Australian VETERINARY JOURNAL
THE JOURNAL OF THE AUSTRALIAN VETERINARY ASSOCIATION LTD
SMALL ANIMALS

CASE REPORT

Primary immune-mediated thrombocytopenia and immune-mediated neutropenia suspected in a 21-week-old Maine Coon cat

MP Best* and DR Fry

Case report A 21-week-old Maine Coon cat presented with an acute-onset coagulopathy. Severe concurrent thrombocytopenia and neutropenia were identified on peripheral blood smears and bone marrow cytology supported a peripheral consumptive process. Other than mild superficial haemorrhage, the cat was clinically well and screening for retroviral diseases, abdominal ultrasound examination, thoracic radiography, haematology and biochemistry panels did not identify an underlying disease. There was no historical pharmaceutical or toxicological trigger noted and the cat was from an area without endemic *Ehrlichia* spp. There was a rapid resolution of both cytopenias following treatment with immunosuppressive doses of prednisolone, though a mild relapse occurred during gradual prednisolone withdrawal and was responsive to a dose increase.

Conclusions This report describes this combination of diseases for the first time in a cat and presents a younger patient than previously described with feline primary immune-mediated haematological disease.

Keywords autoimmune diseases; cats; idiopathic thrombocytopenic purpura; neutropenia; thrombocytopenia

Abbreviations FeLV, feline leukaemia virus; IMN, immune-mediated neutropenia; pIMT, primary immune-mediated thrombocytopenia; RI, reference interval

Aust Vet J 2014;92:250–253 doi: 10.1111/avj.12186

Primary immune-mediated thrombocytopenia (pIMT) is a rare disease in cats, with only 15 rigorously diagnosed cases[1–8] in cats with an age range of 1.5–12 years.[3,4,6–8] In most cases, IMT in cats is considered secondary to other diseases and the proportion of thrombocytopenic cats with pIMT is reportedly only 2–4%.[1,2,5] IMT has been recorded secondarily to and associated with a wide range of primary diseases, including neoplasia, inflammatory diseases, infectious and cardiac disease.[1,2,5] Immune-mediated neutropenia (IMN) has been described very rarely in cats.[9–14] Feline IMN is mentioned in a small number of review articles,[9,12,14] but the single conclusive case report we were able to locate was of IMN occurring secondary to a thymoma.[13] The present report describes a young cat with concurrent pIMT and IMN, with a description of the presenting clinical signs and the initial response to treatment.

*Corresponding author.
Brisbane Veterinary Specialist Centre, Cnr Old Northern and Keong Rds, Albany Creek, Queensland 4035, Australia; matt.p.best@googlemail.com

Case report

A 21-week-old female neutered Maine Coon cat was presented with acute-onset right-sided hyphaema. The cat's previous history was unremarkable, with no recent medications, no known toxin ingestion, last primary vaccination at 19 weeks old and an indoor lifestyle with the exception of occasional supervised harness walks. Physical examination showed that the right eye had blood in the anterior chamber and showed mild uveitis; the left eye displayed no pathology. Additional findings were petechiae on the pinnae and some minor gingival bleeding. The physical examination was otherwise unremarkable.

A comprehensive biochemistry panel did not suggest a cause of the observed cytopenias (Table 1).The packed cell volume was 35% (reference interval (RI), 28–49) and a blood smear examination suggested a severe thrombocytopenia and a moderately severe neutropenia. Activated clotting time was 172 s (RI: 100–160 s; VetScan i-STAT ACT Celite®, Abaxis, Union City, CA, USA) and the citrate activated partial thromboplastin time was 137 s (RI, 65–119 s; Coag Dx™ Analyser, IDEXX, Rydalmere, NSW, Australia). Two in-house feline leukaemia virus (FeLV)/feline immunodeficiency virus antibody (FIV) test kits (Anigen Rapid FIV Ab/FeLV Ag Test Kit, BioNote, Korea; SNAP® FIV/FeLV Combo Test, IDEXX) were run and both were negative for both diseases. For FeLV, the BioNote antigen ELISA has a sensitivity of 94.7% and specificity of 99.7% (vs virus isolation)[15] and the SNAP® kit has a sensitivity of 92.3% and specificity of 97.3% (vs virus isolation).[16] For FIV, the BioNote test has a sensitivity of 96.8% and a specificity of 99.7% (vs Western blot)[15] and the SNAP® test has a sensitivity of 100% and a specificity of 99.6% (vs Western blot).[16] A haematology sample was submitted to QML laboratories in Brisbane and while the results were pending a bone marrow aspirate was collected. The external haematology results (Table 2) confirmed a severe thrombocytopenia (15×10^9/L from a manual count, RI: 200–700) and neutropenia (1.1×10^9/L, RI: 3.8–10.1), but all other parameters were within normal limits. The bone marrow aspirate showed normocellular bone marrow with adequate megakaryocytes and a myeloid left shift suggestive of a peripheral consumptive process involving granulocytes. An abdominal ultrasound examination and three-view thoracic radiography were performed and no disease process was identified.

Initial treatment was oral prednisolone (Pred-X 5 mg Tablets, Apex Laboratories, Somersby, NSW, Australia) at 2 mg/kg twice daily and trimethoprim/sulfadiazine at 120 mg of combined ingredients

Table 1. Biochemical parameters measured in-house[a] on the day of presentation of a Maine Coon cat with an acute-onset coagulopathy, prior to the initiation of treatment

Biochemical parameter	Value prior to initiating treatment (reference interval)
Albumin (g/L)	30 (22–39)
ALP (U/L)	104 (14–192)
ALT (U/L)	82 (12–115)
Amylase (U/L)	896 (500–1400)
Urea (mmol/L)	5.6 (5.7–11.8)
Ca (mmol/L)	2.5 (1.98–2.83)
Cholesterol (mmol/L)	2.29 (1.60–4.93)
Creatinine (µmol/L)	65 (53–141)
GGT (U/L)	<0 (0–1)
Globulin (g/L)	39 (28–48)
Glucose (mmol/L)	6.06 (4.28–8.51)
Lipase (U/L)	170 (40–500)
Phosphate (mmol/L)	2.53(1.45–3.36)
Total bilirubin (µmol/L)	4 (0–15)
Total protein (g/L)	69 (52–82)
Na (mmol/L)	158 (150–165)
K (mmol/L)	3.6 (3.7–5.9)
Cl (mmol/L)	126 (115–126)

[a]Catalyst Dx® Chemistry Analyser (IDEXX, Rydalmere, NSW, Australia). ALP, alkaline phosphatase; ALT, alanine aminotransferase; Ca, total calcium; Cl, chloride; GGT, gamma glutamyl transferase; K, potassium; Na, sodium.

(Tribrissen 20, Jurox, Rutherford, NSW, Australia) once daily. Standard thrombocytopenic nursing care with gentle handling and the feeding of soft food was instituted, with daily monitoring of a blood smear and packed cell volume.

A return to normal numbers of neutrophils was noted 4 days after initiating prednisolone, so the trimethoprim/sulfadiazine was discontinued. A smear examination the next day subjectively showed an increase in platelet numbers but levels were considered inadequate for haemostasis. At 6 days after starting prednisolone, platelet numbers were considered adequate for haemostasis, so the cat was discharged on oral prednisolone at 2 mg/kg twice daily. A haematology sample was submitted to QML at this time and the platelet count was 121×10^{-9}/L (RI: 200–700) and neutrophil count of 4.8×10^{-9}/L (RI: 3.8–10.1), with all other parameters within normal limits. The haematology results over time are shown in Table 2.

The cat remained clinically well and normal on follow-up checks. On day 28, repeat haematology was performed and all parameters were within normal limits except for a mild thrombocytosis at 737×10^{-9}/L (RI: 200–700). Weaning of the prednisolone was initiated, with a 25% reduction in dose every month, and no cytopenia was noted on complete blood counts up to the 2-month recheck. However, a mild neutropenia was noted at approximately 3 months, at which time the prednisolone dose was 5 mg once daily (≈1.6 mg/kg). This neutropenia proved persistent, so the dose was increased to 5 mg twice daily, which achieved resolution.

Discussion

The diagnosis of pIMT relies on a confident diagnosis of thrombocytopenia (a profound thrombocytopenia $<50 \times 10^{-9}$/L is usually anticipated), the exclusion of underlying diseases and the demonstration of antiplatelet antibodies.[1,4,8,17] The latter provides conclusive evidence of the nature of the cell destruction and platelet factor 3 testing,[17] flow cytometry[1,8] and immunofluorescence of the bone marrow for direct antimegakaryocyte antibodies[4,17] have been used to demonstrate this. However, these tests are poorly validated in terms of specificity and sensitivity and are not commercially available.[3,4,6–8] As a result, demonstration of antiplatelet antibodies is usually not pursued and it is accepted that a response to corticosteroid administration is the third diagnostic criterion.[3,6–8]

Equivalent to the situation for IMT, the demonstration of antineutrophil antibodies would be optimal for the diagnosis of IMN and although assays for this purpose have been produced for cats,[10] they have not been used to successfully diagnose IMN and are not widely available or validated. A similar diagnostic challenge exists for dogs and it has been proposed that the diagnosis of IMN in neutropenic dogs requires three of the following criteria: the exclusion of other known causes of neutropenia, presence of concurrent immune-mediated disease, bone marrow cytology supportive of immune-mediated destruction of neutrophils and a prompt response to corticosteroid administration.[18]

For the cat described here, there was no demonstration of antiplatelet or antineutrophil antibodies, because these tests are not readily available within Australia. Both cell lines showed a sequential recovery with immunosuppressive doses of corticosteroids in line with the response expected for pIMT and IMN.

In this case, the prompt response to corticosteroids strongly supports the immune-mediated nature of the disease in the absence of available antibody tests. The diagnosis of idiopathic immune-mediated disease presents the diagnostic challenge of excluding all underlying causes. The extensive testing in the present case addressed all reasonably investigable causes and the findings warranted a diagnosis of idiopathic IMN and pIMT, consistent with published guidelines for the diagnosis of these diseases.[3,6,18] Testing for *Ehrlichia* or other arthropod-borne agents was not part of the disease investigation because these tests are not readily available within Australia and furthermore the diseases were not endemic within the locality of the cat and thus were considered very unlikely.

The presence of recent vaccination should be interpreted with much caution.[19] Although some studies have suggested temporal links between immune-mediated disease and vaccination in humans, these have only been shown to be causal in some very specific situations.[20] Although thrombocytopenia has been noted in humans following certain types of vaccination, it is rare and the associated thrombocytopenia is usually mild and self-resolving[20] and would be unlikely to produce clinical signs if it were to occur in cats. There is no convincing evidence for a link between vaccination and systemic autoimmune

Table 2. Laboratory values for haematological parameters at presentation and during treatment of a Maine Coon cat with acute-onset coagulopathy

Parameter	Day							RI
	0	6	28	65	111	121	135	
Haemoglobin (g/L)	108	99	129	140	144	138	140	80–140
RCC ($\times 10^{12}$/L)	8.6	7.1	9.6	10.7	10.1	9.5	9.9	5.5–10.0
Haematocrit	0.32	0.29	0.38	0.39	0.41	0.38	0.40	0.28–0.45
MCV (fL)	37	41	40	36	40	40	41	40–52
MCH (pg)	13	14	14	13	14	15	14	13–18
MCHC (g/L)	341	337	336	363	353	360	349	310–350
WBC ($\times 10^9$/L)	6.7	9.2	12.0	7.3	6.2	5.3	7.8	6.0–16.0
Neutrophils ($\times 10^9$/L)	1.1	4.8	5.2	4.9	3.3	3.5	4.4	3.8–10.1
Lymphocytes ($\times 10^9$/L)	5.4	3.9	6.0	1.9	2.5	0.7	3.0	1.6–7.0
Monocytes ($\times 10^9$/L)	0.1	0.0	0.5	0.4	0.0	0.7	0.2	<0.7
Eosinophils ($\times 10^9$/L)	0.07	0.55	0.36	0.07	0.37	0.42	0.16	<1.41
Basophils ($\times 10^9$/L)	0.00	0.00	0.00	0.00	0.0	0.0	0.0	<0.11
Platelets (automated) ($\times 10^9$/L)	240	121	737	–[a]	–[a]	662	–[a]	200–700
Platelets (manual) ($\times 10^9$/L)	15							

[a]Not reported because of clumping; pathologist review = clumped, adequate and normal. Analyses performed at QML, Brisbane, QLD, Australia using an Abbott Cell-dyn 3700CS analyser for the complete blood count and Miller's square method for the manual platelet count.
RCC, red cell count; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; RI, reference interval; WBC, white blood cells.

disease in cats[19] and vaccination has not been clearly implicated in IMN for any species. Furthermore, the relapse of neutropenia in the cat in this case many months after vaccination would suggest it was not vaccine-related immune-mediated disease.

FeLV is a common cause of cytopenias in cats and the aetiology can be both bone marrow suppression and the triggering of immune-mediated disease.[21,22] Although most FeLV-symptomatic cats have progressive disease, in a small number of cases of non-regenerative anaemia in regressively infected cats, bone marrow suppression may occur with latent as well as progressive disease.[22] Immune-mediated destruction, however, requires extracellular antigen expression of FeLV and as such requires a progressive or viraemic state.[21,22] In the present case, the bone marrow cytology confirmed a peripheral source of the granulocyte destruction and the rapid response to corticosteroids strongly supports immune-mediated disease. Demonstration of a non-viraemic state is then required to exclude FeLV as the cause of the cytopenias. In this instance, two ELISAs were performed. If the disease prevalence in Australia is extrapolated from an appropriate study of a variety of pet cats,[23] then a disease prevalence of 2% can be used to calculate the negative predictive values for the two tests. Note that this is likely to be a good approximation for the housebound cat reported here. The negative predictive value of the Anigen Rapid FIV Ab/FeLV Ag Test Kit (BioNote) and SNAP® FIV/FeLV Combo Test (IDEXX) would be 99.9% and 99.8%, respectively, compared with virus isolation. The combined negative predictive value is likely to be even greater, although cannot be calculated without knowledge of the consensus between the two tests.

FIV is a rare cause of severe cytopenias in cats and when it occurs it is associated with bone marrow suppression in end-stage disease.[12] In the present case, the cat was too young and asymptomatic to have end-stage FIV,[12] did not have bone marrow suppression and tested negative to two antibody tests, making this virus an extremely unlikely cause of the observed cytopenias.

One pertinent observation was the marginal prolongation of the activated clotting time and the activated partial thromboplastin time. It should be noted that this is a previously noted phenomenon in feline pIMT[3] and may be related to the presence of antiphospholipid antibodies, which are present in 46% of human pIMT cases.[24] It is unfortunate that a prothrombin time was not performed to further qualify this result by definitively excluding a more generalised failure of secondary haemostasis, which would not be anticipated with antiplatelet antibodies.

The initial, automated platelet count (Table 2) was erroneous. A repeat automated count was 150×10^9/L and so a manual count was performed by a pathologist at QML, which concurred with the original in-house assessment. The cause of the inaccurate automated count is most likely the similarity in size between feline red blood cells and platelets.[25] Other minor changes in the clinical pathology values included a persistent marginal microcytosis (Table 2), mild hyperchromia on days 65 and 121 (Table 2) and marginal decreases in urea and potassium concentrations at presentation (Table 1). The significance of these changes is unclear and, given this case was an immature purebred cat, the RIs themselves may be inaccurate.[26] As such, the small variations from general feline RIs must be interpreted in the light of the clinical situation. Other possibilities could include natural variation outside the RIs or spurious results. The marginal thrombocytosis on day 28 may have been corticosteroid induced, as is observed in dogs,[27] or may have been a result of inaccurate

automated platelet count.[25] A quantitative manual platelet count was not performed.

Conclusion

Idiopathic IMN should be considered as a differential diagnosis for neutropenia in cats. The combination of pIMT and IMN, with thrombocytopenia sufficiently severe to cause clinical signs, has been recorded previously in one case study in dogs,[28] but should also be considered in cats. Previous cases of idiopathic immune-mediated haematological diseases have involved older cats, but cats as young as 5 months old should be considered as potentially affected by these diseases. The diagnostic criteria previously proposed for the diagnosis of IMN in dogs were useful and applicable in this case. This cat had minimal clinical signs and a good immediate response to immunosuppressive treatment with corticosteroids.

References

1. Jordan HL, Grindem CB, Breitschwerdt EB. Thrombocytopenia in cats: a retrospective study of 41 cases. *J Vet Intern Med* 1993;7:261–265.
2. Peterson JL, Couto CG, Wellman ML. Hemostatic disorders in cats: a retrospective study and review of the literature. *J Vet Intern Med* 1995;9:298–303.
3. Garon CL, Scott MA, Selting KA et al. Idiopathic thrombocytopenic purpura in a cat. *J Am Anim Hosp Assoc* 1999;35:464–470.
4. Tasker S, Mackin AJ, Day MJ. Primary immune-mediated thrombocytopenia in a cat. *J Small Anim Pract* 1999;40:127–131.
5. Kohn B, Linden T, Leibold W. Platelet-bound antibodies detected by a flow cytometric assay in cats with thrombocytopenia. *J Feline Med Surg* 2006;8:254–260.
6. Bianco D, Armstrong PJ, Washabau RJ. Presumed primary immune-mediated thrombocytopenia in four cats. *J Feline Med Surg* 2008;10:495–500.
7. Öberg J, Tvedten H. Thromboelastography in a cat with steroid responsive thrombocytopenia. *Comp Clin Pathol* 2010;19:429–431.
8. Wondratschek C, Weingart C, Kohn B. Primary immune-mediated thrombocytopenia in cats. *J Am Anim Hosp Assoc* 2010;46:12–19.
9. Chickering WR, Prasse KW. Immune-mediated neutropenia in man and animals: a review. *Vet Clin Pathol* 1981;10:6–16.
10. Chickering WR, Prasse KW, Dawe D. Development and clinical application of methods for detection of antineutrophil antibody in serum of the cat. *Am J Vet Res* 1985;46:1809–1814.
11. Brown MR, Rogers KS. Neutropenia in dogs and cats: a retrospective study of 261 cases. *J Am Anim Hosp Assoc* 2001;37:131–139.
12. Brown RM, Rogers KS. Neutropenia in dogs and cats. *Compend Contin Educ Vet* 2001;23:534–543.
13. Fidel JL, Pargass IS, Dark MJ et al. Granulocytopenia associated with thymoma in a domestic shorthaired cat. *J Am Anim Hosp Assoc* 2008;44:210–217.
14. Schnelle AN, Barger AM. Neutropenia in dogs and cats: causes and consequences. *Vet Clin Small Anim Pract* 2012;42:111–122.
15. BioNote Inc. Anigen Rapid FIV Ab/FeLV Ag: general information. http://www.bionote.co.kr/File/Upload/2013/03/05/2013-03-05(17).pdf Accessed March 2013.
16. Hartmann K, Griessmayr P, Schulz B et al. Quality of different in-clinic test systems for feline immunodeficiency virus and feline leukaemia virus infection. *J Feline Med Surg* 2007;9:439–445.
17. Jain NC, Switzer JW. Autoimmune thrombocytopenia in dogs and cats. *Vet Clin Small Anim Pract* 1981;11:421–434.
18. Perkins MC, Canfield P, Churcher RK et al. Immune-mediated neutropenia suspected in 5 dogs. *Aust Vet J* 2004;82:52–57.
19. Day MJ. Vaccine side effects: fact and fiction. *Vet Microbiol* 2006;117:51–58.
20. Chen RT, Pless R, De Stephano F. Epidemiology of autoimmune reactions induced by vaccination. *J Autoimmun* 2001;16:309–318.
21. Lutz H, Addie D, Belák S et al. Feline leukaemia: ABCD guidelines on prevention and management. *J Feline Med Surg* 2009;11:565–574.
22. Hartmann K. Clinical aspects of feline retroviruses: a review. *Viruses* 2012;4:2684–2710.
23. Malik R, Kendall K, Cridland J et al. Prevalences of feline leukaemia virus and feline immunodeficiency virus infections in cats in Sydney. *Aust Vet J* 1997;75:323–327.
24. Stasi R, Stipa E, Masi M et al. Prevalence and clinical significant of elevated antiphospholipid antibodies in patients idiopathic thrombocytopenic purpura. *Blood* 1994;84:4203–4208.
25. Norman EJ, Barron RCJ, Nash AS et al. Prevalence of low automated platelet counts in cats: comparison of prevalence of thrombocytopenia based on blood smear estimation. *Vet Clin Pathol* 2001;30:137–140.
26. Paltrinieri S, Ibba F, Rossi G. Haematological and biochemical reference intervals of four feline breeds. *J Feline Med Surg* 2014;16:125–136 .
27. Neel JA, Snyder L, Grindem CB. Thrombocytosis: a retrospective study of 165 dogs. *Vet Clin Pathol* 2012;41:216–222.
28. Vargo CL, Taylor SM, Haines DM. Immune-mediated neutropenia and thrombocytopenia in 3 giant schnauzers. *Can Vet J* 2007;48:1159–1163.

(Accepted for publication 17 November 2013)