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Prospective evaluation of oxidative stress and hematologic indices in dogs with systemic inflammatory response syndrome

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Summary

Systemic inflammatory response syndrome (SIRS) is a complex clinical condition associated with systemic inflammation and oxidative stress. This study aimed to assess oxidative stress status and hematological changes in dogs diagnosed with SIRS and to investigate potential relationships between oxidative stress markers and hematological parameters. Dogs diagnosed with SIRS (n = 31; mean age 3.3 years, range 2 months-12 years) and healthy control dogs (n = 9; mean age 4.2 years, range 4 months-10 years) were included in the study. Serum total antioxidant status (TAS) and total oxidant status (TOS) were measured, and complete blood count parameters were evaluated. Serum TAS levels were significantly lower in dogs with SIRS compared to healthy dogs (mean rank: 18.16 vs. 28.56; p = 0.019), whereas serum TOS levels were significantly higher in dogs with SIRS (mean rank: 22.77 vs. 12.67; p = 0.022). Hematological alterations in dogs with SIRS were variable; leukopenia was detected in 35.5% of cases, lymphopenia in 25.8%, and thrombocytosis in 25.8%. Comparison between groups revealed significant differences only in monocyte percentage, which was higher in dogs with SIRS (p = 0.039), and mean corpuscular hemoglobin concentration, which was lower in dogs with SIRS (p = 0.031). Correlation analyses demonstrated weak, non-significant positive associations between TAS and red blood cell count (r = 0.217), hemoglobin concentration (r = 0.304), and hematocrit (r = 0.287), as well as a weak, non-significant negative correlation between TOS and granulocyte count (r = −0.076). These findings suggest that oxidative stress and hematological alterations may occur concurrently in canine SIRS, but are not necessarily directly or linearly related. Repeated and longitudinal evaluation of oxidative stress markers together with hematological parameters may provide further insight into their relationship in dogs with SIRS.

Keywords: canine, systemic inflammatory response syndrome, total antioxidant status, total oxidant status

Systemic inflammatory response syndrome (SIRS) is a condition characterized by abnormalities in hematological and vital signs (6, 10), representing clinical manifestations of a systemic response to infectious (septic SIRS) or noninfectious (nonseptic SIRS) insult (19, 25). Diseases caused by viral, bacterial, fungal and protozoal agents that can progress with sepsis are among the most common infectious causes of septic SIRS (26). Nonseptic SIRS occurs as a result of noninfectious inflammation caused by conditions such as pancreatitis, polytrauma, immune-mediated diseases, neoplasms, burns, heat stroke, or snake bite (21, 26). The increasing severity of hemodynamic and/or metabolic changes during SIRS may lead to a shock condition that can be further complicated by multiple organ dysfunction and may result in death (1, 4). Early identification of potential risk factors or SIRS is important for timely therapeutic interventions and prevention of these life-threatening complications (4).

In canine patients, SIRS is characterized by the presence of at least two of the following clinical abnormalities: alterations in body temperature, abnormal heart rate, abnormal respiratory rate, and deviations in leukocyte count (24). In addition to leukocyte alterations, hemogram abnormalities, such as anemia, elevated hematocrit, and/or thrombocytopenia, may also be encountered in dogs with SIRS (25).

Systemic inflammatory response syndrome represents as an excessive pro-inflammatory cascade triggered by intricate interactions between inflammatory

and endothelial cells, resulting in the production of cytokines, eicosanoids, and free radicals (19). Free radicals and other reactive oxygen species (ROS), when produced in excess and not adequately neutralized, lead to an imbalance that has been redefined over the years as "oxidative stress," a condition capable of causing molecular damage (15, 20). High levels of ROS can damage proteins, lipids, and DNA by collecting unpaired electrons from surrounding molecules (14). Oxidative stress is assessed by the activity of antioxidant enzymes, the presence of oxidative damage byproducts, and endogenous antioxidant concentrations (16). Inflammatory cells, such as neutrophils, eosinophils and macrophages, are reported to be among the endogenous sources contributing to ROS production (18).

Since the measurement of specific antioxidants in blood or tissues can be inconsistent, and species diversity is significant, markers of global antioxidant capacity (total antioxidant capacity), antioxidant enzyme activity, and byproducts of oxidative damage are often used for a more accurate assessment of oxidative stress *in vivo*. In this context, total oxidant status (TOS) and total antioxidant status (TAS) are frequently used parameters to assess overall antioxidant status (16, 17).

In canine practice, numerous studies have investigated oxidative stress levels in various pathological conditions associated with infectious (7, 8, 15, 23) and non-infectious agents (2, 17, 22, 27). Previous studies have shown that TOS is associated with granulocyte-related inflammatory responses (5), whereas TAS has been reported (11) to be related to erythrocyte parameters. This study aimed to determine hematological abnormalities as well as TAS and TOS levels in dogs developing SIRS due to various pathological conditions and to reveal the correlation between them.

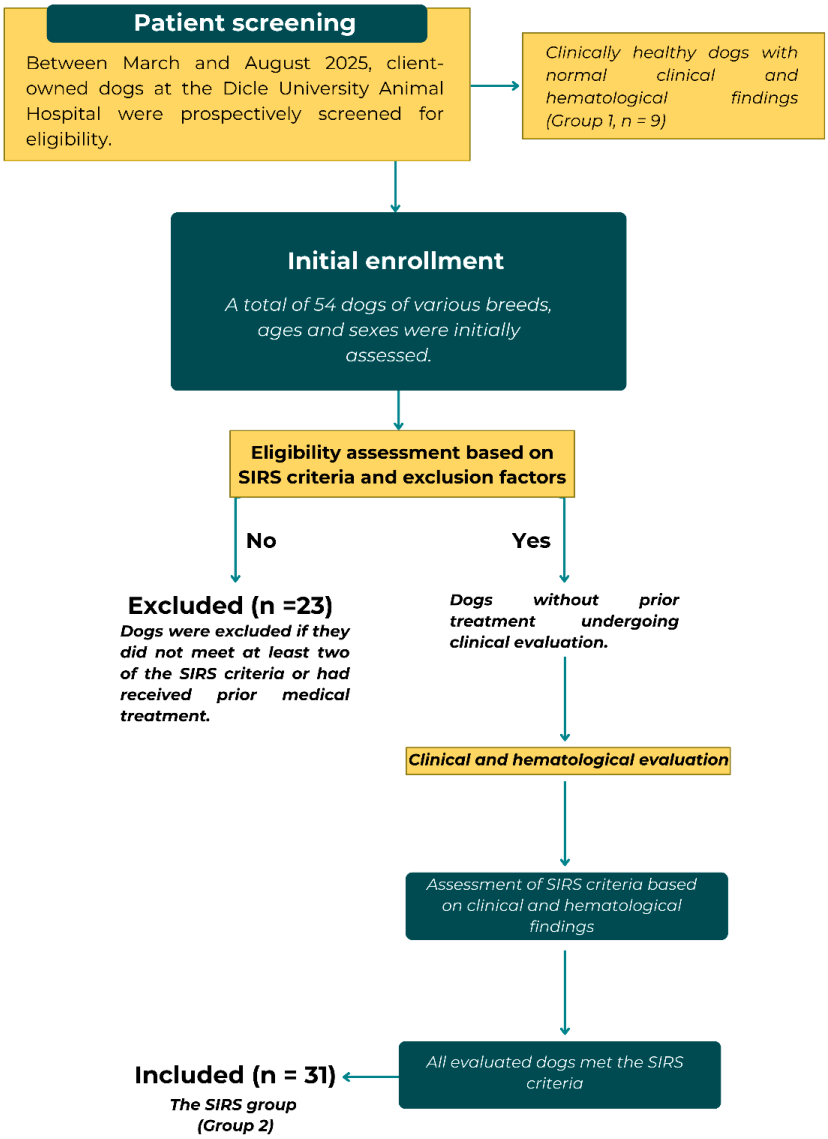

Fig. 1. Study workflow illustrating prospective screening, exclusion criteria, and final group allocation of client-owned dogs enrolled in the study

Material and methods

Ethics statement. Ethical approval was obtained from the Local Ethics Committee for Animal Experiments, Dicle University (08-03/26.03.2025).

Animals and inclusion criteria. Between March and August 2025, a total of 54 client-owned dogs of various breeds and ages and both sexes were prospectively assessed for eligibility at the Dicle University Animal Hospital. Of these, 23 dogs were excluded because they did not meet the SIRS criteria or had received prior medical treatment before presentation. The remaining dogs (n = 31), which had not received any recent treatment and met at least two of the SIRS criteria as defined by Hauptman et al. (12), including abnormal body temperature (< 37.8°C or > 39.4°C), tachycardia (pulse rate > 140 beats per minute), tachypnea (respiratory rate > 30 breaths per minute), and abnormal white blood cell count (< 6,000 or > 16,000 cells/µL), were included in the SIRS group (Group 2). The study workflow, including patient screening, exclusion, and group allocation, is shown in Figure 1.

Dogs presented to the hospital for routine clinical and hematological examinations, with no evidence of systemic infection on physical examination and no hematological abnormalities (n = 9), constituted the healthy control group (Group 1). The owners of all dogs included in the study were informed about the study, and written informed consent was obtained from each owner.

Sample and data collection. The age, breed, sex, clinical findings, and diagnoses of each patient evaluated in the study were recorded. During the clinical examination, each patient's pulse rate, respiratory rate, and rectal temperature were determined. Blood samples were obtained from the cephalic vein and collected into both anticoagulant and non-anticoagulant tubes for complete blood count and serum TAS and TOS analyses. Samples collected into

anticoagulant tubes were immediately analyzed using an automated hematology analyzer (Mindray, BC2800 Vet, China) to determine total leukocyte count (WBC), lymphocyte (Lymph), monocyte (Mon), granulocyte (Gran), total erythrocyte count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT), mean platelet volume (MPV), and eosinophil (Eos) parameters. Samples collected into tubes without anticoagulant were allowed to clot at room temperature and were subsequently centrifuged at 3000 rpm/10 minutes. The serum samples obtained were transferred into Eppendorf tubes and stored at –80°C until the TAS and TOS levels were measured.

Determination of total antioxidant and oxidant status. Serum levels of TAS and TOS were determined spectrophotometrically using commercial ELISA kits (Rel Assay Diagnostics, Mega Tıp, Türkiye) according to the manufacturer's instructions. All assays were conducted under standardized laboratory conditions in strict accordance with the kit protocols. The data obtained were subjected to statistical analyses for further evaluation.

Statistical analysis. Data normality was assessed, and the variables were determined to be non-normally distributed. Comparisons of complete blood count and TAS-TOS data between healthy dogs and dogs with SIRS were performed using the non-parametric Mann-Whitney U test. Spearman's correlation analysis was used to evaluate the relationships between TAS and RBC, HGB, and HCT, as well as between TOS and Gran.

Results and discussion

Breed, age, sex and clinical data of the dogs enrolled. The healthy dogs included in the present study comprised Belgian Malinois (n = 2), mixed breed (n = 1), Anatolian Shepherd (n = 1), German Shepherd (n = 2), Terrier (n = 1), Labrador Retriever (n = 1), and Poodle (n = 1). The mean age was 4.2 years (range: 4 months-10 years). The sex distribution consisted of 7 males and 2 females. The breeds of the dogs with SIRS included in the study were Belgian Malinois (n = 8), mixed breed (n = 5), Anatolian Shepherd (n = 5), German Shepherd (n = 4), Terrier (n = 4), Golden Retriever (n = 3), French Bulldog (n = 1), and Cane Corso (n = 1). The mean age was 3.3 years (range: 2 months-12 years). The dogs with SIRS included in the study comprised 18 males and 13 females. The diagnoses of the dogs with SIRS were as follows: canine parvoviral enteritis (n = 4), canine distemper (n = 2), upper respiratory tract infection (n = 4), acute gastroenteritis (n = 16), chronic gastroenteritis (n = 3), otitis media (n = 1), and oral papillamatosis (n = 1). Figure 2 illustrates the distribution of diseases among the dogs diagnosed with SIRS.

Evaluation of complete blood count. In the evaluation of hematological parameters of the dogs with SIRS, leukopenia was detected in 11 dogs (35.5%) and leukocytosis in 5 dogs (16.1%). Assessment of absolute and percentage lymphocyte parameters revealed lymphopenia in 8 dogs (25.8%) and lymphocytosis in 1 dog (3.2%). Granulocyte assessment indicated granulocytopenia in 4 dogs (12.9%) and granulocytosis in 7 dogs (22.6%). Based on erythrogram indices, anemia was detected in 1 dog (3.2%), while no erythrogram abnormalities were observed in the remaining cases. No thrombocytopenia was detected, whereas thrombocytosis was observed in 8 dogs (25.8%). Additionally, eosinophilia was not detected in any of the dogs. Table 1 presents the mean, minimum, and maximum values of hematological parameters in the healthy dogs and the dogs with SIRS. Comparing hematological parameters between the healthy dogs and the dogs with SIRS, only Mon% and MCHC showed significant differences. Monocyte% was significantly ($p = 0.039$) higher in the dogs with SIRS compared to the healthy dogs, whereas MCHC was significantly ($p = 0.031$) lower in the dogs with SIRS than it was in the healthy dogs.

Serum TAS and TOS levels. In the dogs with SIRS, serum TAS and TOS levels differed significantly higher in healthy dogs (Tab. 2). The mean rank of serum TAS levels was significantly ($p = 0.019$) higher in the healthy dogs (28.56) compared with the dogs with SIRS (18.16). Conversely, the mean rank of serum TOS levels was significantly ($p = 0.022$) higher in the dogs with SIRS (22.77) than it was in the healthy dogs (12.67).

In the dogs with SIRS, correlations between TAS and RBC, HGB, and HCT, as well as between TOS and granulocyte count, were evaluated. Total antioxidant status showed weak positive correlations with RBC, HGB, and HCT ($r = 0.217$, $r = 0.304$, and $r = 0.287$, respectively), but none of these correlations were statistically significant. Total oxidant status also demonstrated a weak negative correlation with granulocyte count ($r = –0.076$), which was not statistically significant.

Systemic inflammatory response syndrome is a complex clinical condition that develops as a result of the excessive release of inflammatory mediators during various disease processes (12). Due to its heterogeneous etiology, SIRS in dogs may arise from a wide range of underlying conditions (13). In the

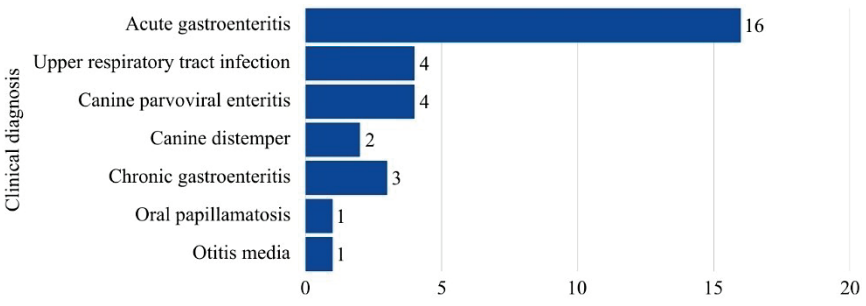

Fig. 2. Distribution of diseases in dogs with SIRS

Tab. 1. Descriptive statistics of hematological parameters in dogs included in the study

Parameters (Unit)	Groups	n	Minimum	Maximum	Mean ± Standard deviation	p-value
WBC (10^9/L)	Healthy dogs	9	8.30	11.10	9.5667 ± 1.0025	0.466
	Dogs with SIRS	31	1.60	50.80	12.6261 ± 10.1377	
Lymph (10^9/L)	Healthy dogs	9	1.10	3.00	1.9667 ± 0.6928	0.795
	Dogs with SIRS	31	0.30	5.30	2.0452 ±1.2476	
Mon (10^9/L)	Healthy dogs	9	0.30	0.60	0.4556 ± 0.1236	0.896
	Dogs with SIRS	31	0.10	1.60	0.4839 ± 0.3474	
Gran (10^9/L)	Healthy dogs	9	6.40	9.00	7.1444 ± 0.784	0.486
	Dogs with SIRS	31	1.10	43.90	10.100 ± 8.8185	
Lymph (%)	Healthy dogs	9	12.40	28.70	20.211 ± 5.7062	0.582
	Dogs with SIRS	31	8.70	51.40	19.996 ± 9.2413	
Mon (%)	Healthy dogs	9	3.00	5.90	4.9333 ± 1.0173	0.039*
	Dogs with SIRS	31	2.50	6.90	4.1839 ± 1.0043	
Gran (%)	Healthy dogs	9	65.90	84.60	74.8556 ± 6.5515	0.315
	Dogs with SIRS	31	44.20	87.50	75.8194 ± 9.6635	
RBC (10^{12}/L)	Healthy dogs	9	4.91	10.28	7.7022 ± 1.4644	0.391
	Dogs with SIRS	31	3.81	11.69	8.0774 ± 1.3483	
HGB (g/dL	Healthy dogs	9	10.40	19.70	16.1111 ± 2.6727	0.627
	Dogs with SIRS	31	7.40	24.20	16.5032 ± 3.0841	
HCT (%)	Healthy dogs	9	37.70	66.10	53.2889 ± 8.36084	0.382
	Dogs with SIRS	31	25.50	83.10	55.6903 ± 10.2095	
MCV (fL)	Healthy dogs	9	64.30	76.80	69.7556 ± 4.5058	0.604
	Dogs with SIRS	31	6.70	74.90	66.9516 ± 11.8370	
MCH (pg)	Healthy dogs	9	19.10	22.20	20.9556 ± 1.0736	0.194
	Dogs with SIRS	31	17.60	22.20	20.3419 ± 1.3455	
MCHC (g/dL)	Healthy dogs	9	27.50	31.00	30.1111 ± 1.0890	0.031*
	Dogs with SIRS	31	28.40	31.10	29.5581 ± 0.6479	
RDW (%)	Healthy dogs	9	12.20	20.80	14.4667 ± 2.6528	0.795
	Dogs with SIRS	31	11.20	16.40	14.0935 ± 1.3783	
PLT (10^9/L)	Healthy dogs	9	192	429	301.0000 ± 101.8368	0.072
	Dogs with SIRS	31	143	708	397.7419 ± 146.3666	
MPV (fL)	Healthy dogs	9	7.10	10.90	8.2111 ± 1.0600	0.399
	Dogs with SIRS	31	6.60	10.30	7.9032 ± 0.8761	
PCT (%)	Healthy dogs	9	0.15	0.44	0.2499 ± 0.1023	0.124
	Dogs with SIRS	31	0.12	0.51	0.3106 ± 0.1103	
Eos (%)	Healthy dogs	9	1.50	6.70	3.8778 ±1.9841	0.116
	Dogs with SIRS	31	0.60	8.60	3.0742 ± 2.4056	

Explanations: Statistically significant ($p < 0.05$) differences between dogs with SIRS and healthy controls are marked with an asterisk (*)

present study, the most frequently observed underlying condition in the dogs with SIRS was acute gastroenteritis (16/31; 51.6%), followed by canine parvoviral enteritis (4/31; 12.9%), and upper respiratory tract infection (4/31; 12.9%). The variety of underlying diseases observed in the present study supports the supposition that oxidative stress may represent a common pathophysiological pathway in dogs with SIRS, regardless of the primary etiology.

Spillane et al. (26) have shown that the SIRS-4 composite criteria perform better in predicting patient outcomes, highlighting the importance of complete blood count parameters in the evaluation of dogs with SIRS, and indicating that SIRS status should not be assessed unless a complete blood count is available. Dogs with SIRS commonly exhibit hematological abnormalities, such as leukopenia and/or leukocytosis, anemia, increased hematocrit values, monocytosis, and thrombocytopenia (24, 25).

In the present study, both leukopenia and leukocytosis were detected in dogs with SIRS, indicating variable leukocyte responses. Granulocytopenia and granulocytosis accompanied the leukogram abnormalities observed in some dogs with SIRS, and changes in circulating granulocyte numbers should be considered when interpreting WBC. Similar variability in leukocyte responses has previously been reported in dogs with SIRS and has been attributed to differences in disease stage and severity (10). Sikora et al.

Tab. 2. Serum TAS and TOS levels in dogs with SIRS and healthy dogs

Parameter (unit)	Healthy dogs			Dogs with SIRS			p-value
	Minimum	Maximum	Mean ± Standard deviation	Minimum	Maximum	Mean ± Standard deviation	
Total antioxidant status (mmol/L)	79.17	87.71	83.51 ± 2.43	1.21	88.71	54.55 ± 32.20	0.019*
Total oxidant status (µmol/L)	80.94	94.00	83.64 ± 3.94	81.31	90.30	83.64 ± 10.95	0.022*

Explanations: Data are presented as minimum, maximum, mean, and standard deviation. A p-value < 0.05 was considered statistically significant, and statistically significant p-values are indicated with an asterisk (*)

(25) suggest that SIRS is associated with neutrophil activation and consumption, which may contribute to the alterations observed in leukocyte counts. Overall, these findings suggest that the leukocyte abnormalities observed in dogs with SIRS reflect a complex inflammatory process.

Although oxidative stress has been reported (11) to shorten erythrocyte lifespan through membrane damage, in the present study erythrogram abnormalities were limited, with anemia detected in only one dog with SIRS. This finding suggests that erythrocyte-related changes may not represent a predominant feature in all cases of SIRS, particularly in the absence of severe hemorrhage or chronic disease (3). Similarly, although thrombocytopenia was not observed, thrombocytosis was detected in 25.8% of the dogs, which is likely to be a reactive response to systemic inflammation. Only significant differences in Mon% and MCHC were observed between the dogs with SIRS and the healthy control dogs. The increase in Mon% observed in the dogs with SIRS may indicate increased monocyte activation during systemic inflammation, consistent with previous reports (22). Conversely, the decrease in MCHC may suggest subtle changes in erythrocyte integrity, possibly caused by inflammatory or oxidative stress-related mechanisms (9).

Although weak correlations were observed between TAS and erythrocyte-related parameters and between TOS and granulocyte count, none of these associations reached statistical significance. These findings suggest that oxidative stress and hematological alteration in dogs with SIRS may occur simultaneously, but not necessarily in a direct or linear manner. Furthermore, the heterogeneous etiology of SIRS and variability in disease severity among affected dogs may limit the detection of potential linear associations between oxidative stress markers and individual hematological parameters, particularly when assessments are based on single time-point measurements.

The fact that no statistically significant correlation was found between serum TAS and TOS levels and hematological parameters in the dogs with SIRS in the present study, is thought to be due to the small sample size and, on the other hand, the wide age range of the dogs included in the study. These factors are considered to be the most important limitations of the study.

In conclusion, the dogs with SIRS exhibited altered oxidative stress status, with decreased serum TAS and increased TOS levels, while hematological changes were limited and weakly associated. Further longitudinal studies with repeated measurements are warranted to clarify this relationship.

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