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R Jasmin
Department of Veterinary Clinical Medicine, Madras Veterinary College, Vepery, Chennai, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

M Balagangatharathilagar
Department of Veterinary Clinical Medicine, Madras Veterinary College, Vepery, Chennai, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

R Edith
Department of Veterinary Parasitology, Madras Veterinary College, Vepery, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu

M Chandrasekar
Department of Veterinary Clinical Medicine, Madras Veterinary College, Vepery, Chennai, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

MG Jayathangaraj
Department of Veterinary Clinical Medicine, Madras Veterinary College, Vepery, Chennai, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

Corresponding Author:
M Balagangatharathilagar
Department of Veterinary Clinical Medicine, Madras Veterinary College, Vepery, Chennai, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

Hematological alterations in canine sepsis

R Jasmin, M Balagangatharathilagar, R Edith, M Chandrasekar and MG Jayathangaraj

Abstract
The aim of this study was to evaluate the hematological alterations in dogs with sepsis in comparison with healthy controls. The present study evaluated the hematological alterations in blood obtained from 103 septic dogs admitted in Critical Care Unit of the Madras Veterinary College Teaching Hospital. The results were compared with 10 healthy dogs. Based on the SIRS criteria, microbial source in blood, organ dysfunction and hemodynamic response the septic patients were identified in the present study. Blood samples were collected aseptically by cephalic venipuncture and analysis was performed using the auto hematology analyzer. The results revealed significant variations in hematological parameters between the control group and the septic groups.

Keywords: SIRS, Sepsis, CCU, Hematology, Dogs

Introduction
Sepsis is one of the leading causes of mortality in human and small animal patients admitted in Intensive Care Units [1]. Early identification of sepsis plays a vital role to decrease mortality and for early implementation of therapies in critically ill patients. The hematologic organ system is a major element in the response to a septic insult and plays a vital role in the resolution phase of severe sepsis [2]. Goyette *et al*. (2004) opined that knowledge of the hematologic manifestations of sepsis can improve diagnosis and therapy of patients with this common and frequently fatal disorder [3]. Piereni *at al.* (2020) have reported that the leucocyte and platelet abnormalities on complete blood count ratios are potential prognostic markers in canine sepsis [4] with this background, the present clinical study was conducted with the objective to determine hematological alterations among dogs presented with sepsis.

Materials and Methods
Study Design: The study was designed as prospective clinical study and was carried out at the Critical Care Unit, Department of Veterinary Clinical Medicine, Madras Veterinary College, Chennai.

Animals: Apparently healthy dogs (n=10) aged between 1 and 5 years presented to the Madras Veterinary College Teaching Hospital for health checkup and vaccination were taken for the control group (Group IV) and they were subjected to a detailed clinical, hematological and various diagnostic tests to assess baseline values of the selected parameters.

Systemic Inflammatory Response Syndrome (SIRS): Dogs fulfilling the SIRS criteria were evaluated for the possibility of an underlying causative septic focus. Dogs presented with two or more than two of the following SIRS criteria were diagnosed as SIRS positive patients. Criteria used for clinical diagnosis of SIRS in dogs (Otto, 2007) [5] were

Temperature	:	< 37.2 °C or >39.4 °C
Heart Rate (beats/min)	:	> 150/minute
Respiratory Rate (breaths/min)	:	> 40/minute
WBC count (cells/cmm)	:	< 5000 or >19000 or > 5% bands

Sepsis: Based on the SIRS criteria, microbial source, organ dysfunction and hemodynamic response, the patients were classified into the following groups.

	Description	Number of cases	Selection criteria
Group 1	Sepsis	36	Confirmed microbial source in blood
Group 1I	Severe sepsis	57	Confirmed microbial source in blood + One or more organ failure
Group 1II	Septic shock	10	Confirmed microbial source in blood + One or more organ failure+ Persistent hypotension
Group 1V	Healthy Dogs	10	Normal vital signs

Etiology of Sepsis: Based on the etiology of sepsis the dogs under study were classified based on the underlying etiology into the following categories Falconer (1981) [4].

	Description	Number of cases	Selection criteria
Category I	Bacterial sepsis	64	SIRS criteria + Universal 16S rRNA PCR and/or Blood culture positive and/or MODS and/or refractory hypotension
Category 1I	Blood parasitic origin of sepsis	39	SIRS criteria + Proven source by PCR and/or blood smear examination + and/or MODS and/or refractory hypotension
Category III	Healthy Dogs	10	Normal vital signs

Blood samples were collected by cephalic venipuncture after aseptic preparation of the site. Blood samples in EDTA tubes were used for routine hematology and clotted blood in plain tubes without anticoagulant were used for assessment of serum biochemistry. The blood samples were subjected to polymerase chain reaction (PCR) using 16S rRNA and blood culture for bacteria. Blood samples were also subjected to PCR for *Ehrlichia canis*, *Babesia* sp. and *Anaplasma* sp. using published primers.

Hematological parameters such as Hemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), White Blood Cell count (WBC count), Platelet count (PLT count) were analyzed using auto hematology analyzer (Mindray – BC-2800 Vet). Peripheral blood smears were prepared, stained with Leishman-Giemsa stain and examined for Differential Leukocyte Count (DLC) and changes in blood picture.

Results

The mean ± S.E, median, minimum and maximum values for haemogram in dogs under various categories by etiological agent are given in Table 1.

The mean ± S.E values of hemoglobin (g/dL) in Category III was 12.76 ± 0.58. The mean ± S.E values of hemoglobin in dogs under Category I and Category II were 9.01 ± 0.50 and 10.36 ± 1.14, respectively. There was no significant difference (P>0.05) in the hemoglobin levels of the Category I and Category II when compared with the Category III. But, the median values of the Category I and II were lower than Category III.

The mean ± S.E values of PCV (%) in dogs under Category I, Category II and Category III were 23.56 ± 1.33, 25.06 ± 1.68 and 34.7 ± 2.13, respectively. A significant (p<0.01) decrease in PCV was observed in the dogs under various categories than Category III.

The mean ± S.E values of RBC count (m/cmm) in dogs under Category I, Category II and Category III were 4.03 ± 0.21, 4.09 ± 0.27 and 6.04 ± 0.43, respectively. A highly significant (p<0.01) decrease in RBC count was observed in the dogs under various categories than Category III.

The mean ± S.E values of WBC count (cells/cmm) in dogs under Category I, Category II and Category III were 28776.06 ± 2428.6, 16038.5 ± 2221.63 and 10,730 ± 822.2, respectively. There was highly significant (p<0.01) increase in WBC count was observed in the Category I than the dogs under other categories.

The mean ± S.E values of platelet count (lakhs/cmm) in dogs under Category I, Category II and Category III were 163906.25 ± 16844.88, 74179.49 ± 13603.00 and 472,000 ± 40239.28, respectively. A highly significant (p<0.01) decrease in platelet count was observed in the dogs under various categories than Category III.

The mean ± S.E values of neutrophil count (%) in dogs under Category I, Category II and Category III were 82.28 ± 1.06, 76.97 ± 1.23 and 74.9 ± 1.28 respectively. A highly significant (p<0.01) increase in neutrophil count was observed in the Category I than the dogs under other categories.

The mean ± S.E values of lymphocyte count (%) in dogs under Category I, Category II and Category III were 12.53 ± 0.81, 15.41 ± 1.03 and 19.10 ± 0.95 respectively. There was a significant (p<0.01) decrease in lymphocyte count of Category I than the Category III and Category II had lymphocyte counts comparable with Category III.

The mean ± S.E values of monocyte count (%) in dogs under Category I, Category II and Category III were 4.60 ± 0.39, 6.31 ± 0.61 and 3.1 ± 0.23, respectively. There was a significant (p<0.01) increase in monocyte count of Category II than the Category III and Category I had monocyte counts comparable with healthy control Category III.

The mean ± S.E values of eosinophil count (%) in dogs under Category I, Category II and Category III were 0.67 ± 0.12, 1.03 ± 0.15 and 2.10 ± 0.18, respectively. A highly significant (p<0.01) decrease in eosinophil count was observed in the dogs under various categories than Category III.

Table 1: Haemogram in dogs under various categories of sepsis

Parameter		Category I Bacterial sepsis (n=64)	Category II Blood parasitic origin of sepsis (n=39)	Category III Control (n=10)	F-value
HB (g/dl)	Mean ± S.E	9.01^{a} ± 0.50	10.36^{ab} ± 1.14	12.76^{b} ± 0.58	2.60^{NS}
	Median	8.4	9.0	12.55	
	Minimum	2.20	2.80	10.70	
	Maximum	17.90	45.57	15.20	
PCV (%)	Mean ± S.E	23.56^{a} ± 1.33	25.06^{a} ± 1.68	34.70^{b} ± 2.13	5.02**
	Median	21.9	22.50	12.55	

	Minimum	4.20	7.90	10.70	
	Maximum	47.10	47.70	15.20	
RBC (m/cmm)	Mean ± S.E	4.03^{a} ± 0.21	4.09^{a} ± 0.27	6.04^{b} ± 0.43	6.40**
	Median	4.15	4.04	5.83	
	Minimum	0.96	1.23	4.39	
	Maximum	7.85	7.16	8.59	
WBC (cells /cmm)	Mean ± S.E	28776.1^{b} ± 2428.6	16038.5^{a} ± 2221.6	10730^{a} ± 822.2	9.97**
	Median	27550	12800	10750	
	Minimum	500	500	4900	
	Maximum	87700	52400	13900	
Platelets (lakhs/cmm)	Mean ± S.E	163906.25^{a} ± 16844.88	74179.49^{b} ± 13603.00	472000.00^{c} ± 40239.28	44.4**
	Median	130500	46000	416000	
	Minimum	9000	9000	355000	
	Maximum	500000	451000	678000	
N (%)	Mean ± S.E	82.28^{b} ± 1.06	76.97^{a} ± 1.23	74.9^{a} ± 1.28	7.52**
	Median	84	74	74	
	Minimum	55	68	67	
	Maximum	97	96	83	
L (%)	Mean ± S.E	12.53^{a} ± 0.81	15.41^{ab} ± 1.03	19.10^{b} ± 0.95	6.08**
	Median	12	15	20	
	Minimum	2	2	12	
	Maximum	28	27	22	
M (%)	Mean ± S.E	4.60^{ab} ± 0.39	6.31^{b} ± 0.61	3.1^{a} ± 0.23	5.32**
	Median	4	5	3	
	Minimum	1	0	2	
	Maximum	21	20	4	
E (%)	Mean ± S.E	0.67^{a} ± 0.12	1.03^{a} ± 0.15	2.10^{b} ± 0.18	10.9**
	Median	0.00	1	2	
	Minimum	0	0	1	
	Maximum	3	2	3	

The values bearing same superscript did not differ significantly.
*p<0.05-significant, **p<0.01-highly significant and ^{NS} p>0.05-non-significant.

Discussion

Sepsis is a common condition and also an important cause of high morbidity and mortality in both human beings and dogs. The hematologic system plays a key-role both in the response to sepsis and its resolution. The present study did not reveal a significant (p>0.05) changes in the hemoglobin level in the septic patients of various categories. But there was a significant decrease in the median value of hemoglobin in septic patients compared to the healthy control. A highly significant (p<0.01) decrease in PCV and RBC was observed in the septic patients of this study. A highly significant (p<0.010) decrease in platelet counts were observed in the dogs with sepsis. A highly significant (p<0.01) increase in the WBC and neutrophil counts were observed in the dogs with bacterial sepsis (Category I) and a highly significant (p<0.01) decrease in lymphocyte count was observed in the Category I whereas Category II is comparable with control. A highly significant (p<0.01) increase in monocyte count was observed in septic patients with blood parasitic origin of sepsis due to *E. canis*. A highly significant (p<0.01) increase in the eosinophil count was observed in the septic dogs of various categories (Table 1).

Rodwell *et al*. (1988) established hematologic scoring system for early diagnosis of neonatal sepsis which includes following parameters abnormal total leukocyte count, abnormal total neutrophil (PMN) count, elevated immature PMN count, elevated immature to total PMN ratio, immature to mature PMN ratio, platelet count and pronounced degenerative changes in PMNs [6].

As erythrophagocytosis occurs with ehrlichiosis, monocytosis was an obvious finding in the present study was concurrent with the earlier finding [7].

Goyette *et al*. (2004) reported that the most common abnormalities of the hematologic system in patients with sepsis are anemia, leukocytosis, thrombocytopenia, and activation of the hemostatic system [3].

In the present study anaemia might be due to the following indirect pathways (i) RBC destruction secondary to development of anti-erythrocytic membrane antibodies, (ii) inhibition of erythrolytic-5-nucleosidase, (iii) development of methemoglobinemia, which is secondary to oxidative stress, (iv) induction of serum hemolytic pattern and (v) increased macrophage erythrocytic activity [8].

Neutrophilia in the present study might be due to the fact that neutrophils respond to variety of signals in the process of chemotaxis, by regulating the migration from blood stream to tissues [9].

Rebar (2015) reviewed various stages of inflammation with the general pattern of leucocytic response *viz*., acute inflammation (characterized by increased WBC, segmented and band neutrophil count, decreased or no change in lymphocytes and variable monocyte count) and chronic inflammation (characterized by increased or no change in WBC, lymphocytes, segmented and band neutrophil count and increased monocytes and variable eosinophil count) [10].

Inflammatory processes may result in an activation of coagulation by an impaired function of natural anticoagulants [11]. Feistritzer and Wiedermann (2007) stated that the activation of hemostasis may finally result in overt Disseminated Intravascular Coagulopathy (DIC) [12]. Brainard

and Brown (2011) opined that patients with systemic inflammatory response might develop hemostatic abnormalities, ranging from subtle and subclinical activation of coagulation to fulminant DIC [13]. DIC results with increased platelet destruction [14]. Bauer and Moritz (2013) documented DIC in 67% of dogs with SIRS [15].

Conclusion

Hematological analysis revealed significantly decreased PCV, RBC and platelet counts and significant leukocytosis. Differential leucocyte count revealed neutrophilia, lymphopenia and monocytosis.

In conclusion, from this study we found that alterations in WBC count, platelet count and hematocrit level in septic patients are cheap and effective tools for early identification and prognosis of sepsis in critically ill patients.

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