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Clinical use of cyclosporine as an adjunctive therapy in the management of feline idiopathic pure red cell aplasia

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The clinical use of cyclosporine is described in a group of client-owned cats diagnosed with idiopathic pure red cell aplasia (PRCA). All 10 cats were treated with combinations of glucocorticoids and cyclosporine. Of the 10 cats, the eight for which follow-up data was available achieved and maintained remission for a median of 31 and 406 days, respectively. Therapy was reduced or discontinued in 7/8 cats; 2/7 maintained remission off therapy and 5/7 cats relapsed. Remission was reinduced in four cats, with 3/4 cats maintained long-term on low dose therapy. Adverse effects associated with cyclosporine therapy were responsive to dose reduction or drug withdrawal. Feline idiopathic PRCA was responsive to combination immunosuppressive therapy with glucocorticoids and cyclosporine. Relapse was common, particularly after drug discontinuation; therefore, most cats required maintenance long-term low dose therapy.

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Pure red cell aplasia (PRCA) has been reported in humans,[1–3] dogs,[4–6] and cats[6,7] as either a primary or secondary hematologic disorder. PRCA is a syndrome characterized by a normocytic, normochromic anemia with a reticulocytopenia in the presence of normal white blood cell and platelet counts. The diagnosis is based on an isolated anemia associated with bone marrow erythroid hypoplasia or aplasia with normal to increased granulopoiesis and megakaryopoiesis.

In humans, the pathophysiology of PRCA is heterogeneous and not completely understood. PRCA can present as a congenital form or an acquired syndrome of either an acute or chronic type. Congenital PRCA is associated with several genetic defects affecting the erythropoietic lineage.[1,2] Acquired PRCA is thought to be an autoimmune disorder and can be secondary to a wide variety of conditions, or a primary hematologic disorder, termed idiopathic PRCA.[1,2] Based on an underlying immune pathogenesis, immunosuppressive therapy is recommended for acquired idiopathic PRCA and secondary PRCA that does not completely respond to treatment of the underlying disease. Optimal immunosuppressive therapy remains controversial in humans, in part due to the lack of controlled clinical trials in an adequate number of patients.

Common immunosuppressive therapies in human patients have included glucocorticoids (GC), cyclophosphamide, cyclosporine A (CsA), or combination therapy. Variable remission rates in humans have been reported for each drug: GC 30–62%, cyclophosphamide 7–20%, and CsA 65–87%.[8–14] GC have historically been the treatment of choice in humans with PRCA,[15] although recently CsA has become more popular as first line therapy.[10,12,13,16]

Similar to the human disease, feline PRCA is an acquired syndrome that arises secondary to a systemic disease (eg, feline leukemia virus (FeLV) subgroup C, PRCA occurs secondary to the cytopathic effect of the virus)[17–21] or as a primary idiopathic hematologic disorder with a presumptive immune-mediated mechanism.[7] A retrospective study reported idiopathic PRCA in nine FeLV negative cats;[7] all cats experienced resolution of their anemia and were considered in clinical remission following combination immunosuppressive therapy, including GC and cyclophosphamide in eight cats and GC and CsA in one cat. The clinical response of these cats to immunosuppressive therapy further supports that as in humans[2] and dogs,[22] feline idiopathic PRCA likely has an immune-mediated pathogenesis.

Little information has been reported on the clinical use of CsA in cats with immune-mediated diseases including PRCA. To date, the reported use of CsA in cats with idiopathic PRCA is limited to two cats,

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and both had a favorable response to combination therapy with GC and CsA.[7,23] Controlled clinical studies comparing immunosuppressive therapies for the treatment of cats with idiopathic PRCA are essentially impossible as this spontaneous disease occurs so rarely in cats. In our hospital CsA has been increasingly used in cats with PRCA as either a first or second line immunosuppressive therapy. The purpose of this study was to retrospectively describe the clinical use of CsA in client-owned cats diagnosed with idiopathic PRCA, specifically focusing on the clinical course, duration of therapy to the induction of remission, the need for maintenance therapy, adverse effects/complications, and outcome.

Materials and methods

Criteria for case selection

A retrospective study was undertaken at the University of Wisconsin, Veterinary Medical Teaching Hospital (UW-VMTH). The computerized medical records and pathology databases were searched from the years 1997 through 2010. Keywords included cat, cats, feline, anemia, PRCA, erythroid hypoplasia, and red cell hypoplasia.

Medical records retrieved by the database search were reviewed. Inclusion criteria were: (1) clinical signs of anemia (pallor, lethargy, weakness); (2) laboratory evidence of a non-regenerative anemia (hematocrit (HCT) $< 25\%$ and an absolute reticulocyte count $< 0.060 \times 10^6/\mu l$) without a concurrent leukopenia (total WBC $> 5000/\mu l$) or thrombocytopenia (platelet $> 150{,}000/\mu l$. An arbitrary cut-off of 150,000 platelets was used to define thrombocytopenia based on the common clinical finding of a mild thrombocytopenia in normal cats; platelet activation by the mechanics of a blood draw commonly result in ex vivo platelet clumping and falsely lowers platelet counts in many cats.); (3) FeLV and feline immunodeficiency (FIV) negative status, as determined by a combination Snap test for FeLV antigen (immunofluorescent antibody, IFA) and FIV antibody (enzyme-linked immunosorbent assay, ELISA) and/or bone marrow IFA testing; (4) a bone marrow aspirate or core biopsy consistent with the diagnosis of PRCA (bone marrow erythroid hypoplasia or aplasia with normal to increased granulopoiesis and megakaryopoiesis), and (5) immunosuppressive therapy that included the use of cyclosporine. The minimal diagnostic evaluation to establish the diagnosis of PRCA and rule out secondary PRCA, included a complete blood count (CBC), reticulocyte count, serum biochemistry profile (chemistry), FeLV/FIV testing (ELISA and/or IFA), and bone marrow aspirate and/or core biopsy. Additional diagnostics were at the discretion of the attending clinician. Exclusion criteria included any cat with an identified underlying primary cause for the anemia. Erythroid hypoplasia was defined as a marked increase in the myeloid:erythroid (M:E) ratio (M:E ratio $> 3{:}1$) in the face of an overall normo- or hypocellular bone marrow as assessed by a single pathologist (JW).[24]

For the cases that met the inclusion criteria, information retrieved from the medical record included signalment, history, clinical signs and physical examination at presentation to the UW-VMTH, results of laboratory testing and imaging, drugs and dosing regimens, CsA trough concentrations where available, adverse reactions/complications, follow-up (short- and long-term), and outcome. Outcome measures calculated included days to remission, duration of remission, and days to relapse. Disease outcomes were described as either lost to follow-up (LTF), complete remission (CR), died (D), or euthanased (E).

To standardize the days to remission, day 0 was considered initial presentation to the UW-VMTH and day 1 was the day the diagnosis of idiopathic PRCA was established and immunosuppressive therapy was initiated. Short-term follow-up was defined as 60 days post-diagnosis and long-term follow-up was defined as >60 days post-diagnosis. Response to treatment was subdivided into cats that achieved: (1) CR, defined as cats with a stable HCT $\geq 25\%$ with resolution of clinical signs; (2) partial remission, defined as cats with an improvement in the HCT by at least 0.3-fold but a HCT $< 25\%$ with improvement or resolution of clinical signs; or (3) no response, defined as cats with no significant increase in the HCT (<0.3-fold increase) that remained transfusion dependent with persistent clinical signs. For the HCT to be considered stable or increased, the change in the HCT could not be directly related to a blood transfusion. Relapse was defined as cats with a decrease in their stable HCT by 0.1-fold.

Statistical analysis

Descriptive statistics were used to summarize all continuous variables; the data is expressed using median and ranges.

Results

Seventeen cases were retrieved from the search of the electronic databases. Of these, 10 cases met the criteria for inclusion. Seven cats were excluded for a variety of reasons including an idiopathic PRCA cat treated with GC and chlorambucil ($n = 1$), regenerative anemia ($n = 1$), concurrent systemic disease ($n = 3$), non-diagnostic bone marrow ($n = 1$), and medical record unavailable for review ($n = 1$).

The diagnostics performed in all cats included a CBC, chemistry profile, reticulocyte count, FeLV/FIV test, bone marrow aspirate or core biopsy, and blood typing. At the discretion of the attending clinician, additional diagnostic tests performed included urinalysis (5/10), Coombs test (6/10), chest radiographs (9/10), abdominal radiographs (2/10),

Table 1. Summary of signalment, history, and physical examination findings of the 11 cats diagnosed with idiopathic PRCA at the time of presentation.

	n	Median	Range
Signalment			
DSH/DLH	7/3		
Males: N/I	6/0		
Females: S/I	3/1		
Age	10	1.4 years	6 months–9 years
Weight	10	3.8 kg	2.5–5.7 kg

DSH = domestic shorthair, DLH = domestic longhair, N = neutered, I = intact, S = spayed.

abdominal ultrasound (9/10), and *Mycoplasma haemofelis* whole blood polymerase chain reaction (PCR) (8/10).

Signalment/history/physical examination

The signalment and weight data for the 10 cats are summarized in Table 1. The presenting complaint for all cats included lethargy, depression, and/or weakness. Other common clinical signs reported by owners were pica ($n = 4$), hiding, vocalization, disappearance, and decreased appetite ($n = 4$). The duration of clinical signs ranged from 2 to 14 days (median 4.5 days). Abnormal physical exam findings for all cats included pale mucous membranes. Other common abnormal physical exam findings included a systolic heart murmur, grade II–IV/VI ($n = 8$), gallop rhythm ($n = 5$), and tachypnea ($n = 4$).

Laboratory results

CBC

The HCTs in all cats were consistent with a severe non-regenerative anemia, ranging from 5 to 10% (median, 7%) with a normal total protein. Other abnormalities included thrombocytosis ($n = 1$), mild lymphopenia ($n = 2$), or lymphocytosis ($n = 5$).

Chemistry

The total bilirubin was very mildly elevated in four cats. Other abnormalities included mild hyperglobulinemia ($n = 3$), hypokalemia ($n = 7$), and mild hyperglycemia ($n = 5$). Table 2 summarizes the significant laboratory results of the 10 cats at the time of initial evaluation, including medians and ranges. CBC results and total protein are presented for all 10 cats, whereas other chemistry results are shown only for those cats in which they were abnormal.

Blood type

All cats were blood typed, 9/10 cats were type A and one cat was type B.

Coombs' test

Six of 10 cats were direct Coombs tested prior to receiving a blood transfusion or GC. Five of six cats

Table 2. Summary of the significant laboratory results at the time of initial evaluation for the 11 cats diagnosed with idiopathic PRCA.

	n	Median	Range	Reference interval
CBC				
HCT (%)	10	7	5–10	27–45
Total protein (g/dl)	10	7.4	6.2–8.3	6–7.8
Mean corpuscular volume (fl)	10	47.3	42.3–59.3	39–55
Mean corpuscular hemoglobin concentration (g/dl)	10	31.5	29.5–40.5	30–36
Reticulocyte count				
Absolute ($\times 10^6/\mu l$)	10	0.014	0.002–0.026	0.004–0.060
Percentage (%)		1.1	0.1–2	0.1–1.2
Platelets ($\times 10^3/\mu l$)	10	395	262–1481	175–600
White blood count ($\times 10^3/\mu l$)	10	13.065	7.66–20.07	5–19.5
Neutrophils ($\times 10^3/\mu l$)	10	7.32	3.11–10.17	2.5–12.5
Lymphocytes ($\times 10^3/\mu l$)	10	4.14	1.07–11.29	1.5–7
Monocytes ($\times 10^3/\mu l$)	10	0.390	0.077–0.99	0–0.85
Eosinophils ($\times 10^3/\mu l$)	10	0.534	0–1.53	0–0.75
Chemistry				
Total bilirubin (mg/dl)	4	0.3	0.27–0.4	0–0.2
Total protein (g/dl)	10	7.3	6.4–8.2	5.9–8.4
Albumin (g/dl)	10	3.7	3.1–4.4	2.3–3.9
Globulin (g/dl)	2	4.1 5.1	–	–
Potassium (mmol/l)	7	2.9	2.3–3.1	3.3–5.4
Glucose (mg/dl)	5	227	196–286	56–153

tested demonstrated a positive result based on reaction with a polyvalent Coombs reagent to IgG, IgM and complement component 3. Quantitative titers were recorded for 3/6 cats and the titers were 1:8 ($n = 2$) and 1:16 ($n = 1$).

Infectious disease testing
All 10 cats were tested for FeLV/FIV using a combination Snap test for FeLV antigen (IFA) and FIV antibody (ELISA). All cats tested serum negative. In 9/10 cats the bone marrow was tested for FeLV (IFA) and all nine cats were negative. All cats were evaluated for *M haemofelis*. Eight of 10 cats were tested for *M haemofelis* via whole blood PCR; 8/8 tested negative. The remaining two cats that were not PCR tested had a blood smear reviewed with no organisms detected.

Imaging results
Nine of 10 cats had thoracic radiographs taken and 8/9 cats had generalized cardiac enlargement with normal pulmonary vasculature and pulmonary parenchyma. The remaining cat had a normal heart, pulmonary vasculature, and lungs. All cats had abdominal imaging performed as part of their work-up. Two of 10 cats had abdominal radiographs taken; one cat was also imaged with an abdominal ultrasound and the other cat's abdomen was only imaged using survey radiographs. The abdominal radiographs in both cats were described as normal. Nine of 10 cats had a complete abdominal ultrasound. Five were interpreted as normal with no ultrasonographic abnormalities and the remaining four cats had non-specific changes. All imaging was performed and evaluated by a board-certified radiologist.

Bone marrow aspirates and core biopsies
All of the cats had bone marrow aspirates and 9/10 cats had bone marrow core biopsies performed. All bone marrow samples were reviewed by a single pathologist (JW). Nine of 10 bone marrow aspirates were considered diagnostic. The one cat with a non-diagnostic bone marrow aspirate had a diagnostic bone marrow core. Bone marrow cellularity was normal in 5/9 cats and decreased in 4/9 cats. Erythroid precursors were absent or present in very low numbers in all cats. When erythroid precursors were present, mostly early stages (rubriblasts, prorubricytes) were noted with normal morphologies; later stages, (rubricytes, metarubricytes, reticulocytes), were rare or absent. Granulocytic precursors were present in all stages and exhibited progressive maturation in all cats. The M:E ratio was increased in all cats due to a marked decrease in the erythroid line, the M:E ratio was 10:1 ($n = 1$), 15:1 ($n = 2$), and >20:1 ($n = 6$). Megakaryocytes were present with normal morphologies in all samples. The number of small lymphocytes was normal in seven cats and increased in two cats. In the cats with increased lymphocyte numbers, the lymphocytes represented 35–60% of the nucleated cell population. Plasma cells and macrophages were present in normal numbers in all cats, although one cat had macrophages that displayed moderate erythrophagocytosis. Eosinophil numbers were mildly increased in one cat. No dysplastic or neoplastic processes were noted on slides examined. Eight of nine bone marrow core biopsies were considered diagnostic. The cellularity of the bone marrow core samples was normal in 3/8 cats and hypocellular in 5/8 cats. Erythroid precursors were absent or present in very low numbers in all cats. The majority of cells were granulocytes and granulocytic precursors in all samples. The granulocytes were present in various stages of development and displayed orderly maturation. Megakaryocytes were present in adequate numbers with normal morphology in all cats. Lymphocyte numbers were normal (6/8) to mildly increased (2/8). For the two cats with increased lymphocytes, the lymphocytes represented 25% of the nucleated cells. Plasma cells and macrophages were present in normal numbers. Eosinophils were mildly increased in one cat. In two cats the bone marrow contained a small amount of regional collagenous tissue (mild myelofibrosis). No dysplastic or neoplastic processes were noted on sections examined.

Initial empirical and supportive treatments (prior to diagnosis)

Supportive treatments administered prior to a definitive diagnosis included blood products in all cats, either packed red blood cells (pRBCs) and/or fresh whole blood transfusions. Six of 10 cats required multiple transfusions; the median number of transfusions per cat was 2 (range 1–2). Three cats were also treated with oxyglobin (dosage 8.2–12.3 ml/kg). All 10 cats were supported with fluid therapy supplemented with potassium chloride and treated with doxycycline, at a median dosage of 5 mg/kg q 12 h (range 5–12.5 mg/kg q 12 h). Additional therapies included fenbendazole ($n = 2$), enrofloxacin ($n = 2$), clindamycin ($n = 1$) and imidacloprid ($n = 1$).

Immunosuppressive and supportive treatments (post-diagnosis)

Immunosuppressive therapies initially prescribed following the bone marrow diagnosis of idiopathic PRCA included GC (prednisone/prednisolone, median dosage 3 mg/kg/day (range 2.5–4.4 mg/kg/day) in nine cats, and dexamethasone at 0.8 mg/kg/day in one cat) and CsA, a median dosage of 8.5 mg/kg/day (range 5–20 mg/kg/day) in all cats. Nine of 10 cats treated with CsA were treated with CsA modified (Neoral; Novartis, Atopica; Novartis, Generic; Ivax, Generic; Pliva) and one cat was treated with CsA in oil (Sandimmune; Novartis). Eight cats also received additional blood products (pRBCs, fresh whole blood, or oxyglobin) 3–29 days (median 8 days) following the initiation of immunosuppressive therapy; two cats required two transfusions.

Short-term follow-up (60 days)

Follow-up data beyond the initial diagnosis were available for 8/10 cats; two cats were LTF within 1–2 weeks of their initial diagnosis. All eight cats achieved CR in a median of 31 days (range 15–51 days). One additional cat was LTF after day 46; therefore 7/10 cats had follow-up data available at 60 days.

Six of eight cats required adjustments in their immunosuppressive therapies prior to remission; an increase in CsA in three cats due to persistent anemia, the withdrawal of CsA in one cat due to an increase in alanine aminotransferase activity (ALT), a decrease in the CsA dose in one cat due to an increased whole blood CsA trough level (>500 ng/ml), a decrease in the dexamethasone dose in a cat following hospitalization for congestive heart failure (CHF), and one cat was changed from prednisone to dexamethasone due to the persistent anemia. Table 3 includes a summary of the reasons and specific dosage adjustments made in these six cats.

The therapeutic regimens that were associated with clinical remission in the nine cats with follow-up data available included prednisone/prednisolone (median dosage 3.2 mg/kg/day, range 2.5–4.4 mg/kg/day) and CsA (median dosage 10 mg/kg/day, range 5.6–20 mg/kg/day) in six cats and dexamethasone (0.4 and 0.54 mg/kg/day) and CsA (9 and 15.4 mg/kg/day) in two cats.

The seven cats with follow-up beyond 60 days remained in CR at day 60. Following remission immunosuppressive therapy was tapered in 4/7 cats before day 60; three cats had a reduction in their GC dose without a change in the CsA dose and one cat discontinued CsA and was maintained on a tapered prednisolone dose.

Long-term follow-up (>60 days)

The seven cats followed to 60 days had additional follow-up data available for a median of 1437 days (range 457–2201 days). All seven cats remained in CR for a median of 406 days (range 128–2186 days). Six of seven cats were weaned completely off all immunosuppressive drugs and remained in CR for a median of 307 days (range 84–1851 days) off therapy. The one remaining cat relapsed 10 days after a dosage reduction in CsA, 155 days after initiating immunosuppressive therapy; immunosuppressive therapy was never discontinued in this cat but was tapered more slowly. Four of the six weaned off medication had a relapse

Table 3. Summary of initial immunosuppressive therapy started, dosage adjustment made prior to remission, day dosage adjustment was made, and reason for dosage adjustment for each of the 10 cats diagnosed with idiopathic PRCA.

Cat ID	Initial therapy (mg/kg/day)	Dosage adjustments (mg/kg/day)	Dosage adjustment (day)	Reason
1	GC − 2.6 CsA − 10	N/A	N/A	LTF, day 7
2	GC − 3 CsA − 6.4	N/A	N/A	LTF, day 9
3	GC − 4 CsA − 20	N/A	N/A	N/A
4	GC − 5.6 CsA − 8	GC − 2.8 CsA − 15.4	38	CHF
5	GC − 2.6 CsA − 5.7	GC − NC CsA − 10.4	6	Persistent anemia
6	GC − 2.5 CsA − 10	GC − NC CsA − discontinued	13	Elevated ALT (1124 U/l)
7	GC − 3.3 CsA − 11.2	GC − NC CsA − 5.6	14	High CsA level (>500 ng/ml)
8	GC − 4.4 CsA − 6.9	N/A	N/A	N/A
9	GC − 2.6 (prednisone) CsA − 9	GC − 3.8 (dexamethasone) CsA − NC	19	Persistent anemia
10	GC − 3 CsA − 5	GC − NC CsA − 8.8	5	Persistent anemia

GC dosage based on potency of prednisone; CsA = cyclosporine A; N/A = not applicable; NC = no change.

of their anemia at a median of 413 days (range 376–1413 days) after beginning initial immunosuppressive therapy, and a median of 236 days (range 84–1173 days) after discontinuing immunosuppressive drugs. During the observation period, the two final cats did not relapse after discontinuing immunosuppressive drugs (197 and 1851 days, respectively).

Of the four cats that relapsed off immunosuppressive therapy, one cat did not respond to reintroduction of prednisone (2.8 mg/kg/day), and died of CHF within 24 h. Remission was successfully reinduced in three cats in a median of 25 days (range 2–26 days) following the reintroduction of GC and/or CsA. Table 4 summarizes the reinduction therapies administered to each of the cats that relapsed.

Final dosages of medications for the cats at last follow-up were prednisolone alone 0.6 mg/kg/day, CsA alone at 0.2 mg/kg every 2 weeks, and CsA (6.25 mg/kg/day) plus prednisolone (0.5 mg/kg q 48 h). One cat was euthanased 80 days following reinduction therapy, after development of diabetic ketoacidosis (DKA) and secondary hepatic lipidosis (HL); the cat's HCT at the time had remained stable.

Tables 4 and 5 summarize the immunosuppressive therapeutic regimens associated with clinical remission, time to remission, duration of remission, days to relapse, reinduction therapy, long-term therapy, outcome, and days of follow-up for all 10 cats. In summary of these 10 cats, three cats were LTF before 60 days and two cats were deceased (one following disease relapse and one due to the development of DKA and secondary HL). Of the remaining cats, two maintained CR off therapy for 197 and 1851 days, respectively and three cats relapsed. All three cats responded to reinduction therapy and maintained remission for a median of 1176 days (range 182–1456 days) on long-term low dose immunosuppressive drugs.

Whole blood trough CsA levels

Five of nine cats treated with CsA modified had whole blood trough CsA levels determined using a high pressure liquid chromatography (HPLC) method; blood was collected 8–19 days (median 13 days) after starting CsA. All five cats were clinically doing well, although 4/5 cats remained anemic. The cats were treated with a median CsA dosage of 8.8 mg/kg/day (range 4–10 mg/kg/day) and the associated median whole blood trough CsA level was 218 ng/ml (range 96–368 ng/ml). The one cat treated with CsA in oil had an elevated trough CsA levels (1602 ng/ml) 14 days after initiating oral CsA therapy; CsA levels decreased in association with CsA dose reduction. Table 6 summarizes the CsA levels measured in each cat.

Table 4. Summary of immunosuppressive therapeutic regimens associated with clinical remission, time to remission, duration of remission, days to relapse, and reinduction therapy in all 10 cats diagnosed with idiopathic PRCA.

Cat ID	Therapy (mg/kg/day)	Remission (days)	Remission duration (days)	Relapse (days)	Reinduction therapy (mg/kg/day)
1	N/A	N/A	N/A	N/A	N/A
2	N/A	N/A	N/A	N/A	N/A
3	GC − 4 CsA − 20	20	13	33	N/A
4	GC − 2.8 CsA − 15.4	51	406	None	None
5	GC − 2.6 CsA − 10.4	15	2186	None	None
6	GC − 2.5 CsA − 10	35	341	376	GC − 3.8 CsA − 12.6
7	GC − 3.3 CsA − 5.6	27	128	155	GC − 0.3 CsA − 2.4
8	GC − 4.4 CsA − 6.9	36	357	393	GC − 3 CsA − 6.25
9	GC − 3.8 CsA − 9	41	1372	1413	GC − 2.8
10	GC − 3 CsA − 8.8	18	415	433	GC − 4 CsA − 8.8

GC dosage based on potency of prednisone; CsA = cyclosporine A; N/A = not applicable.

Table 5. Summary of long-term therapy, outcome, and days of follow-up in all 10 cats diagnosed with idiopathic PRCA.

Cat ID	Long-term therapy	Disease outcome	Follow-up (days)
1	N/A	LTF	7
2	N/A	LTF	9
3	N/A	LTF	46
4	None	LTF	457
5	None	CR	2201
6	GC − 0.6 mg/kg/day	CR	1577
7	CsA − 0.2 mg/2 weeks	CR	1619
8	GC − 0.5 mg q 48 h CsA − 6.25 mg/kg/day	CR	596
9	N/A	D (CHF)	1437
10	N/A	E (DKA)	513

GC dosage based on potency of prednisone; N/A = not applicable; NC = no change; CsA = cyclosporine; D = died; E = euthanized; LTF = lost to follow up; CR = complete remission; DKA = diabetic ketoacidosis; CHF = congestive heart failure.

Adverse effects/complications

Six cats experienced adverse effects/complications during immunosuppressive therapy with GC and/or CsA. Adverse effects included CHF ($n = 2$), upper respiratory infection ($n = 1$), diabetes mellitus ($n = 1$), elevated liver enzymes ($n = 1$), anaphylaxis ($n = 1$), pancreatitis ($n = 1$), DKA with HL ($n = 1$). The adverse effects/complications and outcomes for these six cats are summarized in Table 7.

Discussion

In this group of cats, idiopathic PRCA occurred predominately in young cats; all cats presented with a severe chronic non-regenerative anemia requiring a blood cell transfusion at the time of presentation, with most cats requiring multiple transfusions. The most common physical exam findings were consistent in with a marked anemia including weakness, pale mucous membranes, and an auscultable heart murmur. Approximately half of the cats were hypokalemic at the time of presentation, likely secondary to decreased food intake; long-term potassium supplementation was not required once the cats' appetites returned. None of the cats had a significant hyperbilirubinemia or splenomegaly associated with their anemia suggesting no significant extravascular hemolysis.[1] Thoracic and abdominal imaging was unremarkable, although general cardiomegaly without evidence of heart failure was commonly noted. The

Table 6. Cyclosporine whole blood trough concentrations measured in 5/10 cats treated with oral CsA modified and one cat treated with oral CsA in oil. Information summarized includes dosage of CsA, trough CsA level, day of evaluation after initiation of immunosuppressive therapy, reason for CsA level, and associated dosage adjustments.

Cat ID	Dosage of CsA (mg/kg/day)	Trough CsA levels (ng/ml)	Trough CsA (day)	Reason for CsA level	Dosage adjustment (mg/kg/day)
1	10	N/A	N/A	N/A	N/A
2	6.4	N/A	N/A	N/A	N/A
3	4	N/A	N/A	N/A	N/A
4	4	303	8	RM	NC
5	5.2	177	10	RM	NC
6	10	96	8	RM	NC
7	11.2	1602	14	RM	5.6
	5.6 (CsA in oil)	442	27	Recheck	NC
8	6.9	N/A	N/A	N/A	N/A
9	9	368	19	RM	NC
10	8.8	218	18	RM	NC

N/A = not applicable; RM = routine monitoring; NC = no change; CsA = cyclosporine.

Table 7. Summary of immunosuppressive therapy associated adverse effects/complications and outcomes in six cats.

Cat ID	Post-diagnosis (days)	Adverse effects	Outcome
4	27	CHF	Controlled with furosemide, benazepril, spironolactone, and decreased GC dosage (from 5.6 to 2.8 mg/kg/day)
		URI	Supportive care
5	100	Vomiting/diarrhea	Secondary to high CsA level (791 ng/ml); resolved with CsA dosage reduction (from 10.4 to 5.2 mg/kg/day)
6	2	Diabetes mellitus	Diabetic remission with glargine insulin treatment
	13	Increased ALT (1124 U/l); CsA 96 ng/ml	Resolved with CsA withdrawal
7	1	Anaphylaxis − IV CsA* (8 mg/kg)	Controlled by discontinuation of IV infusion and supportive therapy (IV fluids, diphenhydramine)
9	81	Pancreatitis with second biliary obstruction	Recovered with supportive therapy and a reduction of both GC (from 2.8 to 0.2 mg/kg/day) and CsA (from 9 to 4.4 mg/kg/day) dosages
	1437	CHF	Died
10	511	DKA HL	Euthanased

URI = upper respiratory infection; ALT = alanine aminotransferase ; CHF = congestive heart failure; DKA = diabetic ketoacidosis; HL = hepatic lipidosis.
*CsA (Sandimmune; Novartis).

characteristics of this group of cats with idiopathic PRCA are consistent with those reported previously in a group of FeLV negative cats diagnosed with idiopathic PCRA.[7]

The diagnosis of idiopathic PRCA was established in these cats based on an isolated anemia, the lack of an identifiable underlying systemic disease, and consistent bone marrow findings.[6,7,25] Increased numbers of small lymphocytes were noted in approximately half of the cat's bone marrow samples. A lymphocytosis at the level of the bone marrow has been associated with idiopathic PRCA and idiopathic nonregenerative immune-mediated anemia in dogs[4,26] and cats.[7] In two cats regional mild myelofibrosis was described. Secondary myelofibrosis has been described in humans[27] and dogs[5,28] with PRCA.

The results of this retrospective study support the hypothesis that idiopathic PRCA in cats has an underlying immune-mediated mechanism analogous to that reported in humans,[2,3,29−31] and dogs,[4,5,32] and that disease relapse often occurs following drug withdrawal.[7] All cats with at least short-term follow-up achieved disease remission in association with immunosuppression and 5/6 cats tested in this study were Coombs' test positive. Treatment with combination immunosuppressive therapy was associated with CR in the majority of cats, which is similar to what has been previously reported,[7] albeit with a different therapeutic regimen. In the previous case series of cats with idiopathic PRCA, 6/7 cats with follow-up data achieved CR within 3/5 weeks after initiating treatment with GC and cyclophosphamide, and 3/4 cats with long-term follow-up experienced disease relapse after 3 months to 2 years. This previous report in cats, along with our findings, suggests that both an initial response to immunosuppression and relapse, are common in cats with PRCA.

The clinical course of idiopathic PRCA in cats is similar to that reported in humans; patients with idiopathic PRCA experience initial remission with immunosuppressive therapy, although disease relapse is common.[33] The most common immunosuppressive drugs used to achieve remission in humans with PRCA are GC and CsA, with reported remission rates of 30−62% and 65−87%, respectively.[8−13,34] The use of CsA alone or in combination with GC has higher remission rates, with up to 87% clinical remission, often within 2 weeks of CsA initiation.[10,13] A significant drawback of GC monotherapy in humans is not that remission is infrequent, but that disease relapse and unacceptable side effects are common.[8,13] CsA therapy in humans

is also associated with significantly longer median relapse-free intervals, of 103 months for CsA ± GC, versus 33 months for GC alone.[13] Therefore, CsA has become the leading immunosuppressive therapy used for treating idiopathic PRCA in humans.[12,13] However, without sustained CsA maintenance therapy, relapse within 3 months of drug withdrawal remains as high as 86%.[13,35]

Suggested therapeutic goals for CsA in humans with immune-mediated cytopenias are whole blood trough CsA concentrations of 150–250 ng/ml for a maximum of 3–4 months, followed by maintenance therapy at a minimum dosage as appropriate to maintain remission.[16,36] This recommended CsA trough concentration is empirical, but was chosen by consensus, based on the results of a multi-center randomized study using CsA for the treatment of aplastic anemia in humans.[36] In this study, whole blood trough CsA concentrations were available in five cats and ranged from 96 to 368 ng/ml, at a median CsA dose of 8.8 mg/kg/day, divided q 12 h. All five cats were clinically doing well and achieved disease remission in 31 days (range 15–51 days). Although, initial whole blood CsA trough concentrations were only evaluated in a small number of cats, these levels approximated the therapeutic range recommended for humans with idiopathic immune-mediated cytopenias. Evaluation of a larger number of cats is warranted to determine the therapeutic target for cyclosporine concentrations in cats with immune-mediated diseases including idiopathic PRCA.

One clinical advantage of CsA is its rapid immunosuppressive effect secondary to its inhibition of calcineurin, although this also leads to its toxic effects. The inhibition of calcineurin inhibits T cell activation through the suppression of genes required for B cell stimulation (IL-4 and CD40 ligand) and T cell proliferation (IL-2).[37] The side effects associated with CsA use include anorexia and gastrointestinal signs (vomiting and diarrhea),[38] but it is unclear whether these are associated with high CsA concentrations. However, gastrointestinal side effects of CsA appear to be dose-dependent, and were reversible with dose reduction in one cat in this series.

Hepatotoxicity has been reported in cats treated with CsA, primarily in association with excessive whole blood CsA concentrations (>3000 ng/ml).[39] In this case series, the one cat with an increase in ALT activity had a trough CsA concentration of 96 ng/ml. However, the ALT normalized with discontinuation of the cyclosporine.

Other less commonly reported side effects in cats treated with CsA include secondary infections[38] (seen in two cats in this series), and lymphoproliferative disorders such as lymphoma,[40,41] which we did not observe in this small population.

One cat had clinical signs of anaphylaxis during an IV infusion of CsA (Sandimmune; Novartis), which resolved with immediate discontinuation of the infusion and supportive therapy including IV fluids and diphenhydramine. The cat was subsequently treated with oral CsA in oil (Sandimmune; Novartis) which was well tolerated. In humans, anaphylaxis associated with intravenous CsA is reported to be an IgE-mediated response to the polyoxyethylated castor oil vehicle in the IV formulation.[42–46] Following anaphylaxis, patients have been subsequently treated without incident with oral CsA in oil which is formulated without the polyoxyethylated castor oil carrier.[43,45]

The limitations of this study are the retrospective study design and the small number of cases available. The study design did not allow for an objective evaluation of the effectiveness of CsA versus GC in the treatment of cats with idiopathic PRCA. The low number of cases (11 over a 13-year period) is difficult to overcome, as idiopathic PRCA is a relatively uncommon diagnosis in cats. A randomized controlled prospective clinical study would need to be multi-center in order to objectively evaluate therapeutic efficacy among two or more immunosuppressive protocols in cats with idiopathic PRCA.

In summary, this is the first reported case series describing the clinical use of CsA in cats with idiopathic PRCA. Eight of 10 cats treated with GC and CsA had adequate follow-up and achieved CR in a median of 31 days (range 15–51 days). Two cats maintained long-term disease remission off immunosuppressive therapies. Disease relapse was common, particularly after drug discontinuation, and occurred after a median of 413 days in 4/6 cats that had follow-up beyond 60 days. The cats that relapsed required reinduction therapy followed by long-term low dose immunosuppressive therapy.

Side effects associated with both GC and CsA therapies were relatively common, therefore, therapy needs to be monitored and individualized for each patient. Whole blood trough CsA concentrations may be helpful in cats with clinical signs of CsA toxicity, but are not always increased in cats with side effects. Anaphylaxis to IV CsA is possible, and cats should be monitored carefully during cyclosporine infusions.

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