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Erythrocyte and platelet indices in babesiosis of dogs

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ABSTRACT

In the present study we evaluated the haematological changes in samples of blood obtained from 30 dogs naturally infected with large *Babesia*, before and after therapy with imidocarb dipropionate. The results were compared with those obtained from 50 healthy dogs. The evaluation included red blood cell count (RBC), mean corpuscular volume (MCV), haematocrit (HTC), red distribution width (RDW), platelet counts (PLT), mean platelet volume (MPV) and plateletcrit (PCT). Analyses were performed with an automated blood cell counter (Abbott Cell-Dyn CD 3500). The most common disorders in affected dogs were thrombocytopenia with decreased PCT and increased MPV (suggested activation of platelets). Since RDW did not show any changes in babesiosis (suggested uniform population of RBC's), decreased RBC, MCV and HCT were present in the majority of dogs with babesiosis, before and after therapy. Our results suggest that erythrocyte and platelet indices could provide clinical information about the underlying conditions of anaemia and thrombocytopenia.

Key words: babesiosis, dog, erythrocytes, platelets

Introduction

Babesia canis is intra-erythrocytic protozoan parasite transmitted by ticks to dogs in which parasites induce babesiosis, a disease that resembles human malaria. Since haemolytic anaemia, caused by the destruction of non-infected erythrocytes, is a critical feature of the disease, the most frequent clinical pathological finding in babesiosis is thrombocytopenia (BARIĆ RAFAJ et al., 2005; FURLANELLO et al., 2005; MÁTHÉ et al., 2006; BARIĆ RAFAJ et al., 2008).

The availability of automated blood cell analysers that provide an index of red blood cell distribution width (RDW) has led to new approaches to patients with anaemia. Despite wide acceptance of RDW determination in human medicine, in veterinary medicine there

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is still little information about this parameter in clinical practice. RDW represents the width of erythrocyte distribution using their MCV.

Red cell distribution width is typically elevated in conditions of ineffective red cell production (such as iron deficiency, B_{12} or folate deficiency, and haemoglobinopathies), increased red cell destruction (such as haemolysis), or after blood transfusion. As in regenerative anaemia, the increase is most likely to be seen during the phase of the disease when there are a significant number of normally and abnormally sized erythrocytes present simultaneously (MEYER and HARVEY, 2004). Uniform microcytosis or macrocytosis is not easily recognized in peripheral smears, but can be indicated by the mean cell volume (MCV), value generated by automated haematology analysis of the sample (WALKER, 2008).

The quantification of platelets in peripheral blood is a well-recognized tool in veterinary diagnostics. Recently, new indices related to platelet counts have been provided by haematologic analysers, namely plateletcrit (PCT) and mean platelet volume (MPV), where platelet volume represents a marker of platelet function and activation. In thrombocytopenic dogs, there is frequently a dilemma present: is the low number of thrombocyte the consequence of increased destruction of cells or is there impaired production in bone marrow. If the bone marrow response is intact, as a result of increased production large thrombocytes appeared in the circulation (KAITO et al., 2005; BOMMER et al., 2008). Plateletcrit represents the most relevant physiological parameter of platelet status. Plateletcrit in a blood sample is an indicator of platelet mass in the body, just as HCT is an indicator of total erythrocyte mass in the body (BOMMER et al., 2008).

Although thrombocytopenia is used as a diagnostic sign of babesiosis, changes in values for platelet indices: plateletcrit (PCT) and mean platelet volume (MPV) in response to babesia parasites, and after treatment with imidocarb dipropionate, are still less understood.

The aim of this study was to evaluate platelet and erythrocyte count with their indices concerning cell volume and distribution, in a naturally occurring canine babesiosis, before and after therapy with imidocarb dipropionate.

Materials and methods

Samples of blood were obtained from 50 healthy dogs and 30 dogs with babesiosis, before and after (24-48 hours) therapy with imidocarb dipropionate (6 mg/kg). The diagnosis was made by direct observation of large piroplasms in stained blood smears, recognized as the large species: *Babesia canis*. Polymerase chain reaction analysis confirmed the presence of *Babesia canis canis* subspecies in infected dogs (CACCIO et al., 2002). The samples were transported to the laboratory within 30 minutes of collection, and all analyses were conducted within 1-2 hours of collection. The haematological parameters (RBC, HTC, MPV, RDW, PLT, MPV and PCV) were analysed in whole

EDTA-blood, on Abbott Cell-Dyn CD 3500 automated haematology analyser (Abbot Diagnostic division, Mountain View, CA).

Statistical analysis. The obtained results were compared with results obtained from healthy dogs. Levels of significance were determined by Student t-test (STATISTICA for Windows, ver 5.1. Release, StatSoft, Inc. 1984-1996). Any P value of 0.05 or lower was considered to be significant.

Results

Table 1. Results of statistical processing of all haematological indicators

	N	mean	min	max	SD	P
RBC ($x10^{12}$/L)						
N	49	6.06	4.20	7.80	0.939	
1	30	4.85	1.51	6.92	1.290	0.000007*
2	30	5.17	1.22	7.80	1.440	0.001389*
MCV (fL)						
N	49	74.45	63.00	81.00	3.530	
1	30	71.27	66.00	90.80	4.939	0.001278*
2	30	71.41	66.00	90.20	4.682	0.001567*
HCT (%)						
N	49	45.24	28.14	62.40	8.040	
1	30	34.35	13.71	48.55	8.974	0.000000*
2	30	36.69	11.00	56.16	10.190	0.000087*
RDW						
N	49	15.32	13.80	18.00	1.148	
1	30	15.08	14.00	18.60	0.964	0.341056
2	30	15.10	13.80	18.60	0.949	0.387454
PLT ($x10^{9}$/L)						
N	49	285.76	137.00	548.00	100.104	
1	30	22.23	6.00	81.00	19.624	0.000000*
2	30	46.73	7.00	319.00	61.661	0.000000*
MPV (fL)						
N	49	10.56	7.90	13.50	1.166	
1	29	10.30	5.00	13.10	1.499	0.508831
2	29	11.12	8.00	14.20	1.352	0.037189*
PCT (%)						
N	49	0.300	0.16	0.58	0.104	
1	29	0.02	0.01	0.08	0.019	0.000000*
2	29	0.05	0.01	0.29	0.06	0.000000*

N = healthy dogs, 1 = babesiosis before therapy, 2 = babesiosis after therapy. RBC = red blood cell count, MCV = mean corpuscular volume, HTC = haematocrit, RDW = red distribution width, PLT = platelet counts, MPV = mean platelet volume, PCT = plateletcrit. *P<0,05

Erythrocyte number was significantly decreased in dogs with babesiosis, before and after treatment. As the consequence of erythroyte decrease, haematocrit was also significantly low, compared with the control group. Mean cell volume was significantly decreased before and after treatment, indicating the presence of small erytrocyte population. Despite MCV decrease, RDW remain unaltered before and after treatment. Unaltered RDW was a sign of a uniform population of red blood cells. Platelet number and plateletcrit were significantly decreased during the observation. Mean platelet volume in dogs with babesiosis before the therapy did not show any differences compared with the control group of healthy dogs. After the treatment, mean platelet volume significantly increased. (Table 1).

Discussion

The most common abnormality in the investigated parameters was thrombocytopenia, which was observed in 100% of dogs with babesiosis before therapy, and in 97% of dogs after therapy. Although 67% infected dogs presented with severe depletion of platelets from circulated blood ($<20x10^9$/L), none of them developed haemorrhage. The mechanisms of the thrombocytopenia are not yet fully understood in babesiosis; multiple mechanisms, including platelet sequestration in the spleen, immune-mediated platelet destruction and development of disseminated intravascular coagulation are possible (BOOZER and MACINTIRE, 2003). The contributory effect on thrombocytopenia could have elevated body temperature (OGLSBEE et al., 1999), which was recorded for all infected dogs in this study. Investigating babesiosis in the South African canine population, KETTNER et al. (2003) concluded that the disease is associated with thrombocytopenia in nearly all patients and is severe in the majority of them. In the absence of thrombocytopenia, babesiosis is an unlikely diagnosis. The day after treatment, the number of platelets increases slightly, but still remains low compared with the control dogs ($P<0.05$).

Changes in platelet count and its association with platelet indices may reflect changes in platelet production and reactivity. YILMAZ et al. (2008) concluded that platelet indices have potential value in the diagnosis and monitoring of dogs and humans with endotoxemia.

Modern cell analysers routinely produces platelet indices, as mean platelet volume (MPV), plateletcrit (PCT) and platelet distribution width (PDW), but many clinicians do not utilize them in clinical decision-making. In part this may be attributed to difficulties in their laboratory measurement and the fact that MPV is dependent on a number of variables (time of analysis after venipuncture, method of analysis, anticoagulant used and specimen storage temperature) (JACKSON and CARTER, 1993).

Measured as mean platelet volume, platelet volume is a marker of platelet function and activation (BATH and BUTTERWORTH, 1996). MPV was significantly raised in

dogs with babesiosis after therapy ($P<0.05$). Today it is known that larger platelets are haemostatically more active, and represent a risk factor for developing coronary thrombosis, leading to myocardial infarction in humans (KHANDEKAR et al., 2006). Platelets produced during recovery from acute thrombocytopenia showed a striking increase in volume, with large and even giant forms apparent on the smear as early as 4 hours after the induction of thrombocytopenia (CRAMER, 2001). Macrothrombocytosis indicated active thrombopoiesis, which is associated with the release of immature platelets from the bone marrow during responsive thrombopoiesis in babesiosis in dogs. Intimately tied to inflammatory responses, haemostasis system could be activated in any case, associated with significant inflammation, which is also the case in babesiosis (MATIJATKO et al., 2007). None of our patients with severe PLT depletion showed signs of bleeding, that could be the consequence of their increased activity. ZYGNER et al. (2007) detected thrombocytopenia in 99.5% of dogs with babesiosis, but only 15.3% of examined animals showed an increase in MPV, which suggests a response by the bone marrow. NORTHERN and TVEDTEN (1992) detected macrothrombocytosis in 18 of 31 dogs with immune-mediated thrombocytopenia. Investigating MPV in babesiosis, KETTNER et al. (2003) observed that the mean platelet volume (11.1 fl) for the babesiosis group of dogs was greater than the reference range, which was established from 6-10 fl. NTAIOS et al. (2008) intend for increased MPV and platelet size distribution width to provide a safe diagnosis of idiopathic thrombocytopenic purpura in humans.

Plateletcrit represents the percent of blood volume occupied by platelets. It is well known that the surfaces of cells are essential for clotting reactions to take place (KHANDEKAR et al., 2006) so we examined plateletcrit. PCT were significantly decreased in the babesiosis group before and after therapy ($P<0.05$). This is the consequence of the very low PLT count, where the increase in MPV is only a minor compensation. Platelet indices are also in the focus of human diagnostics; investigating sensitivity and specificity of platelet indices in the diagnosis of immune thrombocytopenia in humans, KAITO et al. (2005) concluded that platelet indices could provide valuable information about the nature of thrombocytopenia, and that more attention should be paid to these indices in the diagnosis of thrombocytopenia. Despite the valuable information given by thrombocyte indices, BOMMER et al. (2008) are of the opinion that platelet volume indices did not aid the interpretation of thrombocytopenia in terms of the underlying pathological processes in the studied dogs.

Babesia initiates a mechanism of antibody-mediated cytotoxic destruction of circulating erythrocytes. Auto-antibodies are directed against components of the membranes of infected and uninfected erythrocytes. This causes intravascular and extravascular haemolysis, which leads to anaemia. Investigating anaemia in dogs with babesiosis, FURLANELLO et al. (2005) noted anaemia in 74% of dogs, in all cases the

anaemia was normocytic and normochromic. Our dogs with babesiosis had significantly ($P<0.05$) decreased RBC, MCV and HTC, before and after therapy. Despite the fall of MCV, RDW remain unaltered, before and after therapy, indicating a homogenous population of erythrocytes.

RDW within normal range should be the consequence of the pre-regenerative status of dogs with babesiosis. Namely, anaemia seen within 3-4 days after haemolysis will show no signs of regeneration in peripheral blood, because of the 4-day production time for red cells (MILLS, 2000).

Measured by most automatic haematology analysers, RDW and MCV may give the first indication of the bone marrow response of an anaemic dog. RBC indices, such as mean corpuscular volume and red cell distribution width, can provide valuable information in establishing a diagnosis in anaemic patients; despite this, these laboratory parameters are frequently overlooked in veterinary clinical practice. RDW increases in all cases of increased anisocytosis, which is usually estimated by stained blood film microscopy.

The 248 dogs investigated with babesiosis, ZYGNER et al. (2007) noted that the most common disorders were anisocytosis and thrombocytopenia.

The most frequent cases of increased RDW occur in regenerative anaemia, because they are followed by an increase in reticulocytes, which are a larger population than mature erythrocytes (CAVALIERE, 2004). RDW could be a useful indicator of the appearance of a younger population, because in some animals increasing of RDW will appear before the MCV exceeds the reference interval. In cases where young erythrocytes become the predominant population, the RDW will begin to decline and may return to the reference interval. This return could appear even though the MCV remains still high. Investigating RDW and MCV, NEIGER et al. (2002) concluded that, by their classification tree, 78% of anaemic dogs with a RDW of 16.25% or less would be expected to have non-regenerative anaemia. Also, the authors concluded that an increase of 1% in the RDW and of 1 fl in the MCV increased the odds of an anaemic dog suffering from regenerative anaemia by factors of 1.3 and 1.14, respectively.

It is well known that babesiosis can cause severe tissue hypoxia and widespread inflammation (JACOBSON and CLARK, 1994). Some new investigations connect red cell distribution width and release of inflammatory cytokines. Namely, pro-inflammatory cytokines have been found to inhibit erythropoietin-induced erythrocyte maturation, which is reflected in part by an increase in RDW (PIERCE and LARSON, 2005).

Conclusions

Decrease of RBC, HTC and MPV occurs in babesiosis, before and after therapy. Despite the fall of MCV, RDW remain unaltered, indicating a homogenous population of erythrocytes. RDW within normal range could be the consequence of the pre-regenerative

status of dogs with babesiosis. Changes in platelet count and their association with platelet indices may reflect changes in platelet production and reactivity. Further studies are needed to evaluate the role of platelet abnormalities and changes in megakaryopoiesis during the course of babesiosis in dogs.

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SAŽETAK

U istraživanju su vrednovane hematološke promjene u uzorcima krvi 30 pasa s babeziozom, prirodno zaraženih, prije i poslije terapije imidocarb-dipropionatom. Rezultati su uspoređeni s rezultatima dobivenim u 50 zdravih pasa. Istraženi su broj eritrocita, prosječni volumen eritrocita, hematokrit, distribucija eritrocita, broj trombocita, prosječni volumen trombocita i trombokrit. Mjerenja su izvedena upotrebom automatskog hematološkog analizatora (Abbott Cell-Dyn CD 3500). Najčešća promjena kod pasa s babeziozom bila je trombocitopenija sa sniženim trombokritom i povećanim prosječnim volumenom trombocita, koji upućuje na aktivaciju trombocita. Do promjena u raspodjeli eritrocita nije došlo kod babezioze (jednolična populacija eritrocita). Sniženi broj eritrocita, prosječni volumen eritrocita i hematokrit bili su prisutni u većine pasa oboljelih od babezioze, prije i poslije liječenja. Rezultati upućuju na to da promjene u broju eritrocita i trombocita mogu biti korisne u pružanju kliničkih informacija o mogućim uzrocima anemije i trombocitopenije.

Ključne riječi: pas, babezioza, eritrociti, trombociti