Primary Immune-Mediated Thrombocytopenia Tentatively Diagnosed in Four Cats

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ABSTRACT

Feline primary immune-mediated thrombocytopenia (pIMT) is uncommon, and to the best of the authors' knowledge, scarcely reported. pIMT is mostly diagnosed based on excluding primary causes inducing secondary IMT, and observing positive response to immunosuppressive therapy. Definitive diagnosis of IMT requires immunoassays, mostly unavailable, demonstrating anti-platelet or anti-megakaryocyte antibodies. Here we describe four severely thrombocytopenic cats, aged 1.5 to 10 years, presented to the Hebrew University Veterinary Teaching Hospital, tentatively diagnosed with pIMT. Clinical signs at presentation included pale mucous membranes, extreme weakness to collapse, and bleeding. Platelet counts raged from 5 to $11x10^3/\mu L$ (reference interval, $156.4\text{-}626.4x10^3/\mu L$). All cats were treated with prednisolone. In two cats, both recovered, prednisolone was the single immunosuppressive agent used. Another cat, partially and insufficiently responded to prednisolone, thus, additionally, received mycophenolate-mofetil (MMF), leading to remission. The remaining cat, with insufficient response to prednisolone, additionally received, over time, MMF, followed by leflunomide, resulting in a partial response. The thrombocytopenia had partially improved, however long-term prednisolone therapy was still required, eventually resulting in diabetes mellitus. This is the first report of MMF treatment in feline pIMT. With only four cases over the 2-year study period, feline pIMT was an uncommon cause of thrombocytopenia in our hospital. In some cats, profound thrombocytopenia at presentation led to severe hemorrhagic anemia, mostly due to gastrointestinal bleeding, posing a serious life-threatening risk. Prednisolone treatment alone induces remission in some cats, but when response to prednisolone is insufficient, or with recurrence, additional immunosuppressive drugs are needed. The overall prognosis in this small cohort was good.

Keywords: Feline; Platelets; Immunosuppressive therapy; Prednisolone; Mycophenolate-mofetil.

INTRODUCTION

Thrombocytopenia is a common laboratory abnormality in cats (1–4). Nevertheless, often, it is a spurious finding (i.e., pseudothrombocytopenia), due to exclusion of platelets from the count for various causes (5), including platelet clumping, and 'threshold failure' (mostly, when the complete blood count is done using impedance particle analyzers), due to overlapping particle volumes of feline red blood cells and platelets (1, 6–7). Low platelet counts, measured by impedance particle analyzers, are common in cats, however, confirmed true thrombocytopenia by blood smear examination occurred in only 3.1% of such cases (6). Microscopic manual platelet counting, or evaluation, are therefore imperative to validate the automated platelet count in feline blood samples, especially in the face of thrombocytopenia (2, 6-8). The prevalence of true thrombocytopenia in cats has been recorded as 5.9% (7).

Generally, true thrombocytopenia results from reduced production, increased destruction, increased consumption and splenic sequestration of platelets, or their combinations (1, 4, 7). In immune mediated thrombocytopenia (IMT), platelets are destroyed by the immune system (3, 4). IMT might be primary (idiopathic; pIMT; also termed immune thrombocytopenic purpura – ITP), or secondary (sIMT) (3, 4). The reported occurrence of IMT in cats highly varies among thrombocytopenic cats, from very low (0.42%) among 41 cats (7), to 11% among 194 cats (1). In cats, most IMT cases are secondary, due to infections (e.g., feline infectious peritonitis [FIP], feline immunodeficiency virus [FIV], feline leukemia virus [FeLV] and pyelonephritis), other immune mediated diseases (e.g., systemic lupus erythematosus), drugs (e.g., griseofulvin, doxorubicin, azathioprine, carboplatin, propylthiouracil, and ribavirin), neoplasia (e.g., lymphoma and leukemia) and blood transfusion (4). Such conditions might trigger antiplatelet antibody formation, with increased platelet destruction by mononuclear phagocytic cells (1, 4, 7). pIMT alone, or combined with immune-mediated hemolytic anemia (IMHA), although common in dogs (4), is very uncommon in cats, with < 30 cases reported in the literature (1, 7, 9-13).

The clinical signs of severe pIMT, as with any severe thrombocytopenia, include oral, gingival, and vaginal mucous membrane and skin epidermal and dermal petechiae and ecchymoses, epistaxis, hematuria, melena, hematochezia, and bleeding secondary to minor trauma (e.g., during grooming) (9, 11–12, 14).

The definitive diagnosis of IMT is challenging, because direct and indirect immunoassays to detect bindable or bound anti-platelet and anti-megakaryocyte antibodies, often based on flow cytometry (9, 14–17), are unavailable under most clinical settings, especially so for cats (14). Therefore, pIMT is diagnosed under most clinical settings by confirming a severe thrombocytopenia, excluding all underlying diseases potentially inducing sIMT, and lastly, observing a favorable response to immunosuppressive therapy (1, 2).

This report describes the clinical and laboratory findings, the treatment and outcome of four cats putatively diagnosed with pIMT, presented to the Hebrew University Veterinary Teaching Hospital (HUVTH) between years 2018 to 2020.

MATERIALS AND METHODS

Selection of cats

This retrospective case-series includes four cats presented to the HUVTH with confirmed severe thrombocytopenia, and tentatively diagnosed with pIMT during years 2018 to 2020. Data was collected from their medical records.

Laboratory Methods

At the HUVTH, blood for complete blood count (CBC; Advia 2120i, Siemens, Erfurt, Germany). FIV antibodies and FeLV antigen (SNAP* Combo Plus, IDEXX Laboratories, Westbrook, ME, USA) were collected in potassium-EDTA tubes. The packed cell volume (PCV) was measured manually by centrifuging blood in capillaries containing heparin, while total plasma protein (TPP) was measured using a clinical refractometer (Atago, Tokyo, Japan). Blood samples for serum chemistry (Cobas 6000, Roche, Mannheim, Germany; at 37° C) were collected in tubes containing no anticoagulant, with gel separators, allowed to clot, centrifuged, and harvested sera were immediately analyzed. Blood samples for hemostatic tests were collected into 3.2% trisodium-citrate tubes. Thromboelastometry (Rotem Delta, TEM, Munich, Germany) was run in citrated whole blood, within 30 minutes from collection, for three hours. Citrated plasma was harvested within 30 minutes from collection. Hemostatic tests, including prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen concentration (Claus method) and antithrombin activity (ATA) were measured using a coagulometric analyzers (ACL-300 Top, Instrumentation Laboratory, Milano, Italy). Citrated plasma D-dimer concentration was measured using a latex particle-enhanced immunoturbidimetric assay (Tina-quant D-dimer Gen 2; Roche, Mannheim, Germany; Analyzer, Cobas 6000, Roche, Mannheim, Germany; at 37° C). All analyses (excluding thromboelastometry) were completed within 60 minutes from collection.

CASE REPORTS

Cat 1

A 6.5-year-old (body weight [BW], 4.5 kg), neutered female domestic shorthair (DSH) cat, living outdoors was referred to the HUVTH due to severe anemia and thrombocytopenia. Two weeks prior to presentation, the cat went missing for

48 hours, and was later found non-ambulatory. She was then presented to the referring veterinarian. Survey radiography showed right hip joint luxation and sacral fracture, suggestive of a previous trauma. The CBC revealed borderline hematocrit (24.47%; reference interval [RI], 24-45%), leukocyte count (WBCC) within RI (12.26x10^3/μL; RI, 5.5-19.5 x10^3/μL) and severe thrombocytopenia (platelets, 44x10^3/μL; RI, 300-800 x10^3/μL). Serum chemistry was unremarkable.

The cat was hospitalized and received supportive care, and was discharged one day later, when the appetite and ambulation had improved. Two days later, when presented for surgical repair of a hip joint luxation, repeat CBC showed severe anemia (hematocrit, 18%). She was then discharged, and prescribed amoxicillin-clavulanic acid (Synulox, Zoetis, Catania, Italy; 15 mg/kg q12h PO) and doxycycline (Doxylin, Dexcel, Or-Akiva, Israel; 10 mg/kg q24h PO). Two days later, with listlessness and decreased appetite, the anemia had worsened (hematocrit, 15%). The cat received two units of matched packed red blood cells (pRBC). Thoracic survey radiography and abdominal ultrasonography findings were unremarkable. Bacterial infection of the tail area due to the trauma was tentatively diagnosed, and the tail was surgically amputated two days prior to presentation to the HUVTH. The cat became anorexic, and with no improvement in clinical signs, was referred to the HUVTH.

At presentation to the HUVTH, physical examination abnormalities included pale mucous membranes, tachycardia (heart rate [HR], 220 bpm), with galloping heart rhythm. CBC showed severe, marked normocytic normochromic non-regenerative anemia (hematocrit, 13 %; RI, 27.7-46%; reticulocytes, 24.8x10^9/L; RI, 15.0-81.0x10^9/L), neutrophilic leukocytosis (WBCC, 39.4x10^3/μL; RI, 6.3-19.6x10^3/μL) and severe thrombocytopenia (platelets, 10x10^3/μL; RI, 156.4-626.4 x10^3/μL), confirmed microscopically (Table 1). Microscopic blood smear examination showed absence of polychromasia, mild neutrophilic cytoplasmic toxicity and moderate neutrophil left shift. Serum creatinine concentration was unremarkable (Table 2).

In-saline slide agglutination test was positive, although this result should be interpreted cautiously in light of the previously administered blood transfusions (Table 1). FIV and FeLV serology was negative (Table 1). The activated partial thromboplastin time (aPTT) was prolonged (infinite; RI, 12.3-16.7 sec) (Table 1). Thromboelastometry showed hypocoagulability (i.e., prolonged clot formation time and decreased α-angle, amplitudes at 10 and 20 minutes, and maximal clot firmness). Abdominal sonography was unremarkable.

The severe thrombocytopenia was then tentatively diagnosed as pIMT, which most likely led to severe gastrointestinal bleeding, and the deterorating anemia, supported by presence of melena and hematochezia noted later, during hospitalization.

Treatment included prednisolone (Danalone, Trima, Maabarot, Israel; 2 mg/kg PO q12h), doxycycline and marbofloxacin (Marbocyl, Vetoquinol, Towcester, UK; 5 mg/kg PO q24h) and an additional pRBC unit. On day 7 of hospitalization, the manual platelet count was 30x10^3/μL. The cat had regained appetite, was alert, with no overt clinical bleeding, and was discharged. Prednisolone, marbofloxacin (5 mg/kg PO q24h), omeprazole (1 mg/kg PO q24h) and mirtazapine gel (0.6 mg/kg topically q24h) were prescribed.

Twenty-one days post-discharge the platelet count further increased (135x10^3/μL), and the hematocrit had normalized (39.7%). With follow-up at the referring clinic, prednisolone dose was gradually (approximately 25-50%, every 2-3 weeks) and progressively tapered, up to complete withdrawal. No recurrence was noted four months post cessation of steroid therapy.

Cat 2

A 1.5-year-old (BW, 2.2 kg), neutered female Scottish fold, strictly indoors cat, with no prior medical history, was presented to the HUVTH following an acute collapse episode. Several hours prior to the presentation, she was found in lateral recumbency, with open-mouth breathing, and was immediately brought to the HUVTH. Physical examination abnormalities included extreme weakness, lateral recumbency, hypothermia, pale mucous membranes, dehydration (7%), tachypnea (respiratory rate, 48 breaths/min), tachycardia (HR, 228 bpm) and severe hypotension (unmeasurable arterial blood pressure).

CBC showed severe thrombocytopenia (platelets, 8x10^3/μL), confirmed microscopically, and non-regenerative anemia (hematocrit, 8.2%) (Table 1). Serum chemistry revealed several abnormalities (Table 2). In-saline slide agglutination test was negative. Serology for FIV and FeLV was negative (Table 1). Abdominal sonography was unremarkable.

The cat was hospitalized, and administered intravenous crystalloids, colloids, fresh frozen plasma and pRBC,

Table 1: Complete blood count, retrovirus serology, clotting times, red blood cell osmotic fragility test and in-saline slide agglutination test results of four cats tentatively diagnosed with primary immune-mediated thrombocytopenia at presentation to the Hebrew University Veterinary Teaching Hospital.

Analyte (Units)	Cat 1	Cat 2	Cat 4	Cat 3	Reference Interval
Leukocytes ($x10^3/\mu L$)	39.4	15.8	28.0	12.7	6.3-19.6
Neutrophils ($x10^3/\mu L$)	35.73	11.86	27.07	7.97	3.0-13.4
Monocytes ($x10^3/\mu L$)	0.26	0.83	0.23	0.45	0.0-1.0
Lymphocytes ($x10^3/\mu L$)	2.44	2.9	0.53	3.88	2.0-7.2
Eosinophils ($x10^3/\mu L$)	0.48	0.09	0.06	0.27	0.3-1.7
Basophils ($x10^3/\mu L$)	0.03	0.01	0.03	0.02	0.0-0.1
Large unclassified cells ($x10^3/\mu L$)	0.46	0.06	0.07	0.07	0.0-0.2
Red blood cells ($x10^6/\mu L$)	2.6	1.9	7.86	1.3	6.0-10.1
RDW (%)	22.7	22.0	19.8	20.4	14.4-19.4
Hematocrit (%)	12.2	8.2	29.4	7.4	27.7-46.8
Hemoglobin (g/dL)	4.1	2.7	9.8	2.1	8.1-14.2
Mean corpuscular volume (fL)	46.5	43.5	37.4	59.4	41.3-52.6
Hemoglobin concentration distribution width (g/dL)	2.85	2.69	3.04	2.66	1.6-2.9
Mean corpuscular hemoglobin (pg)	15.8	14.5	12.5	16.9	12.0-16.0
Mean corpuscular hemoglobin (pg)	15.1	14.4	13.3	17.1	12.0-16.0
MCHC (g/dL)	34.0	33.4	33.3	28.4	27.0-32.8
CHCM (g/dL)	32.6	33.4	35.7	28.9	26.9-33.0
Reticulocytes (%)	0.95	2.2	0.66	10.04	0-1.3
Reticulocytes ($x10^9/L$)	24.8	41.4	52.1	125.6	15.0-81.0
Platelets ($x10^3/\mu L$)	10.0	8.0	11.0	5.0	156.4-626.4
Mean platelet volume (fL)	12.4	16.4	24.0	21.7	8.6-18.9
Platelet crit (PCT) (%)	0.01	0.01	0.03	0.01	0.3-0.8
Platelet distribution width (fL)	106.1	93.9	64.3	61.7	46.3-80.0
Packed cell volume[1] (%)	13.0	8.0	30.0	8.0	31-48
Total plasma solids[2] (g/dL)	6.0	5.0	8.0	6.0	5.9-7.5
Feline immunodeficiency virus antibody	negative	negative	negative	negative	
Feline leukemia virus antigen	negative	negative	negative	negative	
Prothrombin time (sec)	10.3	ND	13.9	17.9	8.7-10.5
Activated partial thromboplastin time (sec)	infinite	ND	71.7	44.1	12.3-16.7
In-saline slide agglutination test	positive	negative	positive	negative	
Red blood cell osmotic fragility test	ND	positive	positive	ND	

RDW, red blood cell distribution width;
MCHC, mean corpuscular hemoglobin concentration;
CHCM, corpuscular hemoglobin concentration;
ND, not done;
[1], Measured manually by centrifuging whole blood in heparinized capillaries;
[2], Measured by refractometry.

Table 2: Serum chemistry results of four cats tentatively diagnosed with primary immune-mediated thrombocytopenia at presentation to the Hebrew University Veterinary Teaching Hospital.

Analyte (Units)	Cat 1	Cat 2	Cat 3	Cat 4	Reference Interval
Creatine kinase (U/L)		1976		585	73-260
Aspartate transaminase (U/L)		41		25	17-58
Alanine transaminase (U/L)		16	327	44	27-101
Alkaline phosphatase (U/L)		10		5	14-71
Gamma-glutamyl transferase (U/L)		<3		<3	0-4
Amylase (U/L)		687		701	500-1800
Triglycerides (mg/dL)		72		66	8-80
Cholesterol (mg/dL)		70.6		84.4	89-258
Total bilirubin (mg/dL)		0.26		0.312	0.0-0.2
Glucose (mg/dL)		459		201	63-118
Albumin (g/dL)		2.2		2.8	2.2-4.6
Total protein (g/dL)		3.91		6.85	6.0-8.1
Globulin (g/dL)		1.71		4.05	2.8-5.4
Urea (mg/dL)		87.2	92.9	42.7	38.5-70.6
Creatinine (mg/dL)	0.78	1.37	0.87	1.34	1.1-1.6
Phosphorus (mg/dL)		2.44		4.47	3.2-6.3
Total calcium (mg/dL)		7.9		8.4	9.0-10.9
Sodium (mmol/L)		146		150	145-158
Chloride (mmol/L)		108.8		111.6	110-126
Potassium (mmol/L)		3.2	3.66	4.16	3.6-4.9
Total CO_2 (mmol/L)		7.5		16.4	15-21
Beta-hydroxybutyric acid (mmol/L)		0.21		0.05	0.0-0.47

norepinephrine (Noradrenaline, Sintetica SA, Mendrisio, Switzerland; 0.05 μg/kg/min CRI, once), amoxicillin-clavulanic acid (Clavenir, Laboratorio Reig Jofre S.A., Barcelona, Spain; 15 mg/kg IV q12h) and enrofloxacin (Baytril, Grovet, Utrecht, Holland; 5 mg/mg, slow IV q24h), which was discontinued later, and replaced with doxycycline. Melena was observed 24 hours post-admission. pIMT was tentatively diagnosed, which appeared to have led to severe gastrointestinal bleeding, anemia, hypoproteinemia, hypoalbuminemia, hypocholesterolemia, and increased serum urea concentration.

On day 5 of hospitalization, with normalized platelets count (175x10^3/μL), the hematocrit increased (26.3%) and with clinical improvement, the cat was discharged. Prednisolone (2 mg/kg q12h PO) and doxycycline (same dose) were prescribed. In subsequent follow-ups, CBC and serum chemistry were completely normal. Prednisolone dose was gradually and progressively tapered (reduced by 25% every 14 days) up to complete withdrawal. During a 2-year follow-up period, there was no recurrence of thrombocytopenia.

Cat 3

A 2-year-old (BW, 4 kg) male neutered indoors-outdoors DSH cat, with no prior medical history, was presented to the HUVTH due to acute anemia. Two weeks before the presentation, he disappeared for five days, and was then found, bleeding from tongue and neck wounds, suspected as bite wounds. One week later, at the referring veterinary clinic, the cat underwent castration, and the distal part of the left pinna was trimmed (commonly done in Israel, to mark the castration of stray cats). Continuous post-op bleeding from the scrotum and the cut in the pinna was noted. Several days later, at the referring clinic, CBC showed severe thrombocytopenia (platelets, 11x10^3/μL; RI, 300-800x10^3/μL) and

anemia (hematocrit, 7%; RI, 24-45%). Serum chemistry showed mildly increased alanine transaminase (ALT) activity (141 U/L; RI, 20-100 U/L), hyperbilirubinemia (bilirubin, 0.7 mg/dL; RI, 0.1-0.6 mg/dL) and increased blood urea nitrogen (50 mg/dL; RI, 10-30 mg/dL). The cat, still under sedation, was referred to the HUVTH.

Physical examination abnormalities at presentation to the HUVTH included depression (likely also due to sedation), weakness, pale mucous membranes, bradycardia (HR, 120 bpm), hypothermia, occipital skin petechiae, blood clot and hematoma on the distal part of the left pinna, necrosis on the right aspect of the tongue, with general tongue swelling, and gingival bleeding and blood clots. The scrotum was swollen, with a blood clot on the surgical incision lines.

CBC showed severe thrombocytopenia (platelets, $5x10^3$/μL), confirmed microscopically, and regenerative anemia (hematocrit, 7.4%, reticulocytes $125.6x10^9$/L) (Table 1). Serum chemistry abnormalities included hyperbilirubinemia and increased ALT activity and urea concentration (Table 2). In-saline slide agglutination test, FIV and FeLV serology (Table 1) and *Mycoplasma haemofelis* whole blood PCR (PCRun, Biogal Galed Lab., Galed, Israel) were negative. The prothrombin time (PT; 17.9 sec; RI 8.7-10.5 sec) and aPTT (44.1 sec) were prolonged (Table 1).

Severe pIMT was tentatively diagnosed, leading to serious bleeding from the surgical sites, resulting in severe hemorrhagic regenerative anemia. The clotting times prolongation was attributed to clotting factor consumption and loss, while the increased ALT was likely reactive, secondary hepatic hypoxemia due to the severe anemia and shock state.

The cat was hospitalized and treated with intravenous fluids, fresh frozen plasma and pRBC (one unit of each), doxycycline, prednisolone (2 mg/kg PO q12h) and mycophenolate-mofetil (MMF; Cellcept, Roche Products Limited, Welwyn Garden City, UK; compounded as syrup; 10 mg/kg PO q12h). The cat improved progressively during hospitalization, but required additional a pRBC unit. On day 5, the platelet count increased to $88x10^3$/μL, while the hematocrit remained stable (18%), and the cat was discharged, and prescribed prednisolone, doxycycline and MMF (same doses). The immunosuppressive drugs were gradually tapered, and later withheld; Further exact details from the medical record were unavailable, as the cat was lost to follow-up.

Cat 4

A 12-year-old (BW, 7.5 kg), strictly indoors, male neutered, British shorthair cat, was presented to the HUVTH due to listlessness that had worsened to extreme weakness and depression. Twelve months prior to presentation, the cat presented due to dyspnea and recurrent vomiting episodes, and was diagnosed with unclassified cardiomyopathy, secondary cardiogenic pulmonary edema, stage-I chronic kidney disease and lymphocytic-plasmacytic inflammatory bowel disease (IBD). Physical examination abnormalities at presentation included marked tachypnea (respiratory rate, 56 breaths/min), fever (rectal temperature, 39.9° C) and 4/6 parasternal mitral systolic murmur.

CBC revealed severe thrombocytopenia (platelets, $11x10^3$/μL), confirmed microscopically, mild borderline microcytic-normochromic, non-regenerative anemia (PCV, 30%) and mature neutrophilic leukocytosis (Table 1). Upon blood smear microscopic examination, mild spherocytosis was suspected. Serum chemistry abnormalities included mild hypocholesterolemia (84 mg/dL; RI, 89-258), presumably from blood loss, and very mild hyperbilirubinemia (bilirubin, 0.312 mg/dL; RI, 0.0-0.2). In-saline slide agglutination and RBC osmotic fragility tests were positive (Table 1), suggestive of extravascular immune mediated hemolytic anemia (IMHA). FIV antibody and FeLV antigen serology were negative (Table 1). The PT (13.9 sec) and aPTT (71.7 sec) were prolonged. D-dimer concentration was increased (1404 ng/mL; RI <250). Fibrinogen concentration was 587.3 (RI, 150-300 mg/dL) (Table 1). Abdominal and thoracic sonography abnormalities included only mild hepatomegaly. Fecal occult blood test (Fecal occult blood, YD Diagnostics, Bangkok Thailand) was positive, suggestive of gastrointestinal bleeding, likely due to the severe thrombocytopenia.

Based on these test results, pIMT was tentatively diagnosed, with secondary hemorrhagic anemia, and with a possible contribution of immune mediated hemolysis. The concurrently present IMHA and IMT are consistent with Evans syndrome (15).

The cat was hospitalized, and treated with intravenous fluids, pimobendane (Cardisure, Eurovet Animal Health B.V., Holland; 0.25 mg/kg PO q12h), pantoprazole (Controloc, London, UK; 1 mg/kg IV q24h), mirtazapine gel, MMF (10 mg/kg PO q12h), amoxicillin-clavulanic acid, maropitant (Zoetis, Girona, Spain; 1 mg/kg IV q24h), sucralfate (Agtech Inc., Manhattan, KS, USA; 250 mg PO q8h) and

prednisolone (2 mg/kg PO q12h). During hospitalization, the cat clinically improved, regaining appetite, while the vital signs and blood pressure normalized. The cat was discharged after 5 days of hospitalization, when the platelet count was $25x10^3/\mu L$, and prescribed prednisolone, mirtazapine gel, MMF (same doses as above) and marbofloxacin (5 g/kg PO q24h).

On recheck, at one-week post-discharge, the platelet count increased ($142x10^3/\mu L$), and normalized ($320x10^3/\mu L$) at three weeks post-discharge. Drug therapy was withheld for 45 days post-discharge from the hospital. Recurrence of the IMT (with confirmed platelet count of $64x10^3/\mu L$) was seen 11 months later. Prednisolone (1.5 mg/kg PO q24h), as a single immunosuppressive agent was reintroduced, and 172 days later, the platelet count normalized ($198x10^3/\mu L$), remaining stable (around $250x10^3/\mu L$) for four months. Ten months later, with ongoing prednisolone therapy, diabetes mellitus (DM) was diagnosed, presumably secondary to the long-term prednisolone treatment. Therefore, prednisolone was tapered, and eventually withheld. The cat remained clinically stable for three additional years, with platelet counts around $60\text{-}80x10^3/\mu L$, when it was diagnosed with septic peritonitis of unknown cause, and euthanized at the owners' request.

DISCUSSION

This report describes four cats putatively diagnosed with pIMT, which is infrequently reported, with < 30 cases reported in the literature to date (1, 7, 9–11, 15–17), thereby significantly adding information of feline pIMT. The severe thrombocytopenia at presentation was confirmed by examination of the blood smear, ruling out pseudothrombocytopenia.

Antiplatelet antibody assays are unavailable in Israel currently, and therefore, in all cats herein, as in most clinical cases (14), pIMT was diagnosed by excluding all primary underlying diseases potentially leading to sIMT, including retroviral and other infections, neoplasia, and inflammatory non-infectious diseases (e.g., pancreatitis), and observing a favorable response to immunosuppressive treatment (9–10, 12, 16). Although absence of anti-platelet antibody assays herein precluded a definite diagnosis of IMT, positive results of such tests cannot differentiate between pIMT and sIMT. Additionally, negative anti-platelet antibody assay results might not necessarily rule out IMT. In humans with pIMT, anti-platelet antibodies might be absent, while other mechanisms of platelet destruction do occur, mediated by antigen-specific cytotoxic T cells, either destroying platelets peripherally, in the spleen, or impairing bone marrow platelet production (18–21), and the complement might potentially participate in the immune-mediated platelet and megakaryocyte destruction (18). Whether this also occurs in cats with pIMT remains to be investigated.

The reported prevalence of all cause thrombocytopenia in cats is 5.9% (1), with hematological, infectious, and neoplastic diseases being the most common thrombocytopenia-associated diseases (1, 16). In a study of 42 thrombocytopenic cats, 50% of cases were positive for platelet-associated antibodies detected by flow cytometry, suggesting that immune-mediated platelet destruction is an important, rather common pathological mechanism in feline thrombocytopenia of various underlying diseases (7). Nevertheless, only 2 cats (5%) were diagnosed with pIMT (7), supporting the observation that pIMT is an uncommon cause of feline thrombocytopenia (1).

In a study of 194 thrombocytopenic cats, pIMT was associated with the most severe thrombocytopenia (1), which was also recorded in this small cohort, with platelet counts ranging between $8\text{-}11x10^3/\mu L$ at presentation. Such severe thrombocytopenia often leads to overt bleeding from the oral mucosa, the gastrointestinal tract (i.e., melena and hematochezia), and surgical and venipuncture sites, as well as cutaneous and scleral petechiae, epistaxis, hyphema and bleeding secondary to minor trauma (12, 14–16). In all four cats in this study, gastrointestinal bleeding was noted, either as a single manifestation of bleeding, or among other bleeding signs. In three cats herein, due to the severe thrombocytopenia, as previously noted in pIMT (1, 7, 9–10), moderate to severe hemorrhagic anemia (hematocrit range 7.4-12.2%) led to weakness, depression, decreased appetite, hypovolemia and shock, in agreement with previous findings (16). In the fourth cat, hemorrhagic anemia did develop later on, and was attributed to gastrointestinal bleeding, possibly aggravated by ongoing IBD and possible concurrent IMHA. The latter was diagnosed by the suspected spherocytosis and positive in-saline slide agglutination and RBC osmotic fragility tests. Additionally, in two cats in which hemostatic tests were performed, clotting times were significantly prolonged, possibly due to developing DIC, secondary to the marked

thrombocytopenia-associated severe hemorrhage, with loss of plasma proteins, as reported previously (9, 16).

The response to immunosuppressive therapy in this case-series was variable. While in two cats, prednisolone as a single agent, induced complete remission, in the other two, although the platelet count somewhat improved, response to prednisolone alone was insufficient. While higher prednisolone doses potentially might have induced remission, the addition of MMF was deemed beneficial, for induction of a quicker response, and as a 'steroid-sparing' drug, decreasing the adverse effects and risks of prolonged high-dose prednisolone therapy (19), allowing earlier prednisolone tapering. MMF treatment has been described in few case reports in cats, in IMHA, hemophagocytic syndrome with IMHA and immune-mediated poly arthritis (20-24), but to the best of the authors' knowledge, this is the first report describing MMF therapy in cats with pIMT or Evans syndrome.

Recurrence of IMT occurred in one of the four cats, requiring prolonged prednisolone therapy to maintain adequate platelet counts, albeit below lower reference limit, to prevent bleeding. This very likely contributed to the development of DM in this cat, inducing insulin resistance (9, 19). Most cats administered high glucocorticoid doses do not develop DM, while those that do, are considered likely with underlying subclinical DM (19). This cat herein, that eventually developed DM was obese (7.5 kg BW), and at presentation, its glucose concentration was 201 mg/dL. Although this hyperglycemia might have resulted from anxiety and stress, possibly, the cat might have had insulin resistance, with subclinical DM. In such obese cats, prolonged prednisolone therapy should be carried out cautiously, and the addition of other immunosuppressive drugs (e.g., MMF), early in the disease course, potentially allowing earlier prednisolone tapering, should be considered favorably. If DM does occur during glucocorticoid therapy, such additional immunosuppressive drugs might allow withholding the glucocorticoid, potentially promoting diabetic remission (19).

Concurrent IMHA and IMT (Evans syndrome) in cats is rarely described (25). Most cases are secondary, with variable background conditions, such as neoplasia, infections (e.g., FIP, FIV and FeLV), systemic lupus erythematosus, adverse drug reaction, and post-bone marrow transplantation reaction (7, 25–26). In contrast to most reported Evans syndrome cases, in Cat 4 herein, Evans syndrome was primary.

In conclusion, pIMT was diagnosed in four cats herein, based on exclusion of underlying primary causes of sIMT, and observing favorable responses to immunosuppressive therapy. pIMT was associated with severe thrombocytopenia, leading to clinically significant bleeding, resulting in anemia, often severe, and hypovolemic shock. The anemia and hypoproteinemia, sometimes with hemostatic derangement suggestive of DIC, required blood component transfusion in some cats. Response to immunosuppressive therapy was favorable, albeit variable, in all four cats. Further studies are warranted to determine the true prevalence of pIMT among thrombocytopenic cats, the utility of additional therapeutic options (e.g., intravenous human immunoglobulins and vincristine), the response to treatment, and the long-term prognosis.

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