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NOTE

Clinical Pathology

Presumptive precursor-targeted immune-mediated anemia concurrent with gastrointestinal lymphoma in a cat

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ABSTRACT. A 10-year-old spayed female mixed-breed cat presented with progressive nonregenerative anemia. Clinicopathological abnormalities included severe nonregenerative anemia (packed cell volume [PCV]: 7%, aggregate reticulocytes: $1.12 \times 10^3/\mu l$) and a hypoechogenic mass well-localized in the stomach. Bone marrow (BM) smears revealed increased particle hematopoietic cellularity with decreased myeloid:erythroid (M:E) ratios, no dysplasia of any lineage, and presence of erythroid precursors phagocytized by macrophages. The cat was diagnosed with presumptive precursor-targeted immune-mediated anemia (PIMA). The stomach mass was consistent with CD 20 positive T-cell lymphoma. The lymphoma was completely resected via surgery, and the PIMA was cured by immunosuppressive therapy. On day 410, both diseases have not recurred without medications. This is the first report of feline PIMA and concurrent gastrointestinal lymphoma.

KEY WORDS: bone marrow, CD20 positive T-cell lymphoma, phagocytosis, stomach, transmural lymphoma

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Precursor targeted immune-mediated anemia (PIMA) is known to immune-mediated destruction of erythrocyte precursors and is associated with bone marrow (BM) erythroid hyperplasia and/or erythroid maturation arrest [2, 8]. Feline nonregenerative anemia with suspected immune-mediated destruction of erythrocyte precursors is called nonregenerative immune-mediated anemia (NRIMA) in the veterinary literatures [3, 7, 17, 21, 23, 25]. However, we consider that the use of term 'NRIMA' can be confusing because it is not always clear whether the pathogenesis is persistently nonregenerative or preregenerative stage of immune-mediated hemolytic anemia (IMHA). The two latest studies of canine PIMA suggested that the suspected immune-mediated pathogenesis for targeting of erythroid precursors called 'PIMA' more aptly describes and characterizes pathogenesis of the condition rather than the term 'NRIMA' [2, 8]. Therefore, we agree with their suggestion and herein use the term 'PIMA' in this report. The definite 'feline PIMA' has not been reported. However, we suspect that some reports of feline NRIMA cases in past must be presumptive PIMA. Feline NRIMA like presumptive feline PIMA are also few reported and little information is available describing cats more than dogs. [3, 7, 17, 21, 23, 25]. Feline IMHA is known as likely concurrent with some lymphomas, but feline PIMA concurrent with lymphoma confined to gastrointestinal (GI) have ever been unreported [17]. To the best of our knowledge, this is the first report of feline PIMA concurrent with gastrointestinal (GI) lymphoma.

A 10-year-old, 3.8-kg, spayed female mixed-breed cat was referred to the authors' private veterinary hospital, Akiyoshi Animal Clinic (Kanagawa, Japan) with a 3-week history of anorexia, lethargy and weight loss, which had progressed to nonregenerative anemia. The cat showed nonregenerative anemia prior to referral to our veterinary hospital. At 19 days and 7 days before presentation, the cat's packed cell volume (PCV) were 19% and 11%, and the aggregate reticulocyte concentrations were $2.4 \times 10^3/\mu l$ and $2.8 \times 10^3/\mu l$, respectively. The aggregates reticulocyte concentrations were counted by manual procedure using new methylene blue (NMB) stain. Hypoproteinemia and hypoalbuminemia were not seen on either day. Nonregenerative anemia was persistent for at least 19 days. During the 19 days immediately before presenting at Akiyoshi Animal Clinic, the cat was being treated with maropitant (2 mg/kg/day) and prednisolone (1.3 mg/kg/day) by subcutaneous injection; however, this treatment did

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Table 1. Day 0 results of complete blood cell counts, coagulation test and blood chemistry

		Unit	Reference interval			Unit	Reference interval
RBC	1.79	$\times 10^6/\mu l$	6.54–12.20	Total proteins	6.9	g/d*l*	5.0–7.8
PCV	7	%	30.3–52.3	Albumin	2.7	g/d*l*	2.6–4.0
Hemoglobin	2.3	g/d*l*	9.8–16.2	ALT	27	IU/*l*	17–78
MCV	39.1	f*l*	35.9–53.1	AST	39	IU/*l*	17–44
MCH	12.8	*p*g	11.8–17.3	ALP	103	IU/*l*	47–254
MCHC	32.9	d/d*l*	28.1–35.8	GGT	1	IU/*l*	5.0–14
Aggregate reticulocytes	1.12	$\times 10^3/\mu l$	>15*	Total bilirubin	0.1	mg/d*l*	0.1–0.8
				Ammonia	75	μg/d*l*	16–78
WBC	20,210	/μl	28,70–17,020	Glucose	202	mg/d*l*	78–128
Neutrophils	16,160	/μl	2,300–10,290	Cholesterol	171	mg/d*l*	115–320
Lymphocytes	3,020	/μl	920–6,880	Triglyceride	45	mg/d*l*	30–133
Monocytes	460	/μl	5–670	Urea	11.6	mg/d*l*	10.0–29.2
Eosinophils	510	/μl	170–1,570	Creatinine	0.6	mg/d*l*	0.4–1.4
Basophils	60	/μl	1–260	Phosphorous	6	mg/d*l*	1.9–5.0
Platelets	785	$\times 10^3/\mu l$	151–600	Calcium	10.8	mg/d*l*	9.3–12.1
PT	10	sec	8.0–11.0	Fe	198	μg/d*l*	53–168
APTT	15	sec	10.2–32	TIBC	210	μg/d*l*	211–458
Fibrinogen	202	mg/d*l*	5–300	UIBC	12	μg/d*l*	40–431
AT	100	%	107–141	SAA	93.5	μg/m*l*	<2.5
FDPs	2.5	μg/m*l*	<5	Sodium	154	mmol/*l*	141.0–152.0
D-dimer	0.8	μg/m*l*	<1.5	Potassium	4	mmol/*l*	3.8–5.0
Folate	16.1	μg/*l*	9.7–21.6	Chloride	118	mmol/*l*	102–117
Cobalamin	555	n*g*/*l*	290–1,000	T4	1.9	μg/d*l*	0.9–3.7
				EPO	10.2	mIU/m*l*	1.9–22.9

*If PCV is <20%, aggregate reticulocyte count is definitely to be more than $15 \times 10^3/\mu l$ [3, 16, 23]. RBC, red blood cell; PCV, packed cell volume; MCV, mean cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cell; PT, prothrombin time; APTT, activated partial thromboplastin time; AT, anti-thrombin, FDPs, fibrin degradation products; EPO, erythropoietin; ALT, alanine aminotransferase; AST, asparate aminotransferase; ALP, alkaline phosphatase; GGT, gammma ghutamyl transferase; Fe, iron; TIBC, total iron-binding capacity; UIBC, unsaturated iron-binding capacity; SAA, serum amyloid A.

not resolve the hematological abnormalities.

On presentation, physical examination revealed a fever (41°C) and pale oral mucosa. CBC results (IDEXX ProCyte Dx Hematology Analyzer; IDEXX Laboratories, Tokyo, Japan) revealed a severe, normocytic, normochromic, nonregenerative anemia (PCV: 7%; mean cell volume (MCV): 39.1 f*l*, MCHC, mean corpuscular hemoglobin concentration (MCHC): 32.9 d/d*l*). The aggregates reticulocyte concentrations were counted by manual procedure using NMB stain. The aggregate reticulocyte concentrations were $1.12 \times 10^3/\mu l$ (Table 1). A routine plasma biochemistry (FUJIFILM DRI-CHEM 7000V; FUJIFILM Co., Tokyo, Japan) revealed no abnormalities; however, the serum amyloid A (SAA) was severely increased (Table 1). Serologic tests were negative for the feline leukemia virus (FeLV) antigen and the feline immunodeficiency virus (FIV) antibody (SNAP, FeLV/FIV Combo, IDEXX Laboratories, Tokyo, Japan). The serologic test for feline pancreatic lipase immunoreactivity (f-PLI) was normal (SNAP, f-PLI Kit, IDEXX Laboratories). Feline infectious peritonitis (FIP) was less likely because the serum antibody value was negative for the feline coronavirus (FCoV) (immunofluorescence assay method, IDEXX Laboratories). Systemic radiographs, urinalysis and a fecal examination were normal. No melena was observed. Ultrasonography revealed evidence of a hypoechogenic mass (2.2 × 1.9 cm) well-localized in the stomach (Fig. 1), an enlarged regional lymph node (1.7 × 0.8 cm) and no abnormalities in the other abdominal organs. Direct Coombs test and saline slide agglutination tests at room temperature and at 4°C were performed at a commercial laboratory (FUJIFILM VET Systems, Tokyo, Japan), and they revealed a negative result. Hemostatic tests were no abnormalities (Table 1). Serum iron, total iron-binding capacity (TIBC), unsaturated iron-binding capacity (UIBC), folate, vitamin $B_{12,}$ and erythropoietin (EPO) were examined at a commercial laboratory. Systemic hemorrhaging including gastrointestinal was unlikely because of the increased serum iron concentrations (198 μg/d*l*), decreased serum UIBC concentrations (12 μg/d*l*) and the normal plasma total protein and albumin concentrations (Table 1). Serum TIBC, folate, vitamin B_{12}, and EPO

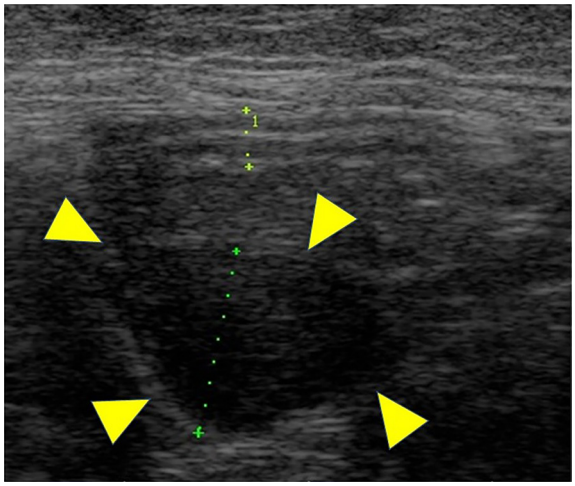

Fig. 1. Ultrasonographic findings of the gastric mass. Arrowheads indicate the hypogenic mass in the stomach.

concentrations were within their normal range of RIs (Table 1). The blood culture examination was negative. Infections with *Anaplasma* spp, *Bartonella* spp, *Cytauxzoon felis, Ehrlichia* spp, feline *Haemoplasma,* FeLV, FIV, and FCoV were ruled out via real-time PCR, which was performed on the peripheral blood at a commercial laboratory (IDEXX Laboratories). No exposure to oxidative toxins was evidenced.

On day 1, the cat received whole blood transfusion (20 m*l*/kg BW, total: 76 m*l*). On the following day, bone marrow (BM) was aspirated at three sites, including the bilateral proximal humerus and proximal femur, and the cat underwent extirpation of the masses in the stomach and regional lymph node (Fig. 2). The stomach mass was removed surgically in the horizontal margin 2 cm and 2 cm part of the omentums in addition to all layer of the stomach in the deep margin.

A 500-cell differential cell counts were performed on each of three BM aspiration smears. The count included mature neutrophils. BM cellularity was high with normal numbers of mature megakaryocytes (5–6 cells per ×100 magnification) [14, 23]. Nine marrow particles and all the particles demonstrated moderate numbers of hemosiderin-laden macrophages, indicating adequate iron stores in this cat [5, 15]. No morphological abnormalities were seen. The myeloid: erythroid (M:E) ratio was low at 0.35:1 (0.35; RI, 1.2–2.2) (Fig. 3). Erythroid series maturation was complete with a moderate left shift characterized by increased rubricytes and prorubricytes. Myeloid series maturation was complete, balanced, and morphologically unremarkable. Polychromatophils were very few observed in the background. Lymphocytes and plasmacytes were within the reference intervals (RI) at 11.3% and 1.5%, respectively [14, 15]. Macrophages occasionally contained phagocytized intact erythroid precursors (Fig. 3). No infectious agents were seen. The real-time PCR of FeLV provirus performed on BM aspiration samples was negative. The cat was diagnosed with presumptive precursor immune-mediated anemia (PIMA). Histologic sections of the gastric mass and gastric lymph node were routinely prepared and examined. Expanding the submucosa and tunica muscularis, several neoplastic tissues proliferated in an individual-to-confluent manner and exhibited coalescing foci (Fig. 4). Each tissue was composed of sheets of slightly atypical round cells with scant eosinophilic cytoplasm, round-to-ovoid hypochromatic nuclei approximately twice the size of a red blood cell, and distinct nucleoli (Fig. 4). The mitotic count was 30 per ten 400× fields. Neoplastic cells resembling centroblasts were infrequently observed (less than 5 per one 400× field). Rarely, neoplastic cells were observed adjacent to the stomach serosa but without breaching it (Fig. 4). No neoplastic invasion was noticed in the blood or lymph vessels. The overlying mucosa was rimmed by hyperplastic, but not neoplastic, mucosa. The gastric mass was diagnosed as transmural lymphoma with large cell types. The surgical margins were achieved completely. The GI lymphoma was completely resected. The gastric lymph node showed no neoplastic changes and was diagnosed with reactive lymph node. Based on these findings, the GI lymphoma was definitively well-localized in the stomach. Immunohistochemical staining was performed for CD3, CD20, and granzyme B using a mouse anti-CD3 monoclonal antibody (1–2 μg/m*l*; Clone, F7.2.38; Thermo Fisher Scientific, Waltham, MA, USA), rabbit anti-CD20 polyclonal antibody (0.1–0.5 μg/m*l*; Thermo Fisher Scientific), and rabbit anti-granzyme B polyclonal antibody (1:100–400 dilution; Spring Bioscience, Pleasanton, CA, USA), respectively. The gastric mass tumor cells

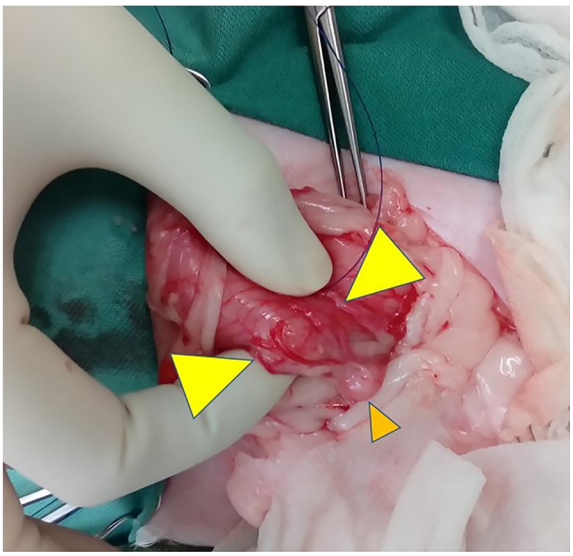

Fig. 2. Macroscopic findings of the gastric mass and the lymph node under open surgery. Yellow and orange arrowheads indicate the gastric mass and lymph node, respectively.

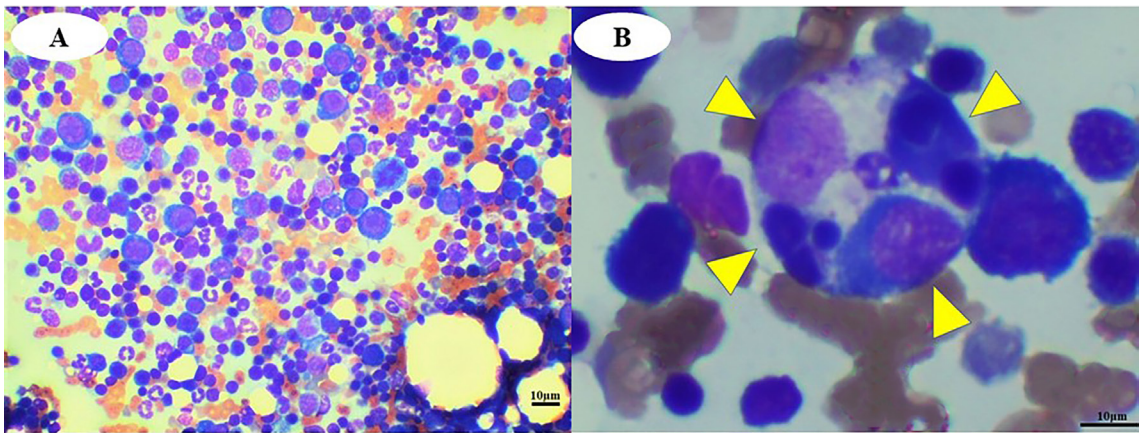

Fig. 3. Cytological smear of the bone marrow (Wright-Giemsa staining). (A) Cellularity is high, with normal-to-high marrow cellularity. Bar=10 μm (B) Arrowheads indicate macrophage phagocytosis. Bar=10 μm.

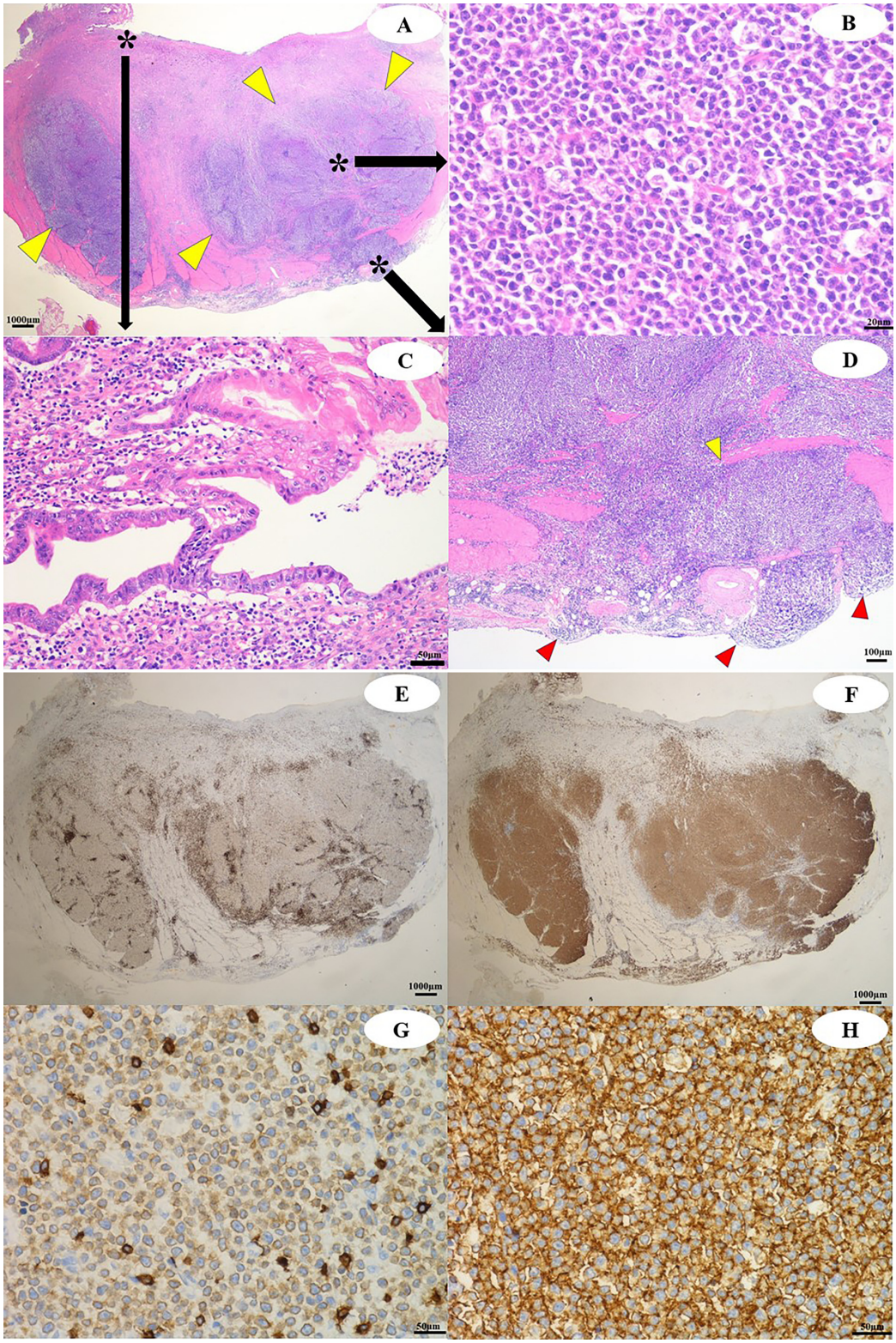

Fig. 4. Histological findings of the gastric mass. (A) Arrowheads indicate several neoplastic tissues. Hematoxylin and eosin (H&E) stain, Bar=1,000 μm. Three asterisks and black arrows detail in (B), (C) and (D), respectively. (B) The lymphoma was composed of sheets of slightly atypical round cells with scant eosinophilic cytoplasm, round-to-ovoid hypochromatic nuclei approximately twice the size of a red blood cell, and distinct nucleoli. H&E stain, Bar=20 μm. (C) The overlying mucosa was rimmed by hyperplastic but not neoplastic mucosa. H&E stain, Bar=50 μm. (D) Yellow and red arrowheads indicate tumor cells expanded to the tunica muscularis and adjacent to the serosa of the stomach, respectively. H&E stain, Bar=100 μm. (E) The hypomagnification and (G) hypermagnification of immunohistochemistry stained by the anti-CD3 antibodies, 3-3′-diaminobenzidine (DAB) chromagen, hematoxylin counterstain. Most neoplastic cells weakly reacted to the anti-CD3 antibodies. (F) The hypomagnification and (H) hypermagnification of immunohistochemistry of immunohistochemistry stained by the anti-CD20 antibody, 3-3′-diaminobenzidine (DAB) chromagen, hematoxylin counterstain. Most neoplastic cells strongly reacted to the anti-CD20 antibody.

reacted strongly to the anti-CD20 antibody and weakly to the anti-CD3 antibody, while no reaction occurred against anti-granzyme B antibodies (Fig. 4). PCR for antigen receptor gene rearrangement (PARR) analysis (Kahotechno Co., Ltd., Fukuoka, Japan) was performed to examine clonal lymphocyte expansion in the GI lymphoma, the reactive lymph node and BM smears. As previously described, genomic DNA was extracted from the GI lymphoma tissue, the lymph node tissue and BM smears [1, 9, 10]. Clonal rearrangements of the T-cell receptor γ (TCRγ) gene were detected on the GI lymphoma only (Fig. 5). No clonal patterns were detected for the immunoglobulin heavy-chain (IgH) gene rearrangement on all samples. The primers and amplification design were based on the previous reports [13, 24]. The GI lymphoma was CD20 positive T-cell lymphoma with transmural morphology.

Consequently, the cat was diagnosed with presumptive PIMA concurrent with primary GI lymphoma. Prednisolone (4 mg/kg, PO, q24 hr) as immunosuppressive therapy and enrofloxacin (8 mg/kg, PO, q24 hr) as the infection prevention after the surgery were administered on day 2 (Fig. 6). The enrofloxacin was stopped on day 16. Chlorambucil (2 mg head, PO, every other day) as immunosuppressive and anti-cancer therapy was administered on day 10 (Fig. 5). On day 30, the anemia and increased SAA were resolved (PCV: 27%, RBCs: 6.65 × $10^6/\mu l$, aggregate reticulocyte concentrations: 19.5 × $10^3/\mu l$; and SAA: 1.2 μg/m*l*) (Fig. 5). PIMA and GI lymphoma did not recur as the prednisolone and chlorambucil were tapered every 2–4 weeks. On day 410, the cat was in good condition without the medications (Fig. 6). The recurrence of PIMA has not been seen and the ultrasonography showed no evidence of GI lymphoma.

This is the first report of a presumptive feline PIMA with definitely T-cell GI lymphoma well-localized in the stomach. PIMA is considered a separate entity within a spectrum of IMHA and is characterized by selective targeting erythroid precursors in the BM [2, 8, 23]. Few reports have been published on canine PIMA, and feline case has not been reported [2, 8, 23]. Therefore, feline PIMA requires further characterization including cause,

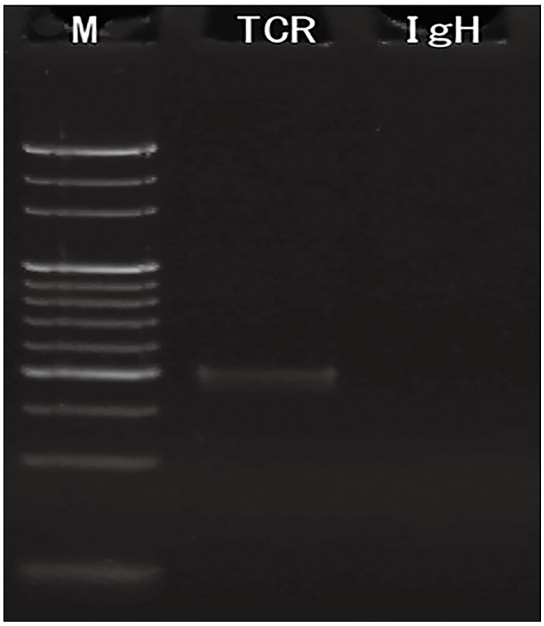

Fig. 5. Electrophoresis of PCR for antigen receptor gene rearrangement (PARR) PCR amplification derived from the gastrointestinal lymphoma tissue. Left lane; M indicates a marker (20 bp DNA ladder, Takara: Kusatsu, Japan, 3409A), Middle lane; T-cell receptor γ (TCRγ), Right lane; immunoglobulin heavy-chain (IgH). A single strict PCR amplification around 100 bp was amplified by TCRγ specific primers, indicating that T cell γ rearrangement was detected, but amplification of IgH was not seen.

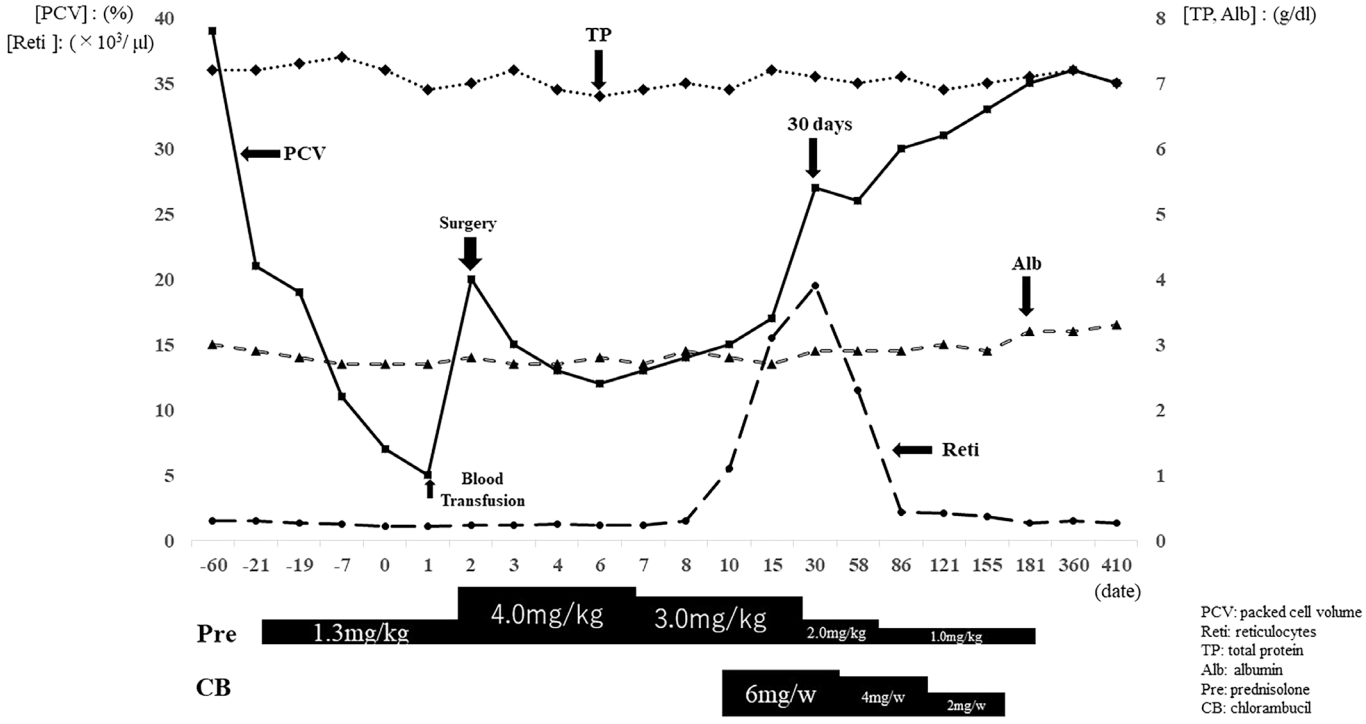

Fig. 6. Clinical course of this case, the graph shows packed cell volume (PCV) transition and each drug's dose transition. Reti indicates aggregate reticulocyte concentrations. Pre and CB indicate prednisolone and chlorambucil, respectively.

clinicopathological finding, therapeutic protocol and prognosis. In this disorder, rubriphagocytosis characterized by phagocytosis of intact erythroid precursors is usually seen in PIMA cases but is nonpathognomonic for this disease [2, 8]. The previous study to characterize canine PIMA characterized it by 1) persistent nonregenerative anemia, 2) ineffective erythropoiesis, and 3) absence of dysplasia of any lineage [2, 8]. Nonregenerative anemia in cats is defined as PCV <20% with aggregate reticulocyte count $<15 \times 10^3/\mu l$ by some previous reports [3, 17, 25]. The present case showed persistent nonregenerative anemia without the presence of aggregate reticulocytosis. Generally, ineffective erythropoiesis is defined as persistent nonregenerative anemia accompanied by increased particles hematopoietic cellularity with decreased M:E ratios in BM and an absence of aggregate reticulocytosis [2, 3, 8, 25]. The cat in the present study had increased cellularity with decreased M:E ratios in the BM and persistent nonregenerative anemia without the presence of aggregate reticulocytosis in peripheral blood. The cat had definitive ineffective erythropoiesis. As other differentiate diagnosis of ineffective erythropoiesis, there are FeLV infection, iron deficiency including gastrointestinal hemorrhage, myelodysplastic syndrome (MDS), BM involvement by neoplastic tumor, and, rarely, folate or cobalamin deficiency [14, 15]. The latter two were excluded out in this cat. FeLV infection was excluded by negative of antigen detection serology, and real-time PCR in the peripheral blood and BM samples, respectively. Gastrointestinal hemorrhage was unlikely since plasma total protein and albumin concentrations were normal range, and clinical signs also were not seen. Iron deficiency was ruled out by increased serum iron concentration, decreased serum UIBC concentration, and presence of numerous BM hemosiderin-containing macrophages, indicating that the BM iron stores were adequate. It is reported that healthy cats lack detectable marrow iron stores, but adequate BM iron stores in cat having immune-mediated anemia (IMA) including IMHA and PIMA are present [15, 17]. We believe that the increased serum iron concentration and decreased UIBC concentration resulted from the PIMA and indicate ineffective BM erythropoiesis in this cat. The present case lacked dysplasia of any lineage and MDS was excluded. Histiocytic diseases including histiocytic sarcoma and hemophagocytic syndrome were excluded based on the absence of malignant findings and a cut-off of <2% hemophagocytic histiocytes [22]. The lymphocytes and plasmacytes in the BM were within the RI [14, 15]. However, PARR was performed and negative in the BM; thus, BM lymphoid neoplasia was excluded. As a result, the present case was diagnosed with presumptive PIMA.

In previous literature, GI lymphomas were classified as B-cell, T-cell, non-B-cell, or non-T-cell lymphomas by immunohistochemistry to detect CD3 and CD79a or CD20 expressions [11, 12, 18, 26, 27]. These GI lymphomas were then further classified as mucosal or transmural lymphomas [12, 26, 27]. The present case was diagnosed as transmural lymphoma with infiltration of large lymphocytes. Feline transmural lymphomas were reported as diffuse large cell B-cell lymphoma (DLBCL), enteropathy-associated T-cell lymphoma (EATCL) type I with/without LGL lymphocytes morphology [12]. This cat was diagnosed with transmural T-cell lymphoma well-localized in the stomach without LGL morphology, because no reaction occurred against anti-granzyme B antibodies and clonal patterns for the TCRγ gene were positive, and IgH gene was negative. In addition, the present lymphoma was CD20 positive T-cell lymphoma by detection of clonal patterns for the TCRγ gene, although the GI lymphoma reacted to both the anti-CD20 antibody (strongly positive) and anti-CD3 antibody (weakly positive). Rarely, it is difficult to made lymphoma classification based on only the result of immunohistochemistry in other species since CD20 positive T-cell lymphoma of GI lymphoma has been reported in humans and dogs [6, 16]. Some studies described that activated T-cell lymphoma was likely reacted to anti-CD20 antibody [6, 16]. The GI T-cell lymphoma of present cat was double-positive in CD20 and CD3.

The present case had good prognosis by combination of complete surgical resection, prednisolone and chlorambucil. The majority of transmural lymphomas in stomach are known as diffuse, large B-cell lymphomas (DLBCL) characterized by centroblastic type and high mitotic rate, and thus have poor prognosis (median survival time: 3.5months) [12]. However, the present case was not DLBCL but CD20 positive T-cell lymphoma with transmural high-grade morphology and has been having long prognosis. The prognosis of feline CD20 positive T-cell lymphoma in stomach is unknown. However, we consider that aggressive resection of discrete GI lymphoma well-localized in the stomach may be a reasonable consideration, even if the GI lymphoma is expected as high-grade lymphoma. The two latest studies reported that feline discrete intermediate- or large-cell GI lymphoma with complete surgical margins may survive longer than with incomplete margin or without surgical lymphoma masses resection [4, 20]. Probably, complete surgical margins in this case contribute to have good prognosis.

Secondary PIMA may result from several causes such as neoplasia, infectious diseases, and inflammatory diseases. The SAA, like C-reactive protein, is an acute-phase protein. Increased serum SAA levels are triggered by physical injury to the host, including infectious diseases, inflammatory diseases and neoplasia [19]. In our case, many infections were most likely ruled out based on PCRs and bacterial culture of the blood. Inflammatory diseases, including cholangitis and pancreatitis, were less likely in the present case, because the liver enzymes and f-PLI were within the reference intervals; therefore, the cause of the increased SAA was considered GI lymphoma. We considered that the present case was not differentiated secondary to lymphoma or primary because there is no reliable test to differentiate between the 2 pathogenesis in veterinary medicine. Therefore, the presence of concurrent GI lymphoma and PIMA in our case may represent two comorbidities rather than PIMA secondary to GI lymphoma.

In the previous report, some lymphomas have been suspected to be likely associated with the occurrence of IMA, such as IMHA [17]. IMHA involves the immune system targeting mature RBCs, which results in hemolysis, and is often accompanied by spherocytes, agglutination, or Coombs' positivity. Swann *et al.* reported that primary IMHA occurred more commonly than secondary and nonregenerative anemia were seen in 25.4% (16/63) and 29.6% (8/27) in primary and secondary IMHA, respectively [17]. Furthermore, erythrophagocytosis in BM was seen in 40% (26/65) with IMHA, and some cats showed erythroid precursors phagocytosis by macrophage [17]. We consider some of those IMHA cases with nonregenerative anemia actually must be PIMA. The numbers of cats diagnosed as PIMA will increase by definitive cytological BM examination, further study will be needed in future.

To the best of our knowledge, this is the first report of concurrent with feline PIMA and GI lymphoma. This case represents a potential novel information for PIMA with GI lymphoma in cats, which resolved following immunosuppressive therapy that

includes anti-cancer efficiency and completely resection of lymphoma. Further cases may be identified with BM aspiration on GI lymphoma, particularly in nonregenerative anemia. Further clinical study and more data are required to clarify a causal link between PIMA and GI lymphoma.

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REFERENCES

1. Akiyoshi, M., Hisasue, M., Neo, S., Akiyoshi, M. and Goto-Koshino, Y. 2019. A case of hemophagocytic syndrome progressing into large granular lymphoma in a dog. *Vet. Clin. Pathol.* **48**: 71–77. [Medline] [CrossRef]
2. Assenmacher, T. D., Jutkowitz, L. A., Koenigshof, A. M., de A Lucidi, C. and Scott, M. A. 2019. Clinical features of precursor-targeted immune-mediated anemia in dogs: 66 cases (2004-2013). *J. Am. Vet. Med. Assoc.* **255**: 366–376. [Medline] [CrossRef]
3. Black, V., Adamantos, S., Barfield, D. and Tasker, S. 2016. Feline non-regenerative immune-mediated anaemia: features and outcome in 15 cases. *J. Feline Med. Surg.* **18**: 597–602. [Medline] [CrossRef]
4. Gouldin, E. D., Mullin, C., Morges, M., Mehler, S. J., de Lorimier, L. P., Oakley, C., Risbon, R., May, L., Kahn, S. A. and Clifford, C. 2017. Feline discrete high-grade gastrointestinal lymphoma treated with surgical resection and adjuvant CHOP-based chemotherapy: retrospective study of 20 cases. *Vet. Comp. Oncol.* **15**: 328–335. [Medline] [CrossRef]
5. Hughes, D. A., Stuart-Smith, S. E. and Bain, B. J. 2004. How should stainable iron in bone marrow films be assessed? *J. Clin. Pathol.* **57**: 1038–1040. [Medline] [CrossRef]
6. Kakinoki, Y., Hashiguchi, J., Ishio, T., Chiba, K., Niino, D. and Ohshima, K. 2015. CD20-positive primary gastric T-cell lymphoma poorly responding to initial treatment with rituximab plus CHOP, and a literature review. *Int. J. Hematol.* **102**: 702–708. [Medline] [CrossRef]
7. Kohn, B., Weingart, C., Eckmann, V., Ottenjann, M. and Leibold, W. 2006. Primary immune-mediated hemolytic anemia in 19 cats: diagnosis, therapy, and outcome (1998–2004). *J. Vet. Intern. Med.* **20**: 159–166. [Medline]
8. Lucidi, C. A., de Rezende, C. L. E., Jutkowitz, L. A. and Scott, M. A. 2017. Histologic and cytologic bone marrow findings in dogs with suspected precursor-targeted immune-mediated anemia and associated phagocytosis of erythroid precursors. *Vet. Clin. Pathol.* **46**: 401–415. [Medline] [CrossRef]
9. Mochizuki, H., Nakamura, K., Sato, H., Goto-Koshino, Y., Sato, M., Takahashi, M., Fujino, Y., Ohno, K., Uchida, K., Nakayama, H. and Tsujimoto, H. 2011. Multiplex PCR and Genescan analysis to detect immunoglobulin heavy chain gene rearrangement in feline B-cell neoplasms. *Vet. Immunol. Immunopathol.* **143**: 38–45. [Medline] [CrossRef]
10. Mochizuki, H., Nakamura, K., Sato, H., Goto-Koshino, Y., Sato, M., Takahashi, M., Fukushima, K., Nakashima, K., Fujino, Y., Ohno, K., Uchida, K., Nakayama, H. and Tsujimoto, H. 2012. GeneScan analysis to detect clonality of T-cell receptor γ gene rearrangement in feline lymphoid neoplasms. *Vet. Immunol. Immunopathol.* **145**: 402–409. [Medline] [CrossRef]
11. Moore, A. 2013. Extranodal lymphoma in the cat: prognostic factors and treatment options. *J. Feline Med. Surg.* **15**: 379–390. [Medline] [CrossRef]
12. Moore, P. F., Rodriguez-Bertos, A. and Kass, P. H. 2012. Feline gastrointestinal lymphoma: mucosal architecture, immunophenotype, and molecular clonality. *Vet. Pathol.* **49**: 658–668. [Medline] [CrossRef]
13. Moore, P. F., Woo, J. C., Vernau, W., Kosten, S. and Graham, P. S. 2005. Characterization of feline T cell receptor gamma (TCRG) variable region genes for the molecular diagnosis of feline intestinal T cell lymphoma. *Vet. Immunol. Immunopathol.* **106**: 167–178. [Medline] [CrossRef]
14. Rizzi, T. E., Clinkenbeard, K. D. and Meinkoth, J. H. 2010. Normal hematology of the cat. pp. 811–820. *In*: Schalm's Veterinary Hematology, 6th ed. (Weiss, D. J. and Wardrop, K. J. eds.), Wiley-Blackwell, Ames.
15. Stockham, S. L. and Scott, M. A. 2008. Bone marrow and lymph node. pp. 323–368. *In*: Fundamentals of Veterinary Clinical Pathology, 2nd ed. (Stockham, S. L. and Scott, M. A. eds.), Blackwell Publishing, Ames.
16. Suwa, A. and Shimoda, T. 2017. Concurrent with T-zone lymphoma and high-grade gastrointestinal cytotoxic T-cell lymphoma in a dog. *J. Vet. Med. Sci.* **79**: 736–739. [Medline] [CrossRef]
17. Swann, J. W., Szladovits, B. and Glanemann, B. 2016. Demographic Characteristics, Survival and Prognostic Factors for Mortality in Cats with Primary Immune-Mediated Hemolytic Anemia. *J. Vet. Intern. Med.* **30**: 147–156. [Medline] [CrossRef]
18. Swerdlow, S. H., Campo, E., Harris, N. L. and Jaffe, E. S. and Pileri, S. A. 2017. WHO classification of tumors of haematopoietic and lymphoid tissues. World Health Organization.
19. Tamamoto, T., Ohno, K., Takahashi, M., Nakashima, K., Fujino, Y. and Tsujimoto, H. 2013. Serum amyloid A as a prognostic marker in cats with various diseases. *J. Vet. Diagn. Invest.* **25**: 428–432. [Medline] [CrossRef]
20. Tidd, K. S., Durham, A. C., Brown, D. C., Velovolu, S., Nagel, J. and Krick, E. L. 2019. Outcomes in 40 cats with discrete intermediate- or large-cell gastrointestinal lymphoma masses treated with surgical mass resection (2005-2015). *Vet. Surg.* **48**: 1218–1228. [Medline] [CrossRef]
21. Weiss, D. J. 2005. Differentiating benign and malignant causes of lymphocytosis in feline bone marrow. *J. Vet. Intern. Med.* **19**: 855–859. [Medline] [CrossRef]
22. Weiss, D. J. 2007. Hemophagocytic syndrome in dogs: 24 cases (1996–2005). *J. Am. Vet. Med. Assoc.* **230**: 697–701. [Medline] [CrossRef]
23. Weiss, D. J. 2008. Bone marrow pathology in dogs and cats with non-regenerative immune-mediated haemolytic anaemia and pure red cell aplasia. *J. Comp. Pathol.* **138**: 46–53. [Medline] [CrossRef]
24. Werner, J. A., Woo, J. C., Vernau, W., Graham, P. S., Grahn, R. A., Lyons, L. A. and Moore, P. F. 2005. Characterization of feline immunoglobulin heavy chain variable region genes for the molecular diagnosis of B-cell neoplasia. *Vet. Pathol.* **42**: 596–607. [Medline] [CrossRef]
25. Winzelberg Olson, S. and Hohenhaus, A. E. 2019. Feline non-regenerative anemia: Diagnostic and treatment recommendations. *J. Feline Med. Surg.* **21**: 615–631. [Medline] [CrossRef]
26. Wolfesberger, B., Skor, O., Hammer, S. E., Flickinger, I., Kleiter, M., Rütgen, B. C., Schwendenwein, I., Tichy, A., Hittmair, K. M., Degasperi, B. and Fuchs-Baumgartinger, A. 2017. Does categorisation of lymphoma subtypes according to the World Health Organization classification predict clinical outcome in cats? *J. Feline Med. Surg.* **19**: 897–906. [Medline] [CrossRef]
27. Wolfesberger, B., Fuchs-Baumgartinger, A., Greß, V., Hammer, S. E., Gradner, G., Knödl, K., Tichy, A., Rütgen, B. C. and Beham-Schmid, C. 2018. World Health Organisation classification of lymphoid tumours in veterinary and human medicine: a comparative evaluation of gastrointestinal lymphomas in 61 cats. *J. Comp. Pathol.* **159**: 1–10. [Medline] [CrossRef]