Clinical Pathology

Note

Monoblastic leukemia (M5a) with chronic basophilic leukemia in a cat

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RUNNING HEAD

AML WITH BASOPHILIC LEUKEMIA IN A CAT
ABSTRACT

A cat was presented with depression and anorexia. The complete blood cell count (CBC) revealed non-regenerative anemia (PCV, 8.5%), marked thrombocytopenia (2,400 /µl), and leukocytosis (32,090 /µl). In the peripheral blood, proliferation of blast cells (85%; 27,276 /µl) and basophils (7.7%; 2,460 /µl) was observed. Bone marrow aspirate showed hyperplasia with 8.8% blasts and 90.2% basophils of all nucleated cells. The blast cells were negative for myeloperoxidase staining and positive for alpha-naphthol butyrate esterase staining, indicating the agranular blasts are monoblasts. Thus, acute monoblastic leukemia (M5a) with chronic basophilic leukemia was diagnosed. Basophils accounted for more than 40% of the bone marrow, and we diagnosed secondary basophilic leukemia. Secondary basophilic leukemia should be included in the differential list when abnormal basophil increases are observed in feline bone marrow.

KEY WORD: acute monoblastic leukemia (M5), basophilic leukemia, cat, feline leukemia virus (FeLV)
Basophilic leukemia (BL) can be classified as acute or chronic, and further classified as primary or secondary [20]. Acute basophilic leukemia (ABL) is a relatively rare form of acute leukemia that accounts for 4–5% of all cases of acute non-lymphocytic leukemia in humans [14, 15, 18]. Conversely, primary and secondary cases of chronic basophilic leukemia (CBL) have been reported in approximately equal numbers [4]. In humans, secondary (acute/chronic) basophilic leukemia is often associated with chronic myelogenous leukemia (CML), especially during acute transformation or with myelodysplastic syndromes [7, 18]. It is rarely secondary to acute myelogenous leukemia, but has been reported to occur secondary to M2 or M4 of acute myeloid leukemia (AML) in the French-American-British (FAB) classification in association with genetic abnormalities such as t(6;9) (p23;q34). No cases of M5 have been reported [8, 9, 17].

In the veterinary field, there have been eight reports of basophilic leukemia in dogs, all of which were of the chronic type [1, 12]. In addition, there has been one case each of basophilic leukemia in cats and horses, all of which were acute forms [3, 6]. All the reports in animals were primary, and no cases of secondary disease have been reported.

Here, we report an extremely rare case of acute monoblastic leukemia (AML-M5a) in a cat that was thought to be secondary to chronic basophilic leukemia.

A nine-year-old, intact female mixed-breed cat was presented to the Sanyo Animal Medical Center with five days of depression and anorexia. The cat was not vaccinated. The patient's history included jaundice and fever approximately one year ago, and blood tests showed severe neutropenia (110/μl) and mild thrombocytopenia (12,200/μl). The patient was treated with antibiotics and recovered. After an ELISA (IDEXX Laboratories, Westbrook, ME, United States) test for feline leukemia virus (FeLV) antigen was positive, and the test for feline immunodeficiency virus (FIV) antibody was found to be negative. The cat had a pale mucous membrane. Fecal examination showed no evidence
of parasitic infection. No enlargement of the body surface lymph nodes was observed. Ultrasonography did not show any enlargement of the liver or spleen. The complete blood count (CBC) revealed non-regenerative anemia (PCV 8.5%; reticulocyte count 2,300 /µl), marked thrombocytopenia (2,400 /µl), and leukocytosis (32,090 /µl) (Table 1). In peripheral blood smears, medium to large round cells accounted for 85% (27,276 /µl) of the cells, which had round nuclei and small amounts of relatively bright blue cytoplasm, sometimes with a few small, well-defined vacuoles. The nuclei were generally round, but sometimes slightly concave. The chromatin was fine and the nucleoli was indistinct, however, some cells had 1–3 small nucleoli. Seventeen percent of the round cells (15% of all nuclear cells: ANC) had distinct nucleoli. There was also an increase in basophils (2,460 /µl) and a decrease in neutrophils (1,177 /µl) (Fig. 1). Very few platelets were detected. A bone marrow examination from the femur was performed using an 18G pediatric Jamshidi biopsy needle (Fuji Systems Co., Ltd, Tokyo, Japan). Ketamine hydrochloride (8.0mg/kg, Ketalar for intramuscular injection, 500 mg, Daiichi Sankyo Pharmaceutical Co., Ltd, Tokyo, Japan) as an analgesic and sedative treatment for the bone marrow examination. The bone marrow was hyperplastic. The percentage of nucleated cells in the bone marrow was 90.2% for cells with purple granules, 8.8% for round cells without granules, 0.4% for neutrophils, and 0.5% for lymphocytes. Few erythroblasts or megakaryocytic cells were observed. Cells with granules were found in various stages of differentiation, ranging from those with analogous round nuclei and nucleoli, to those with concave or bifurcated nuclei. The cytoplasmic granules were relatively large and distinct, and cells with ring-shaped nuclei were scattered in the more differentiated cells. There were many relatively undifferentiated cells, of which 6.1% had distinct nucleoli. Cells without granules had round nuclei and small to moderate amounts of pale blue cytoplasm, with fine chromatin and one or two distinct nucleoli. Occasional depressions and slits were observed. A small number of vacuoles and duplex structures were also observed in the cytoplasm. A few fine azur granules were also observed. The thin
rim of cytoplasm in the cells resembled that of round cells found in peripheral blood. There were no dysplastic findings or hemophagocytic images. A core biopsy could not be performed.

Cytochemical staining, including myeloperoxidase stain (MPO; NewPO-K Staining Kit, Mutoh Chemical KK, Tokyo, Japan), naphthol AS-D chloroacetate esterase stain (CAE; Fukuyama Clinical Research Center, Hiroshima, Japan), alpha-naphthol butyrate esterase stain (NBE; Fukuyama Clinical Research Center), and periodic acid-Schiff stain (PAS; Fukuyama Clinical Research Center) were performed to identify cells in the bone marrow. Cells with round nuclei without granules were negative for MPO and PAS, and positive for NBE (Fig. 2A, B, D), and the NBE activities were inhibited by the addition of NaF (Fig. 2C). These findings suggest that these agranular cells were monoblasts or promonocytes. Cells with granules were MPO- and NBE-negative and CAE- and PAS-positive, which is consistent with the cytochemical findings of cat basophils [5] (Table 2). Basophils included basophilic-myeloblasts (6.1%), basophilic-promyelocytes (16.2%), basophilic-myelocytes (34.1%), basophilic-metamyelocytes (23.1%), band-basophils (6.2%), and segmented-basophils (4.5%). Erythroid and megakaryocytic lineage cells were almost never found (Fig. 3A, B) (Table 3). A polymerase chain reaction for antigen receptor rearrangement (PARR) analysis (canine-lab, Tokyo, Japan) of the peripheral blood and bone marrow samples was performed to detect the clonal expansion of lymphocytes. Clonality was not detected in either sample. The primer used was based on the following literature, but the details were a trade secret [13, 16]. Based on these findings, a diagnosis of acute monoblastic leukemia (M5a) with chronic basophilic leukemia was made.

Symptomatic treatment was started with a whole blood transfusion. A total of four whole blood transfusions of 30 ml each were performed on days 1, 4, 8, and 11. The cat was scheduled with sequential chemotherapy including methotrexate (0.5 mg/kg, IV, Pfizer, Tokyo, Japan), doxorubicin (1 mg/kg, IV, Aspen Japan, Tokyo, Japan), cytosine arabinoside (100 mg/m², SC divided twice daily
for two days, Nihonshinyaku, Tokyo, Japan), vincristine (0.03 mg/kg, IV, Nihonkayaku, Tokyo, Japan), and prednisolone (2 mg/kg, IV daily, Pfizer). The treatment processes are presented in Table 4.

On day 22, there were no blasts or basophils in the peripheral blood and a regenerative response to anemia and thrombocytopenia was observed. However, on day 50, there was an increase in the number of blast cells and progression of anemia and thrombocytopenia. Blast cells in the peripheral blood were medium sized and had round or indented nuclei with dispersed chromatin. The nucleolus was unremarkable. Compared to the initial examination, the blasts showed a tendency to be lobulated and curved in the nucleus, and the cytoplasm was slightly broader, with some having fine azur granules (Fig. 4A). These cells were positive for alpha-naphthylbutyrate esterase (α-NBE) staining (Fig. 4B), and these reactions were inhibited by NaF (Fig. 4C) and negative for CAE and MPO staining. These cells were morphologically and cytochemically considered to be monocytic cells.

On day 90, an enlargement of the left popliteal lymph node was observed. A cytological analysis of the lymph node, which was performed by fine-needle aspiration, revealed the presence of blast cells and occasional basophils (Fig. 5A). The increased number of blasts in the lymph nodes had morphological characteristics of more monocytic cells because they had broad cytoplasm, fine azur granules, and the nucleus tended to be curved or lobulated. The bone marrow consisted of blasts (63%) and basophils (33%) (Fig. 5B). The morphology of the blasts ranged from slightly broader cytoplasm, similar to that of the increasing number of blasts in the peripheral blood, to the same morphology as the thin-rim cytoplasm of the blasts seen in the peripheral blood at the time of the initial diagnosis. Chemotherapy was discontinued and the cat died on day 98. A necropsy was not performed.

In this report, a case with marked basophilia and blast increase was observed, suggesting that the basophilia was caused by acute leukemia. A basophilia is recognized by an increased absolute
basophil count (>200 basophils/μL), and generally occurs with concomitant eosinophilia. Some causes of basophilia in cats include allergic respiratory conditions, heartworm disease, eosinophilic granuloma complex, basophilic leukemia, myeloid leukemia, mast cell neoplasia, and polycythemia vera [21]. In addition, the proposed classification and diagnostic criteria for basophilic leukemia in humans, published in 2017, proposes to refer to hyperbasophilia (HB) when basophils in the peripheral blood are seen continuously above 1000 /μl. Persistent HB is highly suggestive of the presence of neoplastic disease (usually myeloid tumors) when other blood cell count abnormalities are also present, and in most cases, the underlying myeloid tumor is detected or already known before HB develops [20]. The cells with large basophilic granules, in this case, were MPO- and NBE-negative and CAE- and PAS-positive, which is consistent with the cytochemical findings of basophils in cats [5]. In humans, metachromasia with toluidine blue is important in differentiating basophils, but cat basophils stain strongly positive for CAE, a feature not seen in humans or dogs, which facilitates basophil differentiation in cats. This case was also considered to be HB, and because many abnormal cells without the characteristics of acute leukemia were observed in the peripheral blood. In addition, basophils, including immature basophils, accounted for more than 80% of the bone marrow cells. Therefore, these features in this case were attributed to neoplastic growth due to the lack of an underlying condition causing this reactive increase in basophils. Basophilic granules are usually secondary granules and are found in cells at the myelocyte level, however, in this case, based on the degree of aggregation of nuclear chromatin and the presence of nucleoli, basophilic granules were also found in cells considered to be promyelocytes or myeloblasts. Granules have also been observed in human ABL blasts [2, 9, 19], suggesting that basophilic blasts and promyelocytes are most likely the result of neoplastic proliferation rather than normal differentiation into increased basophils. This suggests that the increase in basophils in this case is a neoplastic increase [3], and the patient was diagnosed with basophilic leukemia.
According to the criteria for the diagnosis of basophilic leukemia in humans, secondary basophilic leukemia is diagnosed when an underlying myeloproliferative tumor is identified, and basophils comprise more than 40% of the bone marrow cells or peripheral blood. In patients with ABL, the percentage of blast cells (myeloblasts plus metachromatic blasts) is >20%. In contrast, in patients with CBL, the percentage of blast cells are <20%. If the basophil count is less than 40%, the name of the underlying myeloproliferative tumor is appended with -Baso (e.g., M2-Baso). However, if it can be demonstrated by flow cytometry or other means that the blasts are precursors to basophils, the diagnosis is made as basophilic leukemia or myeloproliferative tumor with basophilic differentiation (e.g., M2 with basophilic differentiation). Following this criterion, the current case was diagnosed as secondary chronic basophilic leukemia because of the presence of underlying AML, a basophil count >40% in the bone marrow, and myeloblasts plus basophilic blasts <20%. In this case, the basophil count in the bone marrow was very high, at 90.5%. In humans, the number of basophils in the bone marrow was found to be clearly higher in secondary CBL than in primary CBL [4], which is consistent with the findings in this case. If the patient's basophil count is less than 40%, the diagnosis would be AML-Baso, if the blasts were basophil precursors; the diagnosis would be AML with basophilic differentiation, if the blasts plus basophils were less than 40%; and it would be basophilic leukemia if the blasts were greater than 40%. Basophilic leukemias have been reported infrequently in the veterinary literature and may occur as a myeloproliferative neoplasm or as an acute process. Both develop from the neoplastic transformation of multipotent bone marrow stem cells and are further subdivided into granulocytic and monocytic leukemias [21]. The underlying diseases in secondary basophilic leukemia reported in humans include CML, the acute transformation of CML, and myeloproliferative tumors that contain myeloblasts, such as M2, M4, and MDS, which occur because of the tendency of myeloblasts to differentiate into basophils. The mechanism by which basophils differentiate from myeloblasts has not been clarified to date, and to the best of our knowledge, no
mechanism has been proposed [18]. In this case, it is unlikely that the monoblasts differentiated
directly into basophils, but it is possible that the tumor arose from an abnormality in
granulocyte-macrophage progenitor (GMP) cells, which are progenitor cells that can differentiate into
bone marrow monocyte progenitor cells (CFU-GM) and basophil progenitor cells (CFU-Baso). It
would be very interesting to clarify if the monoblasts and basophils in this case originated from the
same clone. In humans, flow cytometric analysis has been used to identify basophils using the
CD203c and CD294 markers that are specific for basophils [18]. In addition, the origin of the cells
can be deduced by fluorescence in situ hybridization (FISH) [18]. Bounous et al. also indicated that
even though cytochemical staining suggested that the blasts were basophils, they could not prove that
they were basophilic cells, so they could only say that they were M2-Baso, although acute basophilic
leukemia was highly probable [3].

The hematological changes in basophilia and blast cells in this case were extremely atypical, then the
interpretation of the pathology of this case was complicated. In the peripheral blood at the time of the
first visit, there was an increase in round cells without obvious granules and mature basophils, and
there was no continuity between the two groups. Therefore, they were considered to be different cell
lineages. The round cells had a very high N/C ratio and were morphologically unclassifiable. Among
the round cells, 17% had a clear nucleolus, and the cells without nucleolus had the same chromatin
pattern, cell size, and N/C ratio as the cells with nucleolus, so all round cells were considered to be
blasts. Thus, the percentage of blasts in peripheral blood was 85%. The percentage of blasts in the
bone marrow, including basophil blasts and blasts without granules, was low at 14.9%, however, the
percentage of blasts in the peripheral blood was more than 20% and this led to the diagnosis of AML.
According to the classification criteria for acute leukemia in animals, AML was defined as a blast
ratio of 30% or more in the bone marrow, but the blast ratio in the peripheral blood was not clearly
described [10]. According to the WHO classification of hematological tumors in humans, MDS is
diagnosed when the blast ratio in peripheral blood and bone marrow is up to 19%, and AML is diagnosed when the ratio is higher [22]. In recent years, this classification criterion was used for the diagnosis of leukemia in animals [11]. Cytochemical staining of blasts without granules in bone marrow was negative for MPO, CAE, PAS, and NBE was inhibited by NaF. These results indicated that the blasts were monocytic cells and acute monoblastic leukemia (M5a) was suspected. In the peripheral blood and bone marrow, at the time of relapse, blasts with a thin cytoplasm as seen in the peripheral blood at the time of initial diagnosis were present, along with blasts with a broader cytoplasm and a clearly monocytic morphology, suggesting that these blasts were immature cells of the monocytic lineage with a different degrees of differentiation based on NBE staining. Therefore, the blasts found in the peripheral blood at the time of the initial diagnosis were presumed to be monocytic cells. Thus, this case was diagnosed as secondary CBL in accordance with the diagnostic classification criteria for human BL, judging that the condition was a combination of M5a and CBL.

There were some limitations in this report. First, in this case, we could not perform a core biopsy, so we could not perform immunostaining. Thus, we could not analyze the surface markers by flow cytometry, so we could not prove, other than morphologically, that the round cells in the peripheral blood at the time of initial diagnosis and the round cells without granules in the bone marrow were monocytic cells of the same origin. Second, we could not prove that the round cells in peripheral blood and the round cells without granules in bone marrow were monocytic cells of the same origin, except morphologically. Third, it could not be clarified whether AML preceded CBL or vice versa because the previous course of the disease was unknown. In addition, we diagnosed this case as secondary chronic basophilic leukemia according to the classification and diagnostic criteria for humans, but whether this diagnosis can be directly applied to cats requires further discussion.
In conclusion, this is a case report of a cat showing the presence of juvenile leukemic cells and many purple granules in the peripheral blood and bone marrow tissue. These were thought to be secondary to AML-M5 and the development of CBL. This is an extremely rare report, and it may be necessary to revise the differential diagnosis list for feline basophilia in the future. We hope that this report will contribute to the future development of veterinary hematology.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.
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FIGURE LEGENDS

Fig. 1. Peripheral blood smear findings: Wright-Giemsa stain (×400). (A) Peripheral blood smear showing five unclassified blast cells. (B) Two mature basophils with granules.

Fig. 2. A panel of cytochemical stains was performed on bone marrow. (A) Myeloperoxidase (MPO) (×400). The blasts and basophils were negative for MPO. (B) Naphthol AS-D chloroacetate esterase (CAE) and alpha-naphthyl butyrate esterase (NBE) (Double esterase stains) (×400). The blasts were positive for NBE and negative for CAE (arrow). The basophils were positive for CAE and negative for NBE. (C) The NBE activities of blasts were inhibited by the addition of sodium fluoride (NaF) (×400). (D) Periodic acid-Schiff (PAS) staining (×400). The blasts were negative and basophils were positive for PAS.

Fig. 3. Bone marrow aspirate from the initial presentation. (A) Bone marrow aspirate smear showing blasts with high nuclear-cytoplasmic ratio (arrow), open chromatin, and many immature/mature basophils (×200). (B) Basophilic myeloblast (1), basophilic promyelocyte (2), basophilic myelocyte (3), basophilic metamyelocyte (4), and blast cells (5) (×400).
Fig. 4. A–C: Peripheral blood smear findings on day 50. (A) Increase in immature monocytic cells, Wright-Giemsa stain (×400). (B) Cells positive for naphtylbutylate esterase (NBE) (×400). (C) The NBE activities of cells were inhibited by the addition of sodium fluoride (NaF) (×400).

Fig. 5. A–B: Cytology of lymph node and bone marrow on day 90. (A) Cytology of the popliteal lymph node biopsy. (B) Cytology of the popliteal bone marrow aspirate.
|                         | This case | Reference values   |
|-------------------------|-----------|--------------------|
| Erythrocytes($\times 10^6$) | 1.15      | 6.54-12.20         |
| Hemoglobin(g/dl)         | 2.4       | 9.8-16.2           |
| Packed cell volume(%)    | 8.5       | 30.3-52.3          |
| Reticulocytes(%)         | 0.2       | 3.0-50.0           |
| Platelets($\times 10^3$/μl) | 2.4   | 151-600            |
| Leukocyte(μl)            | 32,090    | 2870-17020         |
| Band Neutrophil          | 0         | 0-300              |
| Segment Neutrophil       | 1,177     | 2300-10290         |
| Lymphocyte               | 749       | 920-6880           |
| Monocyte                 | 321       | 50-670             |
| Eosinophil               | 107       | 170-1570           |
| Basophil                 | 2,460     | 10-260             |
| Blast cell               | 27,276    |                    |
| Total plasma protein(g/dl) | 6.8    | 5.7-7.8            |
| Alubumin(g/dl)           | 2.7       | 2.3-3.5            |
| Alanine aminotransferase (U/l) | 39   | 22-84              |
| Alkaline phosphatase(U/l) | 50     | -58                |
| Total cholesterol (mg/dl) | 67      | 95-259             |
| Glucose(mg/dl)           | 115       | 71-148             |
| Total bilirubin(mg/dl)   | 0.1       | -0.4               |
| Blood urea nitrogen(mg/dl) | 21   | 17.6-32.8          |
| Creatinine(mg/dl)        | 1.5       | 0.9-2.1            |
| Iron(μg/dl)              | 328       | 53-168             |
| Total iron binding capacity(μg/dl) | 337 | 211-458          |
| Transferrin saturation(%) | 97.3     | 28-44              |
| Stain                        | Blast | Basophil |
|------------------------------|-------|----------|
| Myeloperoxidase             | -     | -        |
| Chloracetate esterase       | -     | ++       |
| α-Naphthyl butyrate esterase| +     | -        |
| Periodic acid-Schiff        | -     | +        |
| Myeloid series    | Erythroid series |
|------------------|------------------|
| Myeloblast       | 8.8              |
| Promyelocyte     | 0                |
| Myelocyte        | 0                |
| Metamyelocyte    | 0                |
| Neutrophilic band| 0                |
| Neutrophil       | 0.4              |
| Basophil myeloblast | 6.1         |
| Basophilic promyelocyte | 16.2     |
| Basophilic myelocyte | 34.1        |
| Basophilic metamyelocyte | 23.1    |
| Basophilic band  | 6.2              |
| Basophil        | 4.5              |
| Eosinophil       | 0                |

Lymphocyte       | 0.5              |
Plasma cell      | 0.1              |
Monocyte         | 0                |
M:E ratio*       | 0                |

*M:E ratio: the Myeloid : Erythroid ratio
| Day | 4 | 10 | 17 | 24 | 31 | 43 | 51 | 59 | 66 | 73 | 83 | 90 | 95 | 97 |
|-----|---|----|----|----|----|----|----|----|----|----|----|----|----|-----|
| PCV (%) | 8 | 13 | 14 | 15 | 17 | 28 | 29 | 25 | 20 | 12 | 11 | 10 | 6 |    |
| Plate ($\times 10^3/\mu l$) | 1 | 4 | 7 | 9 | 36 | 24 | 10 | 3 | 4 | 0 | 0 |    |    |    |
| WBC ($/\mu l$) | 5,030 | 7,730 | 2,480 | 4,090 | 1,550 | 5,100 | 25,550 | 100,330 | 143,200 | 223,220 | 179,260 | 232,430 | 1,800 | 25,170 |
| Seg ($/\mu l$) | 302 | 4,096 | 1,340 | 2,290 | 1,010 | 3,540 | 3,679 | 2,007 | 1,432 | 7,815 | 3,585 | 29,054 | 180 | 2,265 |
| Baso ($/\mu l$) | 805 | 77 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4,930 | 2,324 | 180 | 126 |    |
| blast ($/\mu l$) | 1,006 | 387 | 0 | 0 | 0 | 0 | 0 | 0 | 20,235 | 94,812 | 137,949 | 204,246 | 168,504 | 177,809 | 0 | 12,871 |
| MTX (0.5mg/kg, IV) |  |  |  |  |  |  |  |  |  |  |  |  |  | VBL |
| ADM (1mg/kg, IV) |  |  |  |  |  |  |  |  |  |  |  |  |  |    |
| Ara-C (100mg/m²×BID, SC, for two days) |  |  |  |  |  |  |  |  |  |  |  |  |  |    |
| VCR (0.03mg/kg, IV) |  |  |  |  |  |  |  |  |  |  |  |  |  |    |
| Prednisolone (mg/kg) | 2 | 2 | 2 | 2 | 2 | 1 | 1 | 2 | 2 | 2 | 2 | 2 |    |
| Blood Transfusion (35ml) |  |  |  |  |  |  |  |  |  |  |  |  |  |    |

PCV: packed cell volume, WBC: White blood cell, IV: intravenous, BID: bis in die, SC: subcutaneous
MTX: methotrexate, ADM: doxorubicine, Ara-C: cytosine arabinoside, VBL: vinblastine, ND: not done