INFLUENCE OF INVASION OF INTRACELLULAR PARASITES ON PLATELET RESPONSE IN DOGS BASED ON CLINICAL CASES

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Abstract

Blood morphology in dogs infected with intracellular parasites of the genera Babesia and Ehrlichia was examined. The parasites were detected in peripheral blood smears stained by the May-Grünwald-Giemsa method. During the parasitic invasion, a decrease in thrombocyte counts was observed. This was connected with forming platelet aggregates. It was noted that the number of giant platelets simultaneously increased.

Key words: dogs, blood platelets, Babesia, Ehrlichia.

Parasitic infections affect all animal species as well as humans (8). The diagnosis of parasitic diseases poses numerous problems in medical and veterinary practice. In many cases, the developmental or adult forms of parasites may be difficult to determine in the analysed material despite the severity of clinical symptoms.

Invasions caused by parasites, in particular internal parasites, are the source of foreign antigens and exotoxins for all organisms. Parasitic invasions cause local or general inflammations with various stages of progression. The clinical symptoms of inflammation include the general health deterioration, weakness, fever, alternating diarrhoea and constipation (in infections of the gastrointestinal tract), and progressive anaemia. The above symptoms are particularly severe in infections caused by intracellular parasites such as Babesia sp., Ehrlichia sp., and blood sucking parasites, including Gasterophilus intestinalis, horse bot-flies, and roundworms (20). The major vectors of transmission of parasites, like Babesia and Ehrlichia are ticks, but the role of mosquitoes is still discussed (1, 10).

Thrombocytes take an active part in different immunological reactions including antiparasitic immunity, although these cells are not typically considered as part of the immunity system. Examinations of blood morphology in dogs infected with Babesia sp. revealed thrombocytopenia, leukopenia, and anaemia (15). It is worth to emphasise, that very often during babesiosis and ehrlichiosis, no typical changes in blood morphology are observed, which can lead to false diagnosis of the disease.

Taking into account the scarce reference data on platelet reactions during intracellular parasites invasion in dogs, the study was performed to determine these reactions after natural infection with the parasites.

Material and Methods

The study was conducted on 24 dogs of various breeds and ages. The animals were divided into three equal groups. The control group consisted of clinically healthy animals demonstrating normal haematological parameters and an absence of intracellular parasites in peripheral blood.

The second group comprised dogs naturally infected with Babesia sp., diagnosed on the basis of clinical symptoms and results of laboratory tests. Clinical symptoms included an increased body temperature, a higher respiratory rate, an increased pulse rate, yellow discoloration of mucosa and skin, mucosal pallor, apathy, and movement difficulty owing to decreased muscular strength. Morphological analyses revealed a drop in erythrocyte counts, decreased haemoglobin and haematocrit values, and a significant drop in thrombocyte counts. The presence of babesia trophozoites was observed in blood samples stained by the May-Grünwald-Giemsa (MGG) method. The presence of intraerythrocytic parasites of the genus
Babesia was determined in the form of droplets and ameboids in specimens stained by the MGG method. The progression of untreated babesiosis was accompanied by dark-coloured urine due to haemoglobinuria. In two cases, non-regenerative anaemia was reported as a result of bone marrow hypoplasia. Leukopenia and lymphopenia were observed as the disease progressed. The presence of rarely occurring plasmocytes in peripheral blood is connected with haematopoietic disorders caused by bone marrow hypoplasia. Despite the applied antiprotozoan treatment, weakened kidney function and inhibited urination was noted in five dogs with advanced symptoms of the disease.

The third group consisted of dogs naturally infected with *Ehrlichia* sp. Peripheral blood samples were collected and analysed. The presence of ehrlichial morulae was observed in blood samples stained by the MGG method.

The material for analyses was sampled twice from each group. The first collection from infected animals took place on days 2-3 after the clinical symptoms appeared. A diagnosis was established, and the dogs were subjected to the treatment. Dogs with confirmed babesiosis were treated by injection of imidocarb and dogs with ehrlichiosis were treated by injection of oxytetracyclines. The second blood collection was performed a week after the first sampling. Measuring the thrombocyte counts was performed using the ADVIA 2120i analyser. The results were analysed statistically in one factor orthogonal design. The significance of differences between mean values in groups was defined by Student's *t*-test using Statistica 10.0 software.

**Results**

In animals with relatively mild symptoms of babesiosis, a high number of giant platelets were noted with an insignificant drop in thrombocyte counts relative to the reference values for the studied species. A high number of giant platelets and few platelet aggregates were observed in specimens containing ameboids. In blood samples marked by an abundance of parasites at the trophozoite stage, the number of giant platelets remained constant, but higher platelet aggregate counts and a drop in thrombocyte counts were noted. Regardless of the developmental form of the parasite, an improvement in the patient's condition following the administration of antiparasitic drugs was accompanied by a drop in the number of platelet aggregates and giant platelets. An increase in platelet counts was also observed, but the number of thrombocytes never reached the reference values given for the species.

Mean platelet value (MPV) and thrombocyte (PLT) values remained within the reference range in control animals after the first and second sampling (Table 1). In groups with various developmental forms of *Babesia*, an increase in MPV values, a drop in PLT counts, and an increase in the number of giant platelets were noted after the first blood collection. PLT counts increased significantly (P≤0.01) and average MPV values decreased insignificantly in the second sampling.

In the group of animals with anaplasmosis, morula of *Ehrlichia* sp. were found in granulocytes (Fig. 1). In the first sampling, platelet counts initially rose above the reference values, but no changes in MPV were reported (Table 1). The second sampling revealed a significant drop in PLT counts (P≤0.01) and an increase in MPV values (P≤0.01) (Table 1). Numerous platelet aggregates were determined in peripheral blood smears stained by MGG (Fig. 2).

![Fig. 1. Ehrlichia sp. in granulocyte. MGG, 400x.](image1)

![Fig. 2. Aggregated platelets in ehrlichiosis. MGG, 200x.](image2)

**Discussion**

The progression of inflammation is determined by the intensity of parasitic infection and immune efficiency. The immune system activates cellular and humoral responses to the parasitic infection (17), but in most cases, the expression of parasitic antigens is not observed on the surface of host cells. The only exceptions are protozoan infections caused by *Trypanosoma* and *Toxoplasma gondii* in humans (22). In infections caused by other parasites, T lymphocytes induce the differentiation and proliferation of cytotoxic cells through the release of IL-2 and IL-12 cytokines.
Table 1
Correlations between MPV and PLT values in control group and groups with parasitic invasions.

<table>
<thead>
<tr>
<th>No.</th>
<th>Control group</th>
<th>Group with Babesia sp.</th>
<th>Group with Ehrlichia sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MPV (fl)</td>
<td>PLT (x10^3/mm³)</td>
<td>MPV (fl)</td>
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<td>-----</td>
<td>---------------</td>
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</tr>
<tr>
<td>1</td>
<td>9.3</td>
<td>330</td>
<td>19.1</td>
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<tr>
<td>2</td>
<td>9.8</td>
<td>235</td>
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<td>3</td>
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<tr>
<td>Mean</td>
<td>9.5</td>
<td>306</td>
<td>21.5</td>
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* P≤0.01
Immune mechanisms also activate macrophages by increasing their phagocytic ability.

Blood platelets are not an element of the immunocompetent system, but they actively participate in the immune mechanism, including in the antiparasitic immune response (16). Thrombocytes play an active role in primary haemostasis by forming fibrin-bound aggregates and platelet plugs (19). Similarly to macrophages and granulocytes, blood platelets demonstrate phagocytic ability, and by releasing inflammatory mediators, they interact with immune system cells. In the study by Momi et al. (14), mice infected with Trypanosoma equiperdum showed a drop in thrombocyte counts in peripheral blood and a decrease in the levels of thromboxane A2 produced by platelets. Thrombocytopenia reaching 50% of the reference range was observed in mice infected with Trypanosoma cruzi. Platelets are activated following a direct contact with the parasite, and in the presence of IgE, IgG, C-reactive protein, lymphokines, and components of the complement system. Chadderdon (3) has demonstrated the presence of a protein with high levels of anti-platelet activity in the excretions and secretions of hookworms. The discussed protein, a hookworm platelet inhibitor, blocks platelet adhesion and aggregation (3). Ridel et al. (18) observed elevated IgE concentrations and increased platelet cytotoxicity against parasites in rats infected with Toxoplasma gondii. Joseph et al. (8) demonstrated that thrombocytes actively participate in the allergic inflammatory response. Thrombocytes have specific surface receptors that enable them to induce the phagocytosis of cells coated with IgE antibodies and to activate lymphocytes. In a study by Viens et al. (23), blood platelets adhered to Trypanosoma musculi to eliminate the parasites. Platelet cytotoxicity is directly activated by parasites (6, 7). Cyclooxygenase activity and the conversion of arachidonic acid stimulate thromboxane production and the antiparasitic activity of platelets. T lymphocytes play a crucial role in the regulation of platelet cytotoxicity. TNF is one of the lymphokines that activate thrombocytes to directly eliminate Schistosoma mansoni larvae (2). Acute infections caused by the above parasite were also characterised by an increase in IL-5 concentrations. Platelets deliver an antiparasitic effect not only by participating in the process of pathogen elimination (5, 25). The release of inflammatory mediators, coating ability, lysis, and phagocytosis minimise the parasite's adverse impact on the host organism and reduce parasite counts (11, 12). It may be concluded that parasitic infection increases platelet production and enhances the cytotoxic function of thrombocytes. The multidirectional effects of parasitic infections cause haematopoietic disorders and adverse biochemical changes (21, 24).

In anaplasmosis, thrombocytopenia may result from enhanced platelet phagocytosis by macrophages, increased breakdown of platelets in the liver, and platelet damage produced by the immune response (16). According to the source data, pancytopenia observed in ehrlichiosis is often caused by bone marrow hypoplasia. Impaired megakaryopoietic activity may lead to megathrombocytopenia, and platelets, whose size resembles that of normal erythrocytes, appear in peripheral blood (4). The results of other authors' previous work suggest that an initial increase and successive drop in thrombocyte counts and the presence of giant platelets are characteristic of ehrlichiosis (9, 26). The disease is also accompanied by significant changes in peripheral blood morphology and frequent biochemical disruptions.

In the literature, there are few reports on the behaviour of platelets during the course of diseases, such as babesiosis and ehrlichiosis. Zygner et al. (28) demonstrated that a decrease in the number of platelets in the course of babesiosis can be caused by self-destruction of platelets and/or may occur as a result of platelet destruction. Similar observations were made in the present investigation. The formation of platelet aggregates was also observed in other studies (27) and was confirmed in this study. The authors also noted that the formation of giant platelets occurs much more frequently in the course of ehrlichiosis than in the course of babesiosis. That was confirmed by Mikulska-Skupień and Procajło (13) during the observation of ehrlichiosis in two-year-old dog. In the study, it was noted that a decrease in the number of platelets may lead to the formation of giant platelets and platelet aggregates. Undoubtedly, a decrease in thrombocyte counts reduces the efficiency of the coagulation system and may contribute to the formation and development of various disorders such as disseminated intravascular coagulation (DIC).

In conclusion, it should be noted that in the course of parasitic intracellular diseases, a slight decrease in platelet count is observed. It is accompanied by formation of platelet aggregates and giant platelets, which can cause abnormal blood clotting and coagulation disorders.

References