

**BONE MARROW TRANSPLANT IN A DOG WITH BONE MARROW APLASIA  
SECONDARY TO ERLIQUIOSIS: CASE REPORT**

*TRANSPLANTE DE MEDULA ÓSSEA EM UM CÃO COM APLASIA MEDULAR  
SECUNDÁRIO A ERLIQUIOSE: RELATO DE CASO*

Tainá Minuzzo<sup>1\*</sup>; Solimar Dutra da Silveira<sup>2</sup>

1. Médica Veterinária residente do Departamento de Clínica Médica de Pequenos Animais, Universidade Federal do Paraná, Palotina-PR, Brasil.

2. Médico Veterinário residente do Departamento de Clínica Cirúrgica de Pequenos Animais, Universidade Federal do Paraná, Palotina-PR, Brasil.

\*Autor para correspondência: [minuzzotaina@gmail.com](mailto:minuzzotaina@gmail.com)

DOI: 10.4025/revcivet.v7i2.51913

**Resumo:** A aplasia medular é uma alteração extremamente rara em cães e gatos e tem como principal característica uma medula óssea hipocelular em decorrência do tecido hematopoiético ter sido substituído por células adiposas, acarretando assim em um quadro de pancitopenia sanguínea. Sabe-se que uma das principais causas de aplasia medular é a infecção por *Erlichia canis*, doença esta que em fase crônica causa uma hipoplasia de precursores de todas as linhagens celulares e essa anormalidade hematopoiética acarreta inúmeros problemas para o animal, como letargia, anorexia, perda de peso e fraqueza muscular. Uma vez instalado o quadro de anemia, pode ocorrer também diátese hemorrágica resultante do comprometimento de hemostasia primária (trombocitopenia), assim como predisposição a adquirir infecções secundárias, uma vez que esses pacientes comumente apresentam quadro de leucopenia. O objetivo desse trabalho é relatar o caso de um paciente canídeo, diagnosticado com aplasia medular subsequente à erliquiose crônica, onde se institui o tratamento com transplante de medula óssea que acarretou melhora dos valores hematológicos após o procedimento.

**Palavras-chave:** hematologia, pancitopenia, erliquiose crônica.

**Abstract:** Bone marrow aplasia is an extremely rare disorder in dogs and cats and its main characteristic is a hypocellular bone marrow, since hematopoietic tissue has been replaced by fat cells, thus leading to blood pancytopenia. It is known that one of the main causes of bone marrow aplasia is the infection by *Erlichia canis*, a disease that in the chronic phase causes a hypoplasia of precursors of all cell lines and this hematopoietic abnormality causes numerous problems for the animal, such as lethargy, anorexia, weight loss and muscle weakness. Once anemia is present, hemorrhagic diathesis resulting from the impairment of primary hemostasis (thrombocytopenia) may also occur, as well as a predisposition to acquire secondary infections, since these patients commonly present with leukopenia. The objective of this work is to report the case of a canine patient, diagnosed with bone marrow aplasia subsequent to chronic ehrlichiosis, in which treatment with bone marrow transplantation was instituted, resulting in an improvement in hematological values after the procedure.

**Keywords:** hematology, pancytopenia, chronic ehrlichiosis.

## INTRODUCTION

Canine ehrlichiosis is a commonly observed disease in the clinical routine of small animals, caused by gram negative bacteria of the genus *Erlichia*, whose main species diagnosed in Brazil is *Erlichia canis* (LABRUNA and PEREIRA, 2001; VIEIRA et al., 2011). It is believed that the main reservoir of the causative agent of ehrlichiosis is the tick *Rhipicephalus sanguineus*, which ends up transmitting the microorganism to dogs through blood repast. However, it is also known that another possible form of transmission occurs through the blood transfusion of a contaminated animal to another healthy one (SAITO, 2009; WOODY and HOSKINS, 1991; HUXSOLL, 1976).

The definitive diagnosis of ehrlichiosis can be established by visualizing bacterial morulae in circulating monocytes in blood smears, by detecting the bacterial DNA in the blood or spleen by the polymerase chain reaction (PCR) method, through high antibody titers for the bacteria by Elisa method, or even by isolation of the agent in cell cultures (HARRUS et al., 1998; FARIA et al., 2010).

It is known that ehrlichiosis can present itself in three distinct phases: acute, subclinical and chronic, causing clinical, hematological and biochemical changes, varying from mild to severe reactions. Chronic ehrlichiosis usually sets in when the animal has an incompetent immune system and is unable to eliminate the agent, acquiring at this stage particularities of an autoimmune disease, whose clinical signs are severe and can vary from pancytopenia (due to bone marrow aplasia), hemorrhages, glomerulonephritis, neurological disorders, generalized weakness, selective appetite and apathy (WOODY and HOSKINS, 1991; WANER and HARRUS, 2013).

The pathophysiology of bone marrow aplasia subsequent to chronic infection by *Erlichia canis* occurs through immunomediated mechanisms that lead to hypoplasia or aplasia of the cell precursors of all marrow lines. The high mortality rate in this phase is due to this situation, which causes severe pancytopenia resulting in anemia, whose clinical signs include lethargy, hyporexia and weight loss, in addition to hemorrhagic diathesis due to impairment of primary hemostasis (thrombocytopenia) and finally, there is a risk of acquiring secondary infections in view of intense leukopenia (WANER and HARRUS, 2013; WAKI et al., 2010; MYLONAKIS et al., 2010; VECHIATO et al. 2005; MORAES and TAKAHIRA, 2010; THRALL et al. , 2015).

Knowing this, a treatment that is effective for the resolution of the bone marrow aplasia condition is extremely important. However, there are few options available and not all of them demonstrate considerable effectiveness. Currently, one of the treatment alternatives is bone marrow transplantation (BMT). However, there is little information about BMT, as well as few reports of its use in veterinary medicine.

BMT consists of the intravenous administration of hematopoietic progenitor cells, which by tropism, end up re-inhabiting and performing the reconstruction process of all cell lines: erythroid, megakaryocytic and myeloid (FONSECA and SECOLI, 2008; VECHIATO et al. 2005).

There are some restrictive conditions for the institution of bone marrow transplantation, and such factors are: complexity for obtaining sufficient quantities of bone marrow cells (BM), which ends up requiring multiple aspirations of the BM in an aseptic procedure, as well as the time taken to obtain a therapeutic response after transplantation, that is, for there to be the synthesis of new blood cells, on average it takes two to four weeks (PRANKE, 2006).

The objective of this work is to report the case of a canine patient diagnosed with bone marrow aplasia subsequent to chronic ehrlichiosis, in which treatment with bone marrow transplantation was instituted, resulting in an improvement in hematological values after the procedure.

## MATERIAL AND METHODS

A dog, mixed breed, eight years old, weighing 5.7 kg. was assessed at the Veterinary Hospital of the Federal University of Paraná - Sector Palotina, in the small animal medical clinic sector. During the anamnesis, it was reported that the patient had a selective appetite for three months, lethargy for seven days and sudden loss of vision. No history of previous disease was described.

On physical examination, it was observed that the patient was alert, normohydrated, had non-reactive lymph nodes, pink mucous membranes, capillary filling time of two seconds, cardiac auscultation without abnormality with a frequency of 136 beats per minute (bpm), regular pulse, pulmonary auscultation without changes with a frequency of 20 movements per minute (mpm), rectal temperature at 38°C, presence of ectoparasites (ticks), presence of petechiae and ecchymosis along the abdomen and ophthalmic changes (uveitis, hyphema and congestion of episcleral vessels). In view of the reported abnormalities and alterations found in the physical examination, the patient underwent complementary tests, namely: blood count, corrected reticulocyte count, renal biochemical profile (urea, creatinine, sodium, potassium, phosphorus and calcium) and liver biochemical profile (alanine aminotransferase, alkaline phosphatase, albumin and total proteins). There was also prolonged bleeding at the venipuncture site (jugular vein), which suggested a hemostatic disorder.

In the hemogram, a condition of normocytic, normochromic and arregenerative anemia (corrected reticulocytes = 0.6 %) was found, in addition to intense leukopenia and thrombocytopenia (TABLE 1 - 1st day). Biochemical tests were all within the reference range for the species. Due to the pancytopenia associated with the presence of ectoparasite (tick), the rapid ehrlichiosis test (Erlichiose Ac Test/Alere) was performed, obtaining a positive result. Therefore, treatment for ehrlichiosis with doxycycline for 28 days (5mg/kg, BID, PO) and imidocarb dipropionate was instituted, with two applications with an interval of 14 days between them (5mg/kg, SC).

Two days after ehrlichiosis treatment started, a new blood count was carried out, which again demonstrated a normocytic and normochromic anemia, with a slight drop in hematocrit, worsening of the thrombocytopenia and a slight increase in the value of total leukocytes, however, persisting a condition of leukopenia (TABLE 1- 3rd day). In view of these changes, a myelogram was performed, which showed a hypocellular bone marrow in all strains, showing approximately 90 % of fat in its composition, without blast and megakaryocytic cells being observed. In addition, the smears showed rare mature platelets. Approximately 5 % of typical lymphocytes and reactive monocytes were observed, as well as intense lump fibrosis, coinciding then with a condition of bone marrow aplasia.

Eighteen days after the institution of the treatment for ehrlichiosis, a new blood count was performed to monitor the case, which confirmed the persistence of pancytopenia: progressive drop in hematocrit and total leukocytes, slight improvement, but maintenance of thrombocytopenia (TABLE 1 - 18° day). Thus, given the non-responsiveness to conventional treatment, bone marrow transplantation was chosen in order to improve the patient's clinical condition.

The prerequisites established for the choice of bone marrow donor were: being in the age group from 1 to 7 years old and having at least 25 kg, as well as having no clinical history of hemoparasitosis and not having any abnormality in the blood count. After choosing the animal that met all these criteria, a cross-reaction test between donor and receptor was performed, confirming compatibility between both. 10 ml syringes were prepared with the ratio of 1 ml of sodium citrate to 9 ml of bone marrow to be collected. With the donor dog under general anesthesia and in lateral decubitus, trichotomy and antisepsis of the collection site

(proximal tibial epiphyseal region) was performed, and then by direct puncture with a 1.20x40 mm gauge needle, the material was collected, obtaining a final aspirated volume of 57 ml.

After the marrow collection, the material was stored in a sterile bag for transfusion, being administered to the recipient intravenously (peripheral vessel) through a transfusion equipment to remove spicules and fats from the collection site, with a total time of four hours infusion. In order to monitor the patient during the transplant, some parameters were observed: heart rate, respiratory rate and rectal temperature. However, the patient did not show any abnormality during the procedure, thus not requiring intervention.

Fifteen days after the bone marrow transplant, the patient returned for reevaluation, in which the tutors reported that he was more active after the procedure. To monitor the condition, the blood count was repeated, with a considerable increase in hematocrit, leukocytes and platelets, but with persistent pancytopenia (TABLE 1 - 33° day).

The patient returned for reevaluation thirty days after bone marrow transplantation, and a new blood count was performed, which indicated the resolution of the anemia, improvement in platelet values, persistence of thrombocytopenia. Also, total leukocytes remained the same as the last blood count performed (TABLE 1- 48th day).

**Table 1.** Results of blood counts performed before and after bone marrow transplant in a patient diagnosed with bone marrow aplasia

	1° day	3° day	18° day*	33° day	48° day
<b>Erythrocytes (millions/<math>\mu</math>L)</b>	3.91	3.51	2.61	4.38	6.26
<b>Hemoglobin (g/dL)</b>	8.5	8.9	7.2	11.5	13.9
<b>Hematocrit (%)</b>	24	21	19	35	46
<b>MCHC (%)</b>	35	36	36	32	30
<b>MCV (fl.)</b>	61	60	75	79	73
<b>Platelets (<math>\mu</math>L)</b>	11,100	4,000	17,000	35,000	57,000
<b>Leukocytes</b>	800	1,400	700	3,400	3,400
<b>Corrected reticulocyte (%)</b>	0.6	-	-	-	-

\*Bone marrow transplantation

Source: The Authors, 2019.

The patient was monitored for 120 days after the marrow transfusion procedure, showing no concomitant changes and revealing a gradual improvement of the condition. However, the patient will remain under monthly medical supervision, for hematological control and better monitoring of the patient's evolution.

## RESULTS AND DISCUSSION

Chronic ehrlichiosis occurs when the animal has an incompetent immune system and is unable to eliminate the causative agent (*Erlichia canis*), with a milder reaction of clinical signs such as weakness, lethargy and selective appetite being observed in this phase (WOODY and HOSKINS, 1991 ). In addition, the presence of hemorrhagic diathesis due to the impairment of primary hemostasis is very common (MYLONAKIS et al., 2010). The clinical signs described above are consistent with what was demonstrated by the patient, considering the presence of petechiae, ecchymosis and prolonged bleeding after venipuncture, as well as lethargy and hyporexia.

It is known that this phase of the disease can develop with bone marrow aplasia, which is an extremely rare hematological abnormality in dogs and cats and is manifested by severe peripheral pancytopenia, associated with erythroid, myeloid and megakaryocytic hypoplasia due to the replacement of hematopoietic tissue by adipose tissue (in this case hematopoietic tissue occupies less than 25% of the marrow) (THRALL et al., 2015; MORAES and TAKAHIRA, 2010; MYLONAKIS et al., 2010; BRAZZELL and WEISS, 2006). In view of the suspicion of bone marrow aplasia, the patient's myelogram was performed, which indicated a hypocellular bone marrow in all strains, showing approximately 90 % fat in its composition.

It is important to highlight that myelosuppression occasionally occurs in the chronic phase of the disease and can then develop after recovery from the acute phase of the disease or in animals without previous signs of acute infection (MYLONAKIS et al., 2010), the latter being the case of the patient, considering that no clinical history of the disease was described by the owners until the time of the consultation.

A pancytopenia condition is established when there is a decrease in all blood cell lines produced in the bone marrow, and the values used to determine this hematological change are: hematocrit less than 36 %, total leukocytes with values below 6,000/ $\mu$ L and platelets below 200,000/ $\mu$ L (WEISS et al., 1999). Bearing this in mind, it can be said that the patient in the present report showed severe pancytopenia, considering that the blood count values before bone marrow transplantation were well below the values previously mentioned.

However, contrary to what was observed by Weiss (1999) in his retrospective study, the patient in this case did not obtain hematological improvement after treatment for ehrlichiosis with doxycycline and imidocarb dipropionate, given that the animal in the present report had an installed condition of bone marrow aplasia, which occasionally turns out to be unresponsive to conventional treatments, unlike the patients in the study who probably had pancytopenia due to an acute infection.

Bone marrow transplantation (BMT) is a therapeutic option for the treatment of bone marrow aplasia, enabling an increase in patients' survival. First, the animal can only undergo a BMT procedure if it presents a stable clinical condition, without any important dysfunctions of systems or organs (FONSECA and SECOLI, 2008; CASTRO et al., 2001). It is known that the patient reported here did not present changes in other systems that would prevent the procedure.

The pre-transplant phase requires aplasia of the receptor's marrow to facilitate graft picking, where it is commonly done with the administration of myelotoxic drugs (FONSECA and SECOLI, 2008; APPELBAUM, 1996). The patient in the reported case already had aplastic marrow, not needing any procedure that would lead to this condition, so the marrow was already ready to receive the material that would be transplanted.

The prerequisites established for donor selection (age range from 1 to 7 years, have at least 25 kg, have no clinical history of hemoparasitosis and not showing any change in the blood count) are consistent with what is described by Castro et al. (2001).

The donor bone marrow aspiration procedure requires the donor to be under general anesthesia and must be performed aseptically in order to reduce the risk of secondary infections, since it interferes with the donor animal's immunity, as well as causing hematological changes (CASTRO et al., 2001; OLSSON et al., 2009). During the collection in the present report, all the precautions described above were taken in order to mitigate the risks of complications during and after the technique. The total aspirated volume was 57 ml, in line with what is described by Castro et al. (2001), who estimates an average of 10ml/kg of the receptor. After collection, the material was stored in a blood collection bag without anticoagulant, since it had already been added to the sodium citrate syringes at the time of material collection. After that, a transfusion equipment was used to filter the spikes that were in the content that would be transplanted, procedure that corresponds with what is proposed by Castro et al. (2001).

Bone marrow transplantation is performed through the intravenous administration of hematopoietic progenitor cells, which end up lodging in the bone marrow by tropism, initiating the hematopoietic reconstruction process (VECHIATO et al., 2005; FONSECA and SECOLI, 2008). The way in which the material was administered coincides with what was described by the authors previously.

Pranke (2006) described that patients submitted to BMT may present hematological improvements from two to four weeks after the procedure, a fact that was observed in the patient in the present report, given that there was an extremely significant improvement in hematocrit values, as well as leukocytes and platelets.

## CONCLUSION

In view of the report, it can be said that bone marrow transplantation brought good hematological responses in the dog diagnosed with bone marrow aplasia, resulting from a condition of chronic ehrlichiosis, since the anemia condition ended thirty days after the procedure. However, although thrombocytopenia and leukopenia have not ceased, there is a significant increase in leukocyte and platelet values compared to the results of blood counts prior to transplantation, thus demonstrating a tendency to normalization. Thus, it is necessary to monitor the patient in the following months in order to monitor whether there will be a total improvement of the case, considering that the recovery may be long and uncertain due to the scarcity of reports on the subject.

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