Idiopathic pure red cell aplasia and nonregenerative immune-mediated anemia in dogs: 43 cases (1988–1999)

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Objective—To examine clinical features, laboratory test results, treatment, and outcome of dogs with pure red cell aplasia (PRCA) and idiopathic nonregenerative immune-mediated anemia (NRIMA).

Design—Retrospective study.

Animals—43 dogs with severe nonregenerative anemia.

Procedure—Medical records of dogs determined to have PRCA, NRIMA, or ineffective erythropoiesis on the basis of bone marrow analysis between 1988 and 1999 were reviewed. Criteria for inclusion were ≥ 5-day history of severe nonregenerative anemia (Hct < 20%; < 60.0 × 10^3 reticulocytes/μl) with no underlying diseases. Information was retrieved on signalment, clinical signs, laboratory test results, treatment, and outcome.

Results—Median age of the dogs was 6.5 years. Spayed females and Labrador Retrievers were significantly overrepresented. Median Hct was 11% with no evidence of regeneration (median, 1.5 × 10^3 reticulocytes/μl). Direct Coombs' test results were positive in 57% of dogs. Biochemical abnormalities included hyperferremia and high percentage saturation of transferrin. Bone marrow findings ranged from PRCA (5%) to erythroid hyperplasia (55%). Myelofibrosis was common. Dogs were treated with immunosuppressive drugs and the response was complete, partial, and poor in 55, 18, and 27% of the dogs, respectively. Mortality rate was 28%.

Conclusions and Clinical Relevance—An immune-mediated pathogenesis should be considered in dogs with severe, nonregenerative anemia, normal WBC and platelet counts, hyperferremia, mild clinical signs, and no evidence of underlying disease. Bone marrow findings range from the rare PRCA to erythroid hyperplasia. Myelofibrosis is often detected in affected dogs and may prevent bone marrow aspiration. (*J Am Vet Med Assoc* 2000;216:1429–1436)

Immune-mediated hemolytic anemia (IHA) is a common hematologic disorder in dogs, resulting from antibody-mediated (with or without complement) destruction of RBC directly within the circulation or by the monocyte-macrophage system. In most affected dogs, the anemia is idiopathic, although neoplasia (especially lymphoma), immunologic disorders (eg, **systemic lupus erythematosis [SLE]**) and infectious agents (eg, *Babesia canis*) can initiate the disease.[1-3] Classically, IHA is characterized by a moderate-to-severe regenerative anemia with spherocytosis, leukocytosis with a left shift, and a positive Coombs' test result.[3,4] **Pure red cell aplasia (PRCA)**[5-11] and nonregenerative forms of IHA[9,10,12-18] have been recognized in dogs. Lack of regeneration has been attributed to the lag time for development of a regenerative response in dogs with peracute anemia, bone marrow suppression from underlying disease, infiltrative bone marrow disease, inadequate nutrients (eg, iron) for erythropoiesis, and immune-mediated destruction of erythroid progenitors in the bone marrow.[1,7,10] The chronicity of anemia (based on duration of clinical signs) and response to treatment with immunosuppressive drugs in these reports favor an immune-mediated pathogenesis for the nonregenerative nature of these disorders. Up to 33 to 58%[1,a,b] of IHA can be nonregenerative, but in these reports, chronicity of anemia was not clearly established, making it difficult to distinguish those dogs with anemia that were truly nonregenerative from those with anemia of peracute onset. Failure to recognize severe, nonregenerative anemia as being immune-mediated can have serious consequences on clinical outcome.

The purpose of the study reported here was to examine clinical features, laboratory test results, treatment, and outcome of dogs with PRCA and idiopathic **nonregenerative immune-mediated anemia (NRIMA)**. We report on 43 dogs with severe idiopathic nonregenerative anemia that was responsive, in most dogs, to treatment with immunosuppressive drugs. The anemia in 2 of these dogs was caused by PRCA, whereas the remaining 41 dogs had evidence of erythropoiesis in the bone marrow. We used the term NRIMA to describe the anemia in the latter dogs, as reported for other similarly affected dogs.[9,10,12-18]

Criteria for Selection of Cases

The computer database of the Clinical Pathology Laboratory was searched for canine patients with diagnoses of PRCA, NRIMA, or ineffective erythropoiesis made from smears of bone marrow aspirates performed at the Veterinary Medical Teaching Hospital at Cornell University. The terms NRIMA and ineffective erythropoiesis were used interchangeably as diagnostic codes in the computerized records of dogs with severe nonregenerative anemia but with cytologic or histologic evidence of erythropoiesis in marrow. Medical records of dogs retrieved by the search were reviewed. Criteria for inclusion in our study were a minimum 5-day history of clinical signs, a severe nonregenerative anemia (Hct < 20%) with an absolute reticulocyte count < 60 × 10^3/μl (absolute reticulocyte counts 2 or more fold higher than 60.0 × 10^3/μl would be expected in a regenerative anemia), and absence of any identifiable underlying disease that could be the primary cause of the

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severe anemia. Information was retrieved on signalment, clinical signs, laboratory test results, treatment, and outcome.

Procedures

Hemograms, coagulation profiles, serum biochemical analyses, and immunologic tests had been performed on dogs at the time of admission to the teaching hospital. Blood samples collected into tubes with EDTA were analyzed with an automated hematology analyzer.[c] A direct Coombs' test was performed on blood samples that contained EDTA as an anticoagulant. For this test, samples were washed 4 times in physiologic saline (0.9% NaCl), a 2% RBC suspension was serially diluted in saline, then mixed with a canine-specific Coombs' reagent (containing rabbit antiglobulin against canine IgG, IgM, and C3).[d] After incubation at 37 C for 30 minutes, the samples were evaluated microscopically for agglutination. Coagulation panels were performed on blood samples collected into a 3.8% solution of citrate, using a mechanical detection device (fibrometer)[e] and standard methods.[19] Serum **fibrin(ogen) degradation product (FDP)** concentrations were measured using a commercial latex agglutination kit.[f] Biochemical analyses were performed on serum or heparinized blood samples using standard methods with an automated chemistry analyzer.[g]

Proportions of dogs by sex (sexually intact male, castrated male, sexually intact female, spayed female) and breed (those represented by 2 or more dogs) in our study were compared with a standard population (those admitted to the teaching hospital during the same period). Response to treatment was defined as an absolute reticulocyte count > $60 \times 10^3/\mu l$ or increasing Hct (unrelated to blood transfusions). Response to treatment was divided into 3 categories: complete, in which Hct increased to within or just below the reference range; partial, in which Hct did not reach the value necessary for a complete response; and poor (or no) response, in which there was no increase in either absolute reticulocyte count or Hct. Proportions of dogs by response to treatment were compared among the different types of immunosuppressive drugs (corticosteroids alone, corticosteroids and cyclophosphamide, and corticosteroids and azathioprine). To determine whether duration of anemia affected response to treatment, the median duration of anemia was compared among the 3 response categories. To determine whether certain laboratory variables affected response to treatment, proportions of dogs with high **antinuclear antibody (ANA)** titers (> 1:80), positive Coombs' test results, spherocytosis in smears of blood samples obtained from a peripheral vein, and detection of myelofibrosis or erythroid hyperplasia in bone marrow aspirates were compared among the 3 response categories. In addition, median Hct, WBC count, **mean erythrocyte cell volume (MCV)**, platelet count, serum alkaline phosphatase activity, and serum total bilirubin concentration at the time of admission were compared among the 3 response categories.

Statistical Analysis

Proportions of dogs by sex and breed were compared using a 1-group proportions test.[h] Proportions of dogs with high ANA titers, positive Coombs' test results, spherocytosis, myelofibrosis, or erythroid hyperplasia were compared with a 2-group proportions test (which defaulted to a Fisher's exact test when sample sizes were < 40).[h] Medians were compared using the Kruskall-Wallis one-way ANOVA and rank sum test.[h] A value of $P < 0.05$ was used to indicate significance.

Results

Signalment and clinical signs—Fifty-four dogs were retrieved from the search of the database. Of these, 43 met the criteria for inclusion in our study. Ten dogs with concurrent diagnoses of *Ehrlichia canis* infection, neoplasia, or chronic renal failure were excluded. One dog that was being treated with primidone for idiopathic epilepsy was excluded. All these dogs were referred to the teaching hospital for evaluation or treatment of severe anemia.

Dogs ranged from 10 months to 12 years old (median age, 6.5 years), and included 6 (14%) sexually intact males, 8 (19%) castrated males, 6 (14%) sexually intact females, and 23 (53%) spayed females. Spayed female dogs were significantly ($P = 0.01$) overrepresented, compared with the population admitted to the teaching hospital during the same time (of which 34% were spayed females). There were 13 (30%) mixed-breed dogs, 5 (12%) Labrador Retrievers, 2 (5%) Golden Retrievers, 2 (5%) Rottweilers, 2 (5%) Dachshunds, and 1 (2%) Beagle, Boston Terrier, Bull Terrier, Cocker Spaniel, Doberman, English Springer Spaniel, German Shepherd Dog, Great Dane, Irish Setter, Miniature Dachshund, Miniature Schnauzer, Peke-a-poo, Pekingese, Pomeranian, Sealyham Terrier, Tibetan Mastiff, Tibetan Spaniel, and Whippet. Of these breeds, Labrador Retrievers were significantly ($P = 0.002$) overrepresented, compared with the population admitted to the teaching hospital during the same time (of which 3% were Labrador Retrievers).

Dogs were referred with a 5 day to 3 month history of lethargy (29 dogs; 67%), anorexia (14; 33%), pallor (12; 28%), weakness (12; 28%), weight loss (7; 16%), pica (7; 16%), collapse (7, 16%), exercise intolerance (4; 9%), vomiting (3; 7%), fever (3; 7%), syncope (3; 7%), coughing (2; 5%), seizures (1; 2%), and signs of depression (1; 2%). Thirty-one (72%) dogs had clinical signs for more than 7 days prior to referral. Two dogs had nonregenerative anemia that progressively worsened over 3 to 4 weeks and 2 had an initially regenerative anemia, which worsened and became nonregenerative over 1 to 4 weeks before referral. Eighteen dogs (with known vaccination histories) had been vaccinated against distemper virus, canine infectious hepatitis virus, and parvovirus within 6 days to 3 years before referral. One dog had clinical signs for up to 3 weeks before vaccination. Treatment administered by referring veterinarians included corticosteroids (dexamethasone or prednisolone) in 18 dogs, antibiotics (amoxicillin, cephalosporins, enrofloxacin, and doxycycline) in 13 dogs, 1 to 2 blood transfusions in 7 dogs, anabolic steroids in 2 dogs, and recombinant human erythropoietin in 1 dog. Three dogs were being treated for pre-existing conditions, namely hypothy-

roidism (thyroxine), hypoadrenocorticism (dexamethasone and fludrocortisone acetate), and atopy (alternate day administration of prednisolone).

On admission to the teaching hospital, 37 (86%) dogs were bright and alert and 6 (14%) were depressed or listless. Findings on physical examination were mucous membrane pallor (43 dogs, 100%), grade I-IV/VI systolic cardiac murmurs (22, 51%), tachycardia (11, 26%), tachypnea (8, 19%), splenomegaly (6, 14%), and hepatomegaly (2, 5%). Most dogs were clinically stable and appeared tolerant of the anemia. One dog had evidence of pulmonary thromboembolism (dyspnea with ventilation-perfusion mismatch on blood gas results).

Laboratory test results—All 43 dogs were severely anemic (median Hct, 11%), with absolute reticulocyte counts < 60×10^3 reticulocytes/µl (median, 1.5×10^3 reticulocytes/µl; **Table 1**). On admission to the teaching hospital, Hct of the 7 dogs that received blood transfusions before referral ranged from 7 to 26%, whereas their Hct before transfusion ranged from 9 to 14%. The anemia was normocytic normochromic in most dogs. Nucleated RBC (median, 0 nucleated RBC/100 WBC; range, 1 to 7 nucleated RBC/100 WBC) and small to large numbers of spherocytes were observed in Wright's stained smears of blood samples obtained from peripheral veins of 7 (16%) and 23 (54%) dogs, respectively. Other morphologic abnormalities observed in RBC were small-to-moderate numbers of macrocytes (8 dogs, 19%), ovalocytes (6, 14%), fragments (schistocytes or keratocytes; 3, 7%), and agglutination (2, 5%). The WBC counts were within the reference range in 17 (40%) dogs. Twenty-one (49%) dogs had a mild left shift, and 5 dogs had > 1×10^3 band neutrophils/µl. Two (5%) dogs were mildly pancytopenic (Hct values, 11 to 12%; neutrophil count, 3.5 to 3.6×10^3 neutrophils/µl; platelet count, 106 to 133×10^3 platelets/µl). In 11 dogs, platelet counts were not quantified; however, platelet numbers were estimated as adequate in Wright's stained smears of blood samples obtained from peripheral veins. The platelet count was < 30×10^3 platelets/µl (ie, 9×10^3 platelets/µl) in only 1 of the 7 thrombocytopenic dogs. The most common serum biochemical abnormalities were high liver enzyme activities, low bicarbonate concentration, and hyperferremia (**Table 2**). Sixteen of the dogs with high serum alkaline phosphatase activity had been treated with corticosteroids by the referring veterinarian or had hyperbilirubinemia. Results of coagulation profiles were within reference ranges in most dogs, and serum FDP concentrations were < 10 µg/ml. Urine specific gravity ranged from 1.006 to 1.060 (median, 1.020) in 30 dogs. Abnormalities on urinalysis were bilirubinuria (excessive for the urine specific gravity; 10 dogs, 33%), pyuria (3, 10%), moderate proteinuria (3+ reaction with sulfosalicylic acid precipitation, urine specific gravity, 1.054; 1, 3%), and bacteriuria (1, 3%).

Direct Coombs' test results were positive in 20 of 35 (57%) dogs, 5 of which had prior blood transfusions. In many dogs (12/20; 60%), the Coombs' test results were weakly positive (positive at dilutions < 1:4). Of the 15 dogs that had a negative Coombs' test result, 7 had received corticosteroids by the referring veterinarian. Results of serologic testing for *E canis* and *Rickettsia rickettsii* were negative in 21 of 21 and 16 of 16 dogs, respectively. Thirty-one dogs were tested for ANA by indirect immunofluorescence; 14 (45%) were seronegative, 10 (32%) had low titers within the reference range (ANA titers, 1:10 to 1:80; reference range, ≤ 1:80), and 7 (23%) had high titers (ANA titers, 1:160 to 1:640).

Table 1—Results of hematologic testing in 43 dogs with pure red cell aplasia and nonregenerative immune-mediated anemia at time of referral

Variables	Median	Range	No. (%) above reference range	No. (%) below reference range	Reference range
Hematocrit (%)	11	5–26	NA	43 (100)	39–57
RBC count ($\times 10^6$/µl)*	1.6	0.7–3.9	NA	42 (100)	5.6–19.6
Hemoglobin (g/dl) †	3.6	1.5–9.4	NA	40 (100)	13.7–19.6
MCV (fl)*	70	55–87	13 (31)	9 (21)	64–73
MCHC (g/dl)†	35	30–36	0	1 (3)	31–37
ARC ($\times 10^3$/µl)*	1.5	0–37.8	NA	42 (100)	< 60.0
WBC ($\times 10^3$ /µl)	16.9	3.7–45.5	16 (37)	10 (23)	7.5–19.9
Neutrophils	13.4	2.1–39.1	21 (49)	7 (16)	3.9–14.7
Band neutrophils	0.1	0–6.9	22 (51)	NA	0
Lymphocytes	1.4	0–4.3	0	23 (54)	1.5–5.2
Monocytes	0.8	0–5.5	10 (23)	11 (26)	0.3–2.2
Eosinophils	0.2	0–1.9	2 (5)	23 (54)	0.1–1.6
Basophils	0	0–0.7	2 (5)	NA	0–0.1
Platelets ($\times 10^3$/µl)‡	387	9–1,294	9 (28)	7 (22)	179–510
Total protein (g/dl)†	6.9	5.2–9.6	8 (20)	5 (13)	5.9–7.8

*The RBC count (and MCV and absolute reticulocyte count) was measured in 42 dogs (canceled in 1 because of agglutination). †Hemoglobin (and mean corpuscular hemoglobin concentration [MCHC]) and total protein concentration was measured in 40 dogs (canceled in 3 because of lipemia). ‡Measured in 32 dogs.

NA = Not applicable. MCV = Mean cell volume. ARC = Absolute reticulocyte count.

Bone marrow aspiration was performed on 39 dogs within 24 to 72 hours of referral. In the remaining 4 dogs, bone marrow was aspirated within 4 to 8 days of referral, after the Hct continued to decrease, with no evidence of regeneration. In 6 dogs, bone marrow was difficult to aspirate despite attempts at several sites (referred to as a "dry tap"); core biopsy specimens of the bone marrow were obtained in 5 of these dogs for histologic evaluation. In 21 dogs, bone marrow was difficult to aspirate and either no spicules (4 dogs) or spicules that were tough or "rubbery" and did not smear well were obtained. Bone marrow core biopsy specimens were obtained in 11 of these dogs. Bone marrow cellularity could not be determined in 4 dogs because of lack of spicules in the smears of the bone marrow aspirate and no subsequent core biopsy specimen. Cellularity was high in 29 (74%) dogs (estimated from Wright's stained smears of the bone marrow aspirate or H&E stained smears of the core biopsy specimen in 26 and 3 dogs, respectively), normocellular (considering the dogs' age) in 9 (23%) dogs (estimated from the aspirate and core biopsy specimen in 7 and 2 dogs, respectively), and hypocellular in 1 (3%) dog (estimated from the core biopsy specimen). Myeloid-to-erythroid ratios were determined in 37 dogs (excluding 6 dogs with a dry tap) and ranged from > 99:1 to 0.18:1. Erythroid status could not be judged in 1 dog with a dry tap and no core biopsy specimen. Of the remaining 42 dogs, there was erythroid hyperplasia in 23 (55%) dogs, normal erythroid numbers in 6

Table 2—Selected biochemical and hemostatic test results in dogs with pure red cell aplasia and nonregenerative immune-mediated anemia at the time of referral

Variables	No. of dogs	Median	Range	No. (%) above reference range	No. (%) below reference range	Reference range
Biochemical analysis						
Serum alanine aminotransferase (U/L)	41	59	9–1,399	18 (44)	1 (2)	17–85
Serum aspartate aminotransferase (U/L)	41	28	7–171	6 (15)	1 (2)	16–50
Serum alkaline phosphatase (U/L)	41	99	31–4,524	19 (46)	0	12–122
Serum bicarbonate (mEq/L)	41	18	13–27	0	18 (44)	16–26
BUN (mg/dl)	41	19	5–146	12 (29)	3 (7)	8–30
Serum total bilirubin (mg/dl)	40	0.2	0–1.6	9 (23)	0	0.1–0.4
Serum iron (mg/dl)	36	315	90–455	29 (81)	0	46–241
Iron saturation of transferrin (%)	36	95	25–100	32 (89)	0	17–69
Hemostasis						
PT (s)	20	7	5–9	1 (5)	2 (10)	6–8
APTT (s)	20	16	13–24	1 (5)	0	12–21
Fibrinogen concentration (mg/dl)	20	338	180–662	2 (10)	0	105–510
FDP concentration (μg/ml)	9	< 10	< 10	0	NA	< 10

BUN = Blood urea nitrogen. PT = Prothrombin time. APTT = Activated partial thromboplastin time. FDP = Fibrinogen degradation product. NA = Not applicable.

(14%) dogs, erythroid hypoplasia in 11 (26%) dogs, and PRCA (no identifiable erythroid precursors, M:E > 99:1) in 2 (5%) dogs. In 5 dogs, the erythroid maturation sequence was not evaluated because maturation stages could not be identified accurately in core biopsy specimens, insufficient cells were available on smears of the bone marrow aspirate, or there was PRCA. In the remaining 38 dogs, erythroid maturation was complete to polychromatophilic RBC in 24 (63%) dogs, although polychromatophilic RBC were scarce in 13 of these dogs. Erythroid precursors did not mature beyond basophilic rubricytes or metarubricytes in 11 (29%) and 3 (8%) dogs, respectively. There was a left shift in the maturation process (increased proportions of rubriblasts, prorubricytes, and basophilic rubricytes relative to polychromatophilic rubricytes, metarubricytes, and polychromatophilic RBC) in 19 (50%) dogs. Mild dysplasia (megaloblastic cells, cells with blebbing or budding nuclei) was observed in a few rubricytes and metarubricytes in 2 (5%) dogs. Megakaryocyte numbers were judged low (3 dogs) or high (4) in the 7 thrombocytopenic dogs and high in all 9 dogs with thrombocytosis. Myeloid maturation was complete to segmented neutrophils, and maturation was balanced in all dogs. In the 7 neutropenic dogs, numbers of myeloid precursors in the bone marrow were judged within reference ranges (5 dogs) or low (2). Percentages of lymphocytes and plasma cells in marrow were determined in 35 dogs. Small lymphocytes were diffusely distributed throughout the smears and composed 2 to 60% of total marrow cells (median lymphocyte count, 9%), with 15 dogs having > 10% lymphocytes. Plasma cells were associated with bone marrow spicules and composed 0 to 33% of total marrow cells (median plasma cell count, 1%). Examination of marrow smears stained with Prussian blue in 39 dogs revealed small amounts of iron in 2 (5%) dogs, moderate amounts in 9 (23%) dogs, and large amounts in 28 (72%) dogs. Mild-to-severe reticulin fibrosis was observed in Gomori-methamine-silver-stained sections of the bone marrow core biopsy specimens of all 16 (37%) of the 43 dogs, of which 1 also had severe collagen fibrosis on trichrome-stained smears of the bone marrow core biopsy specimen. Most dogs with myelofibrosis had erythroid hyperplasia (9/16, 56%); none had PRCA.

Ultrasonographic and radiographic examination of the abdomen and thorax was performed in 33 dogs. No abnormalities were detected in 9 (27%) dogs; other observations included mild hepatomegaly in 12 (36%) dogs, mild generalized splenomegaly in 6 (18%) dogs, mild hepatosplenomegaly in 3 (9%) dogs, and mild cardiomegaly in 2 (6%) dogs. Endocrine and chronic inflammatory diseases were excluded on the basis of the severity of the anemia. Other underlying diseases capable of causing severe anemia were not identified in any dog.

Treatment—After referral, 5 dogs were treated with prednisolone[i] (0.2 to 3 mg/kg [0.1 to 1.4 mg/lb] of body weight, PO, q 12 h) as the sole immunosuppressant. Twelve dogs were treated with cyclophosphamide[j] (0.5 to 2 mg/kg [0.2 to 0.9 mg/lb], PO, q 24 h, 4 consecutive days a week) in combination with corticosteroids (prednisolone at a dosage of 0.5 to 2 mg/kg [0.2 to 0.9 mg/lb], PO, q 12 h, 11 dogs; or dexamethasone[k] at a dosage of 0.2 mg/kg [0.1 mg/lb], PO, q 24 h for 7 days, then q 48 h, 1 dog). Twenty-six dogs were treated with azathioprine sodium[l] (2 mg/kg [0.9 mg/lb], PO, q 24 h for 5 days, then q 48 h) in combination with corticosteroids (prednisolone at a dosage of 1 to 2 mg/kg [0.5 to 0.9 mg/lb], PO, q 12 h, 24 dogs; or dexamethasone at a dosage of 0.1 to 0.24 mg/kg [0.05 to 0.11 mg/lb], PO, q 24 h, 2 dogs). Dogs were also treated with gastroprotectorants (famotidine at 0.5 mg/kg [0.2 mg/lb], PO, q 24 h, 22 dogs, and sucralfate at 0.5 to 1 g, PO, q 8 to 12 h, 24 dogs), antibiotics (28

Table 3—Response to treatment with immunosuppressive drugs in 43 dogs with pure red cell aplasia and nonregenerative immune-mediated anemia

Immunosuppressive therapy	No. of dogs	Outcome, No. (%)*				Relapses, No. (%)†
		Complete	Partial	None	Unknown	
Corticosteroids	5	1 (25)	2 (50)	1 (25)	1	0
Corticosteroids and cyclophosphamide	12	8 (73)	1 (9)	2 (18)	1	1 (11)
Corticosteroids and azathioprine	26	13 (52)	4 (16)	8 (32)	1	5 (29)

*Percentage of those dogs with known outcome. †Percentage of those dogs that had a partial or complete response to therapy.

dogs; tetracycline hydrochloride at a dosage of 5 mg/kg [2.3 mg/lb], PO, q 24 h; enrofloxacin at a dosage of 2.5 mg/kg [1.1 mg/lb], PO, q 12 h; amoxicillin at a dosage of 20 mg/kg [9.1 mg/lb], PO, q 12 h; or cefadroxil at a dosage of 22 mg/kg [10 mg/lb], PO, q 12 h), acetylsalicylic acid at 0.5 mg/kg [0.2 mg/lb], PO, q 24 h (11 dogs), and ferrous sulfate at 10 mg/kg [4.5 mg/lb], PO, 3 times a week (1 dog). Thirty-three dogs received between 1 to 3 blood transfusions and 1 received hemoglobin glutamer-200 (oxyglobin).

Three dogs were lost to follow up after not responding to treatment within 10 to 11 days after admission **(Table 3)**. Of the remaining 40 dogs, 22 (55%) had a complete response, 7 (18%) had a partial response, and 11 (27%) had no response to therapy. A response to treatment was seen within a median of 2 weeks (range, 1 to 10 weeks) and the Hct was within or just below the reference range within 1 to 10 months after starting treatment in those dogs with a complete response. One dog treated with corticosteroids and cyclophosphamide did not respond after 1 month, but had a complete response, beginning within 1 week, after treatment with azathioprine was added. One dog treated with corticosteroids and azathioprine developed clinical (dyspnea) and laboratory (indication of ventilation-perfusion mismatch on the basis of blood gas tensions) evidence of pulmonary thromboembolism during the first week of treatment, but recovered clinically. Six (21%) of the dogs that responded to treatment relapsed when treatment was tapered or ceased, after initially responding. Three of these dogs responded to an increased frequency or dosage of immunosuppressive drugs. The anemia in 1 dog completely resolved; the dog then had repeated bouts of thrombocytopenia that responded to treatment with prednisolone and azathioprine. Five dogs were weaned completely off medication and were without clinical signs of anemia up to 2 years later. Eleven dogs were treated with corticosteroids or azathioprine on alternate days until last communication with the referring veterinarian (8 months to 3 years later). In 2 dogs (both having a complete response to treatment) with myelofibrosis on initial examination, the myelofibrosis was no longer evident (as determined by histologic examination of serial core biopsy specimens of the bone marrow) with resolution of anemia.

The anemia was present for a median of 7 (range, 5 to 30), 9 (range, 5 to 35) and 7 (range, 5 to 90) days in those dogs responding completely, partially, or poorly to treatment, respectively. These median values were not significantly different from each other ($P = 0.56$). The proportions of dogs in each response category were not significantly different for each treatment. Proportions of dogs with high ANA titers, positive Coombs' test results, spherocytosis, myelofibrosis, or erythroid hyperplasia were not significantly different among the 3 response categories. Similarly, median Hct, WBC and platelet counts, MCV, serum alkaline phosphatase activities, and serum total bilirubin concentrations were not significantly different among the 3 response categories.

The overall mortality rate (resulting from a poor response to treatment or complications with the anemia, including transfusion reactions) was 11/40 (28%), with dogs dying or being euthanatized within 3 days to 10 weeks of referral. Of these 11 dogs, 10 did not respond to treatment with immunosuppressive drugs and were euthanatized within 1 month of admission. Four of these dogs were examined on necropsy; pertinent findings included extramedullary hematopoiesis (1 dog) and splenic thrombosis (2). Underlying diseases were not detected in any of these dogs. An additional 10 dogs were euthanatized for unknown reasons or causes unassociated with the anemia (eg, spinal cord disease, pancreatitis) within 3 weeks to 3 years after admission.

Discussion

Dogs with PRCA and NRIMA in our study were mostly middle aged (ie, median age, 6.5 years), and spayed females were significantly overrepresented. Female dogs are predisposed to developing IHA.[1,3,4,m] In the literature, 66% of dogs with PRCA and NRIMA were female, of which 54% were spayed.[5-18] Labrador Retrievers were significantly overrepresented in our study and in a recent study of 7 dogs with myelofibrosis and severe nonregenerative anemia,[20] suggesting that the breed may be predisposed to this disorder. Breeds commonly affected by IHA (Old English Sheepdogs, Irish Setters, Poodles, Cocker Spaniels, English Springer Spaniels, and Collies[1,4,a,m]) were not overrepresented in our study.

One of the selection criteria for our study was at least a 5-day history of clinical signs. This period was chosen to eliminate dogs not given sufficient time to mount a regenerative response to acute hemolytic anemia. Most dogs had clinical signs of anemia for more than 1 week. At admission, physical examination findings of mucous membrane pallor, tachypnea, tachycardia, hepatosplenomegaly, and murmurs were attributed to the anemia. Most dogs in our study were well tolerant of severe anemia, suggesting that anemia was slowly progressive, allowing amelioration of signs by physiologic adaptation.

All dogs had severe nonregenerative anemia,

which was predominantly normocytic and normochromic. The WBC counts were within reference ranges or mildly high, and band neutrophils were generally found in low numbers. In contrast, WBC counts are generally high with a moderate-to-severe left shift in dogs with acute, regenerative IHA.[3,4] Other common hematologic features seen in the dogs of our report include a lack of circulating metarubricytes, few-to-moderate numbers of spherocytes, and macrocytes. Macro- or microscopic agglutination was observed in 2 dogs. Similar to previous reports,[5-14,16,18] platelet counts were within reference ranges or high in most of our dogs. Thrombocytopenia was observed in 7 (22%) dogs, 1 of which was severely thrombocytopenic. Immune-mediated thrombocytopenia has been described in up to 68% of dogs with IHA.[1,3,a] Three of the reported dogs with NRIMA[10,12,17] were thrombocytopenic, 2 severely (5 to 7 $\times$ 10^3 platelets/µl). Thrombocytopenia was likely a result of platelet destruction at extramedullary sites (eg, liver or spleen) or low platelet production, because both megakaryocytic hyperplasia and hypoplasia were observed in smears of bone marrow aspirates from our dogs. Two dogs were pancytopenic, although neutrophil and platelet numbers were mildly decreased, compared with the severity of the anemia. All hematopoietic cells were present (although megakaryocyte numbers were judged low) in smears of bone marrow aspirates from these 2 dogs, findings compatible with ineffective hematopoiesis (or intramedullary cell death). The neutropenia and thrombocytopenia responded rapidly to immunosuppressive therapy in these 2 dogs, although their anemia remained nonregenerative until they died or were lost to follow-up (within 2 to 3 weeks of starting treatment). Similarly, neutropenia and thrombocytopenia has been reported to resolve more rapidly than anemia in a pancytopenic dog with ineffective erythropoiesis.[17]

Results of coagulation panels were within reference ranges in most of our dogs and all had negative FDP results. In contrast, many dogs with IHA have coagulation abnormalities compatible with disseminated intravascular coagulation and are predisposed to thrombosis, especially in the pulmonary vasculature.[21,n] The precise incidence of pulmonary thromboembolism in IHA is unknown and is likely underestimated because of the difficulty of confirming its presence with routine diagnostic procedures. Pulmonary thromboembolism was detected in 2 dogs in our study, in 1 before and in 1 after treatment. This illustrates that pulmonary thromboembolism, although uncommon, can be observed in dogs with NRIMA, and may be treatment related.

Of dogs reported to have PRCA and NRIMA, 24% had positive Coombs' test results,[5-18] compared with 57% of dogs in our study. Although the positive Coombs' test results could be related to prior blood transfusions in some of our dogs, immune-mediated destruction of mature RBC was likely, a conclusion supported by the mild hyperbilirubinemia and hepatosplenomegaly in 23 and 64% of our dogs, respectively. Prior treatment with corticosteroids by the referring veterinarian could potentially have contributed to negative Coombs' test results in some dogs. Serum iron concentrations and percentage saturation of transferrin were consistently high, and 72% of our dogs had high amounts of bone marrow iron, findings compatible with increased iron turnover secondary to ineffective erythropoiesis or hemolysis of RBC in the circulation.[22] In some dogs, iron variables were measured sequentially after treatment. Serum iron concentrations and percentage saturation of transferrin were consistently high and only decreased to within reference intervals when dogs developed a regenerative response. Other commonly observed serum biochemical abnormalities in our dogs were high alanine aminotransferase (attributable to hypoxic injury to hepatocytes) and alkaline phosphatase (attributable to exogenous corticosteroid administration, stress-related endogenous corticosteroid release, and/or cholestasis) activities, and low bicarbonate concentration (attributable to hypoxia).

High ANA titers were seen in 23% of our dogs, a finding that supports an immune-mediated pathogenesis for the anemia. Similarly high ANA titers have been described in other dogs with PRCA and NRIMA[6,17] and IHA.[1] None of our dogs had other clinical features compatible with SLE (ie, dermatologic lesions, renal disease, or polyarthritis) and hemolytic anemia is an uncommon manifestation of SLE in dogs, occurring in only 13% of affected dogs.[23] This suggests that high ANA titers can be a component of PRCA and NRIMA in dogs and a diagnosis of SLE should not mistakenly be made.

The results of our study indicate that bone marrow findings in dogs with similar hemogram results vary from PRCA to erythroid hyperplasia, emphasizing that bone marrow results cannot be predicted from hematologic findings and the marrow must be examined to determine the status of erythroid cells in the marrow. The diagnosis of PRCA was reserved for those dogs in which rare or no erythroid precursors were observed in the smears of the bone marrow aspirate, whereas those with erythroid precursors were determined to have NRIMA. Pure red cell aplasia was found in 2 (5%) dogs of our report, whereas in previous reports, 40% of the dogs had PRCA.[5-11] Pure red cell aplasia appears to be the most severe expression of NRIMA. Erythroid hyperplasia with complete, although left-shifted, maturation to polychromatophilic RBC was the most common finding in our dogs (63%), compared with dogs (21%) of previous reports.[10,15,17,18] In our remaining dogs, there was an apparent "maturation arrest" at basophilic rubricytes or metarubricytes, a finding that has been reported.[12,13,15,17] Mild dysplastic changes were observed in 2 dogs and were restricted to the erythroid cells. This was not considered diagnostic of myelodysplastic syndrome as both dogs responded to immunosuppressive therapy and mild myelodysplastic change has been observed in the bone marrow of other dogs with intense effective erythropoiesis. Other common features in the bone marrow aspirates of our dogs were a mild-to-moderate lymphocytosis and abundant bone marrow iron stores. Bone marrow lymphocytosis and plasmacytosis has been observed in the bone marrow aspirate of 1 dog with NRIMA, and was considered supportive evidence for the immune-mediated pathogenesis.[16]

Myelofibrosis was confirmed in all 16 (37%) dogs with bone marrow core biopsies and was suspected in

an additional 11 (26%) dogs, indicating that clinicians should be aware that bone marrow may be difficult or impossible to aspirate in dogs with NRIMA. Primary myelofibrosis has not been described in dogs,[24] and the myelofibrosis in the dogs of our report is likely secondary to the pathogenic mechanism responsible for the anemia. In 2 dogs of this report, the myelofibrosis was reversed with resolution of their anemia. Increased production of fibrogenic cytokines, such as transforming growth factor-β and platelet derived growth factor, by macrophages and megakaryocytes[24] could be responsible for the myelofibrosis in this disorder, although the stimuli for cytokine expression remains to be determined. Myelofibrosis has been described in dogs with NRIMA and PRCA.[6,10,14] In 2 reports of dogs with myelofibrosis,[20,25] 20 of 21 had moderate-to-severe nonregenerative anemia, with WBC and platelet counts generally within reference ranges. In these reports, only 1 dog had a positive Coombs' test result and many of the dogs responded to treatment with corticosteroids. These findings are similar to ours, suggesting that the dogs in these previous reports had NRIMA with secondary myelofibrosis. Indeed, the authors of the more recent report hypothesized that the myelofibrosis was secondary to the immune-mediated destruction of red cell precursors.[20]

It must be emphasized that the anemia was considered immune-mediated in our study primarily on the basis of the favorable response to treatment with immunosuppressive drugs, which was seen in 73% of the dogs. The remaining 27% of dogs were included in our study because they fulfilled our selection criteria and had similar clinical and laboratory results to those that did respond to treatment. The lack of response in these dogs may have been the result of an undetected underlying disease, choice of drug, inappropriate drug dosages, or insufficient time given for a response. The severe anemia in 2 dogs of our report with pre-existing endocrine disease (hypothyroidism or hypoadrenocorticism) was responsive to treatment with immunosuppressive drugs. However, most of our dogs were not tested specifically for endocrine disease; therefore, it is possible that such diseases were present in some dogs and may have contributed to a lack of response to immunosuppressive treatment. In addition, some of the dogs that did not respond to treatment may have responded to other drugs, including intravenous administration of γ-globulin[10] or cyclosporine.[o] An association between IHA and modified-live vaccination has been made.[26] It is unlikely, however, that vaccination initiated the anemia in most our dogs, as some dogs had been vaccinated as long as 3 years prior to onset of clinical signs, whereas others were vaccinated after the onset of clinical signs.

The persistent and prolonged reticulocytopenia in the face of a severe anemia is consistent with immune-mediated destruction of erythroid precursors in the bone marrow (ineffective erythropoiesis). This can develop through antibody or cell-mediated mechanisms.[27] Indeed, the IgG fraction of serum was determined to inhibit colony-forming unit-erythroid production in 4 of 8 dogs with PRCA.[7] The exact antigen is unknown; however, on the basis of bone marrow findings, late-stage erythroid precursors (including polychromatophilic RBC and metarubricytes) appeared to be a target in our dogs. The immune-mediated attack may be targeted against a maturation-associated antigen,[28,29] thus only destroying precursors, or at a common antigen on precursors and mature RBC, producing both intramedullary destruction of precursors and concurrent hemolysis in the peripheral circulation. Only in the latter situation would a positive Coombs test result be expected. In 2 of our dogs, the anemia was initially regenerative and became nonregenerative over 1 to 4 weeks prior to referral. Both of these dogs responded to treatment. This progression from a regenerative to nonregenerative anemia has been described previously in dogs with NRIMA,[10,15] and suggests that the antibody specificity can change during the course of disease.

A response to treatment was seen in our dogs within 1 to 10 weeks, with a median of 2 weeks. This indicates that dogs with PRCA or NRIMA may take several weeks to respond to treatment with immunosuppressive drugs and should be given sufficient time to do so. Of 18 dogs with extensive follow-ups, 5 were successfully weaned off all medication within 2 years after initiating treatment, and 9 were treated with corticosteroids and azathioprine on alternate days for up to 3 years. Relapses occurred in 6 (14%) of our dogs, some of which responded to either increased frequency or dosage of immunosuppressive drugs. After successful resolution of the anemia, 1 dog subsequently developed thrombocytopenia that required long-term treatment with corticosteroids and azathioprine. Similarly, immune-mediated thrombocytopenia developed after successful treatment of 2 dogs that had NRIMA,[9,16] suggesting a generalized immune-mediated process in these dogs.

The proportion of dogs with a complete or partial response was similar for all treatments, although the number of dogs within each treatment group may have biased this finding. In previous reports,[5-9,11,13-17] 61% of dogs responded to treatment with corticosteroids alone, whereas 82% treated with corticosteroids and cyclophosphamide responded. One dog in this report did not respond to 4 weeks of treatment with corticosteroids and cyclophosphamide, but did respond within a week when azathioprine was added. Similarly, 2 of the previously described affected dogs responded to corticosteroids and cyclophosphamide after a poor response to corticosteroids alone,[6,16] suggesting corticosteroids alone may be insufficient to treat these disorders in many dogs. Human γ-globulin has been used successfully to treat 5 dogs with PRCA and NRIMA, of which 3 had either not responded or responded partially to prior treatment with corticosteroids with or without azathioprine.[10] In our study, the choice of cyclophosphamide versus azathioprine appears to be historical (because dogs admitted after February 1996 were treated with azathioprine and those before this date, with cyclophosphamide), but may have also been based on the reported superior effects of azathioprine on canine lymphocyte blastogenesis responses in vitro[30] and to improved survival times achieved with azathioprine in dogs with IHA.[m]

Although empirically, a poorer response might be

expected from dogs with myelofibrosis or erythroid hypoplasia or aplasia, the results of our study indicate that these bone marrow findings are not associated with outcome. Furthermore, the median Hct, WBC count, platelet count, serum alkaline phosphatase activity, and serum bilirubin concentration at the time of admission were not significantly different among the 3 response groups, indicating that these variables were not predictive of response to treatment. This contrasts to previous findings in dogs with IHA, in which thrombocytopenia, high serum alkaline phosphatase activity, and high serum bilirubin concentrations were associated with a poor prognosis.[1,m,n] There was no significant difference between the median MCV in the 3 response categories, which contrasts to a previous report, in which the responding dogs had an initial macrocytosis.[20] Additional variables evaluated in our study were high ANA titers, positive Coombs' test results, and spherocytosis, none of which were predictive of response. However, response to treatment in our study was biased by dogs lost to follow up, given insufficient time to respond (several dogs were euthanized within 1 week of admission), or euthanatized (for unknown or unrelated conditions) after they had partially responded to treatment. Results of previous studies suggest that mortality associated with nonregenerative forms of IHA is higher than with regenerative forms of the disease[1,a]; however, in our study, overall mortality was 28%, which is similar or lower than that of regenerative IHA.[3,m,n]

[a]Burgess KE, Rand W, Moore A. Immune-mediated hemolytic anemia in dogs: a retrospective study of 60 cases treated with cyclophosphamide (abstr). *J Vet Intern Med* 1997;11:143.

[b]Kellerman DL, Lewis DC. Immune-mediated hemolytic anemia: a retrospective analysis of 37 cases (abstr). *J Vet Intern Med* 1995;9:189.

[c]Coulter S+IV hematology analyzer, Coulter Electronics, Hialeah, Fla.

[d]Canine anti-globulin (Coombs) reagent, ICN Biomedical, Aurora, Ohio.

[e]BBL Fibrosystem, Becton Dickinson, Cockeysville, Md.

[f]Thrombo-Wellcotest, Murex Diagnostics, Narcross, Ga.

[g]Hitachi 911, Roche-Boehringer Mannheim Corp, Indianapolis, Ind.

[h]Statistix for Windows, Analytical Software, Tallahassee, Fla.

[i]Prednisolone, Zenith Goldline Laboratories, Fort Lauderdale, Fla.

[j]Cytoxan, Bristol-Meyers Squibb, Princeton, NJ.

[k]Dexamethasone azium, Schering Animal Health, Kenilworth, NJ.

[l]Imuran, GlaxoWellcome, Research Triangle Park, NC.

[m]Allyn ME, Troy GC. Immune mediated hemolytic anemia—a retrospective study: focus on treatment and mortality (1988–1996) (abstr). *J Vet Intern Med* 1997;11:131.

[n]Carr AP, Panciera DL. Immune-mediated hemolytic anemia in dogs: a retrospective study with emphasis on hemostatic parameters (abstr). *J Vet Intern Med* 1996;10:172.

[o]Wohl S, Moore AS. Use of single-agent cyclosporine A in dogs with severe immune mediated hemolytic anemia (abstr). *J Vet Intern Med* 1996;10:173.

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