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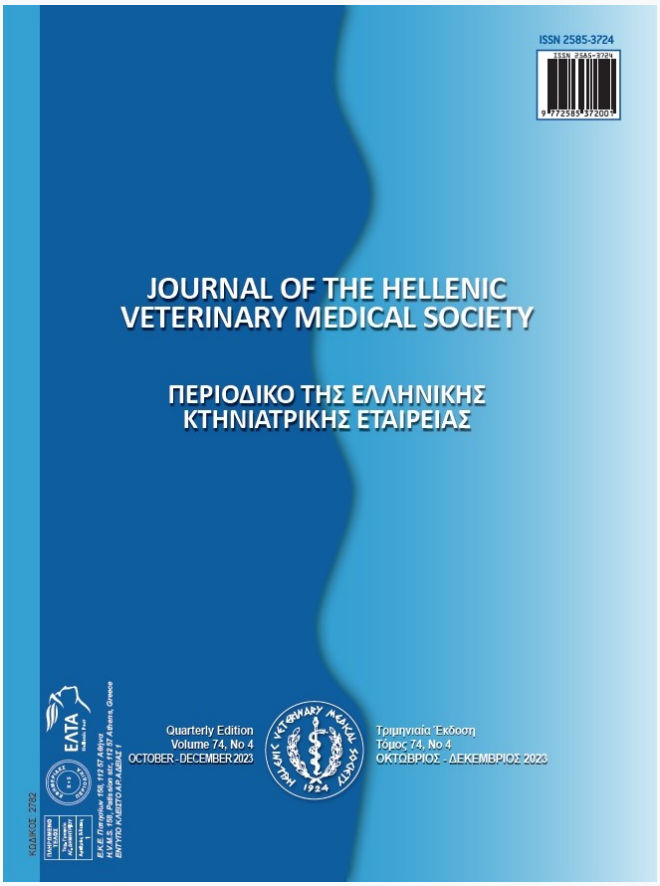

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M Sezer, G Gökce

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Research article
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sTREM-1, MR-Pro-Adrenomedullin, clinical biochemistry and hematology in calves with neonatal sepsis

M. Sezer*, G. Gökce

Kafkas University, Faculty of Veterinary Medicine, Department of Internal Medicine, Kars, Turkey

ABSTRACT: This study aimed to determine the changes in biochemical and hematological parameters and the expression of soluble triggering receptors expressed on myeloid cells-1 (sTREM 1) and mid-regional-pro-adrenomedullin (MR-Pro-ADM) on myeloid cells during treatment in calves with neonatal sepsis. The study group comprised 21 calves, aged 1-16 days, that fit the sepsis criteria. The control group comprised 10 healthy calves of the same age. The clinical examinations of the calves in the study group were performed at certain hours before the treatment (hour 0) and during the treatment (12th, 24th, 48th, and 72nd hours). The blood samples were taken from the vena jugularis before and during treatment from the sick calves and once from the healthy calves. The sTREM-1 levels before treatment (0 h), during treatment (12th, 24th, 48th, and 72nd hours) and the control group was follows 59.17±2.58 ng/L, 57.07±1.65 ng/L, 57.27±2.07 ng/L, 56.64±1.97 ng/L, 57.58±2.18 ng/L and 75.60±2.57 ng/L,39 respectively.The MR-Pro-ADM levels before treatment (0 h), during treatment (12th, 24th, 48th, and 72nd hours) and control group was follows; 117.88±7.75 ng/L, 113.63±5.18 ng/L, 109.97±5.25 ng/L, 114.50±5.35 ng/L, 111,84±5.63 ng/L,65.31±2.81 ng/L, respectively. The serum MR-Pro-ADM levels in calves with sepsis were significantly higher than those in the control group before and during treatment ($P<0.05$). Serum sTREM-1 level was found to be significantly lower before treatment in the sepsis group compared to the control group ($P < 0.05$).MR-Pro-ADM and sTREM-1 were more important, especially in terms of diagnosis in evaluating calves with neonatal sepsis.

***Keywords*:** Biomarker; diagnosis; endotoxemia; prognosis.

Corresponding Author:
M. Sezer, 1Kafkas University, Faculty of Veterinary Medicine, Department of Internal Medicine, Kars, Turkey
E-mail address: sezermert100@gmail.com

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INTRODUCTION

Acute access of pathogens and their toxins to tissues, organs, and systems through the circulation is called sepsis (Silverstein and Otto, 2012;Eroğlu and Kırbaş, 2020). Although sepsis is aggressively treated, its mortality rate is quite high. Neonatal sepsis in calves is mostly caused by Gram-negative bacteria. Sepsis occurs mostly in gastrointestinal tract infections (Costello et al., 2004; Eroğlu and Kırbaş, 2020; Akyüz, 2020). Infectious and noninfectious factors, immune system, and environmental factors play roles in the etiology of diarrhea (Akyüz et al., 2017; Akyüz and Gökce, 2021). Among infectious agents, bacterial pathogens include *Escherichia coli (E. coli)* and its strains, viral pathogens include rotavirus and coronavirus, and parasitic pathogens include *Cryptosporidium parvum* (Al and Balıkçı,2012; Altuğ et al., 2013; Şen et al., 2013;Azkur and Tonbak, 2015; Gülaçtı et al., 2016; Akyüz et al., 2017;Azkur and Aksoy, 2018; Hacıoğlu and Alkan, 2018;Şahal et al., 2018; Bal, 2019). These pathogens are responsible for 90% of diarrhea in calves (Atlı et al., 2018). The clinical symptoms included diarrheal enophthalmos, loss of skin elasticity, stagnation, hyperemia of the mucosal membranes and petechial hemorrhages, tachycardia (but a weak pulse) due to a decrease in blood pressure, loss of appetite, decreased urine output, hypothermia due to fluid loss initially followed by hyperthermia, tachypnea, loss of sucking reflex, prolongation of capillary refill time, darkening of the gums, depression, muscle weakness (Constable, 2007; Akyüz et al., 2017; Topal, 2018; Eroğlu and Kırbaş, 2020),ophthalmitis, and central nervous system disorders if sepsis-related meningitis has developed (Constable, 2007; Akyüz et al., 2017; Topal, 2018; Eroğlu and Kırbaş, 2020). An inflammatory response occurs as a result of the interaction between the organism and the pathogen in the case of sepsis, and biomarkers are released. As early diagnosis and timely effective intervention are important for saving lives. The use of biomarkers has been a focus of research in recent years (Su et al., 2011).

One of the new biomarkers discovered in recent studies is trigger receptor-1 (TREM-1) expressed inmyeloid cells (Latour-Perez et al., 2010). It was discovered by Bouchon et al. in 2000 (Smok et al., 2020). TREM 1 is a 30-kDa glycoprotein. It is located on the surface of neutrophils, monocytes, macrophages, and endothelial cells (Adly et al., 2014). In cases of sepsis, pneumonia, pleural effusion, septic arthritis, meningitis, peritonitis, and metritis, TREM-1 is released into body fluids and blood (Su et al., 2011; Sönmezer and Tülek, 2015). The soluble form of trigger receptor 1, the soluble triggering receptor expressed on myeloid cells-1 (sTREM 1), is used to evaluate the inflammatory response (Su et al., 2011; Gamez-Diaz et al., 2011). The prognostic and diagnostic performance of sTREM-1 varies in cases of sepsis and septic shock (Palmiere and Augsburger, 2014).

Some studies reported that TREM 1 increased before clinical signs of sepsis were seen (Gamez-Diaz et al., 2011) and that sTREM 1 levels remained high in severe infections in which septic shock developed (Su et al., 2011). Other studies, however, found that sTREM-1 acted as a negative acute-phase reactivator in patients with inflammatory bowel disease and that sTREM-1 levels decreased compared with those in the healthy group (Kutlu et al., 2021). Adrenomedullin (ADM) is a new biomarker that can be measured from the bloodstream and can be used in the evaluation of septic patients (Rey et al., 2013). As ADM has 27% similarity to the circulation calcitonin gene-related peptide (CGRP), calcitonin-CGRP was included in the amylin peptide family (Geven et al., 2018). ADM is synthesized and released during sepsis (Jordan et al., 2014). In addition, ADM also has antimicrobial (Garazzino et al., 2019), antifungal, and anti-inflammatory effects (Allaker et al., 2006; Miguel et al., 2011; Bernal-Morell et al., 2018). Mid-regional-pro-adrenomedullin (MR-Pro-ADM) is a 48-amino acid fragment that shows the activity and level of ADM (Stolz et al., 2008; Miguel et al., 2011; Abd Elmouttaleb et al.,2016; Valenzulea-Sanchez et al., 2016).The half-life of MR-Pro-ADM is higher than that of ADM. This duration varies from 2 to 5 days. Therefore, MR-Pro-ADM can be used as a prognostic indicator in patients with sepsis (Valenzuela-Sanchez et al., 2015).

This study aimed to determine the changesin biochemical and hematological parameters and the levels of sTREM-1 and MR-Pro-ADM during treatment in calves with neonatal sepsis.

MATERIALS AND METHOD

The study was started after obtaining the approval of the Kafkas University Animal Experiments Local Ethics Committee (KAU-HADYEK/2020-021).

Animal Material

The study group comprised 21 calves aged 1-16 days, meeting the criteria for sepsis, while the control

group comprised 10 healthy calves in the same age group.

Clinical Examination

The clinical examinations (respiration and pulse rate per minute, rectal body temperature, examination of the conjunctiva, capillary-filling time, mental status, degree of dehydration, and character of stool) of the calves were conducted before the treatment (hour 0) and during the treatment (12th, 24th, 48th, and 72nd hours).

Evaluation of Systemic Inflammatory Response Syndrome and Sepsis Criteria

The existence of at least two of the following criteria were sufficient for the diagnosis of systemic inflammatory response syndrome (SIRS): a total leukocyte count of [($\times 10^3/\mu L$) <5 or >12], a respiratory rate of [(m) >45], a pulse of [(m) <90 or >120], a band neutrophil percentage of [(%) >10], or a body temperature of [(^oC) >39 or <36.6]. If the presence or suspicion of infection was added to SIRS, it was considered as sepsis (Hart and Mackay, 2015; Akyüz et al., 2017; Beydilli and Gökçe, 2019; Eroğlu and Kırbaş, 2020; Akyüz and Gökce, 2021). Neonatal calves with diarrhea meeting the SIRS and sepsis criteria were included in the study.

Determination of Band Neutrophil Percentage

Before treatment, the blood samples were taken from the calves to be included in the study, and a smear was taken in accordance with the technique. After drying, the preparations were stained using May-Grünwald solution (Facepath, Turkey) and concentrated Giemsa solutions (Merck, USA). The dried smears were examined under an Olympus BX53 light microscope. The smears were photographed at 200× magnification with an Olympus DP72 camera. The photographs of each smear were taken from at least 10 different areas. The cells in the photographs were counted using the ImageJ program, and the band neutrophil percentages were determined by calculation.

Agent Detection

A stool rapid test kit (BoviD-5 Ag Test Kit, Bionote Inc., Korea) was used for agent detection in calves with sepsis during the neonatal period. Also, *E. coli* isolation was carried out according to the technique reported by Coura et al. (2015) to detect other strains of *E. coli*.

Complete Blood Count and Biochemical Analysis

A Complete blood count was performed from the whole-blood samples obtained using a hemogram device (VG-MS4e, MeletSchloesing, France). From the serum samples obtained, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), glucose, creatinine (CREA), urea (UREA), total protein (TP), albumin (ALB), creatine kinase (CK), total bilirubin (TBIL), direct bilirubin (DBIL), and globulin values were measured daily with a biochemistry device (Mindray BS120, Mindray MedikalTeknoloji Istanbul, Turkey).

Treatment Protocol

Fluid therapy was applied to the treated calves according to their degree of dehydration. For this purpose, 0.9% NaCl solution (PVC, Eczacıbaşı Baxter, İstanbul, Turkey), 1.3% $NaHCO_3$ (Bikarvil®, Vilsan, Ankara, Turkey), and 5% dextrose (Polifleks, Polifarma, Tekirdağ, Turkey) were administered intravenously (IV). For infection control, enrofloxacin (Baytril 10%®, Bayer, Germany) was administered parenterally at a dose of 2.5-5 mg/kg intravenously for 5 days, and sulfadoxine-trimethoprim (Animar®, Ceva, France) was administered IV for 5 days according to the information leaflet. A powder containing sulfadimidine and oxytetracycline (Tetramezathine®, Ceva, France),used as an oral antibiotic, was given for 5 days according to the information leaflet. As a supplement, vitamin B complex (Berovit B12®, Ceva, France), vitamin C (Maxivit-C®, Bavet, Turkey), vitamin AD_3E (Ademin®, Ceva, France), and sodium selenite + vit E + vit B1 (Yeldif®,Ceva, France) were administered daily according to the information leaflet. Meloxicam (Bavet Meloxicam®, Bavet, Turkey) was administered as a single dose of 0.5 mg/kg via a subcutaneous route for anti-inflammatory purposes. The calves were taken to individual boxes and kept under observation during the treatment.

Evaluation of Passive Immunity in Calves

One of the methods used to evaluate passive immunity in calves was the sodium sulfate precipitation test. For this, 1.9 mL of prepared 14%, 16%, and 18% sodium sulfate stock solutions were put into empty glass tubes. Then, 0.1 mL of the calves' serum was added, and the evaluation was made after waiting for 1 h at room temperature. The presence of turbidity in all tubes indicated that the amount of immunoglobulin G (IgG)was more than 1500 mg/dL, and passive transfer had taken place. The turbidity in only the 16%

and 18% tubes indicated that the amount of IgG was between 500 and1500 mg/dL, and the passive transfer was partially realized. The turbidity in only the 18% tube indicated that the amount of IgG was 500 mg/dL or less, and immunodeficiency occurred (Güngör and Özyurtlu, 2005). The evaluation of passive immunity in calves with sepsis was carried out in accordance with the mention literature.

sTREM-1 and MR-Pro-ADM Analyses

The serum levels of sTREM-1 and MR-Pro-ADM were determined using commercial bovine-specific enzyme-linked immunosorbent assay (ELISA) kits (Bovine sTREM-1 ELISA kit, Bovine MR-Pro-ADM ELISA kit, BT Lab, China). ELISA tests were performed following the manufacturer's protocols, and the optical density was determined in an ELISA reader (Thermo Scientific, USA) at 450 nm wavelength. The regression analysis was performed, and sTREM-1 and MR-Pro-ADM values were read.

Statistical Analyses

The blood data obtained from the sepsis group before and during the treatment and the normal distribution test of the data belonging to the control group were evaluated using visual methods (histogram graph and Q-Q graph) and the Shapiro-Wilk test. One-way analysis of variance (ANOVA) was used for normally distributed data. After evaluating the homogeneity of variances with the Levene test, the Tukey HSD test was applied for*post hoc* comparison. Kruskal-Wallis rank one-way ANOVA was used for multiple comparisons of non-normally distributed data, and paired comparisons of the parameters with the significance level were evaluated with the Mann-Whitney *U* test. After applying Bonferroni correction to the *P* value obtained after this test, the adjusted significance level was considered. The Spearman correlation coefficient was consideredbecause some data did not show normal distribution on blood draw days and control. The data obtained were represented as mean ± standard error (SEM). All analyses were performed using the SPSS (Version 26.0, IL, USA) package program. In statistical analysis, the difference obtained in comparing the data in the groups were considered significant at the $P<0.05$ level.

RESULTS

The clinical examinations (body temperature, respiratory rate/minute, and pulse rate/minute) of the calves with sepsis were performed both before the treatment (hour 0) and during the treatment (12th,

Table 1. Mean values and standard error values of clinical, hematological, and biochemical findings in the study and control groups at different times

Parameters	Sepsis group					Control group	*P*
	Hours						
	*0th	12th	24th	48th	72th		
Body temperature (°C)	36.81 ± 0.52^{b}	38.01 ± 0.15^{ab}	37.97 ± 0.22^{ab}	38.19 ± 0.19^{a}	38.39 ± 0.15^{a}	38.24 ± 0.07^{a}	0.002
Respiration rate (minute)	43.62 ± 4.91^{b}	30.67 ± 2.70^{ab}	28.74 ± 1.88^{ab}	28.63 ± 2.42^{a}	25.05 ± 2.00^{a}	24.80 ± 1.31^{a}	<0.001
Pulse rate (minute)	137.90 ± 6.18^{a}	131.71 ± 4.92^{a}	134.95 ± 2.81^{a}	130.21 ± 3.43^{a}	123.26 ± 4.23^{a}	95.20 ± 1.27^{b}	<0.001
Alanine aminotransferase (IU/L)	96.13 ± 31.86^{ab}	139.07 ± 30.56^{bc}	161.78 ± 34.03^{bc}	369.08 ± 167.20^{c}	186.69 ± 24.08^{c}	32.23 ± 6.00^{a}	<0.001
Total leukocyte count (×10^{3}/µL)	21.01 ± 4.09	23.86 ± 3.20	23.36 ± 3.47	20.87 ± 3.75	30.17 ± 5.66	10.64 ± 0.84	0.128
Aspartate aminotransferase (IU/L)	225.64 ± 88.68^{ab}	495.91 ± 229.20^{bc}	322.66 ± 61.82^{bc}	345.02 ± 76.30^{c}	343.11 ± 59.70^{c}	70.38 ± 4.45^{a}	<0.001
Urea (mg/dL)	96.35 ± 13.69^{a}	87.95 ± 8.12^{a}	65.27 ± 6.16^{ab}	39.87 ± 4.64^{b}	40.70 ± 4.57^{b}	29.56 ± 3.39^{b}	<0.001
Albumin (g/dL)	3.16 ± 0.24^{a}	2.36 ± 0.09^{bc}	2.46 ± 0.10^{bc}	2.24 ± 0.14^{c}	2.31 ± 0.14^{bc}	2.97 ± 0.23^{ab}	<0.001
Creatine kinase (IU/L)	701.28 ± 177.07^{b}	3307.69 ± 206.48^{b}	1095.26 ± 297.96^{b}	1027.30 ± 204.57^{b}	1103.86 ± 426.73^{b}	129.51 ± 19.03^{a}	<0.001
Glucose (mg/dL)	162.67 ± 28.57^{ab}	232.05 ± 20.68^{a}	233.11 ± 23.15^{a}	191.95 ± 18.71^{a}	170.47 ± 16.41^{a}	76.10 ± 3.66^{b}	<0.001
Creatinine (mg/dL)	3.73 ± 0.69^{a}	2.67 ± 0.41^{ab}	1.99 ± 0.30^{ab}	1.36 ± 0.12^{b}	1.32 ± 0.19^{b}	0.98 ± 0.06^{b}	<0.001
Total protein (g/dL)	7.27 ± 0.70^{a}	5.08 ± 0.28^{b}	4.92 ± 0.24^{b}	5.13 ± 0.21^{b}	3.78 ± 0.30^{b}	4.75 ± 0.42^{b}	<0.001
Gamma glutamyl transferase (IU/L)	424.95 ± 126.06	276.41 ± 63.20	250.86 ± 53.87	189.34 ± 37.73	150.52 ± 27.03	319.12 ± 168.90	0.653
Total bilirubin (mg/dL)	0.17 ± 0.09	0.15 ± 0.05	0.08 ± 0.03	0.09 ± 0.03	0.05 ± 0.02	0.09 ± 0.03	0.575
Direct bilirubin (mg/dL)	0.12 ± 0.07	0.10 ± 0.04	0.06 ± 0.02	0.05 ± 0.03	0.02 ± 0.01	0.04 ± 0.02	0.526
Globulin (g/dL)	4.32 ± 0.65^{a}	2.71 ± 0.26^{b}	2.47 ± 0.20^{b}	2.88 ± 0.21^{ab}	1.48 ± 0.22^{b}	1.68 ± 0.32^{b}	<0.001
MR-Pro-ADM (ng/L)	117.88 ± 7.75^{a}	113.63 ± 5.18^{a}	109.97 ± 5.25^{a}	114.50 ± 5.35^{a}	111.84 ± 5.63^{a}	65.31 ± 2.81^{b}	<0.001
sTREM-1 (ng/L)	59.17 ± 2.58^{a}	57.07 ± 1.65^{a}	57.27 ± 2.07^{a}	56.64 ± 1.97^{a}	57.58 ± 2.18^{a}	75.60 ± 2.57^{b}	<0.001

$^{a-c}$Means with different letters on the same line indicate the difference in hours between the patient and control groups ($P< 0.05$).
*Before treatment.

24th, 48th, and 72nd hours). The clinical findings obtained were evaluated statistically. Although the body temperature was low before the treatment, respiration and pulse rate per minute were found to be significantly higher ($P<0.05$, Table 1).Despite fluctuations in the total leukocyte count during the treatment, no statistical significance was determined ($P<0.05$, Table 1).

The values of biochemical parameters ALT, AST, glucose, CREA, UREA, TP, CK, ALB, and globulin were significantly higher before treatment compared with those in the control group ($P<0.05$); however, no statistically significant difference was found in terms of GGT, TBIL, and DBIL values ($P>0.05$, Table 1).

A comparison of sTREM-1 values of the calves with sepsis before treatment (hour 0) and during treatment (12th, 24th, 48th, and 72nd hours) with those in the control group showed that sTREM-1 values were significantly lower in the sepsis group ($P<0.05$, Fig. 1). A comparison of MR-Pro-ADM values of the calves with sepsis before treatment (hour 0) and during treatment (12th, 24th, 48th, and 72nd hours) with those in the control group showed that the values were significantly higher in the sepsis group ($P<0.05$, Fig. 2).

All other parameters in the sTREM-1 and MR-Pro-ADM study of calves with sepsis and their correlation are shown in Table 3. One of the clinical findings, pulse rate, negatively correlated with sTREM-1. The biochemical parameters GGT and glucose negatively correlated with sTREM-1 and positively correlated with ALB. MR-Pro-ADM negatively correlated with GGT and positively correlated with CK.

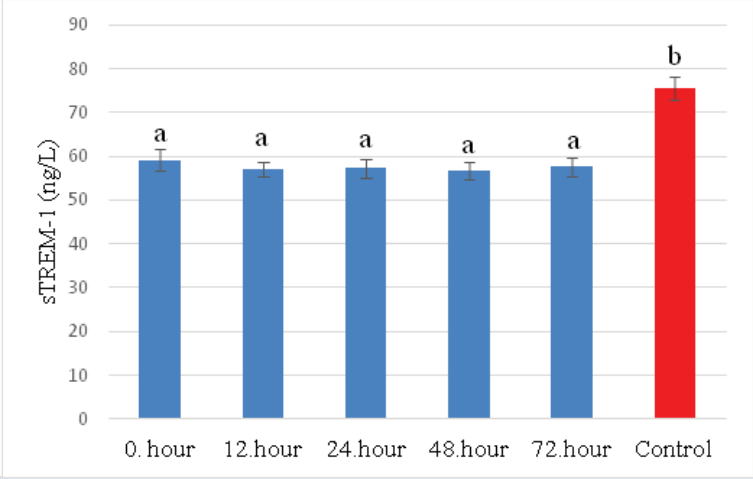

Figure 1. sTREM-1 values determined at different times from the sepsis and control groups. [a, b]Statistical differences in groups ($P<0.05$).

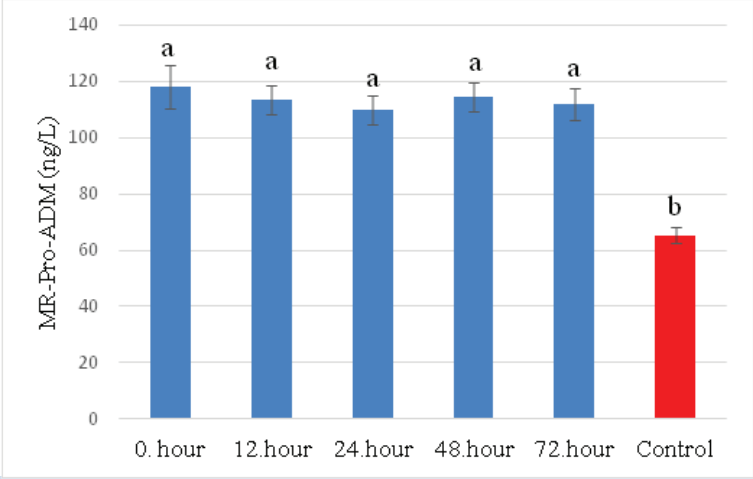

Figure 2. MR-Pro adrenomedullin values determined at different times from the sepsis and control groups.[a, b]Statistical differences in groups ($P< 0.05$).

The hematological parameters white blood cell (WBC) and monocyte counts negatively correlated with sTREM-1 and positively correlated with thered blood cell count and hemoglobin level (Table 3).

The sodium sulfite precipitation test results showed that seven calves did not receive colostrum (Table 2).

Table 2. IgG values and passive transfer status of calves with sepsis (patients $n = 21$)

Calf No	14% solution	16% solution	18% solution	IgG value (mg/dL)	Passive transfer status
1	+	+	+	≥1500	Sufficient
2	+	+	+	≥1500	Sufficient
3	+	+	+	≥1500	Sufficient
4	+	+	+	≥1500	Sufficient
5	-	-	-	≤500	Insufficient
6	+	+	+	≥1500	Sufficient
7	-	-	-	≤500	Insufficient
8	+	+	+	≥1500	Sufficient
9	+	+	+	≥1500	Sufficient
10	+	+	+	≥1500	Sufficient
11	+	+	+	≥1500	Sufficient
12	+	+	+	≥1500	Sufficient
13	-	-	-	≤500	Insufficient
14	+	+	+	≥1500	Sufficient
15	-	-	-	≤500	Insufficient
16	+	+	+	≥1500	Sufficient
17	-	-	-	≤500	Insufficient
18	-	-	-	≤500	Insufficient
19	-	-	-	≤500	Insufficient
20	+	+	+	≥1500	Sufficient
21	+	+	+	≥1500	Sufficient

+, Turbidity in the tubes; -, no turbidity in the tubes.

Table 3. Comparison of all parameters in the study with sTREM-1 and MR-Pro-ADM (patients $n = 21$ and controls $n = 10$)

Parameter	sTREM-1 (ng/L)	MR- Pro-ADM (ng/L)
Alanine aminotransferase (IU/L)	-0.086	0.013
Aspartate aminotransferase (IU/L)	-0.060	0.044
Gamma glutamyl transferase (IU/L)	-0.246*	-0.225*
Creatinine (mg/dL)	-0.093	0.113
Urea (mg/dL)	0.043	0.144
Total bilirubin (mg/dL)	-0.095	-0.035
Direct bilirubin (mg/dL)	-0.095	-0.040
Total protein (g/dL)	0.104	-0.131
Albumin (g/dL)	0.235*	-0.054
Globulin (g/dL)	0.045	-0.050
Creatine kinase (IU/L)	-0.145	0.188*
Glucose (mg/dL)	-0.259**	0.074
Total leukocyte count ($\times 10^3/\mu L$)	-0.228*	0.067
Monocyte count (m/mm^3)	-0.382**	-0.035
Erythrocyte count ($\times 10^6/\mu L$)	0.198*	-0.032
Hemoglobin concentration (g/dL)	0.229*	-0.086
Body temperature (°C)	-0.100	0.066
Pulse (/m)	-0.291**	0.116
Respiration (/m)	-0.031	0.130
sTREM-1 (ng/L)	-	-0.278**

*The correlation was significant at the 0.05 level.
**The correlation was significant at the 0.01 level.

DISCUSSION AND CONCLUSION

Stagnation, tachycardia due to a decrease in blood pressure (but weak pulse), hypothermia due to fluid loss after initial hyperthermia, tachypnea, loss of sucking reflex, depression, enophthalmos, and loss of skin elasticity was seen in calves with sepsis. These results were consistent with previous findings (Constable, 2007; Akyüz et al., 2017; Topal, 2018).

E. coli, rotavirus, coronavirus, clostridial agents, and *Cryptosporidium* play major roles in the occurrence of sepsis in neonatal calves (Akyüz et al., 2017;Azkur and Aksoy, 2018; Hacıoğlu and Alkan, 2018;Şahal et al., 2018; Bal, 2019).Using immunochromatographic test kits, *E. coli* and its strains were detected in 5, viral agents (coronavirus, rotavirus, or both) were detected in 5, and mixed agents (rotavirus, *E. coli*, and strains with coronavirus, *Cryptosporidium*, or all together) were detected in 11calves with sepsis in this study.

Environmental factors (Bal, 2019), immunological factors (Pazarçeviren, 2008), care and feeding conditions and vitamin-mineral and trace element deficiencies (Al and Balıkçı, 2012) are among non-infectious causes of diarrhea. Factors such as failure to give due importance to hygiene in institutions, not giving enough colostrum to calves, and keeping animals of different age groups together are predispositions for diseases (Akyüz et al., 2017). This study showed that the operating conditions of the institution were bad, animals of different age groups were kept together, and seven of the patients did not receive any colostrum, which was in line with the published reports. This information was confirmed by performing the sodium sulfide precipitation test, which was used to determine the passive transfer status in sick calves.

It was important to vaccinate pregnant cows with *E. coli*, rota, and coronavirus antigens (Azkur and Aksoy, 2018). The protection of calves born from unvaccinated cows was found to be insufficient (antibody level of 25%) (Güngör and Baştan, 2004). In this study, the information was given by the owner of the patients that the mothers of the calves did not receive the *E. coli*, rota, and coronavirus vaccines. We believed that this was the reason for the severe infection.

A body temperature of >39°C/<36.6°C, a WBC count of $<5 \times 10^3/\mu L$ / $>12 \times 10^3/\mu L$, a respiratory rate of >45/min, a pulse of <90/>120/min, and band neutrophil of >10% are among SIRS criteria. The presence of two of these criteria is sufficient for SIRS diagnosis (Hart and Mackay, 2015;Akyüz et al., 2017; Beydilli and Gökçe, 2019; Eroğlu and Kırbaş, 2020; Akyüz and Gökce, 2021). The calves included in the study also had at least two of these criteria.

Leukopenia or leukocytosis might be seen in calves with sepsis (Beydilli and Gökçe, 2019; Akyüz and Gökce, 2021). Of the 21 calves in the sepsis group, 2 had leukopenia, 9 had leukocytosis, and 10 were within the reference range. In addition, WBC count in the sepsis group was higher before and during treatment compared to the control group due to infection. These results were similar to those of Akyüz and Gökce (2021).

AST and ALT values were found to be high in calves with sepsis. This was due to liver damage caused by toxemia (Başer and Civelek, 2013; Zeybek, 2013).Also, passive congestion caused by endotoxemia, tension in the stomach, and muscle breakdown affected this increase (Merhan et al., 2016). In this study, the reason why ALT and AST levels were found to be higher in the sepsis group compared to the control group is the decrease in tissue perfusion caused by dehydration and sepsis.

Decreases in blood pressure and glomerular filtration rate were observed in calves with diarrhea due to dehydration. As a result, the renal function deteriorated and acute renal failure developed (Pazarçeviren, 2008; Başer and Civelek, 2013). As a result, the serum urea and creatinine values increased (Özkan and Akgül, 2004; Aycan, 2006;Uzlu et al., 2010; Zeybek, 2013; Başer and Civelek, 2013; Merhan et al., 2016; Bal, 2019; Eroğlu and Kırbaş, 2020). In this study, we think that the reason for the increase in serum urea and creatinine levels in calves in the sepsis group is the deterioration of renal functions due to dehydration ($P<0.05$).

One of the most important complications of sepsis is myopathy. As a result, the serum CK enzyme level increases (Aydın et al., 2018; Akyüz and Gökce, 2021). In this study, as a result of sepsis-related tissue damage and muscle destruction in the patient group, CK enzyme activity increased compared to the control group.

The TP level increases in diarrhea. The reason for this increase is hemoconcentration, which occurs as a result of the decrease in plasma volume due to dehydration (Başer and Civelek, 2013; Eroğlu and Kırbaş, 2020). Other reasons are an increase in serum sialic

acid and hyperfibrinogenemia due to inflammation (Uzlu et al., 2010; Merhan et al., 2016). A study on calves with diarrhea showed a decrease in the albumin level (Silverstein and Otto, 2012). Albumin is also among the albumin-negative acute phase proteins (Gruysve ark 2005). This was an indicator of inflammation (Zeybek, 2013).In this study, an increase in TP level due to dehydration and a decrease in albumin level due to inflammation were determined in the sepsis group compared to the control group.

Hypoglycemia is frequently encountered in calves with diarrhea during the neonatal period (Zeybek, 2013;Trefz et al., 2017; Eroğlu and Kırbaş, 2020). In addition, other studies reported that the serum glucose level did not change in calf diarrhea (Silverstein and Otto, 2012), and sometimes hyperglycemia occurred (Başer and Civelek, 2013; Zeybek, 2013). The reason for the increase was the stress caused by endotoxemia in the animals. Hyperglycemia was predominantly observed before the treatment in the sepsis group in this study. This continued throughout treatment. The reasons for this were the severe conditions of the animals in the study group, lack of oral consumption, state of being hypothermic, and glucose infusion due to the absorption problem caused by damage to the digestive system.

In case of inflammation in the intestines, sTREM-1 activity is suppressed and sTREM-1 levels decrease. This is due to disruption of the intestinal epithelial barrier and tissue destruction. When inflammation occurs in the intestines, neutrophils migrate into the intestinal crypts and mucosal damage occurs. Then, sTREM-1 levels decrease as neutrophils migrate into and adhere to tissues (Kutlu et al., 2021). Coronavirus causes the cubic epithelium to transform into squamous epithelium (Atlı et al., 2018). Rotavirus causes atrophy of villi and proliferation of crypt cells (Bal, 2019). *E. coli* causes lesions in microvilli and destruction of villi (villous atrophy in the small intestines and damage to the lamina propria) (Torres et al., 2005). *C. parvum* causes pathological changes in the intestines (atrophy of villi, hyperplasia of crypts, inflammatory infiltration, and degeneration of microvilli). In this study, sTREM-1 levels in the sepsis group were found to be low for the reasons stated ($P<0.05$).

Adrenomedullin increases during sepsis (Jordan et al., 2014), and the severity of the disease is directly proportional to the level of ADM (Marino et al., 2014; Angeletti et al., 2015; Simon et al. 2016; Geven et al., 2018). When lung injury due to sepsis occurs, ADM cannot be removed from the pulmonary circulation (Abd Elmouttaleb et al., 2016). In addition, ADM levels increase in hepatic and renal failure (Abd Elmouttaleb et al., 2016). Lipopolysaccharides that cause sepsis (Garayoa et al., 2000), proinflammatory cytokines (Ghobrial et al., 2020), hypoxia in sepsis, oxidative stress, and inflammation increase ADM synthesis (Simon et al., 2016; Jordan et al., 2014; Fahmey et al., 2018; Garazzino et al., 2019). Also, ADM has antimicrobial (Garazzino et al., 2019) and anti-inflammatory properties (Akpınar et al., 2014). In this study, MR-Pro-ADM values of calves were found to be higher in the group with sepsis compared to the control group due to hypoxia, oxidative stress, endotoxemia and inflammation.

No studies reported mid-regional-pro-adrenomedullin together with sTREM-1. However, studies on sepsis reported that MR-Pro-ADM levels increased (Fahmey et al., 2018; Garazzino et al., 2019) and sTREM-1 levels decreased (Kutlu et al., 2021) in case of sepsis. Compared to the control group, MR-Pro-ADM increased while sTREM-1 decreased in the group with sepsis. A negative correlation (-0.278) was determined between MR-Pro-ADM and sTREM.

Hence, it was presumed that sTREM-1 was a negative acute phase reactivator incase of inflammation, and MR-Pro-ADM was a positive acute-phase reactivator in case of inflammation. We believed that the levels of MR-Pro-ADM and sTREM-1 did not approach those in the control group during the treatment despite intensive treatment because of the severe course of inflammation and the short follow-up period (3 days). The follow-up period should be longer to see a significant change. In conclusion, MR-Pro-ADM and sTREM-1 are important in terms of diagnosis in the evaluation of sepsis in newborn calves.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this study or its results.

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