A Dog with Acute Myelomonocytic Leukemia

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ABSTRACT. A three-year-old dog with marked leukocytosis, lymphadenopathy, and diarrhea showed an increase in unidentified blasts in the peripheral blood, and they were proliferated in the bone marrow. The dog was diagnosed with myelomonocytic leukemia (M4) because the blast cells were demonstrated by cytochemical staining to be both myeloid and monocytic cells. Although the dog was treated with a multi-combination chemotherapy and induction therapy using vitamin K₂, it died on day 47 after the first admission. This case is the first report of M4 in Japan.

KEY WORDS: canine, cytochemical staining, leukemia.

Acute myeloid leukemia is defined as a hematological malignancy derived from bone marrow hematopoietic cells; the prognosis is poor [3–5, 8]. Jain et al. classified the malignant proliferation of myeloid and monocytic cells in dogs and cats as acute myelomonocytic leukemia (M4) [3]. The definitive diagnosis of M4 is made when myeloblasts and monoblasts together constitute more than 30% of all nucleated cells in the bone marrow, and differentiated granulocytes and monocyes each comprise more than 20% of all nucleated cells. Cases of this type of leukemia have been reported, but the hematological findings, blast cell characteristics, and clinical course of the disease have been unclear [1, 2, 11]. This report describes a dog diagnosed with M4 on the basis of hematological and cytochemical findings, and the clinical course during treatment with several chemotherapeutic agents and induction therapy using vitamin K₂.

The dog was a three-year-old male Welsh Corgi Pembroke with lymphadenopathy and diarrhea. The hematological findings indicated marked leukocytosis (WBC 65,100/µl) and severe thrombocytopenia (PLT 35,600/µl). In peripheral blood smears stained with Wright-Giemsa, numbers of large mature neutrophils, monocyte-like cells, and immature precursors of both cell types were seen (Fig. 1). The immature cells showed variable morphological features, including round, lobed, and horse-shoe nuclei and dark blue cytoplasm. The white blood cell differential count revealed that 37.5% of peripheral blood cells were blast cells. In addition, band and segmented neutrophils, monocytes, and promonocytes were increased (Table 1). Serum biochemical analysis indicated that the liver enzymes were increased slightly (ALT 219 U/l and ALP 698 U/l). Because the case was suspected to be an acute leukemia, bone marrow aspiration was performed immediately. The bone marrow smears were stained with Wright-Giemsa, and more than 500 bone marrow cells were counted. The marrow was hyperplastic, and erythroid lineage cells were almost never found (Table 2). The proportion of large and mononuclear blasts was high (31.1%), and the number of megakaryocytes was decreased. In the Wright-Giemsa stained smears, large, round cells with oval to irregularly round nuclei and blue cytoplasm with azurophilic granules were predominant; the nuclear chromatin was finely stippled, and the nucleus to cytoplasm ratio was high (Fig. 2). These findings suggested that these cells were myeloid or monocytic blast cells. To clarify the character of these cells, peroxidase (New PO-K staining kit; Muto Pure Chemicals), alpha-naphthol butyrate esterase (Esterase staining kit; Muto Pure Chemicals), and alpha-naphthol AS-D chloroacetate esterase (Esterase AS-D staining kit; Muto Pure Chemicals) were used. Most (88%) bone marrow cells were positive for myeloperoxidase staining, and 23% of cells showed a positive reaction to alpha-naphthol butyrate esterase (Fig. 2). A small number (6.5%) of cells, including large blast-like cells, were positive to both naphthol-AS-D chloroacetate and alpha-naphthol butyrate esterase staining (Fig. 2). All alpha-naphthol butyrate esterase staining of cell granules was inhibited by sodium fluoride (NaF), and most of these cells were determined to be monocytic cells. These results indicate that a large number of bone marrow cells were myeloid and monocytic cells and abnormal blast cells that had both myeloid and monocytic characteristics were present. According to these findings, the dog was diagnosed with acute myelomonocytic leukemia (M4).

The dog was treated by multi-drug chemotherapy including cytarabine, doxorubicin, vincristine, and prednisolone based on modified protocol for AML in the initial stage [9]. Vitamin K₂ (Glakay, Eisai) was added as induction therapy, and antibiotics were administered as supportive therapy. Cytarabine (100 mg/m², IV drip; Cylocide, Nippon Shinyo-
aku) was administered from days 2 to 4, and vincristine (0.7 mg/m², IV, Oncovin; Eli Lilly) was given on day 2. However, on day 7, the leukocyte count and blast cell count in the peripheral blood had increased markedly to 95,000/µl and 48,450/µl (51%) respectively. Doxorubicin (20 mg/m², IV, Adriacin; Kyowa) and prednisolone (1 mg/kg, PO, daily, Prednisolone; Sanwa Kagaku Kenkusho) were then administered. On day 14, although the leukocytosis was ameliorated (WBC 24,500/µl), the dog had fever and anorexia. Chemotherapy was discontinued, and the dog was treated with antibiotics and prednisolone. When the general condition had improved on day 22, marked leukocytosis (WBC 132,000/µl) with blast cells was seen, and mitoxantron (5 mg/m², IV, Novantron; Wyeth) was administered intravenously. The therapeutic regime was changed to mild chemotherapy combined with hydroxyurea (50 mg/kg, every other day, Bristol-Myers) and prednisolone, because anorexia and diarrhea were seen after mitoxantron administration. Administration of hydroxyurea was discontinued on day 45 because severe cytopenia were seen, and the dog died on day 47.
Acute myelomonocytic leukemia is rare in dogs, and only a few cases of M4 have been reported [1–3]. Because it is hard to clarify the character of blasts of this type on the basis of morphological features, a specific examination by cytochemical staining and analysis of the immunophenotype using flow cytometry is performed. In fact, Graves et al. described a case with M4 that was misdiagnosed as acute lymphoblastic leukemia, and reported that cytochemical staining was useful to make a definitive diagnosis of M4 [2].

In human medicine, naphthol-AS-D chloroacetate and alpha-naphthol butyrate esterase staining were successful in detecting blasts that had the myeloid and monocytic phenotype [10]. This cytochemical staining is able to demonstrate this characteristic property of blasts in canine M4 because the pathogenesis of M4 is the differentiation of blasts to myeloid and monocytic cells. Furthermore, Villiers et al. reported that when the immunophenotype of a case with M4 was analyzed by using flow cytometry, staining with several antibodies including MPO, MAC387, CD11a, CD11b, and CD11c was positive [11]. Probably, analysis of the immunophenotype using these antibodies is helpful to establish a definitive diagnosis of canine M4 more certainly.

Although the morphological changes in the peripheral blood were quite similar to those of previous reports [3, 11], the bone marrow findings were not characteristic of M4. Most of the blast cells had a small number of distinct magenta (azurophilic) granules in the cytoplasm, and monoblast-like cells comprised only 2.2% in the bone marrow. Although, the percentage of total monocytic cells was 12.4%, indicating that monocytic cells were increased in the bone marrow, the percentage of monocytic cells in the bone marrow was less than 20%. This finding was not consistent with the criteria of M4. However, it was difficult to distinguish myeloid and monocytic cells by cytological evaluation using Wright-Giemsa staining [2]. In fact, a large number of cells were positive to the myeloperoxidase and alpha-naphthyl butyrate esterase stains, indicating that these cells had characteristics of both myeloid and monocytic cells. Therefore, these findings indicated that the case should be diagnosed as M4, not myeloid leukemia (M2) or monocytic leukemia (M5), because a bi-lineal leukemic proliferation including myeloid and monocytic cells was confirmed.

In general, the treatment of canine acute myelomonocytic leukemia by chemotherapy has not been successful; the prognosis is poor and all reported cases have died within a few months [1, 2, 5]. Therefore, the dog was treated with multi-drug chemotherapy plus differentiation-inducing therapy by vitamin K2. Vitamin K2 has been shown to induce differentiation of human myeloid leukemia cell lines and to promote apoptosis of leukemia cells, but it was not effective in this case [6, 7]. Further studies are required to develop an effective therapeutic regimen for acute myelomonocytic leukemia in dogs.

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