Diagnosis of acute erythroid leukaemia (AML-M6) in a dog

We present a case of acute erythroid leukaemia with a myeloblastic component (AML-M6) in a dog. This is the first case reported since the French-American-British classification was adopted. Cytology and electron microscopy of a bone marrow aspirate were used for the diagnosis. Flow cytometry, which is commonly used for the immunophenotyping of canine acute leukaemias, was not suitable for the analysis of this sample, because early erythroid precursors were destroyed by the red blood cell lysis procedure.

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INTRODUCTION

Acute myeloid leukaemias (AML) are classified according to the French-American-British (FAB) system and its revisions.[1,2] In the past, the identification of a cell line of origin was based exclusively on cytological and cytochemical investigations, whereas flow cytometry (FC) is now used.[3,4] The only four cases of acute erythroid leukaemia with a myeloblastic component (AML-M6) in the dog[5,6] were reported before the routine use of FC in veterinary diagnostics and so the utility of this technique in the diagnosis of AML-M6 has not yet been determined. Here we describe a new case of AML-M6 in a dog, in which the neoplastic cells were analysed by FC.

CASE REPORT

A male, mongrel, 10-year old dog, weighing 27 Kg, was referred to us by his veterinarian because of 1-month history of severe pancytopenia unresponsive to treatment with prednisone (Deltacortene® 25 mg tablets, Bruno-Farmaceutici SpA, Rome, Italy) 1 mg/kg/sid, amoxicillin with clavulanic acid (Synulox® 250 mg tablets, Zoetis, Rome, Italy) 20 mg/kg/bid, and doxycycline (Ronaxan® 250 mg tablets, Merial Italia SpA, Milan, Italy) 5 mg/kg/sid. Blood-chemistry examinations, including glycaemia, total proteins, albumin, kidney function tests, and liver enzymes, plain chest X-rays in the three standard projections and abdominal ultrasound were all within the norm. Polymerase chain reaction analysis of whole blood to search for *Ehrlichia canis* and *Babesia spp.* was negative.

Differential diagnosis of the bone marrow failure included: aplasia caused by myelofibrosis/myelonecrosis, leukaemia, myelodysplastic syndrome, immune-mediated pancytopenia or systemic infections (either bacterial or fungal).

On physical examination, the only detectable clinical sign was pallor of the visible mucosae, together with sensorial depression and decreased appetite. The complete blood count (CBC) confirmed the severe

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Table 1 - Complete blood count and differential of a dog with persistent pancytopenia

Parameter	Value	Unit	Reference interval
Red blood cells (N.)	1.79	x10^6/µl	(5.7-8.8)
Haemoglobin	4.3	g/dl	(12.9-18.4)
Haematocrit	13.6	%	(37.1-57.0)
MCV	76	µm^3	(60-77)
MCH	24.0	pg	(19.5-24.2)
MCHC	31.6	g/dl	(31-36)
RDW	20.2		(11.9-18.5)
Reticulocytes (%)	2.7	%	(<1.0)
Reticulocytes (N.)	48.0	x10^3/µl	(<60.0)
Platelets (N.)	73	x10^3/µl	(200-500)
White blood cells (N.)	2.43	x10^3/µl	(6.0-19.5)
Neutrophils (N.)	0.70	x10^3/µl	(3-11.5)
Lymphocytes (N.)	1.14	x10^3/µl	(1-4.8)
Monocytes (N.)	0.10	x10^3/µl	(0.1-1.5)
Eosinophils (N.)	0.49	x10^3/µl	(0.1-1.2)

MCV, mean corpuscular value; MCH, mean corpuscular haemoglobin; MCHC, mean cell haemoglobin concentration; RDW, red cell distribution width.

A bone marrow aspirate from a dog with persistent pancytopenia was analysed by cytology and flow cytometry.

pancytopenia with a non-regenerative normocytic, normochromic anaemia (Table 1). No morphological abnormality was detected at blood smear evaluation. The dog was given a transfusion of fresh, whole blood (450 ml) as palliative treatment while awaiting the results of further diagnostic investigations. After 5 days, the patient became more active and interested in food; the CBC remained stable, but a few medium-sized, atypical cells with a high nucleus/cytoplasm ratio, scant, weakly basophilic cytoplasm and a paracentral, round nucleus containing dispersed chromatin and occasional nucleoli appeared on the blood smear. Cytological examination of a bone marrow aspirate (BM) was performed.

It appeared hypercellular, with a myeloid:erythroid ratio of 0.42. About 60% of the nucleated cells were large, with blue cytoplasm and a clear, perinuclear halo, a generally round nucleus with fine chromatin and single or multiple nucleoli; these were considered to be rubriblasts/prorubricytes (Figure 1).

Among the non-erythroid cells, more than 40% were medium to large sized, with weakly basophilic cytoplasm occasionally containing azurophilic granules, a round nucleus with fine, reticular chromatin and multiple nucleoli (myeloblasts). The predominant population was negative for the immunocytochemically tested markers (CD45, CD18, CD11b, MPO and factor VII). A diagnosis of acute leukaemia was made, probably AML-M6.

In order to confirm the erythroid origin of the prevalent population of cells and exclude an origin from other cell lines (lymphoid or myeloid), flow cytometric analysis of the BM aspirate was performed. As described in the literature,[7] red blood cells (RBC) were lysed by ammonium chloride solution. The sample consisted of small lymphocytes and myeloid cells, and only 23% of the total cells had an erythroid phenotype (negative for CD45, a pan-leukocyte marker, and positive for CD44, expressed by all haematopoietic lineages). A second aliquot of the same sample was treated with density gradient separation (Ficoll-Paque, GE Healthcare, Munich, Germany). This technique, besides separating the erythrocytes from nucleated cells, separates mononuclear cells from polymorphonuclear cells: only the former were analysed by FC. Of these, 81.6% had an erythroid phenotype. In order to compare the results,

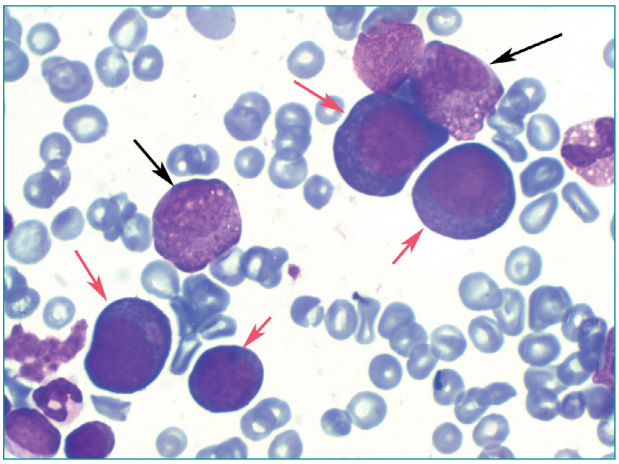

Figura 1 - Bone marrow aspirate of a dog with persistent pancytopenia (May-Grunwald Giemsa, 100X). About 60% of the nucleated cells were large, with blue cytoplasm and a clear, perinuclear halo, a generally round nucleus with fine chromatin and single or multiple nucleoli; these cells were considered to be rubriblasts/prorubricytes (red arrows). Among non-erythroid cells, more than 40% were myeloblasts (medium-sized to large cells, with weakly basophilic cytoplasm ocasionally containing azurophilic granules, a round, eccentric nucleus with fine, reticular chromatin and multiple nucleoli) (black arrows).

the analysis was restricted to the mononuclear cells in the lysed sample, as well: only 54.5% had an erythroid phenotype (Figure 2).

Finally, we used electron microscopy to examine the ultrastructure of cells in the BM aspirate. We analysed 32 cells, which had a rounded-oval shape, diameters of 12-15 mm, nuclei containing dispersed chromatin and one or more evident nucleoli, and cytoplasm containing a high number of polyribosomes and mitochondria and an endoplasmic reticulum with numerous cisternae. The mitochondria contained a high amount of ferruginous micelles, confirming the erythroid origin of the cells.[8] The diagnosis of AML-M6 was, therefore, confirmed (Figure 3).

In view of the poor prognosis, the owners declined clinical staging and chemotherapy and the patient received only palliative treatment with prednisone (Deltacortene® 25 mg tablets, Bruno-Farmaceutici SpA, Rome, Italy) at a dose of 2 mg/kg/sid. Given the progressive worsening of the animal's clinical condition, the owners subsequently accepted the choice of euthanasia for their dog, 10 days after the diagnosis.

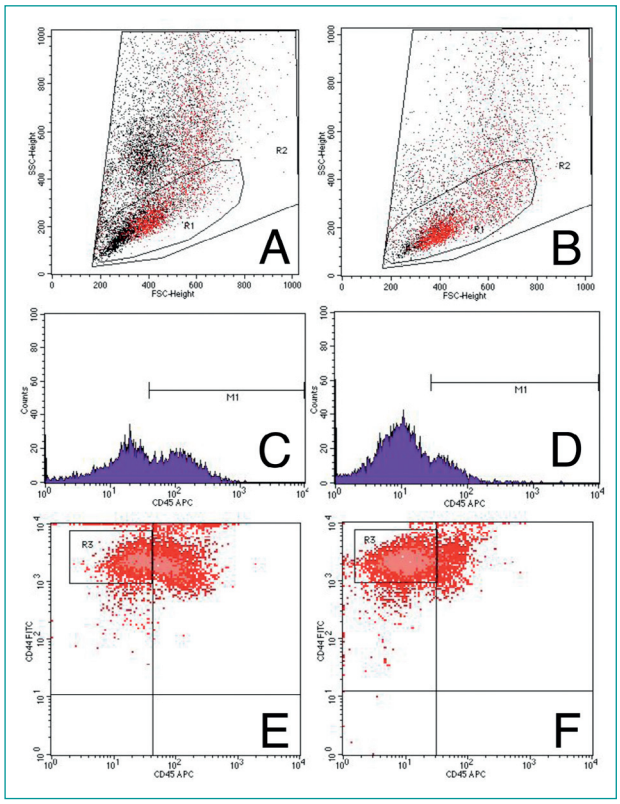

Figura 2 - Flow cytometric analysis of a bone marrow aspirate of a dog with persistent pancytopenia. An aliquot of the sample was treated with red blood cell lysis (A, C, E), whereas a second aliquot underwent density gradient separation (B, D, F). For both samples, a first gate was set to exclude debris (R2) and a second one to include only mononuclear cells (R1). The subsequent analyses were performed only on the cell population included in the R1 gate. The percentage of CD45+ cells among the mononuclear cells was 45.4% in the first aliquot (C) and 18.4% in the second (D). The CD44+CD45- cells in both aliquots were then selected by density plot (E, F), with the purpose of identifying their position in the morphological scattergram (A, B, red dots).

An understanding of the advantages and disadvantages of each diagnostic technique is essential to select the most useful diagnostic test for each, individual case.

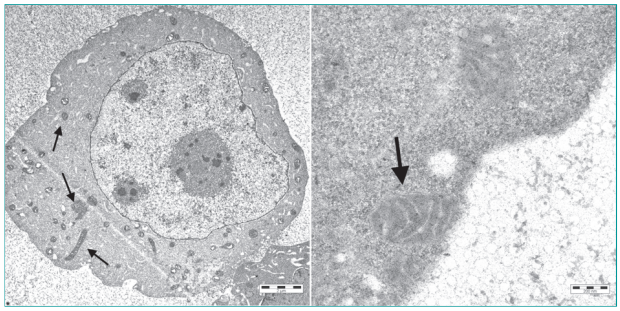

Figura 3 - Erythroblasts in the bone marrow aspirate of a dog with persistent pancytopenia. A: The cells were roundish-oval shaped and contained a round nucleus with finely dispersed chromatin and one or more clearly evident nucleoli. The cytoplasm contained electron-dense material and numerous mitochondria (arrows) (6000X). B: The intracytoplasmic mitochondria contained variable amounts of iron micelles between the lamellae and cristae (arrow) (8700X).

DISCUSSION

AML are not uncommon in the dog; however, since the introduction of the official classification in 1991,[1] only two cases of probable erythroid origin, both apparently pure erythroid leukaemia (AML-M6Er), have been reported,[9,10] whereas the published cases of AML-M6 go back to before this date and the advent of FC.[5,6] Humans with AML-M6 usually have trilineage cytopenia and circulating neoplastic cells are seen in only half of the cases.[11,12,13] The CBC at diagnosis showed anaemia and circulating atypical cells in all dogs with AML-M6 reported in the literature, together with leukocytosis in two cases and thrombocytopenia in one case.[5,6] In the case reported here, the CBC at onset showed trilineage cytopenia, whereas atypical cells appeared in the blood smear only a few days later. Thus, anaemia seems to be the only feature shared by all dogs with AML-M6, whereas circulating neoplastic cells are common, but not always present. Therefore, BM should be evaluated in all dogs with persistent cytopenias of unknown origin, since these could be due to important disorders of the haematopoietic tissue, such as acute leukaemias.

Treatment with corticosteroids alone does not improve the prognosis of dogs affected by AML-M6.

In veterinary medicine the diagnosis of acute leukaemia is usually made following the finding of immature cells in the peripheral blood or from cytological examination of the BM. However, morphology alone is no longer considered sufficient for identifying the lineage of origin of the neoplastic cells: FC is the investigation most commonly used for this purpose.[3,4] In the case reported here, a suspected diagnosis of AML-M6 was made following cytological examination of the BM, which was then analysed by FC. However, neither of the two different methods used yielded results compatible with the morphological appearance of the sample. On one hand, ss expected, there were no polymorphonuclear cells (mature granulocytes) in the aliquot treated with density gradient separation. On the other hand, the aliquot that was lysed contained a clearly lower percentage of erythroid cells than that expected on the basis of cytology. This phenomenon is probably due to the rupture of some of the erythroid precursors together with the mature RBC, as has also been demonstrated to occur in human medicine:[14] indeed, when the analysis was restricted to only mononuclear cells, a clear difference was found in the percentages of erythroid cells between the two aliquots. Both these methods of processing the sample were, therefore, inadequate for confirming the diagnosis of AML-M6. Thus, it was necessary to resort to electron microscopy for the definitive determination of the erythroid origin of the cell population under investigation. This technique, of still limited availability in veterinary medicine, mainly because of its high costs and need for skilled staff, may be fundamental to obtain the definitive diagnosis in selected cases, enabling ultrastructural analysis of the sample of interest. Careful consideration of the clinical case, the differential diagnoses and the strengths and limitations of the individual techniques are, therefore, essential in order to select the most useful diagnostic test in each case.

The prognosis of human patients with AML-M6 is still unsatisfactory, and there are no specific treatments for this subtype of acute leukaemia.[15] In veterinary medicine, the prognosis of animals with acute leukaemia is poor, independently of the phenotype. In particular, all the dogs with AML-M6 reported in the literature died within a few days, but none had received treatment.[5,6] The administration of corticosteroids in the case described here did not improve the patient's outcome. The most appropriate therapeutic protocol for the treatment of dogs with acute leukaemia has yet to be identified.

KEY POINTS

- It would be opportune to evaluate the bone marrow of all dogs with persistent cytopenias of unknown origin.
- Flow cytometry is not always sufficient for determining the cell lineage of origin of acute leukaemias in the dog.
- Effective therapeutic protocols for acute leukaemias in the dog have not yet been identified and the prognosis of affected animals remains poor.

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