



NOTE

Internal Medicine

A cat with myelodysplastic syndrome by administration of the methylation inhibitor Azacytidine

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ABSTRACT. A 5-year-old female cat with nonregenerative anemia and thrombocytopenia was diagnosed with myelodysplastic syndromes (MDS), since peripheral blood and bone marrow (BM) examination revealed various dysplasias and a blast ratio of 19%. Chemotherapy with azacytidine (AZA; 70–35 mg/m², 3–5 days, three cycles) and treatment with prednisolone, antibiotics, and vitamin K₂, and blood transfusion were performed. On day 106, blast cells and dysplasia had decreased in the BM, and the cat remained alive for at least 1,474 days. This report is the first on feline MDS treated with AZA, suggesting appropriate drug dosage, interval and effective combination should be investigated and the pharmacological and cell biological mechanisms needs to be elucidated in the future.

KEY WORDS: cat, chemotherapy, leukemia, myelodysplasia, survival duration

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Myelodysplastic syndrome (MDS) is characterized as a heterogeneous disease in the preleukemia stage, and effective treatments have not been established [8, 9, 11, 23, 25]. In the veterinary field, MDS and acute myeloid leukemia (AML) are rare diseases that occur among young to middle-aged cats infected with feline leukemia virus (FeLV) [2, 8, 10, 18, 23, 25]. The affected cats often show fever, a loss of energy, weakness, opportunistic infections due to immune deficiency, leukopenia, nonregenerative anemia, and thrombocytopenia, and they often transition to acute leukemia [8, 23, 25]. The cytopenic pathology of MDS is caused by clonal expansion of hematopoietic stem cells and their markedly reduced differentiation and maturation ability [10, 15, 16]. An effective therapeutic regimen for feline MDS has not been established, and most cats with MDS show leukemia progression, short survival duration and poor prognosis [8, 23–26]. In particular, our previous report demonstrated that the high-risk group of feline MDS classified into refractory anemia (RA) with excess of blasts (RAEB), RAEB in transformation (RAEB in T), and chronic myelomonocytic leukemia (CMMoL) based on the French-American-British (FAB) classification has a higher probability of transition to acute leukemia and a poorer prognosis than the low-risk groups, including the RA group, suggesting that RAEB is refractory to the therapies used so far [8, 11, 23, 25]. In some previous reports, cats with MDS were treated with blood transfusion, antibiotics, prednisolone, cyclosporine, and erythropoietin [8, 9, 25, 26]. These treatments were supportive therapies, since a detailed case report using chemotherapy has not been reported due to the small number of spontaneous cases of feline MDS.

Azacytidine (AZA), a methylation inhibitor, has recently gained attention because of results showing that it could improve the survival duration in human patients with MDS [12–14, 17, 22]. Therefore, AZA is considered as a potential therapeutic candidate for MDS. AZA is a nucleoside analog in which the carbon atom at the 5-position of the pyrimidine ring of cytidine is converted to a nitrogen atom [13, 14]. It was synthesized in the 1960s, and it has been investigated as a cytarabine analog and a cytotoxic agent for the treatment of AML. The active form of azacytidine binds both RNA and DNA, exerting its cytotoxic effect via interference with RNA transcription and DNA methyltransferase I activity in actively proliferating cells [13, 14, 22]. Recent studies have suggested that the silencing of certain tumor suppressor genes plays an important role in the occurrence of hematological malignancies, and aberrant hypermethylation in the promoter regions affects the epigenetic transcriptional mechanisms of these genes [12–14]. In addition, abnormalities of DNA methylation and transcriptional regulation are prevalent in human MDS; therefore, hypomethylated drugs are expected to be new therapeutic agents for MDS worldwide [12–14].

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In the veterinary field, a study describing the use of AZA in feline MDS has not been reported, and its clinicopathological effects in improving cytopenia or prolonging prognosis are unknown. However, AZA therapy in spontaneous feline tumor cases has not been reported in clinical veterinary medicine. Nevertheless, some studies demonstrated anti-methylation and cytostatic effects in feline lymphoid and mammary tumor cell lines *in vitro*, suggesting that AZA has therapeutic potential in canine and feline tumors as well as in human tumors [5–7]. Therefore, we report the clinicopathological findings and clinical course and prognosis of MDS in a cat that showed an improvement in cytopenia, a reduction in blasts in the bone marrow (BM), and long-term survival with AZA administration.

A 5-year-old, 3.32 kg, spayed female, American short hair cat was referred to Azabu University Veterinary Teaching Hospital with a history of anorexia, nonregenerative anemia, and thrombocytopenia. The cat had been receiving treatment with blood transfusion (40 ml/cat), prednisolone (2 mg/kg/day, PO), doxycycline (5 mg/kg, *semel in die* [SID], PO) and vitamin K2 analog, menatetrenone (2 mg/kg, *bis in die* [BID], PO) for 14 days, but the cytopenia persisted. Physical examination revealed systolic murmur due to anemia, ocular and oral mucosal pallor, and a body condition score of 3 (BCS 3). Systemic lymph node swelling was not observed.

On presentation, physical examination revealed fever (40°C). Complete blood count (CBC) results (Sysmex XT2000, Kobe, Japan) revealed severe nonregenerative anemia; red blood cell (RBC): $3.17 \times 10^6/\mu\text{l}$, hemoglobin (Hb) level: 4.7 g/dl, packed cell volume (PCV): 15.2%, reticulocyte percentage: 1.12% and thrombocytopenia (platelet count: $114 \times 10^3/\mu\text{l}$) (Table 1). The white blood cell (WBC) count was $16,650/\mu\text{l}$ and a differential leukogram revealed neutropenia and an increase in the number of undetermined cells. The results were as follows; band neutrophil count: $487/\mu\text{l}$, segmented neutrophil count: $1,753/\mu\text{l}$, lymphocyte count: $6,426/\mu\text{l}$, monocytes count: $84/\mu\text{l}$, eosinophil count: $97/\mu\text{l}$, myelocytes count: $390/\mu\text{l}$, erythroblast count: $6,580/\mu\text{l}$ and undetermined cells of blast like cell: $390/\mu\text{l}$. The cat showed lack of erythroid regeneration since the number of reticulocytes was $35,504/\mu\text{l}$ (1.12%). Cytological analysis of peripheral blood smears stained by Wright Giemsa revealed characteristic dysplasias of MDS, including erythroblasts without erythrocyte regeneration, megaloblastoid changes, nucleocytoplasmic maturation mismatch of erythroblasts, pseudo-Pelger–Huët anomaly, and micromegakaryocytes (Fig. 1). A few erythroblasts and hematopoietic cells at each stage of maturation from myeloblasts to metamyelocytes were present in peripheral blood. Plasma chemistry (Cobas®6000, Roche Diagnostics K.K., Tokyo, Japan) showed mild azotemia (urea: 46.1 mg/dl) and hyperglycemia (glucose: 211 mg/dl) (Table 1). The results of coagulation and fibrinolysis examinations, including measurements of prothrombin time, activated partial thromboplastin time, antithrombin activity, and fibrin degradation product levels, were normal (COAGE2V, HITACHI, Tokyo, Japan). Thoracic and abdominal radiographs and abdominal ultrasonography findings were unremarkable. Examinations of serum

Table 1. On presentation, the complete blood count, chemistry, and hemostatic test results

	Day 0	Unit	Reference interval		Day 0	Unit	Reference interval
RBC	317	$\times 10^6/\mu\text{l}$	5.65–8.87	Total proteins	6.1	g/dl	5.1–7.7
PCV	15.2	%	37.3–61.7	Albumin	3.6	g/dl	2.5–3.9
Hemoglobin	4.7	g/dl	13.1–20.5	ALT	38	IU	12–45
MCV	47.9	fl	61.6–73.5	AST	19	IU	19–90
MCH	14.8	pg	21.2–25.9	ALP	38	IU	26–150
MCHC	30.9	g/dl	32.0–37.9	Total bilirubin	0.07	mg/dl	0.0–0.3
Reticulocytes	35.504	$\times 10^3/\text{dl}$	10–110	Glucose	211	mg/dl	64–152
RPI	0.182		>1.0	Cholesterol	117	mg/dl	71–234
WBC	16.61	$\times 10^3/\mu\text{l}$	5.05–16.76	Triglycerides	39	mg/dl	8–71
Metamyelocyte	0.39	$\times 10^3/\mu\text{l}$	0	Urea	46.1	mg/dl	15.0–37.0
Band	0.49	$\times 10^3/\mu\text{l}$	<0.3	Creatinine	0.9	mg/dl	0.8–1.8
Neutrophils	1.75	$\times 10^3/\mu\text{l}$	2.5–12.5	Phosphorous	6.4	mg/dl	2.2–6.5
Lymphocytes	6.43	$\times 10^3/\mu\text{l}$	1.5–7.0	Calcium	9.5	mg/dl	8.0–11.1
Monocytes	0.58	$\times 10^3/\mu\text{l}$	0–8.5	Sodium	150.1	mmol/l	145.0–159.0
Eosinophils	0	$\times 10^3/\mu\text{l}$	0–1.5	Potassium	3.8	mmol/l	3.0–4.8
Basophils	0	$\times 10^3/\mu\text{l}$	0	Chloride	115.6	mmol/l	111–125
Blast like cell	0.39	$\times 10^3/\mu\text{l}$	ND	Iron	222	$\mu\text{g}/\text{dl}$	61–240
Erythroblast	6.58	$\times 10^3/\mu\text{l}$	ND	TIBC	360	$\mu\text{g}/\text{dl}$	303–526
Platelets	11.4	$\times 10^3/\mu\text{l}$	148–484	UIBC	138	$\mu\text{g}/\text{dl}$	164–354
				PT	8.6	sec	6.0–9.0
				APTT	15	sec	13–19
				Fibrinogen	306	mg/dl	160–400
				AT	99	%	95<
				FDPs	0	$\mu\text{g}/\text{ml}$	<5

Reference intervals are from our laboratory. PCV, packed cell volume; MCV, mean cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RPI, reticulocyte production index; WBC, white blood cell; PT, prothrombin time; APTT, activated partial thromboplastin time; AT, anti-thrombin; FDPs, fibrin degradation products; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TIBC, total iron-binding capacity; UIBC, unsaturated iron-binding capacity; ND, not determined.

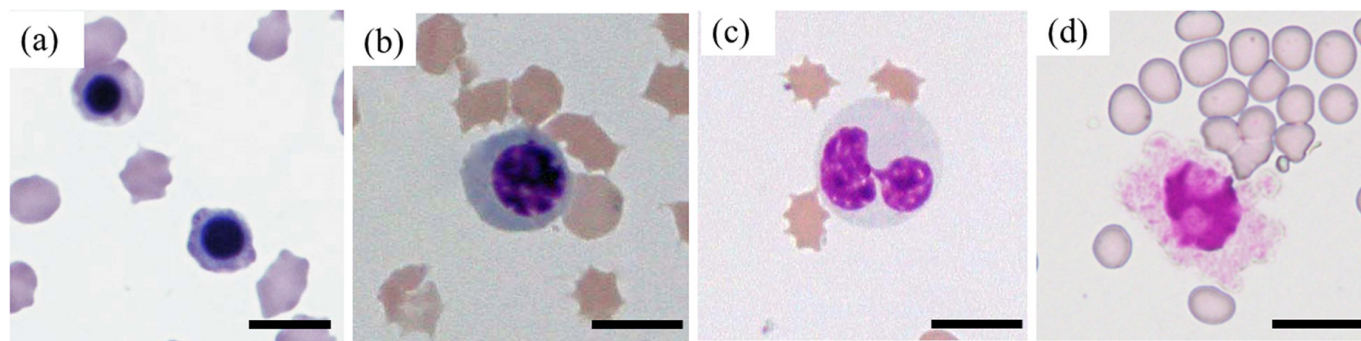


Fig. 1. Abnormal findings in peripheral blood of this case. (a) Appearance of rubriblasts lacking regenerated erythrocytes; This case showed that non-regenerative anemia since polychromatic erythrocytes were scarcely seen and reticulocyte production index (RPI) was low level (0.182), however a small number of erythroblasts (polychromatic and orthochromatic erythroblast) were observed, indicating that the abnormal erythroid cell lineage differentiation and refractory hematopoiesis characteristic of myelodysplastic syndromes (MDS) were occurred. (b) Megaroblastoid change of erythroblasts: orthochromatic erythroblast with a larger nucleoli than normal cell. (c) Pseudo-Pelger–Huët anomaly: neutrophils with chromatin aggregates and eyeglass-shaped nuclei. (d) Micromegakaryocytes: Cytoplasm with platelet-like structure and platelet release are seen (Wight Giemsa stain; Scale bar=10 μ m).

iron and unsaturated iron-binding capacity (UIBC) were performed, excluding iron deficiency anemia and anemia-related chronic disease (Table 1). The serum iron level was 222 μ g/dl, the TIBC was 360 μ g/dl, indicating that iron deficiency anemia and anemia-related chronic disease were unlikely. FeLV antigen and feline immunodeficiency virus (FIV) antibody tests with a test kit (SNAP FIV/FeLV Combo Test, IDEXX, Westbrook, ME, USA) yielded negative findings. In addition, real-time PCR for feline hemotropic mycoplasmas was performed at a commercial laboratory using peripheral blood. For the detection of latent retrovirus infection, a PCR assay of FeLV and FIV provirus in the BM sample was performed by a commercial laboratory (IDEXX laboratory). The findings of all PCR examinations were negative.

Eighteen days before the first admission, BM aspiration was performed at a referral animal clinic. BM specimens were aspirated from the right humerus and left femur to further investigate the potential mechanism underlying the nonregenerative anemia and assess the condition of BM tissue. A bone marrow specimen was sent by a referral veterinarian, and an ACVP board-certified veterinary clinical pathologist evaluated the cytology of BM smears. A 500-cell differential count was performed on multiple BM smears from the right humerus and left femur. All smears showed almost the same findings. BM evaluations showed hyperplasia with increased numbers of blasts including myeloblasts and erythroblasts (Fig. 2). Myelography revealed that the myeloid erythroid (M:E) ratio was 0.23 with erythroid hyperplasia, hypo-segmentation of neutrophils, and the arrest of myeloid cell differentiation and maturation, and the blast ratio in all nucleated cells and non-erythroid cells was 7.2 and 19.0%, respectively (Table 2). Although increase of blast cell and findings to suspect dysplasia were observed, but definitive dysplastic changes were not found in bone marrow. Furthermore, no definitive finding of any immunological destruction showed, since hemophagocytosis by macrophages was not found in the bone marrow. The hematological diseases, non-regenerative immune mediated anemia (NRIMA), immune mediated neutropenia (IMN), and acquired amegakaryocytic thrombocytopenia (AAMT), were excluded for the following reasons. 1) Increase of blast cells in bone marrow was found, indicating that the pathogenesis was considered as erythroleukemia, 2) Peripheral blood smear showed definite abnormal erythroblast maturation; appearance of erythroblastic cells without increased polychromatic erythrocytes that is final product of erythroid hematopoiesis, 3) Typical and significant dysplasia of MDS was seen in myeloid, erythroblast and megakaryocyte cell lineages in peripheral blood. The pathogenesis of this case was considered to be the pre-leukemia stage of erythroleukemia with minimum dysplasia.

On the basis of the FAB classification, this cat was diagnosed with MDS-RAEB [11, 25]. A treatment protocol involving prednisolone and menatetrenone administration, and blood transfusion was performed in this cat for 14 days before the first admission to our hospital, indicating that these treatments were insufficient in improving cytopenia and facilitating a remission. We considered the patient to be refractory to the previous treatment, and we provided the pet owner the following explanation: 1) MDS-RAEB has a poor prognosis based on previous evidence, 2) it showed poor response with previous treatments of prednisolone and menatetrenone, and 3) AZA may be effective for MDS-RAEB based on the evidence for human MDS patients, although the adverse effects of AZA and its therapeutic effects for feline MDS remain unclear. Prior to treatment, the owner gave sufficient informed consent regarding the efficacy and side effects of AZA, and consented to the use of this drug.

The treatment involved the administration of AZA (Vidaza, Nihon Shinyaku, Kyoto, Japan) at 35–70 mg/m^2 SID, SC, for 3–5 days in one cycle and a total of three cycles were carried out (Fig. 3). In addition, corticosteroid treatment (prednisolone; 2 mg/kg , SID, SC), antibiotic administration (enrofloxacin; 2.5 mg/kg , SID, SC), menatetrenone; 2 mg/kg , BID, SC, antiemetic treatment (omeprazole and maropitant; 1 mg/kg , SID, SC, respectively), and whole-blood transfusion (40 ml/1 cat) were performed as necessary. AZA was administered at a dose of 70 mg/m^2 , and decreased activity, vomiting, and diarrhea occurred (day 1). The same amount of AZA was administered, and vomiting was seen with the progression of cytopenia (WBC count: 4,000/ μ l, neutrophil count: 1,230/ μ l, PCV: 12%, platelet count: $102 \times 10^3/\mu$ l) on day 2. In peripheral blood smears, myeloblasts and

Table 2. Myelogram of this case

	Pre (%)	Post (%)
Proerythroblast	3.7	0.2
Basophilic erythroblast	7.2	0.7
Polychromatophil erythroblast	46.1	19.2
Orthoromatic erythroblast	24.6	17.4
Myeloblast	3.5	0.8
Promyelocyte	0.3	1.1
Myelocyte	2.5	5.1
Metamyelocyte	2.4	6.7
Band neutrophil	6.2	17.8
Segmented neutrophil	1.9	27.6
Eosinophil	0.4	2.4
Basophil	0.0	0.0
Monocyte	1.2	0.5
Megakaryocyte	0.0	0.5
ME ratio	0.23	1.67
Blast cell in ANC*	7.2	1.0
Blast cell in NEC**	19.0	2.8
(Lymphoid cells	13.2	12.4)***
(Plasma cells	0.4	0.4)
(Macrophages	1.8	0.6)

*ANC; all nucleated cell, **NEC; non erythroid cell. *** (); indicates the ratio of lymphoid cells, plasma cells, and macrophages in the total cells counted in the bone marrow.

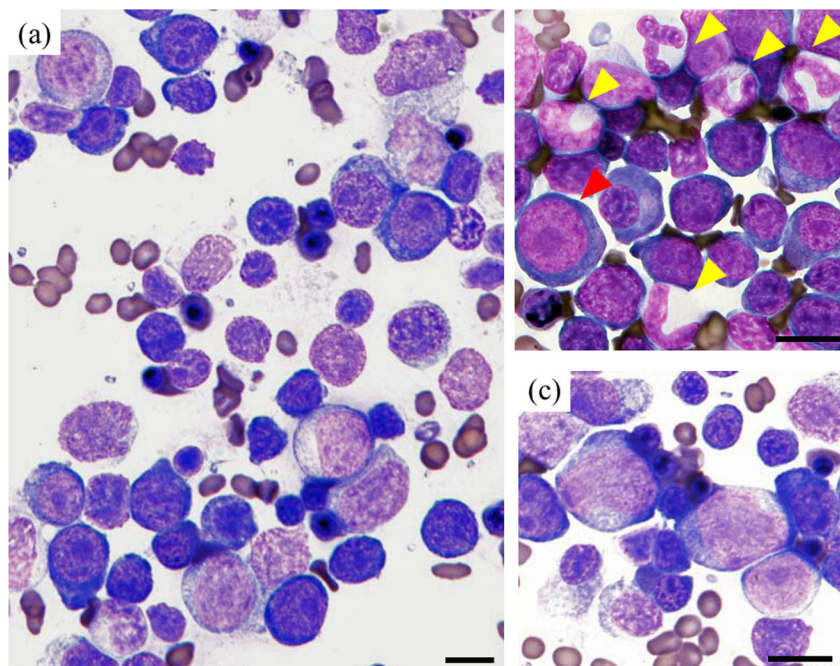


Fig. 2. Bone marrow findings of this case. (a) Hyperplasia erythroid lineage and blast cell increase. (b) Proerythroblast with huge nucleolus was seen (red arrow). Neutrophil differentiation arrest up to metamyelocyte and band neutrophils (yellow arrow) is seen myeloid cells lineage, suggesting hypo-segmentation of neutrophil. (c) Increase of blast cells; the size of the cells was large, the cytoplasm was basophilic, the nucleoli were clear, and chromatin meshes was fine (Wight Giemsa stain; Scale bar=10 µm).

rubricytes disappeared. A combination of antibiotics and granulocyte-colony stimulating factor (G-CSF) (filgrastim: 1.0 mg/kg, SID, SC, 7 times) was administered. The AZA dose was reduced to 35 mg/m² on day 3 due to side effects, including leukopenia (WBC count: 2,860/µl), neutropenia (neutrophil count: 180/µl), vomiting, diarrhea, and severe cytopenia. Subsequently, on day 4, fever disappeared (38.6°C) and digestive symptoms also disappeared. However, neutropenia persisted (neutrophil count range: 0 to 160/µl), and the administration of antibacterial drugs and G-CSF was continued. On day 8, WBC and neutrophil counts were 1,310/µl and 150/µl, respectively, and the progression of cytopenia was not seen. Therefore, whole-blood transfusion (36 ml) was performed, and the patient was discharged from the hospital.

CBC examination on day 29 showed marked hematological improvements: WBC count, 5,300/µl; neutrophil count, 2,430/µl; PCV, 32.1%; and platelet count, 340 × 10³/µl. As second cycle, AZA was administered during hospitalization between day 29 and 32; the dose of AZA was reduced to 35 mg/m²; and the drug was administered three times (on day 29, 31, and 32). On day 29, vomiting and diarrhea occurred, and AZA administration was postponed to day 31 and 32, respectively. During the hospitalization period, hematological adverse effects of AZA administration at 35 mg/m² were not seen, and the leukocyte and neutrophil counts were 5,300 to 14,780/µl and 2,430 to 3,680/µl, respectively. A CBC examination on day 60 showed that the WBC count was 7,010/µl and the neutrophil count was 2,500/µl. The third cycle of AZA treatment was started, wherein AZA was administered at a dose of 35 mg/m² for 3 consecutive days from day 60. On day 63, the cat was discharged since the WBC (7,170/µl) and neutrophil (5,640/µl) counts were within reference range, and only one episode of mild diarrhea was noted. However, severe leukopenia and anemia due to AZA occurred on day 69; the WBC count was 600/µl and PCV was 28.9%. G-CSF (filgrastim: 1.0 mg/kg, SID, SC, 3 times) and antibiotics (enrofloxacin: 5 mg/kg, SID, SC) were administered, and the cytopenia improved on day 74 (WBC count: 4,900/µl, PCV: 28.9%, and PLT count: 410 × 10³/µl). On day 106, hematological remission was achieved (WBC count: 6,000/µl, neutrophil count: 3,790/µl, PCV: 28.9%, and platelet count: 285 × 10³/µl). BM examinations were performed to confirm the condition of the BM tissue and determine the need for further chemotherapy. The BM examination confirmed complete remission, since no abnormalities of differentiation or normal cell maturation were observed, and dysplasia was absent in her BM (Table 2). The blast ratio, which was 19.0% before AZA treatment, decreased to 2.8%, and erythroid hyperplasia (ME ratio: 0.23) was also normalized to myeloid hyperplasia (ME ratio: 1.67). The cat was eventually moved from our hospital to a general veterinary hospital located elsewhere in accordance with the owner's wishes. She continued to receive prednisolone and menatetrenone and was alive for at least 1,411 days after finishing the administration of AZA. The survival duration was at least 1,474 days after AZA administration. The owner then stopped visiting the hospital and the subsequent outcome remains unknown.

To our knowledge, this report is the first showing long-term survival in feline MDS after the administration of AZA. A standard therapeutic regimen for feline MDS has not been reported to date because of the limited number of cases, which was less than

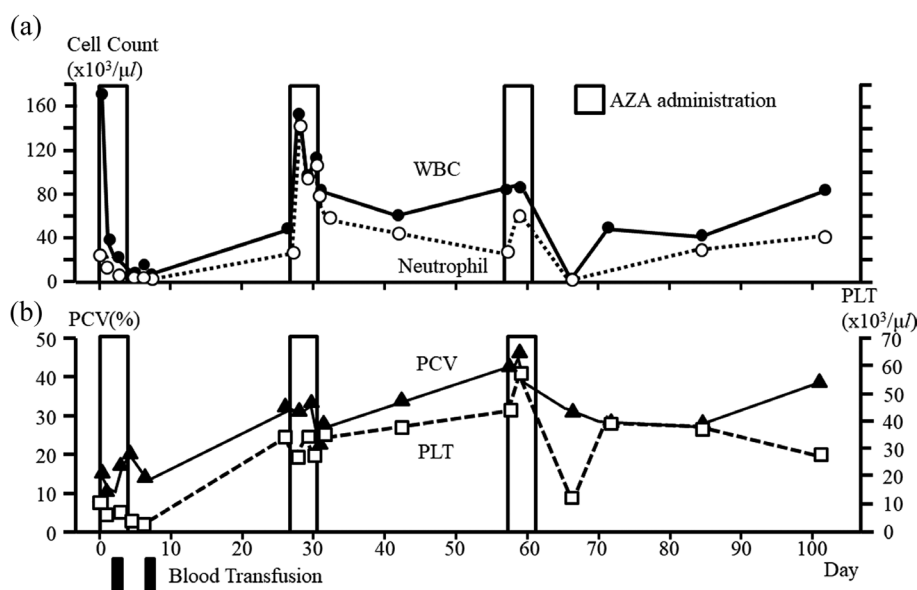


Fig. 3. Clinical course of this case. Hematological changes in the period of Azacitidine (AZA) treatment. Black round dots and solid line; WBC, white circles and dotted line; Neutrophil, black triangles and solid line; PCV, white squares and dotted line; PLT, white column; during AZA administration; black column; blood transfusion.

50 between 1990 and 2020 [2, 8, 11, 23, 24, 26]. In fact, there have been no reports comparing the drug treatment effects in cats with AML and MDS with those in a control group, and the rarity of this disease is an obstacle to finding an effective treatment method. In this study, the administration of AZA to cats with MDS resulted in an improvement in cytopenia, a reduction in the blast ratio, the disappearance of dysplasia, and long-term survival. In a previous study, we showed that high-risk MDS with RAEB had a survival duration of 0.2 to 6 months (median, 2 months) and all patients died [8]. RAEB is considered to be a type similar to leukemia in humans, and the risk of leukemia progression is high with a poor prognosis [8, 11, 23, 25]. In this case, survival was confirmed up to the day, and long-term survival was obtained compared with historical data. There have been reports on the course of treatment in several cats with MDS [8, 11, 23, 25]. In some previous reports, cats with MDS were treated with blood transfusion, antibiotics, prednisolone, cyclosporine, erythropoietin, menatetrenone [8, 9, 25, 26]. Interestingly, in previous studies, cats with MDS that showed long survival periods after receiving cytarabine, which shows the same antimetabolite activity as AZA, which has differentiating and demethylating effects [8]. Therefore, demethylation drugs may be effective for feline MDS. However, methylation status was not analyzed, and the mechanism of action was unknown in this case.

In this case, complete remission of MDS was obtained by using AZA, indicating that it was effective to reduce blast cells and improve cytopenia. AZA exerts its antineoplastic effects via multiple mechanisms of action, including cytotoxic effects on abnormal hematopoietic cells in the bone marrow and the hypomethylation of DNA, although the relative importance of individual mechanisms of action to the clinical outcomes remains to be established [13, 14, 17, 20, 22]. The mechanisms involved in the cytotoxic effects of AZA may include the inhibition of DNA, RNA, and protein synthesis, the incorporation of AZA into RNA and DNA; the induction of apoptosis; and the activation of DNA damage pathways [13, 14, 20]. In this case, the effect of AZA alone was unknown, because prednisolone and menatetrenone were used for therapy. Therefore, the single-agent effect of AZA could not be confirmed, suggesting that it may have had a synergistic effect with the concomitant drugs. In humans, a recent study demonstrated that AZA alone has a negative effect on life prognosis [12, 21]. Currently, research on combination therapy, including AZA with lenalidomide or vorinostat, is underway [21], suggesting that combination therapy with AZA and other drugs will be required to improve feline MDS. Akiyama *et al.* demonstrated that VK2 therapy appears to be promising for the improvement in anemia and thrombocytopenia in patients with low/intermediate-1 MDS. The overall response rate to VK2 monotherapy (45 mg/day) after 16 weeks was 13% (5/38), including four cases that showed improvement in both anemia and thrombocytopenia and one case that showed improvement in thrombocytopenia [1]. Furthermore, Sada *et al.* reported that steroid and xenobiotic receptor (SXR), which has been identified as a receptor of VK2, was present on myeloid progenitors. The major effect of VK2 on myeloid progenitors was the promotion of cell differentiation, suggesting that VK2 may contribute to the improvement in neutropenia [19]. In this case, we used vitamin K2 in addition to AZA, and the synergistic effects of this combination might have resulted in remission. However, the synergistic effect of the combination of AZA and vitamin K2 is unknown, and it will be necessary to study this effect in other cats with MDS in the future.

Thus, when AZA was administered in addition to the existing treatment, a favorable prognosis was obtained. In human MDS, new therapeutic agents such as lenalidomide, venetoclax, the nucleoside analog sapacitabine, and histone deacetylase inhibitors are thought to improve or have a prognostic effect when used in combination with AZA [21, 22]. Therefore, future studies should aim to determine an appropriate drug combination for feline MDS.

In this case, the absence of feline retrovirus infection may have contributed to the improved prognosis. Our previous study suggests that many FeLV infections are associated with MDS and AML and are implicated in their pathology [8]. Since FeLV induces tumorigenesis, it is expected that tumor relapse will occur even if tumor remission is achieved by drug therapy, resulting in a poor prognosis. In fact, feline lymphoma has been reported to show a clearly poor prognosis in individuals infected with FeLV [3]. To date, almost all cases of MDS in cats involved FeLV infections, but outbreaks of FeLV-uninfected acute leukemia have been sporadically reported in recent years [4, 26]. FeLV, an infectious agent that induces tumorigenesis, was not observed in this case, suggesting that the patient's prognosis may be favorable as a result.

There are some limitations that should be discussed in the context of this report. First, the methylation state of the patient's hematopoietic cells could not be confirmed before and after drug administration, and the direct effect could not be evaluated through the assessment of the inhibition of methylation by AZA. Moreover, since vitamin K2 and other therapeutic agents are also used, the effect of AZA alone is unclear. It may be necessary to continue research on this topic to identify appropriate doses and protocols. Second, it is possible that the AZA dose administered in this case was not appropriate, since the cat showed severe neutropenia, fever, vomiting and diarrhea with AZA doses between 35 and 70 mg/m². Fortunately, the cat recovered after the administration of antibiotics and antiemetics and infusion. In human MDS, several studies have described that severe side effects can occur in almost all patients (97% of patients) [20]. The treatment-related adverse events (TRAEs) with AZA treatment occurred during the initial two cycles, resolved with ongoing treatment. The most commonly reported adverse reactions with AZA treatment were hematological events [20]. Recent reports described that the most commonly reported adverse reactions with AZA treatment were hematological events, including anemia, thrombocytopenia, neutropenia [20]. In addition, other reactions were included constipation, diarrhea, nausea, vomiting, as well as injection reactions such as rash/inflammation/pruritus, rash, erythema, and skin lesions [20]. Therefore, the appropriate dose and type and the frequency of side effects in cats should be clarified.

In conclusion, the findings suggest that AZA is one of the treatment options for high-risk feline MDS. However, the pharmacological and cell biological mechanisms underlying the action of AZA, including its effects on methylation, the drug's efficiency, and the appropriate dose of AZA remain unknown. Further research will be required to clarify these issues in the future.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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