Marked thrombocytosis in a dog with digestive signs: High suspicion of AML-M7


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See File 1 (without diagnosis) for initial information in the presentation.

1. Cytological description and differential morphologic diagnoses of atypical cells in blood

Several types of atypical cells were observed in the blood (Figure 2, A/B/C):

- **Figure 2A:** Large round blastic cells with a high nucleocytoplasmic ratio, an ovoid to round nucleus with dense and regular but finely stippled chromatin pattern with inconsistent nucleolus. Their cytoplasm was slightly to moderately basophilic, with borders characterized by villous projections and frequent blebs. Some of these cells showed cytoplasmic extensions, as if the cells produced platelets. The differential diagnosis for these cells includes megakaryocytic/blastic lineage cells, histiocytic/dendritic cells or non-hematopoietic cells.

- **Figure 2B:** Small round cells with a very high nucleocytoplasmic ratio, dense and irregular chromatin, no visible nucleolus, and a very thin basophilic cytoplasmic crown sometimes characterized by small villous projections. These cells could have been atypical small lymphocytes.

- **Figure 2C and 3:** Rare large round cells with a mild to moderate nucleocytoplasmic ratio, ovoid nucleus with irregular chromatin pattern. Their cytoplasm was slightly basophilic with a granular appearance, mimicking granular appearance of platelets. The most probable diagnosis for these cells was micromegacaryocyte.

Thrombocytosis secondary to chronic bleeding (associated with chronic inflammation) cannot be excluded because of the young age of the dog, the digestive signs and the marked anemia. However, due to the very marked morphologic abnormalities of the platelets, associated with the presence of circulating atypical cells compatible with megakaryoblasts and micromegakaryocytes, an acute myeloid leukemia i.e. megakaryoblastic leukemia (AML-M7) or a myelodysplastic syndrome was highly suspected and had to be explored by a bone marrow evaluation.

2. What complementary tests could be interesting to go further in the diagnosis?

A bone marrow aspiration was done. Bone marrow was hypocellular. No erythroid cell was observed. The myeloid lineage showed signs of dysgranulopoiesis (nucleocytoplasmic asynchronism, giant cells). All the platelet precursors were abnormal with signs of dysmegagaryoplasty (dwarf megakaryocytes, nucleocytoplasmic asynchronism). 35% of the nucleated cells were cytomorphologically similar to those
found in the blood (large round blastic cells and small to medium sized blasts with a very high nucleocytoplasmic ratio, a round nucleus with a dense chromatin, and a narrow crown of hyperbasophilic cytoplasm) (Figure 4). The 5 major features that defined AML-M7 cells according to Comazzi et al., were observed in the blood and the bone marrow:

1) Central round nucleus
2) Clear cytoplasmic vacuoles,
3) Cytoplasmic blebs,
4) Bi- or multinucleated cells,
5) Large cytoplasmic fragments/macroplatelets.

Figure 4: Blastic cell showing a finely stippled and highly nucleolated chromatin pattern, a moderately basophilic cytoplasm with characteristic blebbings. Bone marrow smear. May Grunwald Giemsa (x1000).

**Immunocytochemistry:** These cells were positive for **von Willebrand Factor** (vWF) antibodies in immunocytochemistry (Figure 5), confirming the megakaryocytic lineage of these cells. They were negative for myeloperoxidase (cytochemistry).

**All these findings were highly suggestive of AML-M7.**
**Outcome**

Despite the poor prognosis of the disease, the owners decided to bring the dog back home. A blood transfusion was first performed and a corticotherapy was set up (prednisolone 2 mg/kg/day). The bitch was presented for the follow up 2 weeks later: its owner described weakness despite a good appetite and decided to go on corticotherapy even if the anemia had worsened and a thrombopenia appeared. 10 days later, the clinical status deteriorated brutally and the owner elected to euthanize the dog. A necropsy and histology were performed showing multiorganic invasion by the neoplastic cells (brain, liver, spleen, lung, kidney, mediastinal lymph nodes, adrenal glands) (Figure 6, A and B).
Discussion
To date, there are less than 20 cases of canine AML-M7 described in the veterinary literature and to our knowledge, this is the first documented case with observation of cerebral metastasis. Furthermore, most of the described cases mentioned thrombopenia at the time of the first presentation \(^1,3,4,6,8,10,11,14,15\) whereas the first CBC of the present dog revealed a marked thrombocytosis (reported in only 3 cases \(^5,7,9\)). Thrombocytopenia only appeared 2 weeks later. An explanation could be that the diagnosis of the present case of AML-M7 was made early in the evolution of the disease, although thrombocytosis was not found in a reported case of experimentally radiation-induced model \(^15\).

To diagnose AML-M7, cytomorphological characteristics are not sufficient but the presence of 4 of the 5 criteria mentioned above is highly suggestive of the disease\(^3\). Immunocytochemistry and even more immunohistochemistry with platelet markers can allow to go further in the diagnostic. The principle is to highlight megakaryocyte or platelet antigens on cells that do not express lymphocyte or monocyte antigens (to rule out a lymphoid or myeloid origin and to confirm megacaryocytic origin). However, these assays are very time-consuming. Thus, flow cytometric immunophenotyping has been developed and allows to test a lot of markers in a short time period on both blood and bone marrow samples\(^1,3,4\). Since acute myeloid leukemias are rapidly progressive diseases with a poor prognosis, the diagnosis has to be as fast as possible. In this way, flow cytometry is far better than immunohisto/cytochemistry, but is less available in laboratories. Unfortunately, we did not have access to flow cytometry that could have allowed us to further characterize the blastic cells observed in the present case.

To date, the markers (and the reactive cells associated) that have been associated to AML-M7 are:

- CD9 (lymphocytes, monocytes, granulocytes, platelets)\(^1\),
- CD34 (stem cells)\(^6\),
- CD41 (platelets, megakaryocytes)\(^6\),
- CD41/61 (platelets, megakaryocytes)\(^4\),
- CD45 (panleukocytic) \(^3,4\),
- CD61 or GPIIIa (platelets, megakaryocytes, monocytes)\(^1,3,4,5,7,8,10,11,12\),
- CD62p (platelets, endothelial cells)\(^4\),
- CD79a (B lymphocytes)\(^7\),
- Factor VIII-related antigen (megakaryoblasts, platelets)\(^8,10,13\),
- Factor XIII (platelets, hepatocytes, macrophages, monocytes)\(^8\),
- von Willebrand Factor (platelets, megakaryocytes)\(^3,6,7,11\),
References


