotic	Antiemetic	Supplementation
- Isotonic crystalloid (120 mL/kg/d IV) - Dextrose (5%)* - Potassium chloride□	- Ceftriaxone (25 mg/kg IV q12h)	- Maropitant citrate (1 mg/kg IV q24h) - Ondansetron (if it is refractory to maropitant citrate) (0.5 mg/kg IV or SC)	- Duphalyte (50ml / 5 kg body weight) - Filgrastim§ (10 µg/kg SC q24h)

‡ Required amount and the type of fluid therapy were determined by the venous blood gas and electrolyte results

* Dosage is dependent upon the age, weight and clinical condition of the patient as well as laboratory determinations

□ Administered according to the laboratory determinations, not to exceed 0.5 mEq/kg/hr

§ Administered only to the dogs of the Filgrastim group.

Blood sampling

Venous blood samples were collected via *vena cephalica* venepuncture at study admission and for 3 days during the hospitalization period with minimal patient stress. Recorded clinical criteria are vital signs such as rectal temperature, pulse rate, respiratory rate and gingival capillary refill time along with venous blood gas and electrolyte and hemogram analyses results. Blood gas and electrolyte analysis was performed from heparinized venous blood samples with the epoc® Blood Analysis System (Siemens Healthineers, Germany) autoanalyzer. Hemogram analysis was performed from venous blood samples with K_3EDTA using pocH-100i® hematology analyser (Sysmex Corporation, Japan). Blood gases and hemogram analyses were performed within 5-10 minutes after sampling.

Statistical analysis

The data were evaluated using SPSS 25.00 (SPSS for Windows®) statistical software. One sample Kolmogorov-Smirnov test was applied to determine whether all data were parametric or non-parametric. Non-parametric data were evaluated as median (min, max) with Mann-Whitney U, and Kruskal-Wallis test. Statistical significance was considered as $p<0.05$ for all data.

RESULTS

Anamnestic data findings

Baseline anamnestic data including age, body weight, symptom duration prior to enrollment and hospitalization time during the study presented in Table 2.

Table 2 Anamnestic data findings

Parameters	Filgrastim group n: 20 median (min, max)	Standard group n: 20 median (min, max)	p value
Age (days old)	4.5 (3.5, 8)	5 (3.5, 7.5)	0.312
Body weight (kg)	9 (4.5, 12.4)	8.05 (4.6, 11.7)	0.615
Symptom duration (days)*	2.5 (1, 5)	2 (1, 6)	1.000
Hospitalization duration (days)□	5.5 (4, 7)	6 (4, 8)	0.196

* The duration of symptoms prior to enrollment in the study

□ Hospitalization time during the study

Physical examination findings

Respiratory and pulse rates were higher in the Filgrastim group on the 0th day (p=0.030). No statistical difference was observed in terms of other parameters and days. Physical examination findings are presented in Table 3.

Blood gas and electrolyte analysis findings

In the Standard group, pH level was lower than the Filgrastim group of the 1st day (p=0.022). On the 3rd day, BE value was higher in the Filgrastim group (p=0.021). No statistical difference was detected in terms of other parameters and days. Blood gas and electrolyte findings are presented in Table 4. All results of the blood gas and electrolyte analyses are shown in the Supplementary file.

Hemogram analysis findings

The granulocyte count was higher in the Filgrastim group on the 1st and 3rd days than that of the Standard group (p=0.024 and p=0.05, respectively). RBC count was higher in the Standard group on the 1st day (p<0.000). MCV and MCH levels were higher in the Filgrastim group on the 0th, 1st and 3rd days (p<0.05). Hct level was higher in the Standard group on the 1st day (p<0.000). The MCHC level was higher in the Filgrastim group on the 1st and 3rd days (p=0.001 and p=0.003, respectively). Also, the Hb level was higher in the Standard group than in the Filgrastim group on the 1st day (p=0.009). Hemogram analysis findings are presented in Table 5. All results of the hemogram analysis are shown in the Supplementary file.

Outcome and hospital discharge

Dogs were considered available for hospital discharge once clinical signs such as vomiting and diarrhea had resolved, were rehydrated and drinking and eating voluntarily, and hemogram results indicated a rebound from their neutrophil nadir. The median duration of hospitalization for the Filgrastim group was 5.5 (4, 7) and for the Standard group was 6 (4, 8) days (p=0.196). During the hospitalization period, 3 dogs from the Filgrastim group died (2 dogs on the 4th day and 1 dog on the 5th day). In the Standard group, 4 dogs died during the hospitalization period (2 dogs on the 5th day, 1 dog on the 6th day and 1 dog on the 7th day). Anamnestic, blood gas and electrolyte, and hemogram analytes of each group, differing according to survival status, are presented in Table 6, 7, 8 and 9, respectively.

Table 3 Physical examination findings

Parameters	Day*	Filgrastim group n: 20 median (min, max)	Standard group n: 20 median (min, max)	p value
Respiratory rate (breaths/min)	0th	40 (24, 60)	31 (20, 36)	0.005
	1st	34 (22, 45)	27 (16, 51)	0.341
	3rd	32 (15, 37)	29 (21, 35)	0.519
Heart rate (beats/min)	0th	110 (75, 150)	87 (72, 104)	0.030
	1st	112 (70, 142)	86 (80, 110)	0.101
	3rd	110 (70, 160)	90 (80, 112)	0.163
Body temperature (°C)	0th	39.7 (36, 40.1)	39.6 (35.8, 41)	0.988
	1st	37.9 (36.9, 39.5)	38.2 (37, 39.3)	1.000
	3rd	38.05 (37, 39)	38.1 (37, 39.3)	0.892
Capillary refill time (sec)	0th	3 (2, 3)	3 (2, 3)	1.000
	1st	3 (2, 3)	3 (2, 3)	1.000
	3rd	3 (2, 3)	3 (2, 4)	0.660

*0th day refers to the first day of admission to the hospital. 1st and 3rd days are in the hospitalization period during the study

Table 4 Blood gas and electrolyte analysis findings

Parameters	Day*	Filgrastim group n: 20 median (min, max)	Standard group n: 20 median (min, max)	p value
pH	0th	7.31 (7.1, 7.35)	7.28 (6.97, 7.44)	0.423
	1st	**7.38 (7.35, 7.42)**	**7.34 (7.22, 7.44)**	**0.022**
	3rd	7.42 (7.29, 7.45)	7.38 (7.3, 7.45)	0.159
pCO_2 (mmHg)	0th	37.15 (30, 44.8)	34.75 (25, 56.9)	0.659
	1st	37.5 (34.6, 39.2)	38.3 (32.2, 51.7)	0.174
	3rd	42.55 (34.8, 54)	42.25 (31.7, 65.3)	0.349
pO_2 (mmHg)	0th	39.55 (18.7, 47.6)	39.95 (26.9, 55.5)	0.339
	1st	36.3 (25.9, 47.8)	41.9 (29.2, 68)	0.098
	3rd	32.55 (23.1, 48.4)	35.15 (21.6, 62)	0.375
Potassium (mmol/L)	0th	4 (3, 4.7)	3.95 (2.3, 7.3)	0.779
	1st	3.7 (3, 4.7)	4.05 (3.2, 7.44)	0.320
	3rd	4.35 (3.2, 4.75)	3.55 (3, 4.9)	0.177
Sodium (mmol/L)	0th	155.5 (144, 175)	154 (147, 165)	0.789
	1st	147 (142, 164)	146.5 (134.2, 153)	0.234
	3rd	149 (142, 163)	145.5 (135, 156)	0.076
Calcium (mmol/L)	0th	1.08 (0.47, 1.4)	0.95 (0.4, 1.34)	0.384
	1st	1.21 (0.59, 1.44)	1.22 (0.63, 1.59)	0.329
	3rd	0.97 (0.84, 1.58)	1.12 (0.66, 1.45)	0.330
Chlorine (mmol/L)	0th	122 (110, 132)	125.5 (115, 139)	0.205
	1st	115 (112, 122)	115 (111, 120)	0.946
	3rd	115.5 (101, 121)	116.5 (100, 121)	0.451
Glucose (mg/dL)	0th	91.5 (46, 169)	85 (17, 98)	0.077
	1st	103.5 (82, 123)	99.5 (71, 133)	0.541
	3rd	100 (59, 111)	109 (78, 125)	0.208
Lactate (mmol/L)	0th	1.45 (1.2, 6.2)	2.2 (0.9, 3.5)	0.959
	1st	1.45 (1, 3.7)	1.95 (1, 5.5)	0.336
	3rd	1.9 (0.9, 3)	1.95 (1, 5.3)	0.513
Base excess (mmol/L)	0th	-7.95 (-17, -4.4)	-10.4 (-18.4, -2.1)	0.264
	1st	-3.4 (-5.7, 0.1)	-4.15 (-11.3, -0.4)	0.288
	3rd	**2.7 (-3.8, 9.1)**	**0.2 (-5.5, 1.4)**	**0.021**
HCO_3 (mmol/L)	0th	17.7 (10.6, 20.5)	15.8 (12.4, 22)	0.438
	1st	21.65 (20.1, 23.9)	21.3 (15.9, 24.2)	0.317
	3rd	24.75 (20.3, 30.9)	23.8 (20.3, 24.8)	0.080

pH: Power of hydrogen, pCO_2: Pressure of carbondioxide, pO_2: Pressure of oxygen, K: Potassium, Na: Sodium, Ca: Calcium, Cl: Chlorine, Glu: Glucose, Lac: Lactate, BE: Base excess, HCO_3: Bicarbonate. *0th day refers to the first day of admission to the hospital. 1st and 3rd days are in the hospitalization period during the study

Table 5 Hemogram analysis findings

Parameters	Day*	Filgrastim group n: 20 median (min, max)	Standard group n: 20 median (min, max)	p value
WBC (x10^9/L)	0th	9.35 (2.5, 16.45)	11.23 (1.53, 23.53)	0.702
	1st	7.3 (2.89, 13.54)	7.65 (1.02, 12.21)	0.782
	3rd	10.02 (1.62, 16.6)	7.05 (0.76, 11.03)	0.098
Lymphocyte (x10^9/L)	0th	2.77 (0.1, 5.75)	4.32 (0.23, 7.58)	0.243
	1st	1.75 (0.75, 3.97)	2.58 (0.36, 5.56)	0.267
	3rd	2.87 (0.36, 5.45)	2.35 (0.4, 6.03)	0.983
Monocyte (x10^9/L)	0th	1.04 (0.1, 3.6)	2.25 (0.41, 6.95)	0.060
	1st	2.01 (0.15, 2.63)	1.52 (0.25, 4.56)	0.901
	3rd	1.65 (0.6, 4.62)	1.1 (0.13, 3.42)	0.322
Granulocyte (x10^9/L)	0th	5.27 (1.9, 8.38)	2.01 (0.07, 9.3)	0.081
	1st	**3.43 (1.82, 10.12)**	**2.95 (0.69, 6.2)**	**0.024**
	3rd	**3.56 (0.64, 13.62)**	**2.54 (0.23, 5.39)**	**0.050**
RBC (M/mm^3)	0th	6.56 (3.59, 8.29)	6.71 (0.96, 8.74)	0.848
	1st	**4.99 (2.26, 7.6)**	**7.95 (6.46, 9.38)**	**0.000**
	3rd	5.05 (2.17, 9.51)	6.17 (4.93, 8.83)	0.221
MCV (fl)	**0th**	**66.75 (56, 77.8)**	**58.4 (51.9, 69.6)**	**0.015**
	1st	**68.75 (62.3, 73.1)**	**63.55 (59, 71.1)**	**0.050**
	3rd	**64.05 (59.96, 71.2)**	**59.25 (54.8, 68.8)**	**0.008**
Hct (%)	0th	41.3 (25.3, 58.5)	41.05 (27.1, 56.6)	0.601
	1st	**33.6 (15.6, 39.5)**	**48.35 (38.7, 62.4)**	**0.000**
	3rd	33.55 (4.9, 55.7)	37.05 (31, 60.7)	0.340
MCH (pg)	**0th**	**22.55 (15.3, 24.7)**	**19.65 (15.5, 23.5)**	**0.021**
	1st	**22.55 (20.4, 28.4)**	**18.5 (16.2, 20.1)**	**0.000**
	3rd	**21.92 (20.2, 25.1)**	**17.85 (16.3, 19.3)**	**0.000**
MCHC (g/dL)	0th	32.27 (22.2, 35.1)	32.55 (29.3, 34.2)	0.681
	1st	**34.2 (30.8, 39.3)**	**29.2 (28.1, 35.6)**	**0.001**
	3rd	**34.14 (31.5, 36.6)**	**31.55 (26.1, 33.6)**	**0.003**
RDW	0th	10.9 (9, 13.2)	11.9 (9.6, 13.7)	0.124
	1st	9.8 (7.1, 12.7)	11.01 (9.25, 13.6)	0.077
	3rd	11.05 (7.66, 13.2)	11.75 (9.3, 13.7)	0.331
Hb (g/dL)	0th	13.7 (7.7, 19.7)	12.95 (2, 16)	0.346
	1st	**11.2 (4.9, 14.2)**	**14.15 (10.9, 18.7)**	**0.009**
	3rd	11.75 (4.7, 16.7)	11.7 (9.5, 15.9)	0.835

WBC: Leukocyte, Lym: Lymphocyte, Mon: Monocyte, Gra: Granulocyte, RBC: Erythrocyte, MCV: Mean corpuscular volume, Hct: Hematocrit, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW: Reticulocyte distrubition width, Hb: Hemoglobin. *0th day refers to the first day of admission to the hospital. 1st and 3rd days are in the hospitalization period during the study

Table 6 Clinical variables of the Filgrastim group according to the survival status

Parameters	Filgrastim group n: 20 median (min, max)		p value
	Survivor (n: 17)	Non-survivor (n: 3)	
Body weight (kg)	9.7 (4.5, 12.4)	7.85 (7.1, 8.6)	0.483
Symptom duration before hospital admission (days)	2 (1, 5)	3 (1, 5)	0.005
Hospitalization duration (days)	6 (4, 7)	4 (4, 4)	0.883

Table 7 Clinical variables of the Standard group according to the survival status

Parameters	Standard group n: 20 median (min, max)		p value
	Survivor (n: 16)	Non-survivor (n: 4)	
Body weight (kg)	7.2 (4.6, 10)	9.1 (7.3, 11.7)	0.213
Symptom duration before hospital admission (days)	2 (1, 6)	1 (1, 5)	0.357
Hospitalization duration (days)	6 (4, 8)	5 (5, 6)	0.720

Table 8 Statistically significant laboratory variables of the Filgrastim group according to the survival status

Parameters*	Filgrastim group n: 20 median (min, max)		p value
	Survivor (n: 17)	Non-survivor (n: 3)	
Granulocyte (x10^9 /L) (3rd day)	7.3 (2.6, 13.62)	2.6 (1.89, 3.97)	0.044
RDW (1st day)	10.3 (7.1, 12.7)	7.8 (7.4, 8.1)	0.038
Lactate (mmol/L) (3rd day)	2 (0.9, 3)	1.2 (0.9, 1.4)	0.029

RDW: Reticulocyte distribution width. 0th day refers to the first day of admission to the hospital. 1st and 3rd days are in the hospitalization period during the study

*Only statistically significant ($p<0.05$) variables were evaluated

Table 9 Statistically significant laboratory variables of the Standard group according to the survival status

Parameters*	Standard group n: 20 median (min, max)		p value
	Survivor (n: 16)	Non-survivor (n: 4)	
WBC (x10^9 /L) (0th day)	14.43 (10.97, 23.53)	2.54 (1.53, 2.75)	0.001
Lymphocyte (x10^9 /L) (0th day)	6.69 (3.55, 7.58)	1.15 (0.23, 1.4)	0.000
Monocyte (x10^9 /L) (0th day)	5.72 (1.32, 6.95)	0.65 (0.41, 1.14)	0.006
Granulocyte (x10^9 /L) (0th day)	4.66 (1.34, 9.3)	0.81 (0.07, 0.9)	0.014
Granulocyte (x10^9 /L) (1st day)	4.29 (0.77, 6.2)	1.32 (0.69, 2.37)	0.017
Hb (g/dL) (0th day)	10.35 (2, 13.2)	14.3 (13.1, 16)	0.033

WBC: Leukocyte, Hb: Hemoglobin. 0th day refers to the first day of admission to the hospital. 1st and 3rd days are in the hospitalization period during the study

*Only statistically significant ($p<0.05$) variables were evaluated.

DISCUSSION AND CONCLUSION

Canine parvovirus is endemic in many locations and can be carried by non-affected hosts, contributing to the transmission of disease to domestic animals. Although the survival rate reaches 80% in hospitalized patients with appropriate treatment protocols such as administration of fluid to treat dehydration, antiemetics, early nutritional support, broad-spectrum antibiotics, empiric deworming and improving neutrophil counts, the prognosis for survival often depends on the severity of clinical signs at the time that therapy is initiated (Mylonakis et al., 2016; Mazzaferro, 2020). In the present study, it was observed that hG-CSF administration, which was added to the standard treatment protocol, numerically but not statistically reduced the hospitalization duration and mortality rate. Although it was found that hG-CSF administration improved prognosis via red cell indices and increased neutrophils through bone marrow stimulation and mobilization of hematopoietic stem cells, it was also found that the clinical outcome is more dependent on the initial levels of certain analytes, such as WBC and Hb.

Many CSFs, including erythropoietin, thrombopoietin, interleukin (IL)-3, G-CSF, monocyte CSF, and granulocyte macrophage CSF have been purified in the last ten years. Of these CSFs, granulocyte-macrophage CSF and G-CSF have been most extensively studied in veterinary patients (Henry et al., 1998). In the first of three studies evaluating the use of hG-CSF in the treatment of CPE-related neutropenia, it was determined that hG-CSF was neither effective in increasing the neutrophil count nor in shortening the hospitalization duration (Rewerts et al., 1998). A similar result was determined in the second study, and no significant improvement was found either in the survival rate or the neutrophil count in dogs treated with hG-CSF (Mischke et al., 2001). Nevertheless, in contrary to previous studies, Kraft and Kuffer (1995) found that the use of hG-CSF significantly increased the neutrophil count in puppies with CPE. As a result of these aforementioned studies, it was suggested that hG-CSF is not effective in the treatment of CPE-related neutropenia (Duffy et al., 2010). In the present study, it was observed that the Filgrastim group had a higher granulocyte count ($p=0.05$) on the 1st and 3rd days than the Standard group. It is a well-known fact that the administration of G-CSF can mobilize hematopoietic stem and progenitor cells effectively into the peripheral blood (Stroncek and Anderlini, 2001). G-CSF has a role in the production and differentiation of hematopoietic cells as well as mobilization. Therefore, due to the mobilization of CD34+ hematopoietic stem cells, it is widely used in the treatment of chemotherapy-, bone marrow suppression-, and viral disease-related neutropenia. Also, it modulates monocytes and has systemic anti-inflammatory effects along with immune stimulation (Hartung, 1998). Moreover, several studies have reported that G-CSF stimulates the production and release of functional neutrophils in the bone marrow 24 hours after administration (Novotny et al., 1995). In line with these data, the increased granulocyte count of the Filgrastim group of the present study can be explained by the ability of hG-CSF to mobilize hematopoietic stem cells within 24 hours after the administration. Although it has been reported that the body produces neutralizing antibodies against hG-CSF and can cross-react with endogenous cG-CSF (Obradovich et al., 1991), A and B epitope mutation sites, which were identified on the CPV surface, may prevent this antibody neutralization (Wikoff et al., 1994). This fact may promote the use of hG-CSF in CPE (Henry et al., 1998), and thus, hG-CSF administration in addition to standard treatment protocols (such as fluid

therapy, antiemetic and broad-spectrum antibiotic administrations) as the initial treatment of CPE can positively affect the prognosis and clinical outcome since neutropenia is a negative prognostic indicator (Mylonakis et al., 2016; Gülersoy and Naseri, 2022).

Although interest in routine hematological parameters has increased recently, data on clinical and hematological parameters in dogs with parvoviral enteritis are still limited (Gülersoy and Naseri, 2022; Gulersoy et al., 2022). Among these routine hematological parameters, MCV, MCH, and MCHC analytes are used to describe the size of erythrocytes and the amount of hemoglobin. The clinical importance of these analytes is that they are used in the classification of anemia, evaluation of cell morphology and especially hematopoietic response (Sarma, 1990). In dogs with CPE, low levels of RBC, MCH, MCHC and Hb have been reported to be associated with regenerative anemia and/or iron deficiency anemia due to viral persistence in the bone marrow (Gülersoy and Naseri, 2022). In the present study, the dogs of the Filgrastim group had higher MCV and MCH levels on 0^{th}, 1^{st} and 3^{rd} ($p<0.05$), and MCHC levels on days 1^{st} and 3^{rd} days compared to the dogs of the Standard group ($p=0.003$). The dogs of the Standard group had higher RBC ($p<0.000$), Hct ($p<0.000$) and Hb ($p=0.009$) levels on the 1^{st} day than those of the dogs in the Filgrastim group. The higher MCV, MCH and MCHC levels of the dogs in the Filgrastim group of the present study may be associated with the mobilization of blood progenitor cells into the peripheral blood (Olivieri et al., 2004). Anemia due to decreased erythropoiesis as a result of direct damage to the bone marrow by CPV can be concealed in the presence of dehydration, and the higher RBC, Hct and Hb levels of the Standard group may be associated with this fact (Gülersoy and Naseri, 2022).

Previous studies have reported that the overall prognosis for survival rates ranges from 60% to 90% depending on the study, type of therapy, and individual patient response to treatment (Kalli et al., 2010). The present study has a survival rate of 82.5% (7 out of 40 dogs died). In order to improve prognosis and survival rate, in addition to symptomatic treatment, administrations of immunomodulators, cytokines, interferons and antioxidant substances along with enteral feeding are reported to be required (Mazzaferro, 2020). However, the prognosis and mortality rate in CPE may vary depending on the severity of clinical findings at the time of initiation of treatment (Mylonakis et al., 2016). In the present study, 3 dogs from the Filgrastim group died during the hospitalization period between the 4^{th} and 5^{th} days of the hospitalization period. Among the dogs of the Filgrastim group, survivors had higher granulocyte levels ($p=0.044$) on the 3^{rd} day, RDW levels on the 1^{st} day ($p=0.038$) and lactate levels on the 3^{rd} day ($p=0.029$) compared to the non survivors. In the Standard group, 4 dogs died during the hospitalization period between the 5^{th} and 7^{th} days. Compared to the non survivors, the survivors had higher WBC ($p=0.001$), lymphocyte ($p<0.000$), monocytes ($p=0.006$), Hb ($p=0.033$) and granulocyte levels on the 0^{th} and 1^{st} days ($p=0.014$ and $p=0.017$, respectively). Evaluation of routine laboratory tests such as hemogram provide important clinical information for differential diagnosis and monitoring the complications secondary to viral infection (Prittie, 2004). Depending on the tropism of CPV, hemogram findings differ according to the affected cell type and thus detection of hemogram variables with prognostic value can individualize CPE treatment and improve clinical outcome (Mylonakis et al., 2016; Mazzaferro, 2020).

Although the efficacies of additional prognostic indicators such as tumor necrosis factor activity, serum C-reactive protein, serum cortisol, and thyroxine concentrations have been investigated recently at the first admission (Schoeman et al., 2013), routine examination analytes including signalment, patient history, physical examination findings, blood gas and electrolyte findings along with different variables such as vaccination status, presence of diarrhea at first admission, low Hct and neutropenia are reported to be associated with low survival rate (Chalifoux et al., 2021; Muñoz et al., 2021). Among these, the presence of neutropenia has been considered a hallmark for CPE, as CPV attacks actively replicating cells such as bone marrow, thymus, and other lymphoid tissues. It has also been reported that the presence of cytopenia is useful in predicting the clinical outcome during the course of the disease (Castro et al., 2013). In a previous study, it was reported that a total leukocyte count greater than 4500/µL and a lymphocyte count greater than 1000/µL at the time of admission and through 48 hours of hospitalization period were strongly predictive of survival (Goddard et al., 2008). In the present study, leukogram results of the surviving dogs of the Standard group on the 0th day such as higher WBC, lymphocyte, monocyte, and granulocyte counts promoted the fact that prognosis and clinical outcomes in CPE cases were associated with the severity of clinical and laboratory findings at the first admission and at the initiation of the treatment (Kalli et al., 2010). The numerically lower mortality rate and shorter hospitalization time of the Filgrastim group may be related to the immune stimulation and systemic anti-inflammatory effects of filgrastim administration on the day of admission and through the hospitalization period at a dose of 10 µg/kg via subcutaneous route once a day (Hartung, 1998). In addition, this finding can be explained by the ability of homologous G-CSFs to induce sudden and persistent leukocytosis in healthy individuals (Caselli et al., 2016). Although the use of hG-CSF in CPE cases is controversial and the prognosis and clinical outcome of dogs with CPE is dependent on the type of therapy, and individual patient response to treatment (Duffy et al., 2010), the results of the present randomized controlled study (blood gas and electrolyte and hemogram analysis results of all the dogs at the 0th day $p > 0.05$, Tables 3 and 4) indicated that the use of hG-CSF might be associated with a favorable prognosis and clinical outcome.

This study has some limitations. Although hG-CSF was validated to be beneficial during a short hospitalization period of 3 days in this study, the statistical insignificance of some variables related to survival status may be associated with low number of animal material. Also, the lack of investigation of serum biochemistry parameters can be considered as a limitation. Thus, the evaluation of hG-CSF administration with a larger number of animals can demonstrate the prognostic importance of different anamnestic, clinical and laboratory variables, including hospitalization time and survival rate.

Colony stimulating factors (CSFs) are essential molecules that influence blood cell differentiation, proliferation and survival. Although the use of cG-CSF in the treatment of CPE is recommended, the use of hG-CSF is still controversial due to certain notorious changes. However, in this study investigating the short-term effects of hG-CSF in dogs with CPE, no adverse effects such as sudden decrease in WBC and monocyte counts were observed. Thus, the use of hG-CSF can be considered to be of therapeutic importance in cases of CPE. Although the use of hG-CSF at the first admission to the hospital prevents the decrease in the granulocyte count and positively affects the prognosis, it should be kept in mind that the

severity of clinical and laboratory findings at the first admission and at the initiation of the treatment is important in determining the prognosis and clinical outcome of CPE.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTION

Concept – EG, CB.; Design – EG, CB; Supervision – EG, CB; Resources - EK; Materials – AŞ, IG; Data Collection and/or Processing – EK, AŞ, IG; Analysis and/or Interpretation – EG, CB, EK, AŞ, IG; Literature Search – EG, CB, EK, AŞ, IG; Writing Manuscript – EG, CB, EK, AŞ, IG; Critical Review – EG, CB, EK, AŞ, IG.

REFERENCES

Armenise A, Trerotoli P, Cirone F, De Nitto A, De Sario C, Bertazzolo W, et al. 2019. Use of recombinant canine granulocytecolony stimulating factor to increase leukocyte count in dogs naturally infected by canine parvovirus. Vet Microbiol, 231, 177-82. doi:10.1016/j.vetmic.2019.03.015.

Caselli D, Cesaro S, Aricò M. 2016. Biosimilars in the management of neutropenia: focus on filgrastin. Biologics, 10, 17-22. doi:10.2147/BTT.S73580.

Castro TX, Cubel Garcia Rde C, Gonçalves LP, Costa EM, Marcello GC, Labarthe NV, et al. 2013. Clinical, hematological, and biochemical findings in puppies with coronavirus and parvovirus enteritis. Can Vet J, 54(9), 885-8.

Chalifoux NV, Parker SE, Cosford KL. 2021. Prognostic indicators at presentation for canine parvoviral enteritis: 322 cases (2001-2018). J Vet Emerg Crit Care (San Antonio), 31(3), 402-13. doi:10.1111/vec.13052.

Decaro N, Desario C, Campolo M, Elia G, Martella V, Ricci D, et al. 2005. Clinical and virological findings in pups naturally infected by canine parvovirus type 2 Glu-426 mutant. J Vet Diagn Invest, 17(2), 133-8. doi:10.1177/104063870501700206.

Duffy A, Dow S, Ogilvie G, Rao S, Hackett T. 2010. Hematologic improvement in dogs with parvovirus infection treated with recombinant canine granulocyte-colony stimulating factor. J Vet Pharmacol Ther, 33(4), 352-6. doi:10.1111/j.1365-2885.2009.01153.x.

Ford J, McEndaffer L, Renshaw R, Molesan A, Kelly K. 2017. Parvovirus Infection Is Associated With Myocarditis and Myocardial Fibrosis in Young Dogs. Vet Pathol, 54(6), 964-71. doi:10.1177/0300985817725387.

Gerlach M, Proksch AL, Dörfelt R, Unterer S, Hartmann K. 2020. Therapy of canine parvovirus infection - review and current insights. Tierarztl Prax Ausg K Kleintiere Heimtiere, 48(1), 26-37. doi:10.1055/a-1020-3341.

Goddard A, Leisewitz AL, Christopher MM, Duncan NM, Becker PJ. 2008. Prognostic usefulness of blood leukocyte changes in canine parvoviral enteritis. J Vet Intern Med, 22(2), 309-16. doi:10.1111/j.1939-1676.2008.0073.x.

Gulersoy E, Balikci C, Gunal I, Sahan A. 2022. Evaluation of the Efficacy of Blood Gases and Hemogram Parameters in the Diagnosis of non-Neurogenic Distemper and Parvoviral Enteritis in Dogs with Acute Gastroenteritis. Rev Cient Fac Cienc Vet Univ Zulia, 1, 32. doi:10.52973/rcfcv-e32091.

Gülersoy E, Naseri A. 2022. Hematological Status in Septic or Non Septic Dogs due to Parvoviral Enteritis. Dicle Üniv Vet Fak Derg, 15(1), 45-52.

Hammond WP, Csiba E, Canin A, Hockman H, Souza LM, Layton JE, et al. 1991. Chronic neutropenia. A new canine model induced by human granulocyte colony-stimulating factor. J Clin Invest, 87(2), 704-10. doi:10.1172/JCI115049

Hartung T. 1998. Anti-inflammatory effects of granulocyte colony stimulating factor. Curr Opin Hematol, 5, 221-5.

Henry CJ, Buss MS, Lothrop CD. 1998. Veterinary Uses of Recombinant Human Granulocyte Colony Stimulating Factor. Part I. Oncology. Compendium, 20(6), 1-6.

Johannesen S, Budeus B, Peters S, Iberl S, Meyer AL, Kammermaier T, et al. 2018. Biomarker Supervised G-CSF (Filgrastim) Response in ALS Patients. Front Neurol, 26(9), 971. doi:10.3389/fneur.2018.00971.

Kalli I, Leontides LS, Mylonakis ME, Adamama-Moraitou K, Rallis T, Koutinas AF. 2010. Factors affecting the occurrence, duration of hospitalization and final outcome in canine parvovirus infection. Res Vet Sci, 89(2), 174-8. doi:10.1016/j.rvsc.2010.02.013.

Kraft W, Kuffer M. 1995. Treatment of severe neutropenia in dogs and cats with filgrastim. Tierarztl Prax, 23, 609–13.

Mazzaferro EM. 2020. Update on Canine Parvoviral Enteritis. Vet Clin North Am Small AnimPract, 50(6), 1307-25. doi:10.1016/j.cvsm.2020.07.008.

Mischke R, Barth T, Wohlsein P, Rohn K, Nolte I. 2001. Effect of recombinant human granulocyte colony-stimulating factor (rhG-CSF) on leukocyte count and survival rate of dogs with parvoviral enteritis. Res Vet Sci, 70(3), 221-5. doi:10.1053/rvsc.2001.0464.

Muñoz AI, Vallejo-Castillo L, Fragozo A, Vázquez-Leyva S, Pavón L, Pérez-Sánchez G, et al. 2021. Increased survival in puppies affected by Canine Parvovirus type II using an immunomodulator as a therapeutic aid. Sci Rep, 11(1), 19864. doi:10.1038/s41598-021-99357-y.

Mylonakis M, Kalli I, Rallis T. 2016. Canine parvoviral enteritis: an update onthe clinical diagnosis, treatment, and prevention. Vet Med, 7, 91-100. doi:10.2147/VMRR.S80971.

Novotný J, Zvarová M, Prazáková L, Jandlová M, Konvicková L. 1995. G-CSF (Neupogen Roche) v léčběpacientů s chronickouaplastickouanémií s těžkouneutropenií [G-CSF (Neupogen Roche) in the treatment of patients with chronic aplastic anemia with severe neutropenia]. Vnitr Lek, 41(10), 692-5.

Obradovich JE, Ogilvie GK, Powers BE, Boone T. 1991. Evaluation of recombinant canine granulocyte colony-stimulating factor as an inducer of granulopoiesis. A pilot study. J Vet Intern Med, 5(2), 75-9. doi:10.1111/j.1939-1676.1991.tb00935.x.

Olivieri A, Scortechini I, Capelli D, Montanari M, Lucesole M, Gini G, et al. 2004. Combined administration of alpha-erythropoietin and filgrastim can improve the outcome and cost balance of autologous stem cell transplantation in patients with lymphoproliferative disorders. Bone Marrow Transplant, 34(8), 693-702. doi:10.1038/sj.bmt.1704643.

Prittie J. 2004. Canine parvoviral enteritis: a review of diagnosis, management, and prevention. J Vet Emerg Crit Care, 14, 167-76. doi:10.1111/j.1534-6935.2004.04020.x.

Rewerts JM, McCaw DL, Cohn LA, Wagner-Mann C, Harrington D. 1998. Recombinant human granulocyte colony-stimulating factor for treatment of puppies with neutropenia secondary to canine parvovirus infection. J Am Vet Med Assoc, 213(7), 991-2.

Sarma PR. 1990. Red Cell Indices. In Walker HK, Hall WD, Hurst JW (Eds), Clinical Methods: The History, Physical, and Laboratory Examinations (3rd ed., pp. 421-434). Boston,USA: Butterworths.

Schoeman JP, Goddard A, Leisewitz AL. 2013. Biomarkers in canine parvovirus enteritis. N Z Vet J, 61(4), 217-22. doi:10.1080/00480169.2013.776451.

Strom LM, Reis JL, Brown CC. 2015. Pathology in practice. Parvoviral myocarditis in a dog. J Am Vet Med Assoc, 246(8), 853-5. doi:10.2460/javma.246.8.853.

Stroncek D, Anderlini P. 2001. Mobilized PBPC concentrates: a maturing blood component. Transfusion, 41(2), 168-71. doi:10.1046/j.1537-2995.2001.41020168.x.

Wikoff WR, Wang G, Parrish CR, Cheng RH, Strassheim ML, Baker TS, et al. 1994. The structure of a neutralized virus: canine parvovirus complexed with neutralizing antibody fragment. Structure, 2(7), 595-607. doi:10.1016/s0969-2126(00)00062-9.

UPOTREBA Hg-CSF KOD PSEĆEG PARVOVIRUSNOG ENTERITISA: EFEKAT NA KLINIČKE I LABORATORIJSKE VARIJABLE

SAŽETAK

Pseći parvovirus predstavlja jedan od najvažnijih patogenih virusa. Pseći parvovirusni enteritis (CPE) je veoma kontagiozna i često fatalna bolest pasa. Prisustvo neutropenije se smatra osnovnom karakteristikom CPE obzirom da predstavlja negativan prognostički indikator. Faktori stimulacije kolonije granulocita (G-CSF) su esencijalne molekule koje kontroliraju diferencijaciju krvnih stanica, proliferaciju i preživljenje. Cilj ovog istraživanja jeste ispitati efikasnost filgrastima, koji je humani G-CSF, na kliničke i laboratorijske varijable u usporedbi sa standardnim terapijskim protokolom kod CPE. Od 40 pasa oboljelih od CPE, u svaku terapijsku grupu u istraživanju je randomizirano 20 pasa, Pored standardnog terapijskog protokola, psi u Filgrastim grupi su jednom dnevno primali hG-CSF subkutano u dozi od 10 µg/kg u trajanju od 3 dana. Na početku istraživanja i tokom tri dana hospitalizacije su obavljani fizikalni i laboratorijski pregledi. Najvažniji nalaz je predstavljao visok broj granulocita u Filgrastim grupi izmjeren 1. i 3. dan, a u odnosu na Standardnu grupu ($p=0.024$ i $p=0.05$,). Iako upotreba hG-CSF pri prijemu spriječava pad broja granulocita ii ma pozitivan učinak na prognozu, treba imati na umu da su težina kliničke slike i laboratorijskih nalaza pri prijemu i započinjanje terapije važni za prognozu i klinički ishod CPE. Kao rezultat je zaključeno da upotreba hG-CSF može imati terapijski značaj kod CPE

Ključne riječi: Krvna slika, pas, prognoza, terapija