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Cytochemical staining for diagnosis of acute myeloid leukemia in a canine

Coloração citoquímica para diagnóstico de leucemia mieloide aguda em um canino

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ABSTRACT: Acute myeloid leukemia (AML) is a malignant neoplasm arising from the proliferation of hematopoietic cells in the bone marrow, and is considered uncommon in dogs. Due to this, the objective of this study is to report a case of AML of possible myelomonocytic lineage in a canine, highlighting the importance of performing cytochemical staining on blood smears to obtain a definitive diagnosis. A canine, Rottweiler, male, 10 years old, weighing 25.1kg, was treated with a main complaint of apathy, indisposition, anorexia and adipsia for three days. On physical clinical examination, during auscultation he presented muffled heart sounds, pale mucous membranes and cachexia. The blood count showed the presence of anemia, thrombocytopenia and leukemia. The blasts were greater than 30% in relative count, consistent with acute leukemia. The slides were stained using cytochemistry with periodic acid-Schiff and peroxidase, being compatible with cells of myeloid origin (granulocytic and/or monocytic), obtaining a definitive diagnosis of AML of possible myelomonocytic lineage. An abdominal ultrasound (US) was performed, which showed an image suggestive of neoplasm in the liver and spleen. From fine needle cytology guided by US, material was collected from the liver, which showed leukemic infiltration. The patient died within five days and the necroscopic examination was not authorized by the person responsible. It is concluded that a thorough clinical examination associated with the blood count and cytochemical staining of circulating blasts were crucial for obtaining an early and definitive diagnosis of AML of possible myelomonocytic lineage in the patient in this report.

KEYWORDS: Blood count; myelomonoblasts; malignancy.

RESUMO: A leucemia mieloide aguda (LMA) é uma neoplasia maligna decorrente da proliferação de células hematopoiéticas na medula óssea, sendo considerada incomum em cães. Devido a isso, o objetivo deste estudo é relatar um caso de LMA de possível linhagem mielomonocítica em um canino, destacando a importância da realização de coloração citoquímica em esfregaços sanguíneos para obtenção de um diagnóstico definitivo. Um canino, Rottweiler, macho, 10 anos, peso 25,1kg, foi atendido com queixa principal de apatia, indisposição, anorexia e adipsia há três dias. Ao exame clínico físico, à ausculta apresentava bulhas cardíacas abafadas, mucosas pálidas e caquexia. O hemograma mostrou presença de anemia, trombocitopenia e leucemia. Os blastos foram superiores a 30% na contagem relativa, consistente com leucemia aguda. As lâminas foram coradas por citoquímica com ácido periódico de Schiff e peroxidase, sendo compatíveis com células de origem mieloide (granulocítica e/ou monocítica), obtendo-se diagnóstico definitivo de LMA de possível linhagem mielomonocítica. Foi realizada ultrassonografia (US) abdominal, que evidenciou imagem sugestiva de neoplasia em fígado e baço. A partir de citologia com agulha fina guiada por US, foi coletado material do fígado, que evidenciou infiltração leucêmica. O paciente evoluiu para óbito em cinco dias e o exame necroscópico não foi autorizado pelo responsável. Conclui-se que um exame clínico minucioso associado ao hemograma e à coloração citoquímica dos blastos circulantes foram cruciais para a obtenção de um diagnóstico precoce e definitivo de LMA de possível linhagem mielomonocítica no paciente deste relato.

PALAVRAS-CHAVE: Hemograma; mielomonoblastos; malignidade.

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INTRODUCTION

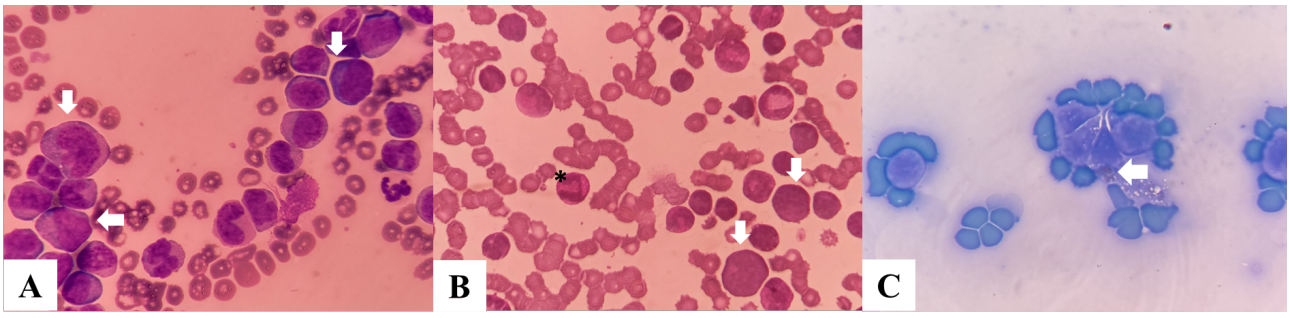
Acute myeloid leukemia (AML) is a malignant neoplasm arising from the proliferation of hematopoietic cells in the bone marrow (Hayashi *et al.*, 2011). It is acute when in the presence of immature myeloid cells above 20% in the blood, bone marrow or extramedullary organs (Stokol *et al.*, 2017). Its classification according the World Health Organization (WHO) subclassifies AML into groups: Minimally differentiated AML, AML – myeloid lineage, AML – myelomonocytic lineage, AML – monocytic lineage, AML – erythroid lineage and AML – megakaryocytic lineage (Vardiman; Harris; Brunning, 2002).

AML is considered uncommon and mainly affects middle-aged dogs, between seven and eight years old, with a predominance in males (Novacco *et al.*, 2016; Davis; Hume; Stokol, 2018). Most animals present nonspecific clinical signs, such as lethargy and inappetence. Therefore, the diagnosis of AML is generally made through morphological findings in peripheral blood and cytochemical staining of blasts (Davis; Hume; Stokol, 2018; Avery, 2020). Cytochemistry has been used for several years to distinguish between AML and acute lymphocytic leukemia (ALL) in animals (Jain *et al.*, 1991; Facklam; Kociba, 1985). Bone marrow evaluation is not included in the initial diagnostic investigation if there is the possibility of the diagnosis being made with peripheral blood (Avery, 2020). The prognosis of AML is unfavorable, even with aggressive chemotherapy (Novacco *et al.*, 2016). Thus, this study aims to report a case of acute myeloid leukemia of possible myelomonocytic lineage in a canine, highlighting the importance of performing cytochemical staining on blood smears to obtain a definitive diagnosis.

CASE REPORT

A Rottweiler male, neutered, 10 years old, weighing 25.1kg, was seen with a history of apathy, indisposition, anorexia and adipsia for three days. Clinical examination revealed the presence of muffled heart sounds on auscultation, capillary refill time (CRT) of 3 seconds, pale and sticky mucous membranes, strong and regular pulse, underweight, 6% dehydration and rectal temperature of 37.3°C. From this, a blood count was performed, which showed anemia (hematocrit: 27% reference: 37-55%, hemoglobin 10.0g/dL reference: 12-16g/dL), thrombocytopenia 82,000/µL (reference: 200,000-500,000/µL) and leukocytosis 223,500/µL (reference: 6,000-17,000/µL). In the absolute differential count, presence of 87,165/µL (reference: zero) blasts presenting cytoplasm with varying degrees of basophilia, rare evident Golgi areas, nuclei mostly cleaved and convoluted with coarse chromatin and expressing nucleoli in numbers of one to three with mild anisonucleoliosis (figure 1A), 4,470/µL metamyelocytes (reference: zero), 6,705/µL rod neutrophils (reference: 0-300), 69,285/µL segmented neutrophils (reference: 3,000-11,500), 11,175/µL lymphocytes (reference: 3,000-11,500), 11,175/µL lymphocytes (reference: 1,000-4,800) and 44,700/µL monocytes (reference: 150-1,350). Toxic neutrophils (2+) were still observed. Cytochemistry of the blood smear was carried out, with negative staining of the blasts with periodic acid-Schiff (PAS) (figure 1B) and positive for peroxidase, being compatible with cells of the myeloid lineage (granulocytic and/or monocytic), obtaining a diagnosis of acute myeloid leukemia (AML) of possible myelomonocytic lineage (Villiers *et al.*, 2006).

Furthermore, biochemical tests were performed to evaluate renal function and liver profile, which showed an increase in creatinine 2.25mg/dL (reference: 0.5-1.5 mg/dL), urea 189.0mg/dL (reference: 21- 60 mg/dL), higher serum activities of alkaline phosphatase 2.900.0U/L (reference: <156U/L) and alanine aminotransferase 186.0 U/L (<102U/L), in addition to a decrease in albumin 15.4g/ L (reference: 26-33 g/L). Abdominal ultrasound (US) revealed preserved dimensions in the liver, regular contour, with the presence of an oval, hyperechoic, discretely heterogeneous structure, vascularized in a network on Doppler study, measuring approximately 5.96x3.95cm, located in quadrate lobe topography/ left medial suggestive of neoplasm. The spleen has preserved dimensions, regular contour, and a rounded, hypoechoic, homogeneous, well-defined structure,



Source: Author, 2022.

Figure 1. Peripheral blood smear from a canine showing circulating blasts (arrows). Fast panoptic staining, 1000x magnification **(A)**. Blasts with negative cytochemical staining for periodic acid Shiff (PAS) (arrow) and neutrophils with positive staining used as technique control (asterisk), 1000x magnification **(B)**. Blood smear showing positive cytochemical staining for peroxidase, blasts compatible with cells of the myeloid lineage (granulocytic and/or monocytic), magnification of 1000x **(C)**.

measuring approximately 0.92x1.01cm, located in the splenic tail and other organs without changes. Cytology was performed with a 25 x 0.7 mm needle, guided by US of the mass found in the liver, which showed generalized leukemic infiltration. Two days after the initial exams, a new blood count was performed, which showed persistence of anemia, thrombocytopenia 50.000/µL (reference: 200.000-500.000/µL), total leukocyte count 417.800/µL (reference: 6.000-17.000/µL) and creatinine measurement which presented a value within the reference range for the species. The patient was hospitalized to receive medication based on maropitant citrate [Cerenia® at a dose of 2mg/kg 1x a day, subcutaneously], dexamethasone disodium phosphate [Biodex® at a dose of 0.10mL/kg every 12 hours, intravenous (IV)], dipyrone [Dipirona sodium® at a dose of 1 drop/kg, every 8 hours, IV], metronidazole [Metronidazol® at a dose of 15mg/kg, every 12 hours, IV] and methadone [Methadone® at a dose of 0.3mg/kg, every 6 hours, IV]. The patient's clinical condition worsened during hospitalization, and cardiorespiratory arrest progressed to death, lasting five days. The guardian did not authorize a necroscopic examination.

DISCUSSION

AML can affect middle-aged to elderly dogs of both sexes, being described more frequently in castrated males (Epperly *et al.*, 2018), which aligns with the present report. The canine in this study was a Rottweiler breed, and although German shepherds are considered the most affected (Tasca *et al.*, 2009; Stokol *et al.*, 2017), this disease has already been described in Rottweiler (Stokol *et al.*, 2017). The clinical picture with an acute five-day evolution presented by the patient is expected in AML, caused by the infiltrative nature of neoplastic cells or, more commonly, as a consequence of changes in hematopoiesis (Bennett *et al.*, 2017).

In the present case, performing a blood count was crucial to obtaining a definitive diagnosis of the patient. Through hematological analysis, leukemias are broadly classified as myeloid or lymphoid and then categorized as acute or chronic according to cell count and differentiation of neoplastic cells (Marino; Tran; Stokol, 2017). In the present study, it was initially possible to observe that the leukemia was acute due to a relative count of more than 30% of circulating blasts. The next step consisted of differentiation and confirmation of the cell lineage present. However, at first, it was difficult to determine the origin of the blasts as being of the lymphoid or myeloid lineage solely by cytomorphological characteristics.

According to Stokol *et al.* (2017), characteristics of myeloid differentiation may be visible in neoplastic cells associated with dysplasia of these cells, serving as additional support for AML; however, it is not confirmatory for the disease. Thus, for the differentiation of blasts, the first cytochemical staining performed on the blood smear was periodic acid-Schiff (PAS), responsible for staining the glycogen in the cytoplasm of the cells. Blasts of the myeloid lineage show negative PAS staining, while lymphoid, erythroid and megakaryocytic lineage blasts show positive staining (Snower *et al.*, 1991). Positive control of PAS staining occurs by visualizing positivity in the cytoplasm of mature neutrophils in the blood smear (Paessler; Helfrich; Wertheim, 2017).

Thus, we were initially able to target the diagnosis of leukemia as being of myeloid origin in the general classification. In the case of the patient, this classification can include either the granulocytic, monocytic or myelomonocytic lineage, requiring that at least 3% of the blasts be positive for peroxidase using enzymatic cytochemistry (Villiers *et al.*, 2006), which was observed. Furthermore, we believe that the patient's AML has a possible myelomonocytic lineage due to peroxidase staining not differentiating the granulocytic stem from the monocytic stem (Villiers *et al.*, 2006), as well as the presence of marked monocytosis. Therefore, to confirm the subclassification of AML proposed by the World Health Organization (WHO), in addition to the techniques mentioned above, it is necessary to use immunophenotyping by immunocytochemistry or cell flow cytometry, or even classification by molecular biology (Vardiman; Harris; Brunning, 2002).

The presence of cytopenias identified in the patient may be justified due to myelophthisis or leukemia preventing new blood cells from being produced sufficiently for circulating replacement (Novacco *et al.*, 2016). In a study by Stokol *et al.* (2017), most dogs presented bicytopenia or pancytopenia associated with leukemia with >20% of circulating blasts. Typical findings include nonregenerative anemia, thrombocytopenia, and leukocytosis (Stokol *et al.*, 2015). Regarding thrombocytopenia, one of the hypotheses is that the presence of neoplastic cells in the bone marrow may be responsible for suppressing thrombopoiesis (Graff *et al.*, 2014). Likewise, concomitant inflammation, increased peripheral consumption, and platelet destruction may also contribute to this change (Phillips; Naskou; Spangler, 2022).

The significant increase in the total leukocyte count denotes leukemia, mainly due to the rapid proliferation of immature and abnormal cells in the bone marrow (Meuten, 2015). In the biochemical profile, the azotemia observed may be of pre-renal origin due to mild dehydration. However, complementary tests such as urinalysis and the urinary protein creatinine ratio should have been performed for better clarification (Meuten, 2015). The elevation of alkaline phosphatase and alanine aminotransferase are related to cholestasis and hepatocyte injury, respectively (Stockham; Scott, 2011), in this case, possibly caused by generalized leukemic infiltration in the liver. The patient's hypoalbuminemia can be justified by decreased protein intake due to anorexia. However, functional changes in the liver must also be considered (Meuten, 2015).

Performing an abdominal ultrasound, in this case, made it possible to observe nodules in organs such as the liver and spleen. It also allowed to collect cytology using a fine needle guided to clarify the diagnosis better. It is known that in many dogs with AML, tumor cells infiltrate extramedullary tissues, such as the spleen, liver and lymph nodes, resulting in lymphadenopathy, hepatomegaly and splenomegaly (Stokol *et al.*, 2017), as observed in patient. There was no time to start the

chemotherapy protocol in this case due to clinical worsening even after hospital stabilization. Despite this, we point out that cytochemical stains made it possible to determine the cellular origin of the patient's leukemia, which allows the stipulation of more appropriate chemotherapy. The unfavorable prognosis, in this case, is in line with that proposed in the literature, as despite advances in the classification of types of leukemia using more modern diagnostic techniques, dogs with AML have a survival time of a few weeks to months, varying on average from 25 to 50 days (Avery, 2020; Vail; Pinkerton; Young, 2020).

CONCLUSION

Acute myeloid leukemia is not commonly diagnosed in dogs. A thorough clinical examination associated with the blood count and cytochemical staining of circulating blasts were crucial for obtaining a definitive and early diagnosis in this case. Furthermore, imaging tests need to be considered due to the high capacity of leukemias to infiltrate hematopoietic organs. Thus, early diagnosis of AML through cytochemical techniques can enable the institution of an appropriate chemotherapy protocol to increase patient survival.

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