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 × 10^3/µl) 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

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 × 10^3/µl and 2.8 × 10^3/µl, 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
RBC 1.79 × 10⁶/µl  6.54–12.20  Total proteins  6.9 g/dl  5.0–7.8
PCV 7 %  30.3–52.3  Albumin  2.7 g/dl  2.6–4.0
Hemoglobin 2.3 g/dl  9.8–16.2  ALT 27 IU/l  17–78
MCV 39.1 fl  35.9–53.1  AST 39 IU/l  17–44
MCH 12.8 pg  11.8–17.3  ALP 103 IU/l  47–254
MCHC 32.9 d/dl  28.1–35.8  GGT 1 IU/l  5.0–14
Aggregate reticulocytes 1.12 × 10³/µl  >15*  Total bilirubin 0.1 mg/dl  0.1–0.8
WBC 20,210 /µl  28.70–17.020  Glucose 202 mg/dl  78–128
Neutrophils 16,160 /µl  2,300–10,290  Cholesterol 171 mg/dl  115–320
Lymphocytes 3,020 /µl  920–6,880  Triglyceride 45 mg/dl  30–133
Monocytes 460 /µl  5–670  Urea 11.6 mg/dl  10.0–29.2
Eosinophils 510 /µl  170–1,570  Creatinine 0.6 mg/dl  0.4–1.4
Basophils 60 /µl  1–260  Phosphorous 6 mg/dl  1.9–5.0
Platelets 785 × 10³/µl  50–1,000  Calcium 2.5 mg/dl  1.8–2.9
Fibrinogen 202 mg/dl  5–300  IUBC 12 µg/dl  40–431
AT 100 %  107–141  SAA 93.5 µg/ml  <2.5
D-dimer 2.5 µg/ml  <5  Sodium 154 mmol/l  141.0–152.0
D-fib 0.8 µg/ml  <1.5  Potassium 3.8–5.0
Folate 16.1 µg/l  9.7–21.6  Chloride 118 mmol/l  102–117
Cobalamin 555 ng/l  290–1,000  T4 1.9 µg/dl  0.9–3.7

*If PCV is <20%, aggregate reticulocyte count is definitely to be more than 15 × 10³/µl [3, 16, 23].

The aggregate reticulocyte concentrations were 1.12 × 10³/µl (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 coronavirus (FCoV) (immunofluorescence assay method, IDEXX Laboratories). Systemic hemorrhaging including gastrointestinal bleeding was unlikely because of the increased serum iron concentrations (198 µg/dl), decreased serum UIBC concentrations (12 µg/dl) and the normal plasma total protein and albumin concentrations (Table 1). Serum TIBC, folate, vitamin B₁₂, and EPO 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 fl, MCHC, mean corpuscular hemoglobin concentration (MCHC): 32.9 d/dl). The aggregates reticulocyte concentrations were counted by manual procedure using NMB stain. The aggregate reticulocyte concentrations were 1.12 × 10³/µl (Table 1).

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Feline Pima with GI lymphoma

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, *Cytotauxzoon 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 ml/kg BW, total: 76 ml). 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. Polychromatophilis 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].

Macrofages 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/ml; Clone, F7.2.38; Thermo Fisher Scientific, Waltham, MA, USA), rabbit anti-CD20 polyclonal antibody (0.1–0.5 µg/ml; 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. 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 × 10⁶/µl, aggregate reticulocyte concentrations: 19.5 × 10³/µl; and SAA: 1.2 µg/ml) (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,
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 lymphomas 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. Infectious 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, erythropagocytosis 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 various diseases. J. Vet. Diagn. Invest. 25: 428–432. [Medline] [CrossRef]

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