Clinical features of precursor-targeted immune-mediated anemia in dogs: 66 cases (2004–2013)

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OBJECTIVE
To characterize the clinical features of dogs with precursor-targeted immune-mediated anemia (PIMA).

ANIMALS
66 dogs with PIMA.

PROCEDURES
Electronic record databases of a teaching hospital were searched to identify dogs with a diagnosis of nonregenerative anemia between 2004 and 2013. Inclusion criteria included persistent nonregenerative anemia (Hct ≤ 30% and reticulocyte count < 76,000 reticulocytes/μL), cytologic findings supportive of ineffective bone marrow erythropoiesis, and absence of underlying disease. Information regarding clinical signs, clinicopathologic findings, treatment, and outcome was extracted from records of eligible dogs. A regenerative response was defined as a reticulocyte count > 76,000 reticulocytes/μL or sustained increase in Hct of > 5%. Remission was defined as a stable Hct ≥ 35%.

RESULTS
The median Hct was 13%, and reticulocyte count was 17,900 reticulocytes/μL. Rubriphagocytosis was identified in bone marrow aspirate samples from 61 of 66 dogs. Collagen myelofibrosis was detected in bone marrow biopsy specimens obtained from 31 of 63 dogs. Immune-mediated targeting of mature erythrocytes was uncommon. All dogs received immunosuppressive therapy. Fifty-five dogs developed a regenerative response at a median of 29 days, and 40 of those dogs went into remission at a median of 59 days after PIMA diagnosis. Thromboembolic events were confirmed for 9 dogs and were associated with a decreased survival time. Median survival time was 913 days for all dogs.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated that most dogs with PIMA responded to prolonged immunosuppressive therapy. Studies to determine optimal immunosuppressive and thromboprophylactic protocols for dogs with PIMA are warranted. (*J Am Vet Med Assoc* 2019;255:366–376)

Nonregenerative anemia with a suspected immune-mediated pathogenesis has been recognized in dogs for nearly 40 years.[1] Although > 250 dogs with the condition have been described, the terminology and criteria used to diagnose the condition have varied. Frequently, affected dogs were reported as a subset of a larger population of dogs with IMHA, which led to the condition being called nonregenerative IMHA.[1–9] This characterization can be confusing because it was not always clear whether those dogs were in a persistently nonregenerative or preregenerative stage of IMHA. Additionally, some dogs that were classified as having nonregenerative IMHA did not have definitive evidence of hemolysis.[2–4,8] The more general term NRIMA accounts for the lack of peripheral erythrocyte targeting in many cases but does not differentiate dogs with and without evidence of immune-mediated hemolysis (eg, dogs with preregenerative IMHA vs dogs with impaired erythropoiesis).

Comparisons among affected dogs remain complicated not just by terminology, but also by diagnostic criteria. Previous studies[1–3,8,10,11,a] have grouped dogs with PRCA together with dogs with typical IMHA. The hallmark of this disorder is not simply nonregenerative anemia, as the terms nonregenerative IMHA or NRIMA imply, but ineffective erythropoiesis owing to suspected targeting of erythroid precursors by the immune system. Ineffective erythropoiesis is recognized by one of several erythropoietic response patterns, most commonly marked erythroid hypercellularity concurrent with persistent nonregenerative anemia and often maturation arrest or a mild left shift in the erythroid

ABBREVIATIONS

aPTT	Activated partial thromboplastin time
FDP	Fibrin degradation product
IMHA	Immune-mediated hemolytic anemia
MSU	Michigan State University
NRIMA	Nonregenerative immune-mediated anemia
PIMA	Precursor-targeted immune-mediated anemia
PRCA	Pure red cell aplasia
PTE	Pulmonary thromboembolism
PVT	Portal vein thrombus

lineage.[12] The immune-mediated pathogenesis for targeting of erythroid precursors is supported by the condition's association with concurrent IMHA, apparent response to immunosuppressive therapy, relapses after cessation of immunosuppressive therapy, and cytologic evidence of selective phagocytosis of apparently intact erythroid precursors, which has been labeled rubriphagocytosis.[12] Consequently, at our institution and for the purpose of the study reported here, we use the term PIMA for the condition because we believe it more aptly describes and characterizes the pathophysiology of the condition. Diagnosis of PIMA is made on the basis of a history of persistent nonregenerative anemia, absence of underlying disease to explain the anemia, and bone marrow findings supportive of ineffective erythropoiesis.

The histologic and cytologic findings in bone marrow specimens from 25 dogs with PIMA that were treated at our institution have been described.[12] However, the number of dogs in that study[12] was fairly small because the inclusion criteria for the study resulted in the exclusion of some dogs that had a clinical diagnosis of PIMA. Also, thromboembolic complications were not assessed for the dogs of that study.[12] The purpose of the study reported here was to expand on the previous study.[12] Specifically, the primary objective of the study was to characterize the signalment, clinical signs, clinicopathologic results (including cytologic and histologic findings for bone marrow aspirate and core biopsy specimens), treatment, and outcome (including thromboembolic complications) for dogs with PIMA. A secondary objective was to determine whether thromboembolic complications were associated with PIMA and contributed to the death of dogs with the condition.

Materials and Methods

Case selection criteria

The electronic medical record database of the MSU Veterinary Medical Center (teaching hospital) and bone marrow database of the MSU Clinical Pathology Laboratory were searched to identify dogs that received a diagnosis of nonregenerative anemia between 2004 and 2013. Dogs with nonregenerative anemia (Hct ≤ 30% and reticulocyte count < 76,000 reticulocytes/µL [ie, the lower limit of the reference range for the absolute reticulocyte count established by the MSU Clinical Pathology Laboratory]) for ≥ 5 days as determined on the basis of case history or in-hospital documentation and cytologic or histologic evidence of ineffective bone marrow erythropoiesis were included in the study. Ineffective erythropoiesis was defined as the presence of one of several erythropoietic response patterns (eg, erythroid hypercellularity or an increase in early-stage erythroid precursors with maturation arrest) that could not be attributed to a preregenerative response or other disease process (eg, myelodysplastic disease or drug reaction) with or without evidence of rubriphagocytosis.[12] Dogs were excluded from the study if they did not undergo cytologic evaluation of a bone marrow specimen or had an identifiable underlying disease that could cause anemia. Also excluded were dogs that received erythropoietin prior to examination at the teaching hospital and dogs with PRCA (ie, dogs with rare or no erythroid precursors and without expansion of early stages of erythroid precursors and with no rubriphagocytosis). Dogs that were referred for the acquisition of a bone marrow sample but were not treated at the teaching hospital were likewise excluded from the study. Most of the 25 dogs with PIMA that were evaluated in a previous study[12] were included in this study, although some were excluded because of the lack of follow-up information.

Medical record review

For each dog enrolled in the study, information extracted from the medical record included signalment, history, clinical signs, diagnostic test and imaging results, treatments (including previous blood transfusions and adjunctive medications), complications (including thromboembolic events and relapses), and outcome. Common diagnostic tests performed included a CBC (including manual cell differentiation and microscopic evaluation of a blood smear); serum biochemical analysis; hemostasis profile; Coombs test; commercial ELISA[b] or serum tests for detection of antibodies against *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Rickettsia rickettsii* (the serum antibody titer was quantified for any dog with a positive ELISA test result); cytologic evaluation of a bone marrow aspirate specimen; and cytologic and histologic evaluation of a bone marrow core biopsy specimen. Diagnostic imaging procedures most commonly performed were thoracic radiography, CT, and abdominal ultrasonography.

Suspected thromboembolic events were diagnosed on the basis of clinical signs, clinicopathologic data, and diagnostic imaging. Specifically, PTE was defined as acute onset of tachypnea, hypoxemia (as determined by arterial blood gas results or pulse oximetry), and absence of evidence of other causes of respiratory distress on thoracic radiographs. Computed tomographic angiography was performed to confirm PTE when possible but was not required for diagnosis of PTE. Thromboemboli in the abdominal vasculature (eg, portal vein, splenic vein, or caudal vena cava) were suspected for dogs with abdominal distension, vomiting, or anorexia, and abdominal ultrasonography or CT angiography was used to confirm the presence of thrombi or abnormal blood flow in the portal, splenic, or vena caval vasculature.

The number of days that each dog received immunosuppressive doses of corticosteroids until detection of a regenerative response and disease remission, resolution, and relapse were recorded when applicable. A regenerative response was defined as a reticulocyte count > 76,000 reticulocytes/µL or, when the

reticulocyte count was unavailable, a spontaneous increase in the Hct of > 5%. Disease remission was defined as a stable Hct ≥ 35%. For dogs in remission, disease resolution was defined as the point at which administration of all immunosuppressive medications was discontinued without recurrence of nonregenerative anemia. Disease relapse was defined as recurrence of nonregenerative anemia during or after the gradual discontinuation of immunosuppressive medications. Outcome at the time of data collection was recorded as alive or dead (regardless of whether death was from natural causes or the result of euthanasia). For each dog, survival time was determined by contacting the owner or primary care veterinarian.

Cytologic and histologic examination of bone marrow specimens

All bone marrow specimens submitted for cytologic and histologic examination underwent routine diagnostic evaluation at the time of submission by board-certified veterinary clinical pathologists. All original reports were reviewed by 1 clinician (TDA) and 1 board-certified veterinary clinical pathologist (MAS) to confirm the presence of ineffective erythropoiesis and an erythropoietic response pattern consistent with PIMA. If the presence of ineffective erythropoiesis and an erythropoietic response pattern consistent with PIMA could not be determined from the original clinical pathology report, the corresponding cytologic or histologic specimen slides were retrieved from the archives and reviewed by a board-certified veterinary clinical pathologist (MAS). That clinical pathologist also reviewed all archived slides of bone marrow core biopsy specimens for collagen myelofibrosis and all archived slides from study dogs for the presence and predominant stage of rubriphagocytosis. Collagen myelofibrosis was subjectively graded as mild, moderate, or marked on the basis of the extent of medullary replacement in the most affected region of the specimen. Predominantly early-stage rubriphagocytosis was defined as phagocytosis of primarily intact rubriblasts and prorubricytes. Predominately mid-stage rubriphagocytosis was defined as phagocytosis of mostly early basophilic and polychromatophilic rubricytes. Predominately late-stage rubriphagocytosis was defined as phagocytosis of mostly polychromatophilic rubricytes through metarubricytes.

Statistical analysis

Descriptive statistics were generated. The sex distribution and proportions of Labrador Retrievers and Dachshunds (the 2 most commonly represented breeds among dogs with PIMA) among the study dogs were compared with those of a standard population (ie, the hospital population during the study period) by use of Fisher exact or χ^2 tests. Comparisons involving categorical variables were also performed with Fisher exact or χ^2 tests. The data distributions for continuous variables were assessed for normality by means of the D'Agostino-Pearson omnibus normality test. Normally distributed continuous variables were assessed with a 1-way ANOVA, and nonnormally distributed continuous variables were assessed with a Kruskal-Wallis test. The Kaplan-Meier method was used to compare survival time and times from diagnosis to a regenerative response, remission, resolution, and relapse between dogs with PIMA that did and did not develop thromboembolic complications and those that did and did not have collagen myelofibrosis; dogs that were lost to follow-up or alive at the time of data collection were censored in the analyses. Kaplan-Meier curves were compared with the log-rank test. All analyses were performed with statistical software,[c] and values of $P < 0.05$ were considered significant.

Results

Dogs

The record search identified 189 dogs with nonregenerative anemia. Sixty-eight dogs were excluded from the study because of an underlying disease, such as chronic renal disease or neoplasia, that was likely the primary cause of the anemia. Twenty-four dogs were excluded because neither a bone marrow aspirate nor bone marrow core biopsy was performed to obtain specimens for cytologic or histologic examination. Fifteen dogs had preregenerative anemia, 3 dogs were misclassified (ie, were not anemic), and 10 dogs were not treated at the teaching hospital and did not have any follow-up information available. Two dogs were excluded owing to prior administration of erythropoietin, and 1 dog was excluded because of poor bone marrow specimen quality. Thus, 66 dogs met the inclusion criteria and were evaluated in the study.

The 66 study dogs had a median age of 6.5 years (range, 1 to 15 years) and included 39 spayed females, 2 sexually intact females, 19 castrated males, and 6 sexually intact males. The female-to-male ratio was approximately 2:1 for the study population. Spayed females were significantly ($P = 0.001$) overrepresented in the study population when compared with the standard hospital population during the same observation period. The study population consisted of 15 mixed-breed dogs, 7 Labrador Retrievers, 5 Dachshunds, 3 Pembroke Welsh Corgis, 2 Boston Terriers, 2 Bichons Frises, 2 Chihuahuas, 2 Great Danes, 2 Italian Greyhounds, 2 Miniature Schnauzers, 2 Shih Tzus, 2 Standard Poodles, and 1 each of the following: Airedale Terrier, Alaskan Malamute, American Pit Bull Terrier, Beagle, Boxer, Catahula, English Toy Spaniel, Fox Terrier, German Shepherd Dog, German Shorthair Pointer, Golden Retriever, Greyhound, Jack Russell Terrier, Polish Tatra Sheepdog, Pug, Rat Terrier, Rottweiler, Shetland Sheepdog, Soft-coated Wheaton Terrier, and Yorkshire Terrier. Dachshunds were significantly ($P < 0.001$) overrepresented in the study population, compared with the hospital population.

History and clinical signs

The duration of clinical signs prior to examination at the teaching hospital ranged from 1 to 240 days, with

the majority (52/66 [79%]) of dogs having a history of clinical signs for ≥ 7 days. The most commonly reported clinical signs were lethargy (n = 56 [85%] dogs), anorexia (44 [67%]), pale mucous membranes (20 [30%]), collapse (15 [23%]), and vomiting (14 [21%]). Nine dogs had received immunosuppressive doses of corticosteroids (prednisone, > 2 mg/kg [0.9 mg/lb]) for a median of 3 days (range, 1 to 26 days) prior to initial examination at the teaching hospital; only 2 of those 9 dogs received immunosuppressive doses of corticosteroids for > 4 days before examination at the teaching hospital. Four of the 9 dogs were receiving anti-inflammatory doses of corticosteroids, the corticosteroid dose was unknown for 2 dogs, and 1 dog had received various regimens of corticosteroids at doses ranging from 1 to 2 mg/kg (0.45 to 0.9 mg/lb) for 243 days. Additional treatments prescribed by referring veterinarians included antimicrobials (doxycycline, n = 6 dogs; amoxicillin, 3; metronidazole, 3; cephalosporins, 2; minocycline, 1; and ampicillin and enrofloxacin, 16), aspirin (2), clopidogrel and aspirin (1), and blood transfusions (7).

Clinicopathologic findings

Hematologic and serum biochemical data were summarized **(Table 1)**. Study dogs typically had severe nonregenerative anemia (median Hct, 13%) at the time of admission to the veterinary teaching hospital. The anemia was generally characterized as normocytic and mildly hypochromic, although macrocytosis was not uncommon. Red blood cell morphological changes commonly associated with immune-mediated hemolysis were usually absent. Mild spherocytosis was reported for 2 dogs, one of which was the only dog that had evidence of agglutination reported. Two dogs had low numbers of ghost cells. Coombs test results were positive for 3 of the 19 dogs for which the test was performed. The most common morphological change was mild to moderate elliptocytosis in 11 dogs, with another 5 dogs having too few elliptocytes to meet reporting criteria but enough to be noted in the comment section of the hematology report.

Serum total bilirubin concentration was increased from the reference range for 23 of the 64 (36%) dogs for which it was evaluated, but that increase was generally mild, and only 4 (6%) dogs had a serum total bilirubin concentration > 1 mg/dL (reference range, 0.1 to 0.4 mg/dL). Among those 4 dogs, 3 had an abnormally high ratio of unconjugated bilirubin to conjugated bilirubin, which was supportive of hemolysis. The remaining dog had moderate peritoneal effusion and a predominance of conjugated bilirubin, which was suggestive of a hepatobiliary cause for the hyperbilirubinemia. That dog was suspected to have a PVT, but the thrombus could not be identified ultrasonographically.

Thirty-two of 64 (50%) dogs that had a serum biochemical analysis performed at the time of hospital admission were hypoalbuminemic (< 2.8 g/dL), and the hypoalbuminemia was typically characterized as mild to moderate. Severe hypoalbuminemia was rare, with only 1 dog with an albumin concentration < 2 g/dL. Only 7 of the 32 (22%) dogs with hypoalbuminemia had received immunosuppressive doses of corticosteroids prior to hospitalization.

Hemostasis was evaluated for 45 of the 66 (68%) dogs, and hemostasis abnormalities were common in tested dogs. Prothrombin time and aPTT were determined for all 45 dogs, fibrinogen concentration was determined for 25 dogs, antithrombin III activity was assessed for 24 dogs, and FDP and D-dimer concentrations were measured for 23 and 19 dogs, respectively. Hypocoagulability (as defined by a prolonged prothrombin time or aPTT) was uncommon in the evaluated population; only 7 (16%) and 3 (7%) dogs had a mildly prolonged prothrombin time and aPTT, respectively. None of the 25 dogs for which the fibrinogen concentration was determined had hypofibrinogenemia (< 108 mg/dL). Fourteen (56%) dogs had hyperfibrinogenemia (> 287 mg/dL), and the median fibrinogen concentration for those dogs was 515 mg/dL (range, 342 to 1,200 mg/dL). For 6 of the 24 (25%) dogs assessed, the antithrombin III activity was < 60% that expected in the plasma of healthy

Table 1—Summary statistics for select hematologic and serum biochemical variables at the time of hospital admission for 66 dogs with PIMA.

Variable	Reference range	Median (range) for all dogs	No. (%) of dogs with results increased from the reference range	No. (%) of dogs with results decreased from the reference range
Hct (%)	40–55	13 (4–28)	0	66 (100)
MCV (fL)*	61–70	68 (54–100)	25 (39)	2 (3)
CHCM (g/dL)*	34–36	33.5 (27–38)	15 (23)	35 (55)
Reticulocytes (X 10^3 cells/μL)†	12–76	17.9 (1.9–75.0)	0 (0)	65 (100)
Platelets (X 10^3 platelets/μL)	160–401	351 (11–871)	27 (41)	6 (9)‡
Leukocytes (X 10^3 cells/μL)	6.1–12.0	11.2 (3.5–55.9)	30 (45)	11 (17)
Neutrophils (X 10^3 cells/μL)	4.0–8.2	8.8 (2.3–38.4)	34 (52)	10 (15)
Band neutrophils (X 10^3 cells/μL)	0.0–0.1	0.15 (0.0–7.9)	35 (53)	0 (0)
Total bilirubin (mg/dL)*	0.1–0.4	0.3 (0.1–8.8)	23 (36)	0 (0)
Albumin (g/dL)*	2.8–4.0	2.8 (1.1–3.7)	2 (3)	32 (50)

*Results available for only 64 of the 66 dogs. †Results available for only 65 dogs. ‡2 of the 6 samples (dogs) had clumped platelets, but subsequent blood samples from those 2 dogs evaluated 2 to 4 days later revealed a low platelet count without platelet clumping.

CHCM = Cell hemoglobin concentration mean. MCV = Mean corpuscular volume.

dogs. Concentrations of FDPs and D-dimers were frequently increased from the respective reference ranges. Fourteen of 23 dogs had an FDP concentration > 5 µg/mL (reference range, < 5 µg/mL), and 14 of 19 dogs had a D-dimer concentration > 250 ng/mL (reference range, < 250 ng/mL).

A commercial ELISA or serum tests for detection of antibodies against tick-borne diseases (*B burgdorferi*, *A phagocytophilum*, *E canis*, and *R rickettsii*) were performed for 42 of the 66 (64%) dogs, of which 13 had positive results consistent with low to moderately high antibody titers against one or more of the tested organisms. Briefly, 10, 3, 2, and 1 dogs were positive for antibodies against *B burgdorferi*, *A phagocytophilum*, *R rickettsii*, and *E canis*, respectively. However, none of the dogs with low to moderately high antibody titers had clinical signs consistent with Lyme disease, anaplasmosis, rickettsial disease, or ehrlichiosis.

Diagnostic imaging

Thoracic radiography was performed for 54 of the 66 (82%) dogs, and all of the radiographs were reviewed by a board-certified veterinary radiologist. Thoracic radiographic findings were unremarkable for 45 dogs, whereas 6 dogs had evidence of cardiomegaly, 2 dogs had pleural effusion, and 1 dog had a dorsocaudal to diffusely distributed pulmonary infiltrate. Abdominal ultrasonography was performed at some point during treatment for 54 dogs. Ultrasonographic findings were unremarkable for 17 (31%) dogs. For the remaining 37 dogs, remarkable ultrasonographic findings included hepatomegaly (n = 17), peritoneal effusion (15), splenomegaly (13), splenic nodules (6), splenic infarct (2), and pleural effusion (1). Samples of the peritoneal effusion fluid were obtained and submitted for complete fluid analysis for 11 of 15 dogs. The fluid was classified as a proteinaceous effusion for 10 dogs and a transudate for 1 dog. For the proteinaceous effusion samples, the median nucleated cell count was 395 cells/µL (range, 50 to 1,833 cells/µL) and the median protein concentration as determined by a refractometer was 4.1 g/dL (range, 3.2 to 5.7 g/dL).

Bone marrow findings

Bone marrow aspirate samples were obtained for all 66 dogs, and a bone marrow core biopsy specimen was obtained for 63 dogs. Rubriphagocytosis was reported in the original clinical pathology report for only 28 dogs; however, after review of archived specimens, it was identified in specimens from 61 of 66 (92%) dogs. Rubriphagocytosis was most commonly detected in bone marrow aspirate samples and only occasionally in bone marrow core biopsy specimens. It was usually stage selective, with phagocytosis typically targeting early-stage, mid-stage, or late-stage erythroid precursors **(Table 2)**. In rare cases, rubriphagocytosis overlapped early-mid or mid-late stages, and there were too few intact phagocytized cells to know which stage predominated. In those cases, the dog was classified as having the latest stage with good marrow representation. Erythroid cellularity usually reflected the stage of precursor being targeted, with erythroid hypocellularity most often present with early-stage precursor destruction and erythroid normocellularity and hypercellularity present with mid- and late-stage precursor destruction, respectively. Most exceptions to those patterns occurred when myelofibrosis limited the extent of overall erythroid cellularity despite expanded erythroid islands in active areas. Of the 5 dogs with suspected but not confirmed rubriphagocytosis, only 1 had high-quality aspirate preparations; collagen myelofibrosis in the other 4 dogs likely affected sample quality. Collagen myelofibrosis was present in 31 of 63 (49%) core biopsy specimens and was subjectively graded as severe, moderate, and mild in 7, 15, and 9 dogs, respectively. The presence of collagen myelofibrosis was not significantly ($P = 0.13$) associated with survival. The median survival time for dogs with collagen myelofibrosis (578 days) did not differ significantly ($P = 0.13$) from that for dogs without collagen myelofibrosis (1,429 days).

Treatment

All 66 dogs were treated with prednisone; the median prednisone dosage administered was 2.1 mg/kg/d (0.95 mg/lb/d; range, 0.7 to 3.7 mg/kg/d [0.32 to 1.7 mg/lb/d]). Thirty-eight dogs received adjunctive immune-modulating therapy during initial hospitalization at the teaching hospital. Specifically, 32 dogs received azathioprine (median dosage, 2 mg/kg/d; range, 1.3 to 2.6 mg/kg/d [0.59 to 1.2 mg/lb/d]), and 3 dogs received cyclosporine (median dosage, 8.4 mg/kg [3.8 mg/lb], q 12 h; range, 6.7 to 20.8 mg/kg [3.0 to

Table 2—Frequency distributions for erythroid cellularity pattern, predominant stage of rubriphagocytosis, and severity of collagen myelofibrosis in bone marrow aspirate samples or bone marrow core biopsy specimens for 66 dogs with PIMA.

Erythroid cellularity pattern	No. of dogs	Predominant stage of rubriphagocytosis observed (No. of dogs)					Severity of collagen myelofibrosis (No. of dogs)			
		Not observed	Early	Mid	Late	All stages	Not observed	Mild	Moderate	Severe
Hypocellularity	11	0	11	0	0	0	9	1	1	0
Normocellularity	7	1	1	4	1	0	3	1	3	0
Hypercellularity	41	2	0	7	31	1	23	7	11	0
Severe myelofibrosis	7	2	0	0	5	0	0	0	0	7
Total	**66**	**5**	**12**	**11**	**37**	**1**	**35***	**9**	**15**	**7**

*Bone marrow core biopsy specimens were not obtained for 3 of these dogs.

9.5 mg/lb], q 12 h). One dog received mycophenolate mofetil only, 1 dog received mycophenolate mofetil and azathioprine, and 1 dog received azathioprine and cyclosporine. For the dogs that received mycophenolate mofetil, the median dosage was 36 mg/kg (16.4 mg/lb, q 12 h; range, 25.9 to 46.1 mg/kg [11.8 to 21.0 mg/lb], q 12 h). The dog that received both mycophenolate mofetil and azathioprine was discharged from the hospital after 7 days, at which time the mycophenolate mofetil had been discontinued and the dog was receiving prednisone and azathioprine. The dog that received azathioprine and cyclosporine while hospitalized was prescribed prednisone and both adjunctive immune-modulating agents after hospital discharge.

Twenty-nine dogs were prescribed an antithrombotic at the time of hospital admission. Three of those dogs received unfractionated heparin while hospitalized and were subsequently transitioned to aspirin (n = 2) or clopidogrel (1) at hospital discharge. Nineteen dogs were treated with aspirin only, 6 dogs were treated with clopidogrel, and 1 dog was treated with both aspirin and clopidogrel. Unfractionated heparin was administered as a constant rate infusion titrated to prolong the aPTT to 1.5 to 2 times the baseline (initial) value. For the 22 dogs that received aspirin, the median aspirin dosage administered was 0.8 mg/kg/d (0.36 mg/lb/d; range, 0.5 to 1.6 mg/kg/d [0.23 to 0.73 mg/lb/d]). For the 8 dogs that received clopidogrel, the median clopidogrel dosage administered was 2.15 mg/kg/d (0.98 mg/lb/d; range, 1.5 to 4.5 mg/kg/d [0.68 to 2.0 mg/lb/d]).

Sixty-one of the 66 (92%) dogs received packed RBC transfusions, and those dogs were administered a median of 2 transfusions/dog (range, 1 to 10 transfusions/dog). Thirty dogs received a packed RBC transfusion only during the initial hospitalization, 15 dogs returned in the following 1 to 3 weeks after initial hospitalization for a transfusion, and 16 dogs received a packed RBC transfusion ≥ 1 month after diagnosis of PIMA and initiation of treatment. The median volume of packed RBCs administered to dogs during the initial hospitalization and follow-up examinations was 22 mL/kg (10 mL/lb; range, 8 to 172 mL/kg [3.6 to 78.2 mL/lb]).

Outcome

The number of dogs that developed a regenerative response and disease remission and resolution and the median number of days for each outcome to occur were summarized **(Table 3)**. Fifty-five of the 66 (83%) dogs developed a regenerative response. Among the 11 dogs that did not develop a regenerative response, 6 were euthanized between 9 and 49 days after hospital admission, 2 dogs died after the owners declined additional blood transfusions at 12 and 17 days after hospitalization, and 3 dogs were lost to follow-up between 1 and 25 days after PIMA diagnosis. The reason reported for euthanasia was a lack of response to treatment for 4 dogs and was not specified for the remaining 2 dogs.

Forty of the 55 (73%) dogs that developed a regenerative response achieved clinical remission. Two of the 15 dogs that did not achieve remission were lost to follow-up at 74 and 105 days after diagnosis. Of the remaining 13 dogs that did not achieve remission, 3 died and 10 were euthanized between 11 and 270 days after initial hospital admission. All deaths were related to confirmed or highly suspected thromboembolic events. The reason reported for euthanasia was a perceived lack of response to treatment for 3 dogs, gastrointestinal bleeding for 3 dogs, complications related to thromboembolic events for 3 dogs, and not specified for 1 dog.

Thirty-one of the 40 (78%) dogs that achieved remission were eventually weaned from all immunosuppressive medications and achieved disease resolution. Among the remaining 9 dogs, 7 were still receiving immunosuppressive therapy at the time of data collection, 1 died from an unknown cause 91 days after diagnosis, and 1 was lost to follow-up 156 days after diagnosis.

Eight of the 40 (20%) dogs that achieved remission had a relapse event at a median of 302 days (range, 179 to 1,311 days) after the initial diagnosis of PIMA. Two of those dogs relapsed while they were being weaned from immunosuppressive therapy, 3 dogs relapsed between 71 and 170 days after immunosuppressive therapy was completely discontinued, and 3 dogs did not have sufficient follow-up information beyond the fact that a relapse event had occurred. Each of 3 dogs had ≥ 2 relapse events.

The times from diagnosis to a regenerative response (time to a regenerative response) and from diagnosis to clinical remission (time to remission) did not differ significantly between dogs with and without collagen myelofibrosis. Additionally, neither severity of collagen myelofibrosis nor stage of rubri-

Table 3—Summary statistics for various positive outcomes for 55 of the 66 dogs with PIMA that developed a regenerative response.

Outcome	No. of dogs that achieved outcome	No. of dogs for which the time to outcome was documented	Median (range) time to outcome (d)*
Regenerative response	55	51	29 (2–111)
Remission	40	33	59 (12–177)
Resolution	31	18	178 (28–385)

*Includes only dogs for which the time to outcome was documented; all other dogs were excluded from the calculation.

phagocytosis had a significant effect on time to a regenerative response or time to remission.

Nine of the 66 (14%) study dogs developed confirmed thromboembolic events in various vessels. Two dogs developed thrombi in the splenic vein, 3 had PTE, 1 had a PVT, 1 developed a thrombus in the caudal vena cava, 1 dog developed a splenic infarct, and 1 developed a splenic vein thrombus and PVT. Another 5 dogs were suspected of having a thromboembolic event; a PTE was suspected in 2 dogs, PVT in 2 dogs, and cerebellar infarct in 1 dog. The thromboembolic event could not be confirmed in those 5 dogs because of a lack of detailed records, owners declining additional diagnostic testing such as abdominal ultrasonography, or inability to visualize a thrombus. The respective associations between thromboembolic events in dogs with PIMA and leukocytosis, thrombocytopenia, hyperbilirubinemia, hypoalbuminemia, and the presence of collagen myelofibrosis were assessed. The association between thromboembolic events in dogs with PIMA and thrombocytosis was not assessed because only 1 of the 9 dogs with a thromboembolic event had thrombocytosis (platelet count, 832,000 platelets/μL), which was classified as mild. Hyperbilirubinemia (total bilirubin, > 0.5 mg/dL) was the only one of those variables that was significantly ($P = 0.036$) associated with thromboembolic events in dogs with PIMA.

The median time between initial diagnosis of PIMA or a disease relapse and identification of a thromboembolic event was 31 days (range, 0 to 72 days). Three dogs had a thromboembolic event detected during the initial examination at the teaching hospital. Five dogs had a thromboembolic event occur within 2 to 8 weeks after diagnosis and treatment initiation, and 1 dog had a thromboembolic event occur following a relapse. Two dogs were receiving aspirin prior to the thromboembolic event, whereas 2 dogs were not receiving immunosuppressive doses of corticosteroids at the time of the event. Among the 9 dogs with confirmed thromboembolic events, 3 died and 3 were euthanized as a result of the thromboembolic event. Of the remaining 3 dogs, 2 were euthanized within 90 days after hospital discharge because of persistent anemia and concern about adverse effects associated with continued administration of immunosuppressive doses of corticosteroids, and 1 was euthanized 417 days after hospitalization because of aspiration pneumonia and sepsis. The median survival time for dogs with thromboembolic events (54 days) was significantly ($P < 0.001$) shorter than that for dogs without a thromboembolic event (1,365 days; **Figure 1**).

The median survival time for all 66 dogs with PIMA was 913 days. Thirty-eight (58%) dogs remained alive, 7 (11%) dogs were lost to follow-up, and 21 (32%) dogs died or were euthanized during the first 6 months after PIMA diagnosis. Of the 21 dogs that did not survive for 6 months after diagnosis, 2 died and 7 were euthanized because of a perceived lack of response to treatment, 3 died and 3 were euthanized because of confirmed or suspected thromboembolic events, 2 were euthanized because of gastrointestinal bleeding, and 1 died and 3 were euthanized for unknown reasons. Neither the presence nor severity of collagen myelofibrosis was significantly associated with survival.

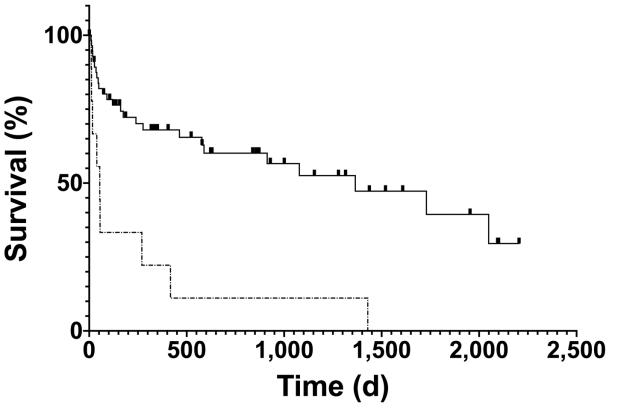

Figure 1—Kaplan-Meier survival curves for PIMA-affected dogs that did (dashed line; n = 9) and did not (solid line; 57) develop a confirmed thromboembolic event. The median survival time for dogs that developed a confirmed thromboembolic event (54 days) was significantly ($P < 0.001$) shorter than that for dogs that did not develop a confirmed thromboembolic event (1,365 days). Vertical hashmarks indicate dogs that were censored from the analysis.

Discussion

The dogs with PIMA evaluated in the present study required immunosuppressive therapy for weeks to months before a regenerative response and disease remission were achieved. Despite the prolonged duration of therapy, the median survival time for the dogs of this study (913 days) was approximately 2.5 years. Collagen myelofibrosis was observed in bone marrow core biopsy specimens from 31 of 63 (49%) dogs with PIMA, but it did not significantly affect probability of survival or time to a regenerative response. Among the 66 dogs evaluated in this study, thromboembolic events were confirmed in 9 (14%) and suspected in 5 (8%), and the median survival time for dogs with PIMA that had a thromboembolic event (54 days) was significantly shorter than that for dogs with PIMA that did not have a thromboembolic event (1,365 days). To our knowledge, the present study was the first to find an association between thromboembolic events and survival time for dogs with PIMA.

Nonregenerative anemia owing to a suspected immune-mediated mechanism had been reported in dogs prior to the present study.[1–13,a] The varied terminology and lack of consistent criteria for diagnosis of PIMA have hindered comparisons among affected dogs and consequent treatments. We believe that use of the term PIMA to refer to the condition more accurately characterizes the disorder by emphasizing the targeting of erythroid precursors, mostly within the bone marrow. The defining feature of the disorder is ineffective erythropoiesis associated with erythroid

precursor destruction rather than the resulting nonregenerative anemia.

Emphasis on the specific target of the immune system as it relates to the resulting anemia can help better characterize immune-mediated anemia as a spectrum of related disorders. At one end of this spectrum is PRCA, by which the immune system is suspected to suppress erythroid colony formation or target the earliest erythroid precursors resulting in complete or nearly complete absence of erythroid precursors.[13,14] At the other end of the spectrum is IMHA, by which the immune system targets RBCs resulting in hemolysis, often accompanied by spherocytosis. The various erythropoietic patterns of PIMA fall in the middle of that spectrum. Characterization of immune-mediated anemias along a spectrum helps emphasize that, although there can be similarities and occasional overlap in various disease processes along that spectrum, PIMA is a distinct clinical disorder.

In a previous study by Stokol et al,[11] the clinical features and outcomes for 43 dogs with NRIMAs, including 2 dogs with PRCA, were described. The disease characteristics for the dogs of that study[11] differed from those of the dogs of the present study, likely owing to the different inclusion criteria used for the 2 studies. Nonregenerative anemia was defined as an Hct < 20% and a reticulocyte count < 60,000 reticulocytes/µL in the Stokol et al study,[11] whereas it was defined as an Hct ≤ 30% and a reticulocyte count < 76,000 reticulocytes/µL in the present study. Moreover, although bone marrow aspirate samples were obtained and cytologically evaluated for all 43 dogs of the Stokol et al study,[11] results for 27 of those samples were nondiagnostic and 2 were classified as PRCA. Conversely, the presence of a bone marrow pattern indicative of ineffective erythropoiesis and PIMA was a requirement for inclusion of dogs in the present study. Finally, the proportion of dogs with evidence of immune system targeting of mature erythrocytes (ie, spherocytosis, n = 23/43 [53%]; positive Coombs test results, 20/35 [57%]) in the Stokol et al study[11] was substantially greater than that in the present study (spherocytosis, 2/66 [3%]; positive Coombs test results, 3/19 [16%]). Thus, the dogs of the present study were less likely to have concurrent IMHA and represented a population in which the disease pathogenesis appeared to be directed at erythroid precursors.

Most of the dogs with PIMA evaluated in the present study were middle-aged spayed females. The fact that the preponderance of dogs with PIMA were middle-aged was consistent with the findings of other studies of dogs with NRIMA[8,11,12] as well as other forms of immune-mediated anemia such as PRCA[14] and IMHA.[7,15] Spayed females were overrepresented in the present study and the Stokol et al study[11] involving dogs with idiopathic NRIMA. Labrador Retrievers were overrepresented in the Stokol et al study[11] but not in the present study. Dachshunds, however, were overrepresented in the present study.

Rubriphagocytosis was reported in the original cytology report for 28 of the 66 (42%) dogs of the present study, but review of archived bone marrow specimens revealed the presence of rubriphagocytosis in 61 of 66 (92%) dogs. That discrepancy reflected interobserver variation related to awareness of rubriphagocytosis, the approaches used to search for it, and recognition of the condition. Regardless, this finding suggested that rubriphagocytosis may be more common in dogs with PIMA than previously reported.[12]

The extent of erythroid cellularity in bone marrow varies from low to high in dogs with immune-mediated anemias.[2,3,8,11,12,c] Results of the present study augmented those of a previous study[12] that suggested erythroid cellularity typically mirrors the predominant stage of rubriphagocytosis. The clinical relevance of that stage selectivity remains unknown because the predominant stage of erythroid precursor undergoing rubriphagocytosis was not significantly associated with the time to a regenerative response or overall survival.

Thirty-one of the 63 (49%) dogs of the present study that underwent collection of a bone marrow core biopsy specimen had evidence of collagen myelofibrosis. That finding provided additional support for an association between collagen myelofibrosis and PIMA in dogs and was consistent with findings of other studies.[3,11,12,14,16] The underlying mechanism for myelofibrosis in dogs with PIMA remains unknown. It has been hypothesized that the development of collagen myelofibrosis in dogs may be similar to that in human patients with autoimmune myelofibrosis, which occurs secondary to primary autoimmune conditions, such as systemic lupus erythematosus and autoimmune hemolytic anemia, or a chronic inflammatory state within the bone marrow.[4,12,17,18]

The relationship between collagen myelofibrosis and outcome in dogs with PIMA is unknown. It was suspected that dogs with severe collagen myelofibrosis may have a more prolonged time to a regenerative response owing to a decrease in erythroid production capacity within the bone marrow. In a previous study[12] of 25 dogs with PIMA conducted by our research group, collagen myelofibrosis was present more frequently in dogs that failed to develop a regenerative response than in dogs that achieved a regenerative response. However, 7 of the 12 dogs that did not develop a regenerative response in that study[12] died or were euthanized within 45 days after diagnosis of PIMA and therefore may not have had sufficient time to develop a response. Three of those dogs were euthanized because of a lack of response to treatment,[12] and the perception of a poor prognosis may have contributed to the decision to euthanize. For the dogs of the present study, the presence of collagen myelofibrosis was not significantly associated with the time to a regenerative response or survival, although the median survival time for dogs without collagen myelofibrosis (1,429 days) was > 2 times that for dogs with collagen myelofibrosis (578 days). Nev-

ertheless, some dogs with collagen myelofibrosis did achieve disease remission and resolution. Whether collagen myelofibrosis persists or subsides with remission of PIMA is unknown because it has not been systematically assessed. The present study was the first to specifically evaluate the severity of collagen myelofibrosis relative to the time to a regenerative response and survival for dogs with PIMA, and no significant associations were identified.

All dogs of the present study received immunosuppressive therapy (ie, prednisone). The fact that 3 dogs achieved remission and then had a disease relapse event after being completely weaned from immunosuppressive therapy suggested that the pathogenesis of PIMA (with little or no evidence of a hemolytic component) has an immune-mediated component. Many dogs of the present study received adjunctive immune-modulating therapies, but owing to the retrospective nature of the study and the various types and dosages of medications administered, statistical analyses to assess the effect of such treatments on the time to a regenerative response and survival were not performed.

Fifty-five of the 66 (83%) dogs evaluated in the present study developed a regenerative response at a median of 29 days (range, 2 to 111 days) after PIMA diagnosis. It can take dogs with PIMA several weeks to months to respond to immunosuppressive therapy, and owners should be informed of that fact so they can be prepared for and have realistic expectations about the time required for a regenerative response to be achieved. In the present study, 10 dogs died or were euthanized partly because of a perceived lack of response to treatment; 8 of those dogs died or were euthanized within 45 days after PIMA diagnosis. Financial considerations might have also factored into the decision to euthanize some of those dogs, but the retrospective nature of the study prevented that information from being ferreted out from the medical records. The median times to remission (59 days; range, 12 to 177 days) and resolution (178 days; range, 28 to 385 days) were also fairly long for the dogs of this study.

Thirty-five of the 66 (53%) dogs of the present study had a left shift, which was typically mild, but the leukocyte and neutrophil counts for those dogs were generally within or only slightly increased from the reference limits. Dogs with IMHA often have an inflammatory leukogram characterized by moderate-to-marked leukocytosis, neutrophilia with a left shift, and monocytosis.[7,19,20] For the small proportion of dogs with neutropenia (10/66 [15%]), the low neutrophil count was attributed to ineffective neutropoiesis or severe myelofibrosis. Those leukogram findings further indicated that the pathogenic mechanisms of IMHA and PIMA differ, although they can occur concurrently and potentially overlap and may actually represent a continuum in the pathogenic spectrum from PRCA to IMHA.

Of the 66 dogs with PIMA evaluated in the present study, 9 and 5 developed confirmed or suspected thromboembolic events, respectively. Thromboembolism has been reported in dogs with NRIMA, although in only 2 of 41 dogs in one study[11] and 1 of 25 dogs in another.[12] Thromboembolic events are the most common complications for dogs with IMHA, with a reported prevalence as high as 80% as determined at necropsy.[6,7,20–22] The underlying mechanism of a prothrombotic state in dogs with IMHA has not been elucidated. For dogs with IMHA, thromboembolic events are significantly associated with reduced survival, and the hypercoagulable state often resolves with treatment.[7,22,23,d]

Assessment of coagulation and characterization of the prothrombotic state in dogs with PIMA remain limited. Secondary hemostasis abnormalities were commonly identified when such testing was performed for the dogs with PIMA in the present study. Fourteen of 19 (74%) dogs evaluated in this study had abnormally high D-dimer concentrations, and 14 of 23 (61%) dogs were hyperfibrinogenemic. However, most study dogs did not undergo coagulation testing, and coagulation was not serially evaluated in any of the dogs. Nevertheless, assessment of hemostasis might be warranted as a part of the baseline diagnostic workup for dogs with suspected PIMA given the high frequency of alterations in coagulation identified for the dogs that underwent such testing in this study.

For the dogs of the present study, hyperbilirubinemia (bilirubin, > 0.5 mg/dL) was significantly associated with the occurrence of a thromboembolic event. To our knowledge, this was the first study to assess the prevalence of thromboembolic events in dogs with PIMA in conjunction with other concurrent diagnostic findings. The clinical relevance of hyperbilirubinemia and its association with thromboembolic events in dogs with PIMA is unclear. In dogs with IMHA, hyperbilirubinemia is often severe and is positively associated with the risk of death and occurrence of thromboembolic events.[7,20] It has been hypothesized that abnormally increased bilirubin concentrations might be associated with an abnormally increased rate of hemolysis, hypoxemia-induced centrizonal hepatic necrosis, or intrahepatic thrombi in dogs with IMHA.[5,20,24] Because most dogs with PIMA evaluated in the present study had little or no evidence of hemolysis despite severe anemia, the mechanism for hyperbilirubinemia and its positive association with the development of thromboembolic events warrant further investigation. Potential mechanisms for hyperbilirubinemia in dogs with PIMA include low-grade destruction of erythrocytes, release of hemoglobin within destroyed erythroid precursors, and turnover of hemoglobin from erythrocytes within thrombi.

Fifteen dogs of the present study had peritoneal effusion, and 10 of the 11 peritoneal effusion samples that underwent evaluation were classified as proteinaceous effusions, with the remaining sample classified as a transudate. Five dogs with peritoneal effusions (4 with proteinaceous effusions and 1 with a

transudate) had confirmed thromboembolic events. The thromboembolic events in the 4 dogs with proteinaceous peritoneal effusions included a splenic vein thrombus (n = 1), splenic infarct (1), PVT (1), and PTE with a strongly suspected PVT (1). The dog with the transudate had a PVT. The mechanism for the peritoneal effusion in the other 10 dogs was not established, but unidentified thrombi or vasculitis could not be excluded. Thirty-two of 64 (50%) dogs with PIMA were hypoalbuminemic at hospital admission. However, the hypoalbuminemia was not considered severe enough in any of those dogs to cause transudation, and only 1 peritoneal fluid sample evaluated was classified as a low-protein transudate. Moreover, only 1 dog had severe hypoalbuminemia (albumin < 2.0 g/dL), and that dog did not have peritoneal effusion.

The underlying mechanism for hypoalbuminemia was not determined for the 32 dogs of the present study with the condition. The hypoalbuminemia was generally mild and unlikely to have substantially contributed to the formation of peritoneal effusion. However, the proteinaceous effusions might have contributed to hypoalbuminemia because 8 of the 10 dogs with proteinaceous peritoneal effusions were also hypoalbuminemic. Hypoalbuminemia in dogs with PIMA might also result from a negative acute-phase protein reaction. No obvious differences were identified between the leukograms of dogs with and without hypoalbuminemia, but the absence of an inflammatory leukogram does not exclude a negative acute-phase protein response, and a high macrophage count and cytophagia, when present, support an inflammatory response.

Two of the 9 dogs with a confirmed thromboembolic event in the present study were receiving antithrombotic treatment in the form of aspirin at the time the event occurred. To our knowledge, no studies have been conducted to assess the efficacy of antithrombotic treatment in dogs with PIMA. Large-scale prospective studies regarding optimal antithrombotic treatment in dogs with IMHA are also lacking, although administration of unfractionated heparin when appropriately titrated on the basis of anti-Xa activity is associated with longer survival times, compared with administration of a fixed dose of heparin.[25] It is unknown whether administration of unfractionated heparin would have a similar effect in dogs with PIMA because the underlying mechanism for thromboembolic events in those dogs may differ from that for dogs with IMHA. Nevertheless, nearly half of the dogs with PIMA evaluated in the present study were prescribed an antithrombotic agent despite the lack of evidence regarding the clinical efficacy of such agents in affected dogs. Because thromboembolic events were significantly associated with death for dogs evaluated in this study, further studies are necessary to determine the optimal antithrombotic treatment regimen for dogs with PIMA.

The median survival time was approximately 2.5 years for the PIMA-affected dogs of the present study. That finding suggested that dogs with PIMA can do well if appropriately treated and managed. Although multiple variables, such as the presence of collagen myelofibrosis and stage of predominant rubriphagocytosis, were evaluated for an association with survival, development of a thromboembolic event was the only variable identified as having a significant association with death. Among the 21 dogs that died within 6 months after PIMA diagnosis, the most common cause of death was euthanasia or death after treatment was discontinued, most commonly owners declining additional blood transfusions, owing to a perception that the dog was not responding to treatment. Given current therapeutic approaches, dogs with PIMA require prolonged treatment for the development of a regenerative response and a commitment by the owners to months of therapy, often with multiple blood transfusions and recheck appointments. Thus, owners should be appropriately informed regarding the therapeutic regimen so that they will have realistic expectations and not discontinue treatment or euthanize the dog before it has a reasonable chance to achieve a regenerative response.

Limitations of the present study were primarily associated with its retrospective nature. Data were obtained from medical records available at a single veterinary teaching hospital and through communication with the primary care veterinarians of study dogs, who often were involved in follow-up appointments. Seven dogs were lost to follow-up within 6 months after initial diagnosis of PIMA, which might have affected the median survival time and times to a regenerative response and remission. Additionally, the times to all outcome variables of interest (regenerative response, disease remission, disease resolution, and death) were calculated from admission to the teaching hospital to outcome occurrence. Sixteen dogs received prednisone prior to admission to the teaching hospital, but the number of days the dogs received prednisone prior to hospital admission were not factored into time calculations for the outcome variables because the dosage regimen administered was not always appropriate for immunosuppression and was not provided for 2 dogs. The median times to a regenerative response, remission, and resolution might have been longer had it been possible to factor in prednisone administration prior to hospital admission.

The timing and frequency of recheck appointments and the acquisition of follow-up information may also have confounded the calculation of times to a regenerative response and remission. Several dogs that achieved a regenerative response, remission, and resolution were excluded from the calculations of the median times to those outcomes owing to the lack of timely follow-up information to determine exactly when dogs achieved each outcome.

Similarly, only the 9 dogs with confirmed thromboembolic events were included in the analyses of factors associated with the occurrence of those events or the effect of thromboembolic events on outcome variables even though an additional 5 dogs

were strongly suspected of having thromboemboli. Confirmatory diagnostics for thromboemboli often involve advanced imaging such as CT angiography or ultrasonography, which owners often decline owing to the expense of such procedures. It is possible that the true prevalence of thromboembolic events in dogs with PIMA was underestimated in this study.

Another limitation was that the study population was fairly small. Twenty-four dogs were excluded from the study because bone marrow specimens were not obtained for evaluation even though the clinical signs for those dogs were consistent with PIMA and many were treated with immunosuppressive doses of corticosteroids and responded to that treatment in a manner similar to that of PIMA-affected dogs. The inclusion of more dogs in the study might have affected our results, especially given that the association between the presence of collagen myelofibrosis and survival approached significance.

Results of the present study indicated that many dogs with PIMA respond to prolonged immunosuppressive therapy. Owners should be prepared to invest in weeks to months of therapy to achieve a regenerative response and clinical remission. Although many dogs with PIMA had evidence of collagen myelofibrosis, it was not significantly associated with the time to a regenerative response or survival. Thromboembolic events were associated with death and a decrease in survival time. Further prospective studies are warranted to determine optimal immunosuppressive and thromboprophylactic protocols for dogs with PIMA.

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Footnotes

a. McManus P. Marrow and erythrocyte changes associated with canine nonregenerative immune-mediated anemia: 44 cases (1996–2000) (abstr). *Vet Pathol* 2000;37:526.
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c. Prism, version 7.04, GraphPad Software, San Diego, Calif.
d. DuHadway M, Jutkowitz LA, Scott M, et al. Resolution of the prothrombotic state in dogs treated for immune mediated hemolytic anemia (abstr). *J Vet Emerg Crit Care* 2013;23:S5.

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