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SHORT COMMUNICATION

Pathological and immunohistochemical aspects of acute megakaryoblastic leukaemia in a cat – Short communication

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ABSTRACT

An adult, mixed-breed, feline leukaemia virus (FeLV-) positive female cat was presented with mucosal jaundice and a history of anorexia and constipation for three days. Physical examination revealed splenomegaly, cachexia, and dehydration. Humane euthanasia was conducted, followed by postmortem examination. Grossly, the cat was icteric, and presented hepatomegaly with multifocal white spots and splenomegaly. Histologically, the bone marrow was nearly completely replaced by a proliferation of megakaryocytes and megakaryoblasts, and there was a proliferation of fibrous connective tissue. Similar neoplastic proliferation was observed infiltrating the liver, lymph nodes, spleen, kidney, skeletal muscle, and lungs. Immunohistochemistry was performed for von Willebrand Factor (VWF), CD79α, CD3, feline immunodeficiency virus, FeLV, and CD61. Marked cytoplasmic labelling was observed in the neoplastic cells for FeLV, VWF and CD61, corroborating the diagnosis of acute megakaryoblastic leukaemia.

KEYWORDS

acute myeloid leukaemia, feline, FeLV, myelofibrosis, CD61

Leukaemias are malignant disorders that originate from the haematopoietic tissue, and are classified into groups such as myeloid and lymphoid, according to their clonal cell of origin (Valli et al., 2017). Acute myeloid leukaemia (AML) is characterised by the substitution of one or more normal myeloid cell lineages by neoplastic cells of the bone marrow (Valli et al., 2017). The main feature of acute leukaemia is the presence of blast cells in the bone marrow at an early stage of maturation, while chronic leukaemia is characterised by the neoplastic transformation of cells at a more advanced stage of maturation (Adam et al., 2009; Harvey, 2012).

Acute megakaryoblastic leukaemia (AMKL) is a malignant clonal proliferation of immature haematopoietic cells of the megakaryocytic lineage, the platelet-producing cells that reside in the bone marrow (Von Boros and Korenyi, 1931). The aberrant proliferation of cells of this lineage and their emergence in the peripheral blood, associated with certain clinicopathological changes, can assist in the identification of this condition (Miyamoto et al., 1996). There are few descriptions of AMKL in humans (Oki et al., 2006; Zhao et al., 2018), dogs (Holscher et al., 1978; Comazzi et al., 2010), and cats (Michel et al., 1976; Schmidt et al., 1983; Holscher et al., 1983; Colbatzky and Hermanns, 1993; Burton et al., 1996). This study aims to describe the pathological and immunohistochemical aspects of a rare case of AMKL in a cat positive for feline leukaemia virus (FeLV).

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An adult, mixed-breed female cat was presented with mucosal jaundice and history of anorexia and constipation for a period of three days. The status of feline immunodeficiency virus (FIV)/FeLV infections was unknown. During clinical evaluation, the cat showed marked cachexia and 8–10% dehydration (moderate loss of skin turgor, dry mucous membranes, weak and rapid pulse, and enophthalmos) (Davis et al., 2013). Hepatomegaly and splenomegaly were identified by abdominal palpation and ultrasonography. Other tests, such as complete blood count (CBC), were not performed due to lack of resources from the owners. Due to the poor prognosis, the cat was euthanised with intravenous ketamine and xylazine administration (0.4 mg/kg, and 2 mg/kg, respectively), followed by an overdose of barbiturate (thiopental). Grossly, the cat exhibited jaundice of the oral and ocular mucosa. The liver was enlarged, with yellow discolouration and multifocal white spots on the capsule, measuring approximately 0.5 cm in diameter. Splenomegaly was observed. The remaining organs did not show any gross abnormalities. Samples from the main organs of the thoracic and abdominal cavities, the brain and the femoral bone marrow were collected and fixed in 10% neutral buffered formalin. Tissues were processed routinely and embedded in paraffin wax. Sections (3–5 µm) were stained with haematoxylin and eosin (HE).

Histologically, the bone marrow was nearly completely replaced by a neoplastic proliferation of cells of the megakaryocytic lineage (blast and mature), arranged in sheets (Fig. 1A). These cells were round, measured 15 µm–40 µm, with marked nuclear and cytoplasmic pleomorphism. The cells had abundant and patchy eosinophilic cytoplasm, with single or multiple nuclei with irregular and often fused

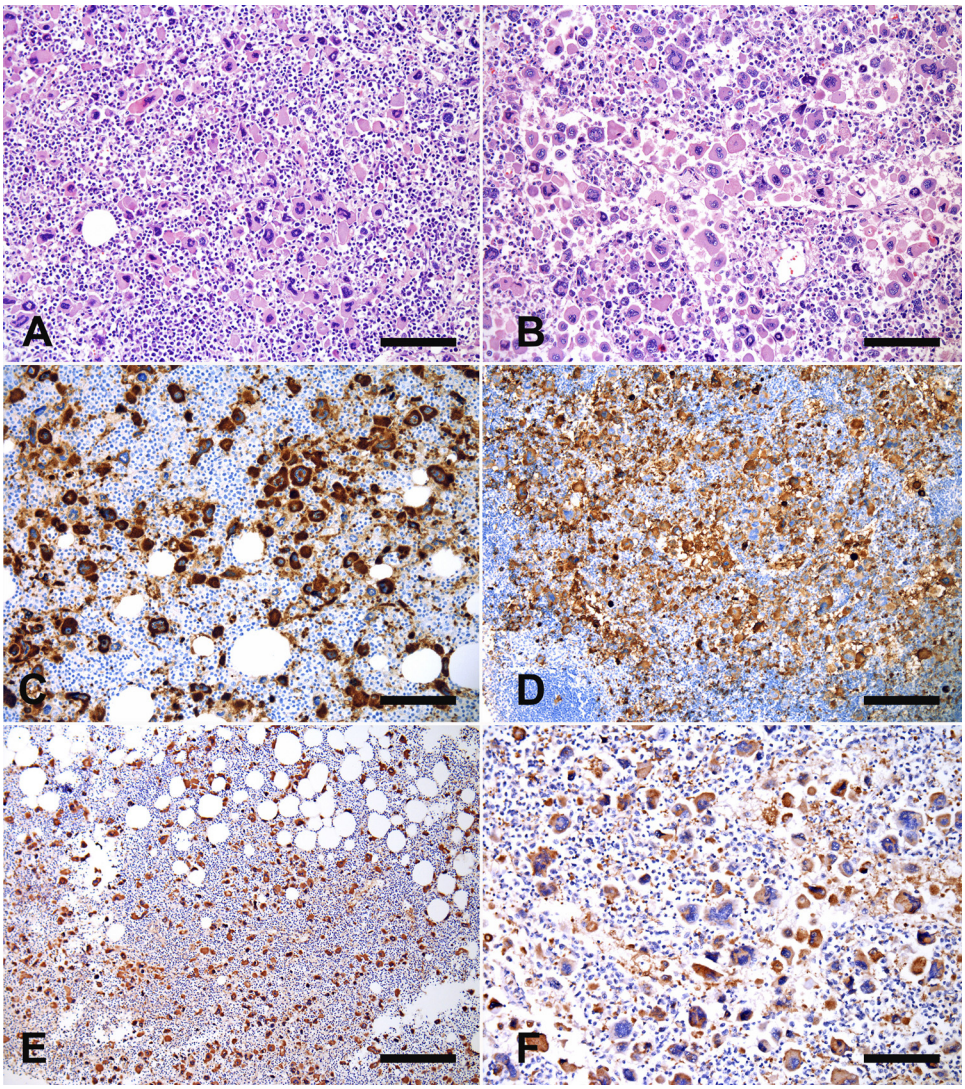

Fig. 1. Acute megakaryoblastic leukaemia in a cat. A and B: Bone marrow and mesenteric lymph node, respectively, replaced by a neoplastic proliferation of cells of the megakaryocytic lineage (blast and mature), arranged in sheets. Haematoxylin and eosin (HE), bar = 100 µm. C and D: Neoplastic cells of the megakaryocytic lineage exhibit marked cytoplasmic labelling for CD61 in the bone marrow and mesenteric lymph node. Immunohistochemistry (IHC), 3,3′-Diaminobenzidine (DAB) staining, bar = 100 µm and bar = 400 µm, respectively. E and F: Neoplastic cells of the megakaryocytic lineage exhibit marked cytoplasmic labelling for von Willebrand factor in bone marrow and mesenteric lymph node. IHC, aminoethyl carbazole (AEC) staining, bar = 400 µm and bar = 100 µm, respectively

lobulations, and stippled chromatin. Marked anisocytosis and anisokaryosis were observed. The mitotic count was zero (2.37 mm^2). Mild fibrous connective tissue proliferation (myelofibrosis) and multifocal moderate inflammatory infiltrate of lymphocytes were observed amidst the neoplastic cells. Similar neoplastic cells were observed infiltrating the lymph nodes (Fig. 1B), liver, spleen, kidney, skeletal muscle, and lungs. Besides the neoplastic cells in the liver, portal areas presented marked multifocal to coalescent infiltrate of lymphocytes and plasma cells, with moderate proliferation of the biliary duct epithelium and fibrous connective tissue (chronic cholangiohepatitis).

Immunohistochemical (IHC) analyses were performed on serial sections of the bone marrow, mesenteric lymph node, liver, spleen, kidney, skeletal muscle, and lungs, using the peroxidase-labelled universal polymer method for von Willebrand Factor (VWF) and CD61. Also, CD79α, CD3, FIV, and FeLV antibodies were used on serial sections of the bone marrow and mesenteric lymph nodes. Positive and negative controls were employed, and sections were counterstained with Harris's hematoxylin. Table 1 describes the antibodies and immunohistochemical protocols applied.

The neoplastic cells of the megakaryocytic lineage exhibited marked cytoplasmic labelling for CD61 (Fig. 1C, D) and VWF (Fig. 1E, F) in the bone marrow, lymph nodes, and all the other affected organs. Few non-neoplastic lymphocytes among the megakaryocytes were positive for CD3 and CD79α. Marked cytoplasmic labelling for FeLV was observed, while FIV results were negative in the bone marrow.

The diagnosis of AML with megakaryoblastic differentiation was based on the histopathological features of severe infiltration of neoplastic megakaryocytes and megakaryoblasts in many organs, as previously described (Burton et al., 1996; Valli et al., 2017), associated with the negative labelling for lymphocyte markers (CD3 and CD79α), leading to the conclusion that our case was not derived from the lymphocytic lineage (lymphoma/lymphoid leukaemia). In addition, the IHC exhibited marked cytoplasmic labelling for platelet glycoprotein IIIa (CD61) and VWF, which is in accordance with the results of other studies on megakaryoblastic leukaemia (Colbatzky and Hermanns, 1993; Park et al., 2006; Rochel et al., 2018).

Clinical signs observed in cats with AMKL, as in our study, are characterised by anorexia and jaundice. Some reports describe progressive weight loss, and pale mucous membranes (Schmidt et al., 1983; Burton et al., 1996). In dogs, there is a description of spontaneous epistaxis, related to severe thrombocytopenia (Colbatzky and Hermanns, 1993). In humans with AMKL, clinical signs such as anaemia, fever and bleeding from the skin or the mucous membranes are common, and related to pancytopenia (Zhao et al., 2018). The case we presented had a clinical progression of three days, similarly to a case previously reported in the literature, in which one week elapsed from the onset of clinical signs until the death of the cat (Burton et al., 1996).

In the present study, marked infiltration of neoplastic cells into the liver was observed which, along with the severe cholangiohepatitis, may have caused difficulty in eliminating bile through the bile ducts, causing intrahepatic jaundice (Boland and Beatty, 2017; Cristo et al., 2019).

The gross findings in the present study are similar to those described in the literature, in which the liver may present nodules comprised of aggregates of neoplastic cells (Burton et al., 1996); however, in our case it was not possible to affirm that the nodules observed were exclusively neoplastic, since concomitant cholangiohepatitis was detected. Besides hepatic nodules, previous studies have described hepatomegaly and/or splenomegaly in cats affected by this condition, related to the severe neoplastic infiltration in the parenchyma of these organs (Schmidt et al., 1983; Burton et al., 1996). In humans with this condition, lymphadenopathy, hepatomegaly and splenomegaly are also described as clinical and/or autopsy findings (Zhao et al., 2018).

Histological lesions reported in cats with megakaryoblastic leukaemia are consistent with a large number of blast cells replacing the bone marrow tissue. These are large round cells compatible with megakaryoblasts (Schmidt et al.,

Table 1. Antibodies and immunohistochemistry protocols

Antibody	Antigen retrieval	Dilution	Detection method	Chromogen	Positive controls
Polyclonal rabbit anti-VWF[a]	Protease XIV RT	Ready-to-use	MACH 4	AEC	Cutaneous hemangioma
Mouse anti-human CD79α (HM47/A9)[b]	HIER-Tris EDTA buffer pH 9.0	1:100	MACH 4	DAB	Lymph node
Polyclonal rabbit anti-human CD3[a]	Protease XIV RT	1:250	MACH 4	AEC	Lymph node
Monoclonal mouse anti-FIV (p24gag)[c]	HIER-Tris EDTA buffer pH 9.0	1:100	MACH 4	AEC	Bone marrow previously tested (Leite-Filho et al., 2019)
Monoclonal mouse anti-CD-61 (2f2)[d]	HIER-Citrate buffer pH 6.0	1:400	EnVision[a]	DAB	Bone marrow
Monoclonal mouse anti-FeLV (gp70)[c]	HIER-Tris EDTA buffer pH 9.0	1:250	MACH 4	AEC	Lymph node previously tested (Leite-Filho et al., 2019)

a: Dako; b: Biocare Medical; c: Bio-Rad Laboratories Brasil; d: Cell Marque; HIER: heat-induced epitope retrieval; MACH 4: Universal HRP-Polymer kit (Biocare Medical); Envision (Dako); AEC: Romulin AEC chromogen kit (Biocare Medical); DAB: 3,30-diaminobenzene (Dako); RT: room temperature.

1983; Burton et al., 1996). These cells are frequently observed infiltrating and replacing the parenchyma of other organs, including the liver, spleen, kidney, lymph nodes, lung, and brain (Schmidt et al., 1983; Burton et al., 1996). These findings are similar to those described in our study.

While in humans AML is frequently related to Down syndrome, and is characterised by *GATA*1 mutation that co-operates with trisomy 21, followed by additional somatic mutations (Gruber and Downing, 2015), in cats the disease is associated with FeLV infection (Fujino et al., 2008). FeLV is an important disease in Brazil, with frequency rates ranging from 0.33% to 31% (Almeida et al., 2012; Costa et al., 2017), mainly because most cats are unvaccinated and they commonly have free access to the outdoors, which are important risk factors of infection (Biezus et al., 2019). The virus can cause insertional mutations, initially in lymphocytes, that can lead to tumour formations (Fujino et al., 2008). It is estimated that this virus is related to the occurrence of lymphoid and myeloid tumours in 60–80% of the cases (Hardy, 1981; Essex, 1982). In this case, we observed positive immunolabelling for FeLV, which is in accordance with the fact that it can be the cause of myeloid leukaemia.

The differential diagnosis of AMKL includes progressive myelofibrosis, which is characterised by fibrosis but also by megakaryocytic hyperplasia in the bone marrow (Messick et al., 1990), since fibrosis and consequently bone marrow insufficiency are commonly observed in cases of AMKL, as we saw in our case. We were able to distinguish these two conditions by CD61 IHC associated with tumour presence in several organs. Finally, a diagnosis of megakaryoblastic leukaemia in a FeLV-positive cat was established. IHC, especially the CD61 marker, was considered a decisive complementary test for confirming the diagnosis.

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