

Clinical, histopathological and immunohistochemical findings in a case of megakaryoblastic leukemia in a dog

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Abstract. The clinical, hematological, and histopathologic features of megakaryoblastic leukemia (M7) were investigated in a 10-year-old female Shih-Tzu dog. Megakaryoblastic leukemia was diagnosed using anti-human platelet glycoprotein (GP IIIa) and anti-human von Willebrand factor (vWF) antibodies. The expression of CD antigen on megakaryoblasts was also assessed using a CD79a monoclonal antibody. Immunological markers allowed visualization of neoplastic megakaryocytes. Antibodies against platelet GP IIIa were demonstrated to be the most useful for the diagnosis of megakaryoblastic leukemia of paraffin-embedded canine tissues. Hematological and histological data coupled with immunohistochemical reactivity for platelet GP IIIa, vWF, and CD79a antigen in blast cells confirmed a diagnosis of M7 megakaryoblastic leukemia.

Key words: Anemia; dog; immunohistochemistry; megakaryoblastic leukemia; M7; pathology.

Acute megakaryoblastic leukemia is a rare form of myeloid leukemia first described in 1931.²¹ Due to insufficient specific morphologic description of the disease and lack of specific immunological reagents, it is not fully characterized in the current literature. Precise diagnostic criteria were added to the French, American, and English (FAB) classification system, which currently designates the disease as FAB megakaryoblastic leukemia.³ Later on, the human classification system was modified and a scheme derived for use in classifying the myeloproliferative disorders in dogs and cats.^{8,11,12} To distinguish the various subtypes of acute myeloid leukemia (AML), the Animal Leukemia Study Group proposed a different set of criteria for classification of AML in dogs and cats.⁹ However, the classification of acute leukemias in animals remains difficult, especially for megakaryoblastic leukemia (M7) and other related myeloid leukemias (M0, M1, and M5).

Megakaryoblastic leukemia is generally accompanied by rapidly progressive myelofibrosis and bone marrow failure. By 1996, only 16 cases of naturally occurring megakaryoblastic leukemia had been reported in dogs.^{4,5,6,9,10,12,15,16,17,19} Affected animals exhibit anorexia, progressive weight loss, and occasionally spontaneous epistaxis. Thrombocytopenia and anemia are frequently observed.^{4,5,10,17}

For diagnosis of megakaryoblastic leukemia in bone marrow, 50% or more of the cells should be of megakaryocytic lineages as demonstrated by ultrastructural studies or immunologic markers.^{6,7,10,13,18} Monoclonal antibodies to platelet glycoproteins Ib, IIb/IIIa, and IIIa (anti-human CD61, clone Y2/51) are the most widely used antibodies to detect cell surface antigens and classify myelogenous leukemias in humans and animals.^{5,6,10,13,14,20} It has been reported that megakaryoblasts may also react with CD79a antibody.¹⁵ However, these findings have not yet been documented in the diagnosis of M7.

In this study, GP IIIa and vWF, and anti-human CD79a antibodies were used to identify neoplastic immunoreactive antigens of megakaryocytic cells in the bone marrow, spleen, lymph node, and liver. The use of immunological markers to membrane surface antigens and cytoplasmic components was essential for the detection of megakaryoblast leukemia cell lineages. These findings will contribute to the understanding of myeloid leukemias and their pathogenesis and diagnosis in dogs.

A 10-year-old female Shih-Tzu dog was presented to the Veterinary Teaching Hospital, Konkuk University, Seoul, South Korea with a history of anorexia, depression, and generalized weakness of 3 weeks duration. Clinical examination revealed emaciation, pale mucous membranes, abdominal distension, and presence of dental tartar. On auscultation, tachycardia and systolic murmurs were detected. Abnormal laboratory findings included neutrophilic leukocytosis (WBC 45,400/ μ l; reference range, 6,000 to 17,000/ μ l), severe anemia (PCV 9.6%; reference range, 37% to 55%) and thrombocytosis (612,000/ μ l; reference range, 200,000 to 500,000/ μ l) (Table 1). The blast cells resembling megakaryoblasts were also observed on peripheral blood smears (data not shown). The animal died despite supportive therapy. A necropsy was performed. Marked hepatomegaly was observed; the spleen also was

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Table 1. Profiles of complete blood cell counts in megakaryoblastic leukemia.

	Day 0 (9/13)	Day 2 (9/14)	Reference range
WBC (μl)	45,400	70,140	6,000~17,000
Monocytes	4,540	3,507	150~1,350
Lymphocytes	6,356	4,909	1,000~4,800
Neutrophils	32,234	55,410	3,000~11,500
Band neutrophils	2,270	6,312	0~300
Eosinophils	0	0	100~1,250
Basophils	0	0	rare
RBC (μl)	1,710,000	3,680,000	5,500,000~8,500,000
Hb (dm/dl)	2.7	7.0	12~18
PLT (μl)	612	820	200~700
MCV (fl)	55.9	58.5	60~77
MCH (pg)	15.8	19.0	19.5~24.5
MCHC (%)	28.1	32.6	32~36
PCV (%)	9.6	21.5	37~55

greatly enlarged, friable, and dark red. Visceral lymph nodes were enlarged. The bone marrow of the femur had a brownish-red appearance.

Samples of bone marrow, spleen, lymph node, liver, kidney, lung, stomach, and heart were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 μm , and stained with hematoxylin-eosin (HE) for evaluation by light microscopy. For immunohistochemical characterization of leukemic cells, a set of monoclonal and

polyclonal antibodies against anti-human platelet glycoprotein IIIa (GP IIIa), rabbit anti-human von Willebrand factor (vWF, Factor VIII-related antigen), and monoclonal mouse anti-human CD79a (precursor B-cell type lineage) were applied to tissue sections using a 2-step Envision system-AP (alkaline phosphatase)^a and Envision system-HRP (peroxidase)^a technique. Antibodies were used at a dilution of 1 : 50 (GP IIIa, CD61, clone Y2/51)^a or 1 : 100 (vWF and CD79a)^a. Tissue sections were first

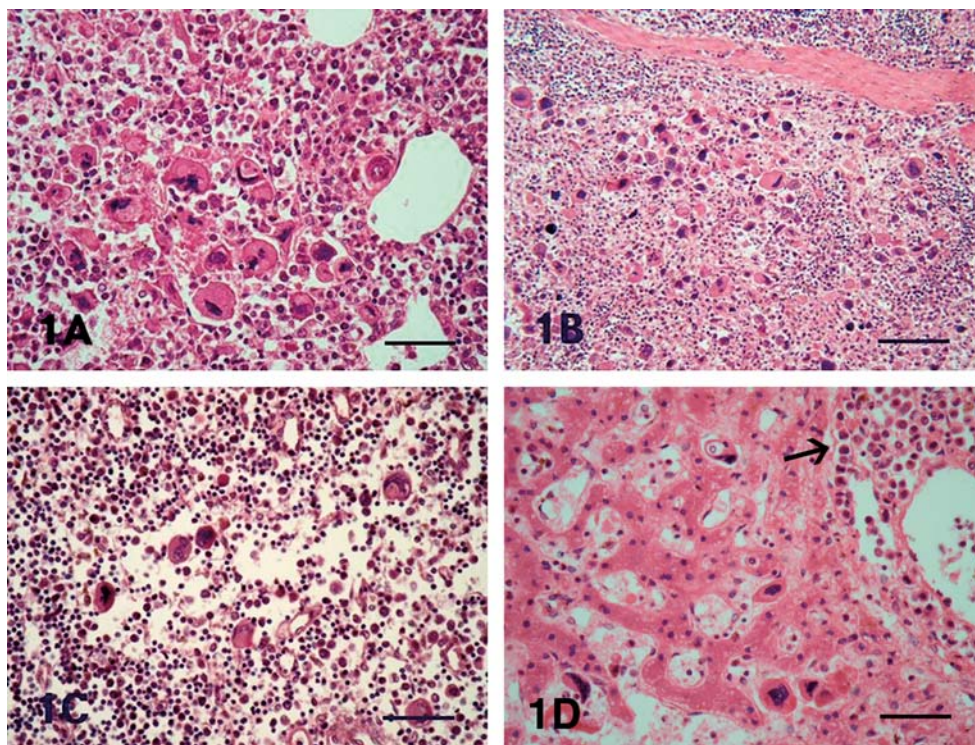


Figure 1. Acute megakaryoblastic leukemia in bone marrow, spleen, lymph node, and liver from a dog with anemia. **A**, Bone marrow: the marrow is markedly hypercellular, due principally to an increased in neoplastic megakaryocytes and megakaryocytic blast cells. HE stain. Bar = 30 μm . **B**, Spleen: predominant population of blasts with numerous scattered neoplastic megakaryocytes. HE stain. Bar = 55 μm . **C**, Lymph node: predominant population of poorly differentiated blast cells with mature megakaryocytes. HE stain. Bar = 30 μm . **D**, Liver: Neoplastic megakaryocytes noted in sinusoidal spaces and many blast cells evident in periportal area (arrow). HE stain. Bar = 30 μm .

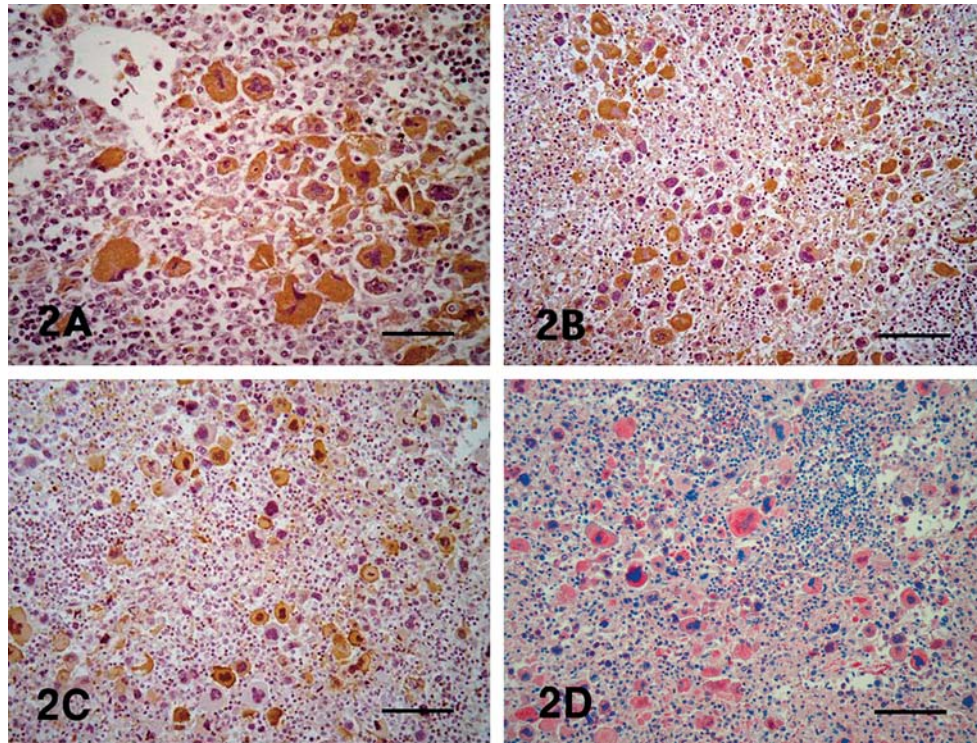


Figure 2. Immunohistochemical detection of platelet glycoprotein IIIa (GP IIIa), CD79a, and von Willebrand factor (vWF). Envision System-HRP (GP IIIa, CD79a), DAB substrate, Envision System-AP (vWF), Fast red substrate-chromogen, Harrison hematoxylin counterstained. **A**, Bone marrow: Stained for GP IIIa. A high percentage of the neoplastic megakaryocytes are positive. Bar = 30 μ m. **B**, Spleen: Stained for GP IIIa. A high number of neoplastic megakaryocytes and megakaryocytic blast cells exhibit positive signal. Bar = 55 μ m. **C**, Spleen: Stained for CD79a. A majority of neoplastic megakaryocytes are strong positive. Bar = 55 μ m. **D**, Bone marrow: Stained for vWF. A high percentage of the neoplastic megakaryocytes are moderately to strongly positive. Bar = 55 μ m.

treated with 5% H_2O_2 in PBS for 20 minutes at room temperature (RT), followed by 3 PBS washes and heat-induced epitope retrieval in a Coplin jar containing 10 mmol/L citric acid monohydrate buffer (pH 6.0) in a microwave oven^b (high power) for 10 minutes, and cooled to RT before being washed 3 times in PBS. Sections were subsequently incubated with primary antibodies in Phosphate Buffered Saline (PBS) for 2 hours at RT. After 3 5-minute washes with PBS, slides were incubated with Envision alkaline phosphatase-conjugated polymer (for vWF) or Envision peroxidase-conjugated polymer (for GP IIIa and CD79a) for 30 minutes. After 3 5-minute washes with PBS, slides were incubated with substrates for each system, and the color reaction was stopped by washing slides in deionized water. All slides were counterstained with Harrison hematoxylin.

Microscopic examination of bone marrow, spleen, lymph node, liver, and kidney revealed the presence of broad sheets of large blast cells which obliterated normal tissue architecture. Many of the blast cells resembled megakaryocytes and megakaryocytic precursors. A consistent feature was presence of immature erythropoietic cells intermingled with neoplastic megakaryocytes. Nuclear and cytoplasmic morphology of blast cells varied considerably. Round to oval nuclei were often centrally located, and the cells were frequently multinucleated. Many cells

had a lymphoid appearance, with sparse cytoplasm and dense nuclear chromatin patterns. As reported for other forms of megakaryoblastic leukemia, the number of blast cells in the bone marrow and spleen were 30% or greater of the population. The most outstanding lesion observed in the bone marrow and spleen was the presence of numerous neoplastic megakaryocytes, all of which had highly pleomorphic nuclei. Sternal and femoral bone marrow aspirates were highly cellular and devoid of fat, and had elevated numbers of neoplastic megakaryoblasts and megakaryocytes (Fig. 1A). The spleen contained numerous poorly differentiated blasts and megakaryocytic cells. Few recognizable lymphoid follicles remained (Fig. 1B). Lymph nodes contained predominantly blast cells, which effaced normal nodal architecture. Scattered megakaryocytes were present (Fig. 1C). Hepatic sinusoids and portal areas contained numerous blast cells (Fig. 1D). The numbers of megakaryoblasts in both lymph node and liver were much less than observed in bone marrow and spleen.

In an attempt to confirm the identities of blast cells, IHC was performed on sections of tissues using monoclonal and polyclonal antibodies specific for detection of neoplastic megakaryocytes or their precursors. The most intense and consistent IHC positive signal was observed in bone marrow and spleen. Positive cells typically displayed dark brown-stained (GP IIIa, CD79a) and red-stained (vWF)

deposits in the cytoplasm. The location and morphology of the positive cells in tissues indicated that they were neoplastic megakaryocytes and megakaryocytic blast cells. Intense and specific GPIIIa-positive staining was seen in cytoplasm of various stages of neoplastic megakaryocytes and megakaryocytic blast cells in bone marrow and spleen (Fig. 2A, 2B). Positive signal was detected in lymph node and liver by use of GP IIIa antibody. Intense staining to CD79a and vWF was detected in neoplastic megakaryocytes and megakaryocytic blast cells in spleen (Fig. 2C) and bone marrow (Fig. 2D). In contrast, no staining was observed in the absence of primary antibody using the same paraffin sections (data not shown). For positive controls, paraffin sections of normal archival tissues of bone marrow were used (data not shown).

This study provides evidence that megakaryoblastic leukemia is associated with severe anemia in dog. Megakaryoblastic leukemia is an uncommon disease in humans, composing only 0.5%–1.2% of adult AML.² Clinical studies have shown that megakaryoblastic leukemia is also uncommon and rarer in dogs.^{10,22} It occurs with less frequency than other types of myeloproliferative disorders.²² The etiology of canine megakaryoblastic leukemia is obscure. It is known that genetic and environmental factors, including exposure to radiation, certain drugs, and toxic chemicals, may play a role in developing megakaryoblastic leukemia in dogs.⁴ No causative viral agent has been identified in humans or dogs, in contrast to cats, where feline leukemia virus has been associated with various myeloproliferative disorders.^{10,22}

The diagnosis of megakaryoblastic leukemia was established on the basis of hematological, morphological, and immunohistochemical data. Clinical signs were nonspecific, which is consistent with other reports in dogs.^{5,14,15,16,17} Anemia, neutropenia, and thrombocytopenia are common findings with megakaryoblastic leukemia.^{5,22} However, in this case only anemia was observed. In contrast to most published reports,^{5,22} the patient exhibited neutrophilia and thrombocytosis. This may be due to blast cells infiltrating bone marrow, as has been previously reported.¹⁶ The diagnosis of megakaryoblastic leukemia was confirmed by evidence of neoplastic megakaryocytes and megakaryoblasts in bone marrow and other organs and detection of strong specific immunohistochemical staining for platelet GP IIIa, vWF, and CD79a in neoplastic megakaryocytes. Previous reports indicate that platelet GP IIIa can be recognized in both mature megakaryoblastic and immature myeloid lineages.^{5,6}

In this study, the pattern of the positive signal for platelet GPIIIa and anti-human CD79a were similar and were recognized consistently in neoplastic megakaryocytes located in bone marrow, spleen, lymph node, and liver. Platelet GPIIIa antigen was rarely detectable in immature myeloid cells. Comparative analysis of the platelet markers used in this study demonstrate that antibodies to CD79a have a high degree of specific intense staining for neoplastic megakaryocytes. It has been reported that CD79a is a marker for the majority of acute leukemias of precursor B cell types and in some acute myeloid leukemias.^{1,10} Because of a high degree of conservation in the intracytoplasmic sequence of CD 79a, anti-human CD79a antibodies have been shown to cross-

react with other species, including cats, cattle, chickens, dogs, horses, and sheep.¹⁰ A comparative analysis of the megakaryocytic marker used in this study demonstrated that CD79a proved to be as effective as GP IIIa in detecting neoplastic megakaryocytes.

The vWF antigen was detected in a variety of myeloid cells, including megakaryoblasts and neoplastic megakaryocytes. This suggests that the vWF may be a unique antigen of myeloid lineage cells during blast cell maturation. Antibodies to vWF (factor VIII-related antigen) are less sensitive in the identification of neoplastic megakaryocytes. However, our present results have indicated that compared with GP IIIa and CD79a antibodies, which mainly recognize neoplastic megakaryocytes, vWF demonstrated that both neoplastic megakaryocytes and megakaryoblasts could be detected.

This article describes megakaryoblasts leukemia in a dog. Several immunologic markers to platelet glycoproteins were used to identify neoplastic megakaryocytes.^{6,7,14,15,16,17,18} Immunohistochemical identification of megakaryoblastic cells and their lineage cells in paraffin-embedded, routinely processed hematopoietic tissues including bone marrow was demonstrated by the use of antibodies to GPIIIa, vWF, and CD79a. Utilization of immunohistochemistry allowed unequivocal identification of megakaryoblastic leukemia.

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Sources and manufacturers

- a DakoCytomation, Inc, Glostrup, Denmark.
- b Samsung, Inc, Seoul, Korea.

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