

## CASE REPORT

# Treatment of acute erythroleukaemia with high-dose cytarabine in a cat with feline leukaemia virus infection

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## Abstract

Erythroleukaemia is a malignant neoplasm of the erythroid lineage that rarely occurs in cats. It is associated with the feline leukaemia virus (FeLV), and owing to the poor prognosis, treatment is rarely reported. A 4-year-old female Korean domestic short-hair cat was presented with hyporexia, fever, lethargy, severe anaemia and rubricytosis. An FeLV antigen test was positive, but a subsequent polymerase chain reaction test was negative. Serum biochemistry analysis results were normal, except for slightly elevated alanine aminotransferase. The patient was tentatively diagnosed with acute erythroleukaemia, and single high-dose (600 mg/m<sup>2</sup>) cytarabine chemotherapy was administered via constant rate infusion for 12 h a day for 5 days. After the first cytarabine administration, the clinical signs and anaemia improved, though no change was noted to other haematological parameters. The patient died of shock 16 days after the second cytarabine administration; the total survival time after diagnosis was 67 days. Post-mortem cytological evaluation of bone marrow aspiration revealed that the myeloid/erythroid ratio was 0.49, the erythroid progenitor cells were 64% of all nucleated cells and the blast cells were 84% of the non-erythroid cells. Histopathology images indicated that the spleen was diffusely expanded by atypical round cells, possibly erythroid precursors. This is the first case report on the prognosis and effects of high-dose cytarabine chemotherapy for acute feline erythroleukaemia with FeLV infection. Although the clinical signs improved, the treatment was not effective. Further studies on erythroleukaemia chemotherapy protocols are required.

## KEYWORDS

acute erythroleukaemia, chemotherapy, cytarabine, feline leukaemia virus, feline

## 1 | INTRODUCTION

Acute erythroleukaemia is a rare and malignant neoplasm of erythroid lineage in humans and cats; it is a subtype of acute myeloid leukaemia (AML), defined as AML-M6 in the French-American-British (FAB) scheme developed by the American Society for Veterinary Clinical Pathology Animal Leukaemia Study Group (Jain et al., 1991). The criteria for AML-M6 diagnosis are that erythroid precursor cells con-

stitute  $\geq 50\%$  and non-erythroid blast cells  $\geq 30\%$  of the bone marrow or peripheral blood (Bennett et al., 1985; Jain et al., 1991). In addition, AML-M6Er is a subtype of acute erythroleukaemia in which rubriblasts are predominant in the blast cells, and the blast cell count, including rubriblasts, is  $\geq 30\%$  of all nucleated cells (Jain et al., 1991).

Haematopoietic tumours, including leukaemia, are associated with feline leukaemia virus (FeLV) infection in cats (Hartmann & Hofmann-Lehmann, 2020). The unique long terminal repeat sequence of FeLV

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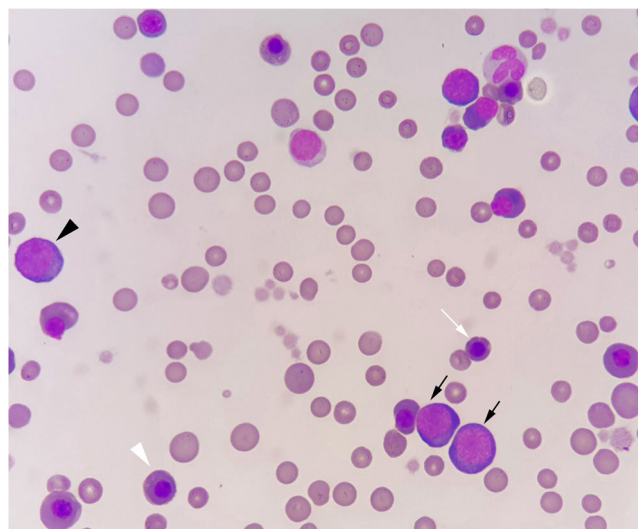
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induces leukaemia in cats (Hisasue et al., 2009). Nonspecific clinical signs such as anemia, lethargy, fever and weight loss are common. Most cases of acute erythroleukaemia occur in young to middle-aged cats, and the prognosis is grave.

Chemotherapy for AML is usually combined with cytarabine and anthracycline during the induction and consolidation phases in humans (Wiernik et al., 2013). In veterinary medicine, treatment of AML, including acute erythroleukaemia, is rarely reported because most cases result in euthanasia due to the grave prognosis. However, AML treatment with high-dose cytarabine, only or in combination, with other medications has been reported in several human medicine cases, and long-term management using cytarabine has been reported in dogs with AML-M7 (Willmann et al., 2009). This case report describes the diagnosis and treatment of AML-M6Er in a cat using high-dose cytarabine.

## 2 | CASE REPORT

A 4-year-old, 5.9-kg spayed female Korean domestic shorthair cat presented with hypoxemia, fever and lethargy. On physical examination, the patient was febrile at 40°C, and the mucous membranes were pale and dry. The other physical findings were unremarkable including palpation of peripheral lymph nodes that was normal. A complete blood count (CBC) revealed a severe normocytic, normochromic anaemia (haematocrit 10.5%, reference range 27.7%–46.8%), lymphocytosis (278,000/ $\mu$ l, reference range: 1500–7000/ $\mu$ l) and monocytosis (135,900/ $\mu$ l, reference range: 0–900/ $\mu$ l; Siemens ADVIA 2120 Haematology System; Siemens). The peripheral blood smear showed numerous nucleated erythrocytes, including blast cells with deeply basophilic cytoplasm, high N:C ratio, round nuclei (two to three times the size of an red blood cell [RBC]), coarse chromatin, and one to two distinct nucleoli, suggestive of rubriblasts with erythroid precursor origin (Figure 1). Myeloid and lymphoid precursor cells were also present. Most erythroid precursor cells were counted as lymphocytes and monocytes by the CBC machine (Siemens ADVIA 2120 Haematology System; Siemens). The lymphocyte and monocyte counts were 4170/ $\mu$ l and 2380/ $\mu$ l in differential peripheral blood smear. Using new methylene blue stain to evaluate reticulocytes, the patient exhibited a non-regenerative anaemia with an aggregate reticulocyte count of less than 15,000/ $\mu$ l (8280/ $\mu$ l; Willard & Tvedten, 2003). The feline immunodeficiency virus antibody test was negative, while the FeLV antigen test was positive (FIV-FeLV Snap Combo Test, FeLV Antigen by enzyme-linked immunoassay [ELISA]; IDEXX). The polymerase chain reaction (PCR) test for detecting FeLV RNA or proviral DNA was negative (RealPCR, IDEXX Laboratories). The serum biochemistry (BS-330, Mindray Bio-Medical Electronics) analysis results, including electrolytes and urinalysis, were within the reference range, except for slightly elevated alanine aminotransferase (200 U/L, reference range: 28–106 U/L). An abdominal radiograph revealed severe homogenous splenomegaly, but the echotexture of spleen was normal and visceral lymph node enlargement was not detected by ultrasonography. The patient was tentatively diagnosed with AML-M6Er.



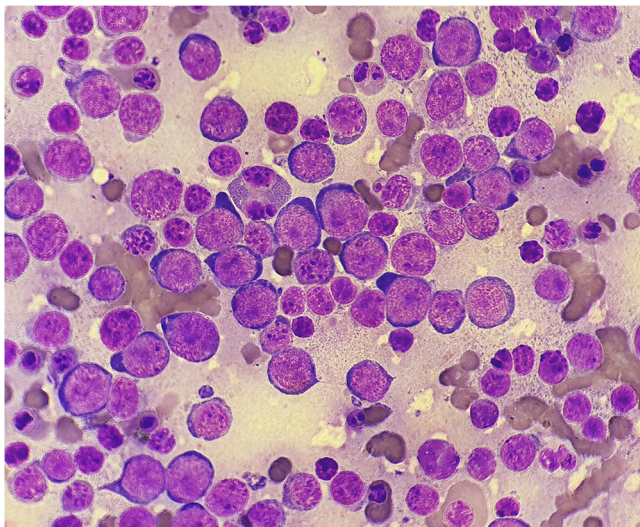
**FIGURE 1** Peripheral blood smear of a cat with erythroid leukaemia and feline leukaemia virus (FeLV) infection. Numerous erythroid precursors, including rubriblasts (black arrowhead), prorubricytes (black arrow), rubricytes (white arrowhead) and metarubricytes (white arrow) are shown (Diff-Quik, X100 objective)

A blood transfusion was performed, the total volume was 120 ml divided into 2 days because of the severe anaemia, and the nucleoside analogue, zidovudine (5 mg/kg PO twice a day), was administered along with supportive care including ferrous sulphate (100 mg/cat PO once a day), mirtazapine (1.88 mg/cat PO once a day), to treat the FeLV infection. However, the clinical signs deteriorated, and the medication was changed from zidovudine to prednisolone (2 mg/kg PO once a day). Regardless of this change in medication, the fever, hypoxemia and lethargy did not improve during the treatment. Moreover, the anaemia did not improve despite several blood transfusions. Therefore, cytarabine chemotherapy was initiated at a total dosage of 600 mg/m<sup>2</sup> for 5 consecutive days. Supportive care using mirtazapine (1.88 mg/cat PO once a day) and ferrous sulphate (100 mg/cat PO once a day) was continued during chemotherapy. Five days post chemotherapy, the haematocrit and clinical signs, including anorexia, lethargy and fever improved. However, other haematological abnormalities, including rubricytosis, did not change (Table 1). The second dose of cytarabine was administered 21 days after the first round. The fever, tachypnea, lethargy and hypoxemia recurred during the second cytarabine treatment. Two weeks later, the patient showed diarrhoea in addition to the previous clinical signs and her blood pressure and body temperature decreased, and she died of shock. The total survival time was 67 days after the AML-M6Er diagnosis.

The necropsy revealed no specific findings except for gross splenomegaly. The size and colour of the liver were not specific. Bone marrow aspiration revealed high cellularity and a low myeloid/erythroid ratio of 0.49 (reference range: 1.21–2.16; Harvey, 2012). The erythroid precursors were 64%, and the total blast cells (including rubriblasts) were 38% of all nucleated cells, while the blast cells were 84% of the total non-erythroid cells (Figure 2). Histologic examination of the spleen revealed that the stroma was diffusely

**TABLE 1** Haematological changes in high-dose cytarabine treatment in a cat with erythroleukaemia and feline leukaemia virus infection

	Before first cytarabine treatment	Seven days after first cytarabine treatment	13 days after second cytarabine treatment
RBC ( $10^3$ cell/ $\mu$ l)	3.63	6.06	4.14
Hematocrit (%)	16.3	27.4	18.9
Haemoglobin (g/dl)	7.3	10.7	6.8
Absolute reticulocyte ( $10^3$ cells/ $\mu$ l)	10.89	11.22	12.42
White blood cell ( $10^3$ cells/ $\mu$ l)	16.99	42.05	32.32
Neutrophils ( $10^3$ cells/ $\mu$ l)	11.57	31.86	27.15
Lymphocytes ( $10^3$ cells/ $\mu$ l)	2.72	3.82	1.94
Monocytes ( $10^3$ cells/ $\mu$ l)	2.04	3.82	1.29
Eosinophils ( $10^3$ cells/ $\mu$ l)	0.68	0	1.29
Nucleated erythroid cells ( $10^3$ cells/ $\mu$ l)	270.48	231.42	228.18
Platelets ( $10^3$ cells/ $\mu$ l)	273	181	249

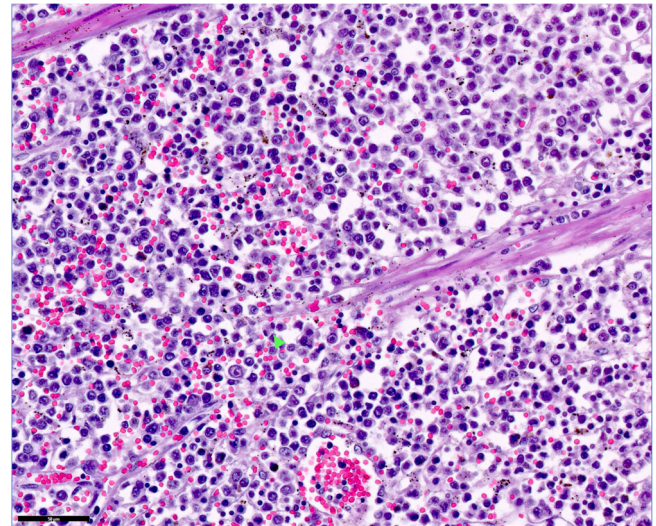


**FIGURE 2** Bone marrow aspiration of a cat with erythroid leukaemia and FeLV infection. Cellularity was high, and most cells were erythroid precursors at varying stages of development (Diff-Quik, X100 objective)

expanded by atypical round cells suspected to be erythroid precursors (Figure 3). A bone marrow biopsy could not be performed because of the small sample size. The peripheral blood and bone marrow cytology and spleen histopathology were significantly suggestive of acute sub-type AML-M6Er erythroleukaemia.

### 3 | DISCUSSION

Erythroleukaemia is a rare haematological neoplasm in humans, dogs and cats. In the FAB scheme, the criteria for acute erythroleukaemia



**FIGURE 3** Histologic examination of the spleen revealed stroma diffusely expanded by myeloid and erythroid precursors, frequent atypical round cells with distinct cell borders, scant to minimal eosinophilic cytoplasm, and irregular round, centrally eccentric nuclei with condensed coarse chromatin and prominent nucleoli. Anisocytosis and anisokaryosis were moderate and mitoses were scattered throughout atypical round cell population. Mitosis indicated with the green arrow head (haematoxylin & eosin, X40 objective, bar = 50  $\mu$ m)

are erythroid precursors  $\geq 50\%$  and non-erythroid blast cells  $\geq 30\%$  of the bone marrow (Bennett et al., 1985; Jain et al., 1991). The World Health Organisation system divides AML into several groups and deals with prognostic factors using genetic abnormalities in humans, which is not routinely used in veterinary medicine (Harvey, 2012). If the patient shows numerous blast cells in a blood smear, a presumptive diagnosis is possible (Nelson and Couto, 2019). In this case, bone marrow aspiration showed that the erythroid precursors were 64%, the total number of blast cells was 38% of all nucleated cells and the blast cells were 84% of the total non-erythroid cells. The patient was diagnosed with AML-M6Er.

FeLV infection is classified into three groups: progressive, regressive and abortive (Little et al., 2020). A progressive infection is characterised by viremia and clinical signs, such as lethargy and fever, and can be infectious. A regressive infection indicates that the FeLV provirus and antigen levels are low; therefore, it is not infectious, and clinical signs are absent or minimal. An abortive infection is characterised by the complete elimination of the virus due to an effective immune response (Hartmann & Hofmann-Lehmann, 2020; Little et al., 2020). If the cat's immune status is inadequate to regulate a regressive FeLV infection, it can change to a progressive infection, and the antigen and provirus levels would increase. Therefore, cats with progressive infection can infect other cats (Rojko et al., 1982). The FeLV p27 protein was measured by ELISA, and the unique region of the FeLV long terminal repeat was detected by real-time PCR (Little et al., 2020; Torres et al., 2008). In this case, the FeLV antigen test by ELISA was positive, but the PCR test for RNA and proviral DNA in blood

was negative. These results indicated a regressive infection (Beall et al., 2019). However, the FeLV subgroup C associated with AML-M6 arises by mutation of a commonly infected form and might not be detected by real-time PCR (Hofmann-Lehmann et al., 2001; Little, 2012). Moreover, considering that the clinical signs and bone marrow disorders are generally associated with progressive infection, it is more likely that the PCR result was a false negative (Little, 2012; Little et al., 2020).

Immunohistochemical staining was performed on the spleen that exhibited CD3 and Pax5 in the lymphoid cells. The results showed that CD3 was regionally highlighted, but this was suspected to represent the normal background-T-cell lymphocyte population of the spleen. Although analysis using other immunohistochemical stains against the myeloid lineage could not be performed, the diagnostic laboratory's pathologists agreed that the combined findings of the histopathologic features, haematological parameters and cytological results indicated AML-M6Er.

Cytarabine is one of the most commonly used medications for AML treatment in humans (Wiernik et al., 2013). Its mechanism in treating leukaemia is via the effects of its metabolites on cancer cell death by interference with DNA and RNA synthesis. Furthermore, the cytarabine metabolites inhibit the G0/G1 phase of the cell cycle and then promote apoptosis. The conventional cytarabine dosage in humans is 100–200 mg/m<sup>2</sup> for 7–10 h, but 2000–3000 mg/m<sup>2</sup> twice daily for 6 days is superior to the conventional dosage in the induction or consolidation phase (Bishop et al., 1996; Herzig et al., 1985). Cytarabine has been used in veterinary medicine for various diseases at varying dosage; it has been reportedly used at 200–300 mg/m<sup>2</sup> in meningoencephalitis of unknown aetiology (at 400–600 mg/m<sup>2</sup> in lymphoma or other neoplasia (Lowrie et al. 2013; Withrow 2020). Therefore, cytarabine at 600 mg/m<sup>2</sup> (considered 'high-dose') was used to treat AML-M6Er in this case.

Cytarabine is commonly injected subcutaneously. However, constant rate infusion (CRI) is potentially more effective than subcutaneous injection because a steady concentration is easily achieved and maintained (Crook et al., 2013). Because of this pharmacokinetic characteristic, cytarabine was administered via CRI for 12 h/day for 5 days in our case.

In human medicine, haematologic complete remission is satisfied by these criteria: blast cells < 5%, absence of circulating blasts and extramedullary disease, neutrophil count  $\geq 1000/\mu\text{l}$ , platelet count  $\geq 100,000/\mu\text{l}$ , blood transfusion independent and absence of disease signs or symptoms (De Greef et al., 2005; Döhner et al., 2017). The high-dose cytarabine treatment was not effective in this patient because the number of blast cells was not significantly decreased. However, the haematocrit and clinical signs such as fever and anorexia improved after the first chemotherapy session. In addition, the patient's activity improved, and the owner was satisfied during this period. Given the grave prognosis of this disease, high-dose cytarabine treatment might be considered a treatment option for erythroid leukaemia.

## 4 | CONCLUSION

To the best of our knowledge, this is the first case report of AML-M6Er in a cat with FeLV infection treated with high-dose cytarabine. The prognosis for the patient was poor despite an improvement in clinical signs and anaemia after the first chemotherapy session; the survival time after diagnosis was 67 days. Further studies on the treatment of acute feline erythroleukaemia with high-dose cytarabine are required.

## ACKNOWLEDGEMENT

We thank the support of the 'Cooperative Research Program of Center for Companion Animal Research (Project No. PJ01404502); Rural Development Administration, Republic of Korea' for the publication of this study.

## CONFLICT OF INTEREST

The authors declare no conflict of interest

## ETHICS STATEMENT

The owner of the cat in this study gave permission for the publication of this manuscript.

## AUTHOR CONTRIBUTION

*Conceptualisation, data curation, formal analysis, investigation, methodology, writing-original draft:* Da Sol Park. *Data curation, methodology, visualisation, writing-review and editing:* Jongbok Lee: *Writing-review:* Kun-Ho Song. *Conceptualisation, data curation, project administration, resources, supervision, writing-original draft, writing-review and editing:* Kyoung Won Seo.

## DATA AVAILABILITY STATEMENT

All data supporting our findings are included in this study.

## PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/vms3.646>

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**How to cite this article:** Park, D. S, Lee, J., Song, K.-H., & Seo, K. W. (2022). Treatment of acute erythroleukaemia with high-dose cytarabine in a cat with feline leukaemia virus infection. *Veterinary Medicine and Science*, 8, 9–13. <https://doi.org/10.1002/vms3.646>