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MYELOGRAM AS A DIAGNOSIS OF MARROW BONE HYPOPLASIA IN DOGS

MIELOGRAMA COMO DIAGNÓSTICO DE HIPOPLASIA MEDULAR EM CÃES

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ABSTRACT

The Marrow Bone hypoplasia is an undetected alteration in domestic animals, characterized by a decrease in one or more percussive cell lines in the bone marrow and consequent cytopenia in peripheral blood. Among the most common causes of this pathological process are infectious, drug-induced and radiation causes. The myelogram is characterized by the quantification of the cell lines in the bone marrow and should always be interpreted in conjunction with the changes observed in the hemogram. However, both the collection of bone marrow and the performance of the myelogram are not routine exams in the veterinary clinic, even presenting great diagnostic power for numerous diseases. Thus, the objective of the present study was to perform a literature review addressing the relevant topics on bone marrow collection, preparation of slide and realization of Myelogram, as well as to provide the reader with a collection of images that can assist in the interpretation of the topics covered.

Key words: Cytopenia, Bone Marrow, Canine

INTRODUCTION

The bone marrow is the largest hematopoietic organ in the body, able to produce red blood cells, platelets and leukocytes. In young animals, active hematopoiesis occurs inside long, flat bones. As the animal grows older, the spinal canal is replaced by adipose tissue, confining the active marrow in places where there is spongy bone, that is, epiphyses and bone metaphysis (CURY, 2003).

Medullary hypoplasia is characterized by cytopenia in the peripheral blood with a decrease in one or more cell lines (red blood cells and/or platelets, and/or leukocytes), resulting in the replacement of hematopoietic tissue with adipose tissue (WEISS, 2003).

The main causes of hypoplasia of bone marrow cell lines in dogs are the result of the destruction of stem cells or medullary progenitor cells. These include infectious diseases such as: leishmaniasis, ehrlichiosis and parvovirus, radiation (WEISS, 2003), estrogen intoxication (SANPERA et al., 2002), chemotherapy medication, phenylbutazone, sulfadiazine/trimethoprim). Other drugs like quinidine, griseofulvin, cephalosporins, phenothiazine, chloramphenicol and captopril are less mentioned (WEISS and KLAUSNER, 1990), as well as idiopathic causes (WEISS, 2006).

The diagnosis begins at the clinic, with a good anamnesis performed by the veterinarian, excluding possible causes of cytopenia and looking for information on the animals exposure to drugs, among other causes. However, it's essential to perform bone marrow puncture followed by myelogram to confirm the diagnosis of spinal cord hypoplasia in dogs (ALENCAR et al., 2002).

Nevertheless, bone marrow aspiration collection is not yet a routine procedure in veterinary clinics, just as there are few professionals trained to read the collected material correctly, making a good diagnosis difficult. (STACY and HARVEY, 2017).

That being said, due to the great importance of the exam and the lack of professionals to perform it, the present study aims to review the literature on the diagnosis of spinal hypoplasia in dogs using the myelogram, addressing the main issues inherent to spinal puncturing technique, making the slides and performing the myelogram, in addition to providing the readers with a collection of images that can assist them in the interpretations.

COLLECTION TECHNIQUE AND MAIN MEDULAR PUNCTURE SITES

Correct collection and preparation of the material is essential for a successful diagnosis. In the spinal cord evaluation, attention must be given to the collection time in the case of *post-mortem* diagnosis, which must be performed within 30 minutes. Even in live animals, sample preparation must be carried out immediately, because the cells tend to suffer significant morphological distortions leading to the misdiagnosis of neoplasms and other disorders (COWELL et al., 2009).

The choice of the puncture site varies according to the animal and the veterinarian's experience. In dogs, medullary aspirate can be obtained in the epiphysis of long bones and in the regions of the ileum, such as the iliac crest or acetabular border (SLATTER, 1998). The sternal bone is also the region of choice for some veterinarians (MASSUMOTO et al., 1997).

The dog's size and it's amount of body fat can influence and hinder collection in some points of the animal's body. Therefore, in small dogs, the collection of spinal samples are performed with less effort in the trans-iliac or proximal regions of the femur (SLATTER, 1998; ZAMPROGNO, 2007). In obese animals, the cranio-lateral portion of the greater humerus tuberosity is the most indicated (ZAMPROGNO, 2007).

Generally speaking, the supplies for bone marrow collection are used on a daily basis and at low costs. The use of needles suitable for this purpose is indicated, although there are professionals who prefer the hypodermic needle 40x12 to perform the technique (LARUE, 2005). Several models of needles are described, with Komiyashiki, Ilinois and Bierrnan being the most used (ALENCAR et al., 2002).

Cury (2003) pointed out the Jamshidi needle as the one of choice for obtaining bone marrow samples. The size of the needle used varies according to the species, the size of the animal in question and the skill of the veterinarian (TVEDTEN, 1989). In addition, Muller et al. (2009) recommend the use of mandrels on needles during processing, to prevent clogging of the bevel with bone bridges.

Whereas the authors of this review have a good result with the collection in the external manubrium, using the 40x12 hypodermic needle, for bone marrow collection in dogs, except in cases of very large or obese animals, in which the needle cannot reach the region bone. In this case, the use of longer catheters could be an option for collection. However, it takes great caution and experience for performing the collection, due to the region's closeness to vital organs.

For spinal aspiration, the animal must be positioned according to the location of choice for performing the technique. It is essential to perform trichotomy and surgical asepsis on the spot, trying as much as possible to prevent contamination at the time of collection. The syringe should be filled with approximately 0.5 mL of ethylenediamine tetraacetic anticoagulant acid (EDTA), in a concentration of 3%, diluted in saline solution to prevent the material from clotting in the needle (COWELL et al., 2009).

Bacigalupo et al. (1992) also describe the use of Heparin (10,000 IU) to wash the suction circuit alongside with 0.9% saline solution, thus avoiding the clotting of the bone marrow collected before the preparation of the slides.

There are also authors who report the non-use of anticoagulants for bone marrow collection to perform the myelogram, however, it must be performed quickly and the slides must be executed immediately, to avoid sample coagulation. (THRALL et al., 2015). Depending on the animal's temperament, in addition to restraining, sedation may also be required, and the choice of drugs is at the discretion of the veterinarian. (FREEMAN, 2000).

To facilitate the introduction of the needle, a small incision in the skin can be made to facilitate penetration into the bone. The area where the needle is going to be introduced must be stabilized with the hand and must be under pressure, exerted with rotating movements in order to penetrate the bone (EURIDES et al., 2010). Injuries to soft tissues are among the main barriers of the technique, as well as the absence of material during aspiration and/or collection of inappropriate samples (LARUE et al., 2005).

After making sure that the needle is firmly attached to the bone, strong negative pressure is applied by pulling the plunger of the syringe several times. If after aspiration, no medullary material is observed in the syringe, it must be removed and the needle relocated (MULLER et al., 2009).

PREPARING THE BLADES

After the collection, for the samples collected without anticoagulants, the material must be deposited on microscopic slides, and then a "Squash" must be performed, or a blood smear, and then the samples must be fixed in methanol for five minutes and stained with Romonowsky type dyes. In some situations, other colorations may be necessary, such as Prussian Blue, for a better evaluation of medullary iron, and special cytochemical colorations (Sudan Black, peroxidase, etc.) (THRALL et al., 2015).

In samples collected with anticoagulants, the collected material should be transferred to a Petri dish to better observe the presence of spinal cord spicules. Once checked, they must

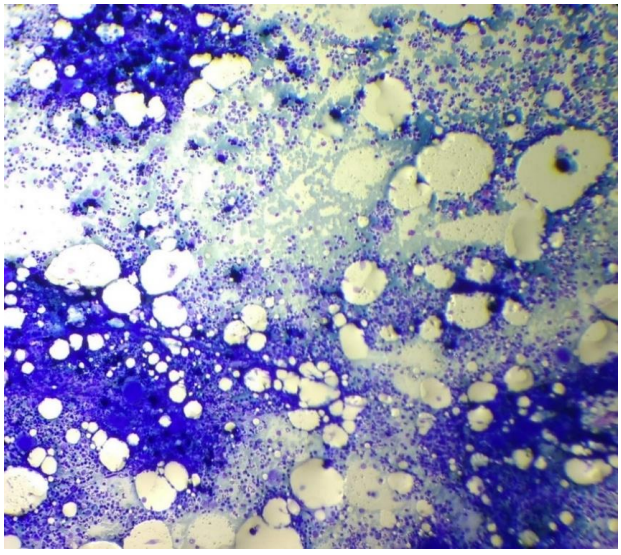
be collected with a capillary tube. The aspirated content must be transferred to a microscope slide where the “Squash” will be performed smoothly (COWELL et al., 2009).

INTERPRETATION AND DIAGNOSIS

The evaluation of the bone marrow begins at the aspiration. An easy collection, with a large amount of lumps, viscous appearance and the formation of a dense smear, suggests a cellular or hypercellular marrow. If the collection technique is acurate, but if there are difficulties in aspirating the material, in addition to the absence of lumps or the presence of small lumps in low quantity, there is evidence of a hypocellular marrow (STACY and HARVEY, 2017).

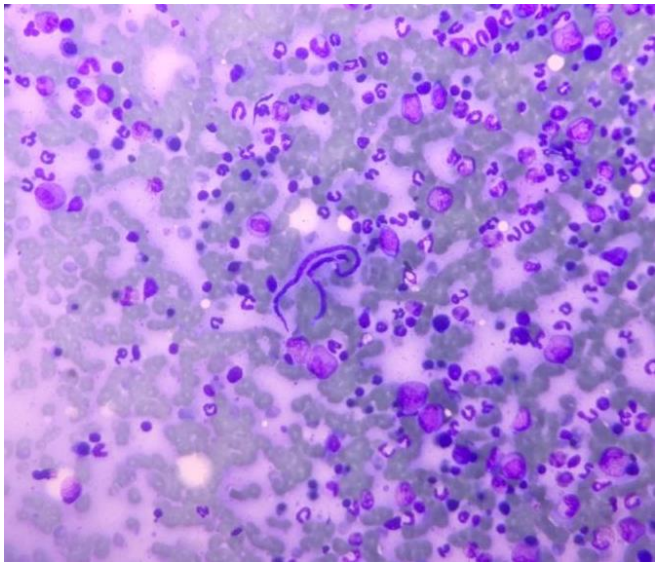
The 10x ocular lens should be used for a first scan of the bone marrow blade in order to assess the general cellularity of the content. However, the general cell evaluation can be subjective, especially in those samples hemodiluted by the use of anticoagulants (STOCKHAM and SCOTT, 2011).

The main principle of the evaluation of spinal cord cellularity is the estimation of the proportion of hematopoietic precursor cells in comparison with the amount of fat present in the slide. According to Thrall et al. (2015) the ideal ratio would be 1: 1 between adipose tissue and precursor cells, which may vary according to age. In young animals the proportion tends to be lower and in very elderly animals it can be higher, because of the replacement of the active spinal tissue by fat with age. (Pic.1).



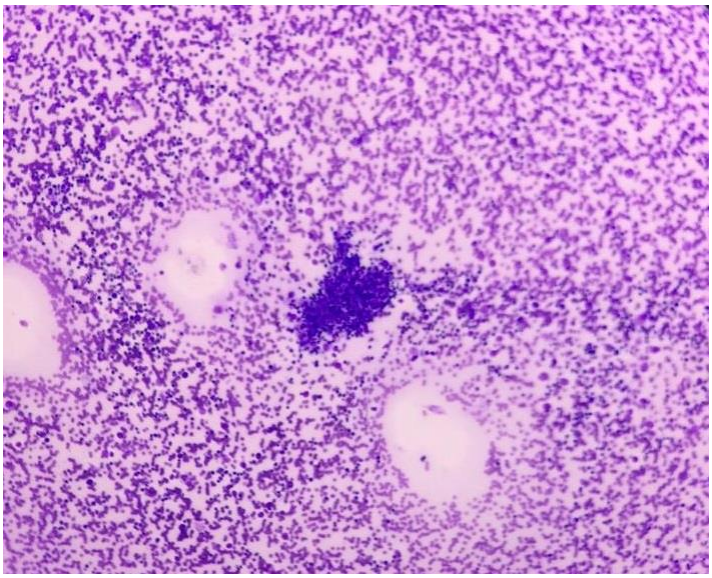
Picture 1. Dog’s bone marrow ideal cellularity, characterizing normal bone marrow, presenting a good cellular proportion in relation to the amount of fat. Original magnification 100x, panotic coloring. Source: Author

Generalized bone marrow hyperplasia occurs when the amount of percussion cells are greater in relation to the amount of fat (WEISS, 2008). An increase in cellularity can occur when there is excessive production of the myeloid, erythroid and/or lymphoid lineage (Pic. 2), according to the need of the organism, whether in cases of infection, neoplasms, autoimmune disorders, regenerative anemias, among others (STOCKHAM and SCOTT, 2011).

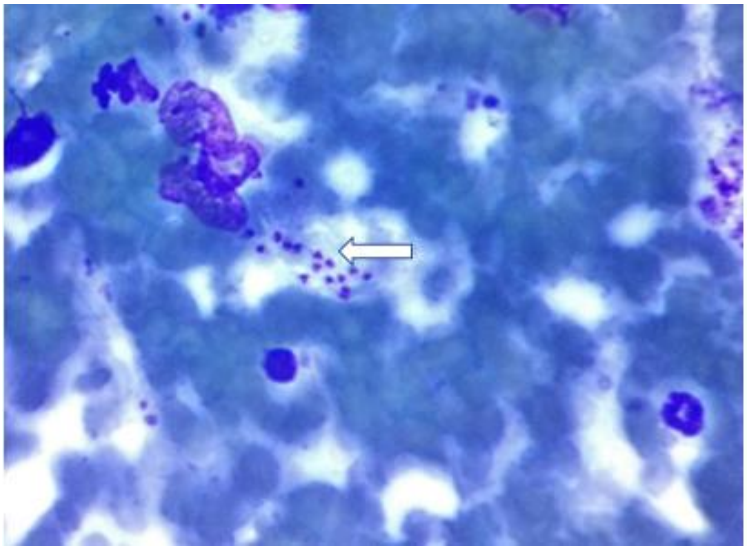


Picture 2. Dog's bone marrow with lymphocytic proliferation, resulting from infection by microfilariae, detected during cell quantification during the myelogram. Animal presented chronic lymphocytosis in peripheral blood. Original 400x magnification, panotic coloring. Source: Author

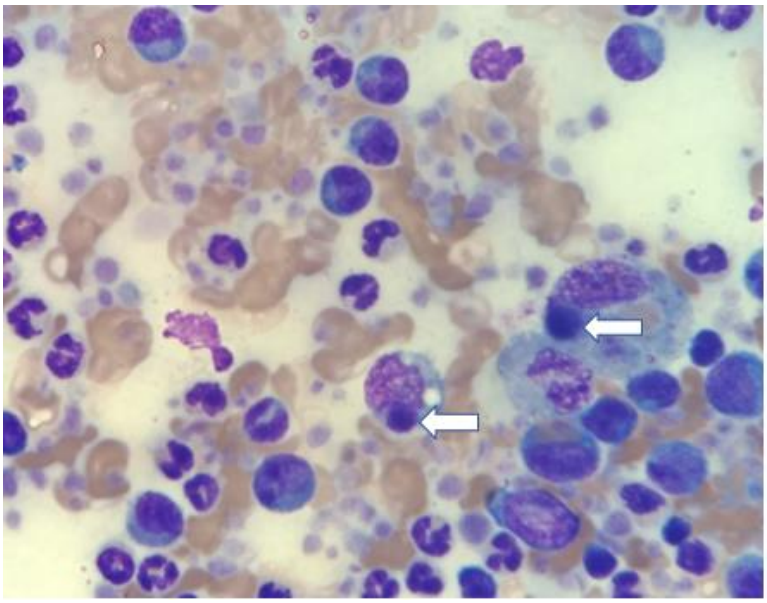
In the evaluation of medullary hypocellularity, the percussive cells will be present in low quantity. It can be defined by the presence of cytopenia in peripheral blood and one or more under-represented or absent cell lines (pic.3) (HARVEY, 2011). In dogs, diseases such as leishmaniasis (Pic. 4) (TURINELLI et al., 2015) ehrlichiosis (Pic. 5) (MYLONAKIS et al., 2006) and parvovirus are among the main infectious causes of hypoplasia and/or spinal aplasia followed by estrogen poisoning and chemotherapy (THRALL et al., 2015).



Picture 3. Dog's bone marrow spinal hypoplasia. A decrease in the amount of percussive cells in the bone marrow is noticeable. The patient had persistent pancytopenia in peripheral blood, in addition to a decrease in all strains in the myelogram. Original magnification 100x, panotic coloring. Source: Author



Picture 4. Amastigote forms of Leishmania sp. in a dog's bone marrow with spinal cord aplasia (arrow). Original magnification 1000x, Panotic Coloring. Source: Author

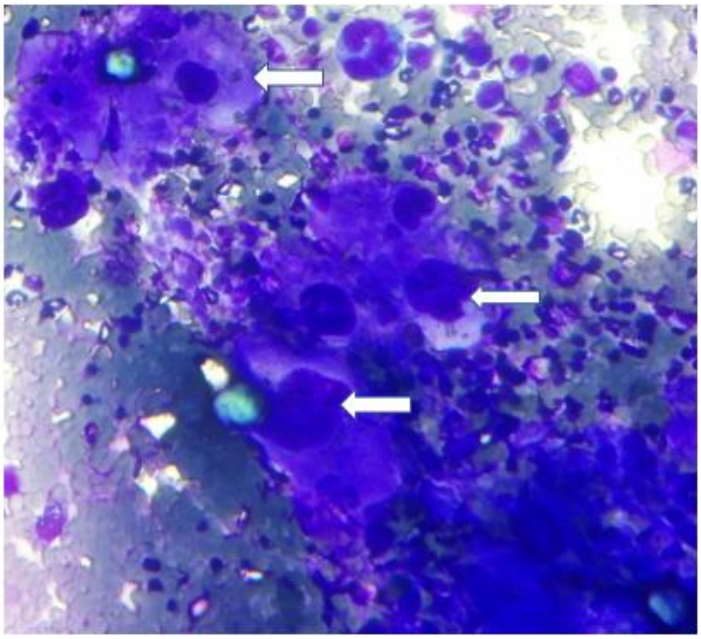


Picture 5. Morulas of Ehrlichia sp. (arrows) in a dog's bone marrow with pancytopenia in peripheral blood. Original magnification 1000x, panotic coloring. Source: Author

EVALUATION OF THE MEGAKARYOCYTIC SERIES

Unless there is excessive hemodilution due to the use of anticoagulants, megakaryocytic hypoplasia should be defined when there is thrombocytopenia and less than 2 megakaryocytes per spike (the usual is 2 to 8 per spike) (Pic. 6). Megakaryocytic hyperplasia is defined by the presence of more than 8 megakaryocytes per spike (GRINDEM *et al*. 2002).

Megakaryocytic hypoplasia with no erythroid and myeloid hypoplasia is considered rare, and may be caused by immune-mediated destruction of megakaryocytes (WEISS E KLAUSNER, 1990).



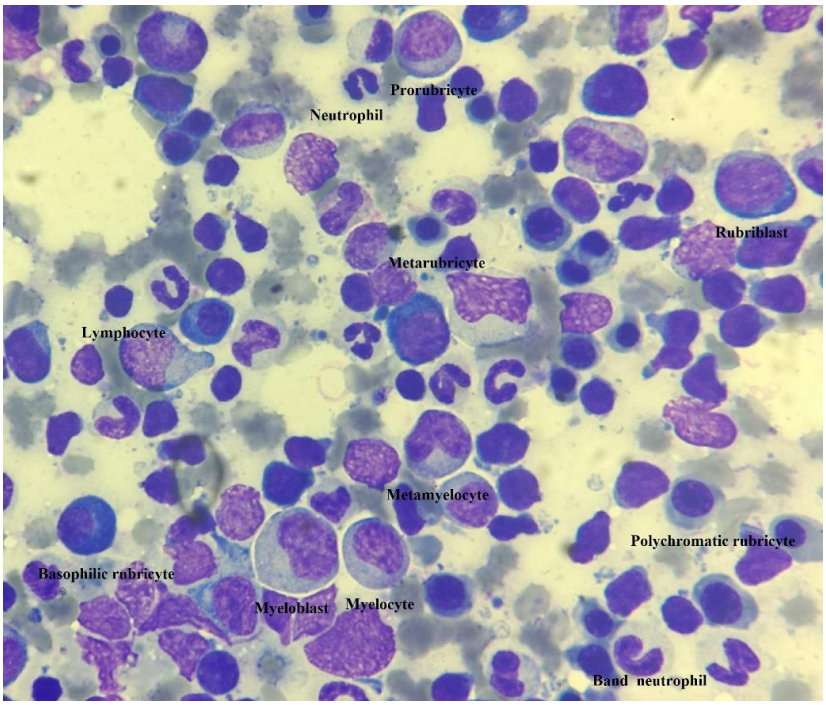
Picture 6 - Dog’s bone marrow with ideal amount of megakaryocytes (arrows). Original 400x magnification, panotic coloring. Source: Author.

ERYTHROID SERIES EVALUATION

The amount, proportion and morphology of the cells of the erythroid lineage can be evaluated in several ocular objectives, depending on the examiner's experience. Erythroid precursors are generally smaller when compared to myeloid percussors. They exhibit more spherical nuclei with dark and condensed nuclear chromatin, darker cytoplasm that can be deeply basophilic (THRALL et al., 2015).

The more mature erythroid cells become smaller, the nuclei progressively condense and the cytoplasm changes from basophil to orange pink due to the accumulation of synthesized hemoglobin. When cell maturation is ordered, the nucleus is extruded before the cells are fully mature (FREEMAN, 2000).

The erythroid series is composed of rubriblast, prorubricyte, basophilic rubricyte, polychromatic rubricyte, metarubricyte and reticulocytes (Pic. 7) (COWELL et al., 2009).



Picture 7. Photomicrograph of a dog's bone marrow, showing some of the main percussion cells that can be found. Original magnification 1000x, panotic coloring. Source: Author

According to Harvey (2011), immature erythroid cells (rubriblasts and prorubricyteytes) should correspond to between 1.1 to 3.3% of the total cells. On the other hand, mature erythroid cells (basophilic rubricyte, polychromatic rubricyte, metarubricyte and reticulocytes) should correspond to between 28.4 to 51.5% of the total cells.

EVALUATION OF THE GRANULOCYTIC, LYMPHOCYTIC AND MONOCYTIC SERIES

The granulocytic series has larger cells when compared to erythroid cells (Pic. 7). It is composed of myeloblast, promyelocyte, myelocyte (neutrophilic, eosinophilic and basophilic), metamyelocyte (neutrophilic, eosinophilic and basophilic) segmented neutrophil and band neutrophil (THRALL et al., 2015).

The mature granulocytic precursors, reduce a little in size, the nucleus condenses and undergo cytoplasmic color and shape changes. The cytoplasm changes from basophil gray to almost colorless in neutrophils, primary cytoplasmic granules become invisible in promyelocytes, and secondary cytoplasmic granules become evident from the myelocyte, appearing colorless in neutrophils, red in eosinophils and blue in basophils (HARVEY, 2011).

The lymphoid and monocytic series can also be reduced in animals with spinal cord (COWELL et al., 2009). The lymphoid series is composed of lymphoblasts, pro-lymphocytes and small lymphocytes. The monocytic series is composed of the monoblast, pro-monocyte and monocyte. Immature myeloid cells must represent between 1.5 and 3.4% of the total cells, while mature myeloid cells (myelocytes, metamyelocytes, segmented) must represent between 36.7 to 62.7% of the total cells. Small to intermediate lymphocytes, on the other hand, should represent between 1.7 to 4.9% of the total cells (HARVEY, 2011).

MYELOID:ERYTHROID RATIO

The myeloid:erythroid ratio (M:E) is the ratio between myeloid cells and nucleated erythroid cells in 1000x magnification. This ratio is determined by the differential count of 500 cells (MISCHKE and BUSSE, 2002).

Lymphocytes, plasma cells, macrophages and stromal cells are excluded from the differential myeloid count. This count, in addition to being exhaustive, is considered frustrating by some professionals, due to the difficulty of differentiating the initial cell lines. (COWELL et al., 2009).

The normal M:E ratio differs between species. In dogs, the range between 0.75:1 to 2.53:1 is considered normal for the M:E ratio (HARVEY, 2011). Any change in response to a physiological disorder that causes less or greater production of cell lines changes the M:E ratio and such changes must be interpreted together with the results of the blood count, in particular the hematocrit, reticulocyte and neutrophil count (THRALL et al., 2015)

Lower M:E ratio may indicate increased red blood cell production, such as that seen in regenerative anemia, as well as decreased neutrophil production or a combination of these two conditions. The increase in the M:E ratio may indicate greater granulocyte production (ie, myeloid hyperplasia) and/or decrease in the production of red blood cells (ie, erythroid hypoplasia). In general, granulocytic hyperplasia occurs in inflammatory processes (TURINELLI et al., 2015).

EVALUATION OF IRON RESERVES

The marrow assessment of iron reserves may require special dyes such as Prussian blue. However, brownish-black iron aggregates can be seen in Romonowsky stains. Hemosiderin can be observed as blackish blue material inside macrophages when iron stores are normal in the dog (HARVEY, 2001).

Increased iron reserves may appear in cases of hemolytic anemia, multiple blood transfusions, elderly animals, hemochromatosis and hemosiderosis in addition to parenteral iron administration. Decreased iron stores are common in young and newborn animals, nutritional iron deficiency and chronic bleeding (COWELL et al., 2009).

FINAL CONSIDERATIONS

The myelogram is a test that really helps the clinician, and can provide a variety of informations both in the diagnosis of spinal aplasia in dogs and in the diagnosis of other diseases. However, for the correct confirmation of the diagnosis, in addition to the quantification of the medullary cells, serial hemogram evaluations, reticulocyte counting, and also a complete anamnesis are necessary, observing whether or not the patient may have been in contact with the possible factors that are able to trigger the disease.

In addition, this complex and subjective examination requires great knowledge and technical training from the veterinary clinical pathologist so that it can be successfully performed, in a way that it contributes to the confirmation of the applicant's diagnosis and clinical conduct.

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