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White blood cell count and neutrophil to lymphocyte ratio in uncomplicated and complicated canine babesiosis caused by *Babesia canis canis*

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ABSTRACT

Canine babesiosis caused by *B. canis* is very common cause of morbidity and mortality of dogs in Croatia. Although white blood cell populations play a crucial role in the SIRS it has been poorly described in canine babesiosis. Therefore the aim of this study was to investigate serial changes in circulating total white blood cells and relationship between circulating neutrophil and lymphocyte counts in uncomplicated and complicated canine babesiosis caused by *B. canis canis*. The study was performed on two groups of animals naturally infected by *B. Canis canis*. Group 1 consisted of 30 dogs with uncomplicated babesiosis while group 2 consisted of 30 dogs with various complications. The blood samples for the analyses were collected at the day of the admission, and the second and seventh day after the administration of imidocarb dipropionat. White blood cell count (WBC) was determined using an automatic haematology analyzer (System 9120; Serono Baker Diagnostic, Pennsylvania, USA). The ratio of neutrophil to lymphocyte count was acquired from relative percentages of neutrophils and lymphocytes that were manually determined from Romanowsky stained blood smears. The percentage of parasitemia was determined by counting the number of parasitized erythrocytes on one thousand erythrocytes. The differential count was determined manually in Romanovsky-stained blood smears. The WBC counts, relative number of neutrophils and lymphocytes, neutrophil to lymphocyte ratio and levels of parasitemia were compared between the two groups using the Mann-Whitney U test. White blood cell count was significantly higher on the seventh day after the antibabesial treatment in the group of complicated babesiosis. The relative number of neutrophiles was significantly higher on the second and seventh days after the antibabesial treatment in complicated babesiosis and the relative number of lymphocytes was significantly lower in the group of complicated babesiosis on the second day after the antibabesial treatment. The neutrophil to lymphocyte ratio was significantly higher on the second day after the treatment in the complicated babesiosis.

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Parasitemia levels were significantly higher in complicated canine babesiosis. On the basis of these results it may be concluded that there is a significant correlation between the severity of clinical course and increase in neutrophils and decrease in lymphocyte percentage. The ratio of neutrophil and lymphocyte counts (in absolute or relative values) is an easily measurable parameter which may express the severity of the affliction and serve as a good prognostic marker.

Key words: dog, babesiosis, parasitemia, white blood cell count, neutrophils, lymphocytes

Introduction

Canine babesiosis caused by *B. canis* is very common cause of morbidity and mortality of dogs in Croatia (CACCIO et al., 2002). Canine babesiosis is a tick borne disease caused by the intra-erythrocytic protozoan parasites *Babesia canis*, *Babesia gibsoni* and *Babesia microti-like* piroplasms (UILENBERG et al., 1989; TABOADA and MERCHANT, 1991; CAMACHO et al., 2001). There are three subspecies of *B. canis*: *B. canis canis*, *B. canis vogeli* and *B. canis rossii* (UILENBERG et al., 1989). The disease can be clinically classified into uncomplicated and complicated forms. Dogs with uncomplicated babesiosis typically present with signs relating to acute haemolysis, including pale mucous membrane, fever, anorexia, depression, splenomegaly and water hammer pulse (TABOADA and MERCHANT, 1991; MATIJATKO et al., 2007). The clinical manifestation of the complicated form is variable and it is related to the complication developed. The complications of canine babesiosis are acute renal failure, cerebral babesiosis, coagulopathy, icterus and hepatopathy, immune-mediated haemolytic anaemia (IMHA), peracute babesiosis, acute respiratory distress syndrome (ARDS), haemoconcentration and shock (LOBETTI, 2000).

The clinical presentation of canine babesiosis caused by *B. canis* is highly variable. The many and varied clinical manifestations of canine babesiosis are difficult to relate to an organism that is solely restricted to the erythrocyte. Some authors have proposed that although the clinical manifestations are diverse, the mechanism promoting them is probably uniform (JACOBSON and CLARK, 1994; LOBETTI, 1998; WELZL et al., 2001). They consider systemic inflammation as a major feature in the pathophysiologic mechanisms of this disease and suggest that systemic inflammatory response syndrome (SIRS) and the subsequent multiple organ dysfunction syndrome (MODS) provide the underlying pathophysiologic mechanism within which apparently unrelated aspects of babesiosis form a predictable pattern.

Two studies have documented SIRS and MODS in complicated canine babesiosis (WELZL et al., 2001; MATIJATKO, 2003). SIRS is the clinical expression of the action of complex intrinsic mediators of the acute phase reaction (PANNEN and ROBOTHAM, 1995; NYSTRÖM, 1988). Since babesiosis, like human malaria caused with *Plasmodium falciparum*, can be classified as protozoal sepsis (BONE et al., 1992; JACOBSON et al., 2002), it is likely that the inflammatory mechanisms in this disease are similar to those of other septic conditions that lead to SIRS and MODS.

White blood cell populations play a crucial role in the SIRS to the severe infection, injury, multiple trauma and shock. The differential white blood cell counts are an easily measurable, available and reliable parameter which can be used as a severity index of SIRS and sepsis (ZAHOREC, 2001). Recently, general immune response to endotoxaemia has been described by an increased number of circulating neutrophils and decreased counts of lymphocytes (JILMA et al., 1999). The total and differential white blood cell counts have been poorly described in canine babesiosis. White blood cell counts were reported in studies about canine babesiosis caused by *B. canis rossi* (ABDULLAHI et al., 1990; REYERS et al., 1998; LOBETTI, 1998). Therefore the aim of this study was to investigate serial changes in circulating total white blood cells and the relationship between circulating neutrophil and lymphocyte counts in uncomplicated and complicated canine babesiosis caused by *B. canis canis* and to test whether sequential measurement of total and differential white blood cell counts would prove valuable in assessing the response to antibabesial treatment.

Materials and methods

Animals. The study was performed on two groups of animals naturally infected by *B. canis canis* admitted to the Clinic for Internal Diseases, Faculty of Veterinary Medicine, University of Zagreb, Croatia, with the clinical signs of acute babesiosis. All of them were from one to 10 years old and both sexes were included in about equal percentages. The diagnosis was confirmed by demonstration of the parasites within infected erythrocytes in a Romanowsky-stained thin blood smears.

One dose (6 mg/kg) of imidocarb dipropionat (Imizol[®], Shering-Plough) was administered to all dogs subcutaneously on the day of the admission.

After the antibabesial treatment the dogs were divided into groups 1 and 2 on the basis of clinical presentation, laboratory data and response to antibabesial treatment. Group 1 consisted of 30 dogs with uncomplicated babesiosis. These 30 dogs presented with classical signs of babesiosis (fever, pale mucous membranes, listlessness, water hammer pulse, anorexia) and after the antibabesial treatment all of them showed improvement in their clinical symptoms within 24 hours after only antibabesial treatment. Group 2 consisted of 30 dogs with various complications (multiple organ dysfunction syndrome, acute renal failure, immune-mediated haemolytic anaemia, icterus and hepatopathy and haemoconcentration and shock and acute respiratory distress syndrome) and after the antibabesial treatment their clinical status required additional therapy according to the complications developed (fluids, whole blood transfusion, oxygen supplementation, antibiotics). Five of them died due to complicated babesiosis.

Samples. The blood samples for the analyses were collected from the cephalic vein at the day of admission, and the second and seventh day after the administration of imidocarb

dipropionat. The samples were placed in tubes with EDTA for haematological analysis and tubes without anticoagulant for biochemical analyses.

White blood cell count (WBC) was determined using an automatic haematology analyzer (System 9120; Serono Baker Diagnostic, Pennsylvania, USA). The ratio of neutrophil to lymphocyte count was acquired from relative percentages of neutrophils and lymphocytes that were manually determined from Romanowsky stained blood smears. The percentage of parasitemia is determined by counting the number of parasitised erythrocytes per one thousand erythrocytes.

The biochemical profile was performed according to standard methods, using an automated biochemistry analyzer (Olympus AU 600, Olympus diagnostic GMBH, Hamburg, Germany) with original reagent kits. Biochemistry included blood urea nitrogen, creatinine, alanin aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, creatinine phosphokinase, amylase, total proteins and albumins. These data were used in patient classification as uncomplicated or complicate case.

Statistical analysis. The WBC counts, relative number of neutrophils and lymphocytes, neutrophil to lymphocyte ratio and percentages of parasitemia were compared between the two groups using the Mann-Whitney U test. P values <0.05 were considered to be significant (LE, 2003).

Results

The WBC count, relative number of neutrophils, relative numbers of lymphocytes and the neutrophyl to lymphocyte ratio before the treatment and on the second and seventh day after the antibabesial treatment in uncomplicated and complicated babesiosis are presented in Fig. 1. and Table 1.

There was no significant difference in WBC count between the complicated and uncomplicated babesiosis before and on the second day after the antibabesial treatment while WBC count was significantly higher on the seventh day after the antibabesial treatment in the group of complicated babesiosis.

The relative number of neutrophiles was significantly higher on the second and seventh day after the antibabesial treatment in complicated babesiosis in comparison with uncomplicated babesiosis (Table 1) and the relative number of lymphocytes was significantly lower in the group of complicated babesiosis on the second day after the antibabesial treatment compared to uncomplicated babesiosis (Table 1). Neutrophil to lymphocyte ratio was significantly higher on the second day after treatment in the complicated babesiosis than in the group of dogs with uncomplicated babesiosis (Fig. 1).

Table 1. WBC, relative number of neutrophils, relative number of lymphocytes and the neutrophil to lymphocyte ratio in the uncomplicated and complicated canine babesiosis before and the second and the seventh day after the antibabesial treatment

		WBC ($\times 10^9$) (ref. value 6-17)		Neutrophils (%) (ref. value 60-78)		Lymphocytes (%) (ref. value 12-33)		N/L ratio	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD.
Uncomplicated	Before treatment	6.73	3.01	69.71	16.10	27.76	15.42	4.14	4.20
	2 nd day after treatment	12.93	4.03	43.19	13.36	50.62	11.83	1.00	0.70
	7 th day after treatment	11.31	3.42	59.57	11.64	30.90	11.74	2.60	2.13
Complicated	Before treatment	7.18	2.23	71.60	11.04	23.13	18.84	4.97	5.47
	2 nd day after treatment	12.27	5.09	62.58*	17.19	32.42*	15.54	2.71*	1.97
	7 th day after treatment	21.73*	12.44	73.63*	15.75	20.50*	10.56	4.49*	2.05

* indicates significant difference between the data obtained from group of dogs suffering from complicated form of babesiosis compared to group with uncomplicated form of babesiosis at the same day. Significance was set at $P < 0.05$.

Parasitemia levels were significantly higher in complicated than in uncomplicated canine babesiosis (Table 2.)

Table 2. Parasitemia level before treatment in uncomplicated and complicated canine babesiosis.

Form of babesiosis	Range	Median	Level of significance
Uncomplicated	0.01 - 0.47 %	0.10	P<0.05
Complicated	0.05 - 21.00 %	0.53	

Relative proportions of lymphocytes and neutrophils are shown in Fig. 1.

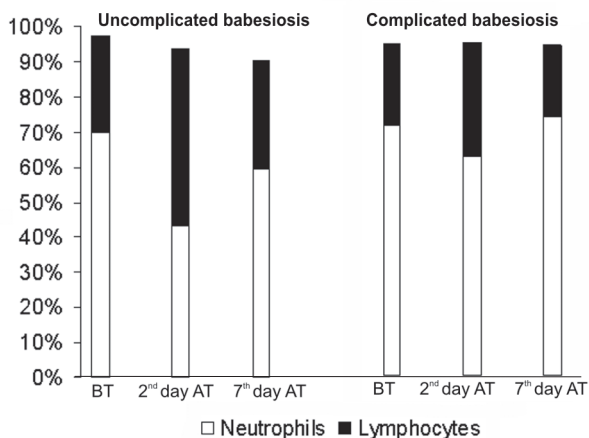


Fig. 1. Relative numbers of neutrophils and lymphocytes in complicated and uncomplicated babesiosis before treatment (BT) and the second and the seventh day after the antibabesial treatment (2nd day AT and 7th day AT). Bars show the average value (mean) of relative number of neutrophils (white) and relative number of lymphocytes (black) in complicated and uncomplicated babesiosis.

Discussion

Canine babesiosis is a potentially life-threatening disease caused by intracellular protozoa, the pathophysiology of which is yet not completely understood. Our results showed that the percentage of parasitemia was significantly higher ($P < 0.05$) in the complicated group than in the uncomplicated group (Table 2). In the uncomplicated group all the cases had parasitemia below 1%, while in the complicated group the percentage of parasitemia was from 0.05 to 21%. It can be concluded that low parasitemia does not guarantee an uncomplicated form of the disease, while the parasitemia above 1% strongly predicts the development of a complicated form of the babesiosis.

Complicated babesiosis has been suggested to be the consequence of the development of SIRS and MODS, both of which are cytokine mediated phenomena (JACOBSON and CLARK, 1994). WELZL (2000) documented the presence of SIRS in 87% and MODS in 48% of dogs with babesiosis caused by *B. canis rossi*, while documented the presence of SIRS in 90% and MODS in 23.3% of dogs with babesiosis caused by *B. canis canis*. These results suggest that complicated babesiosis could be the consequence of dysregulation of the pro-inflammatory and anti-inflammatory mechanisms of the acute phase reaction, resulting in development of fulminate and uncontrolled inflammatory response.

SIRS, sepsis, severe sepsis and septic shock are clinical entities that represent the progression of inflammatory response. Sepsis is defined as SIRS due to confirmed infection, sepsis with MODS is termed severe sepsis, and severe sepsis with refractory hypotension is classified as septic shock (BONE et al., 1992). Due to the fact that canine babesiosis is a protozoal infection and SIRS has been documented in babesiosis (WELZL et al., 2001; MATIJATKO, 2003), this disease can be classified as protozoal sepsis, as human malaria caused by *P. falciparum*. Moreover, because MODS and hypotension have also been documented in cases of babesiosis (JACOBSON et al., 2000; WELZL et al., 2001; MATIJATKO, 2003), inflammatory response in canine babesiosis can progress to severe sepsis and septic shock.

Immuno-competent white blood cell populations (lymphocytes and neutrophils) play a crucial role in the systemic inflammatory response to the severe infection, injury, polytrauma and shock (ZAHOREC, 2001). Recently, general immune response to endotoxemia has been described as an increased number of circulating number of neutrophils and decreased counts of lymphocytes (JILMA et al., 1999). Leucocytosis is together with fever, tachycardia and tachypnea the common clinical sign of sepsis. However, hypovolemic and hypotensive septic patients often initially suffer from hypothermia and leucopenia, but, after resuscitation all such patients develop fever and leukocytosis (NYSTRÖM, 1998).

Leukopenia, like leukocytosis, has been frequently reported in canine babesiosis (PAGÉS and TROUILLET, 1984; ABDULLAHI et al., 1990; REYERS et al., 1998; LOBETTI, 1998). The results of this study showed 43% dogs with leucopenia in the group of uncomplicated cases, and 39% leucopenic cases in the group of complicated babesiosis on the day of the admission. There was no significant difference in WBC count between the two groups at the day of the admission and on the second day after treatment, but the seventh day after treatment the WBC count was significantly higher in the group of complicated babesiosis ($P < 0.05$, Table 1). Therefore the WBC count is not a very sensitive marker in differentiating complicated and uncomplicated canine babesiosis.

ZAHOREC (2001) demonstrated that the ratio of neutrophil to lymphocyte counts, in absolute or relative values, is a simple, rapid and reliable method of evaluating the extent of systemic inflammation. Neutrophilia and lymphocytopenia are typical phenomena of the innate immune response to various insults (ZAHOREC, 2001) that have frequently been reported in canine babesiosis (ABDULLAHI et al., 1990; REYERS et al., 1998). The highest neutrophil/lymphocyte ratio in this study in the uncomplicated group was on the day before the antibabesial treatment (Fig. 1). The improvement of the clinical status as a response to antibabesial treatment resulted in a gradual increase in the lymphocyte percentage and concomitant decrease in the neutrophil percentage in the group of uncomplicated babesiosis, while in the group of complicated babesiosis there was no

such increase in the lymphocyte count and the concomitant decrease in neutrophil count on the second day after treatment.

These results support the conclusion made by ZAHOREC (2001) that there is a significant correlation between the severity of the clinical course and the amount of neutrophilia and lymphopenia. The ratio of neutrophil and lymphocyte counts (in absolute or relative values) is an easily measurable parameter which may express the severity of the affliction and serve as a good prognostic marker.

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SAŽETAK

Babezioza pasa prouzročena parazitom *B. canis* čest je uzrok uginuća pasa u Hrvatskoj. Leukociti imaju ključnu ulogu u nastanku sustavnoga upalnoga odgovora koji je temeljni patofiziološki mehanizam u babeziozi. Cilj ovoga istraživanja bio je odrediti promjene u ukupnom broju leukocita te odnos između neutrofila i limfocita u jednostavnoj i kompliciranoj babeziozi pasa, prouzročenoj vrstom *B. canis canis*. Istraživanje je provedeno na dvije skupine prirodno invadiranih pasa. Unutar prve skupine bilo je 30 pasa oboljelih od nekomplikirane, a unutar druge 30 pasa oboljelih od različitih oblika komplicirane babezioze. Uzorci krvi prikupljeni su trokratno;

na dan pregleda te drugi i sedmi dan nakon primjene antibabezijskoga sredstva (imidokarb dipropionat). Ukupan broj leukocita određen je hematološkim brojačem (System 9120; Serono Baker Diagnostic). Diferencijalna krvna slika dobivena je brojenjem udjela segmentiranih i nesegmentiranih neutrofilnih granulocita, eozinofilnih i bazofilnih granulocita, monocita i limfocita na 100 leukocita u krvnim razmazima. Omjer neutrofila i limfocita izračunat je iz postotka neutrofila i limfocita izbrojenih u obojenom krvnom razmasku. Postotak parazitemije određen je brojenjem parazitiranih eritrocita na tisuću eritrocita. Ukupni broj leukocita, postotak neutrofila i limfocita, omjer neutrofila i limfocita i postupak parazitemije između istraživanih skupina uspoređen je Mann-Whitney-evim U testom. Ukupni broj leukocita bio je značajno viši sedmi dan nakon antibabezijske terapije u skupini pasa oboljelih od kompliciranoga oblika bolesti. Udio neutrofila bio je značajno viši drugi i sedmi dan nakon terapije, dok je udio limfocita bio značajno niži drugi dan nakon terapije, isto u skupini pasa oboljelih od kompliciranoga oblika bolesti. Međusobni omjer neutrofila i limfocita bio je značajno viši drugi dan nakon terapije u pacijenata u kojih se razvio komplicirani oblik babezioze. Isto tako je i postotak parazitemije bio značajno viši u pacijenata s komplikacijama. Zaključno možemo ustvrditi da u pasa oboljelih od babezioze postoji signifikantna koleracija između težine kliničkih znakova te neutrofilije i limfopenije. Međusobni odnos neutrofila i limfocita je dostupan i lako odrediv pokazatelj koji odražava jačinu oštećenja i može poslužiti kao dobar prognostički pokazatelj.

ključne riječi: pas, babezioza, parazitemija, ukupni broj leukocita, neutrofilii, limfociti
