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Idiopathic Immune-Mediated Hemolytic Anemia: Treatment Outcome and Prognostic Factors in 149 Dogs

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Background: Canine idiopathic immune-mediated hemolytic anemia (IMHA) is associated with a high mortality, especially in the 1st 2 weeks after diagnosis despite treatment.

Objectives: To determine treatment outcome and identify prognostic variables in order to define areas of future research.

Animals: One hundred forty-nine dogs with hematocrit <30% and either a positive Coombs' test or spherocytosis and with no evidence of disease that can trigger IMHA were included.

Methods: Retrospective cohort study. All dogs were treated with prednisolone and azathioprine according to a standard protocol. Survival analysis was performed by the Kaplan-Meier method. Variables recorded at the time of diagnosis were tested as possible prognostic variables in a univariate and multivariate Cox proportional hazard model.

Results: The main predictors for mortality in dogs with idiopathic IMHA are the presence of increased plasma urea concentration, bands, thrombocytopenia, and petechiae at the time of diagnosis. The estimated Kaplan-Meier half-year survival was 72.6% (95% confidence interval [CI]: 64.9–81.3%). Mortality occurred mostly within the 1st 2 weeks. Cox proportional hazards analysis indicated that increased plasma urea concentration, icterus, and petechiae were the major independent predictors of mortality in the 1st 2 weeks. In most dogs that survived IMHA, a 3-month protocol of azathioprine with prednisolone maintained clinical remission. The estimated half-year survival for dogs that survived the 1st 2 weeks was 92.5% (95% CI: 86–99.3%).

Conclusions and Clinical Importance: If the dogs survived IMHA, a 3-month protocol of prednisolone and azathioprine was effective with regard to survival and clinical outcome. Future research should be directed at identifying whether thrombotic tendency in dogs with IMHA is the main contributor to the development of increased plasma urea concentration, icterus, thrombocytopenia, and petechiae.

Key words: Azathioprine; Prednisolone; Prognosis; Spherocytosis; Therapy.

In immune-mediated hemolytic anemia (IMHA), red blood cells are destroyed as a consequence of antierythrocyte antibody production. Immunoglobulin M (IgM)-mediated hemolysis is caused mainly by intravascular complement activation and subsequent intravascular hemolysis. This is in contrast with the IgG-mediated IMHA where hemolysis is mainly caused by macrophages in liver, spleen, or both.[1] IMHA may be primary or secondary in nature. Secondary IMHA occurs when an underlying disease such as neoplastic and chronic infectious diseases, or exposure to drugs, toxins, and vaccines, leads to attachment of immunoglobulins to erythrocytes.[2,3] Sixty to 75% of IMHA cases in dogs are thought to be primary or idiopathic in origin.[2,4]

Clinical signs of IMHA include lethargy, inappetence, pigmenturia, tachycardia, pale mucous membranes, and fever.[2] Diagnosis is based on the demonstration of hemolytic anemia together with evidence of immune-mediated destruction of red blood cells. In some cases, the antierythrocyte antibodies lead to autoagglutination, and in other cases the presence of antierythrocyte antibodies can be confirmed by the Coombs' test.[2,5] Spherocytosis is considered to be a strong indicator of immune-mediated hemolysis.[5] Spherocytes are formed when macrophages remove a portion of the erythrocyte membrane that is coated with antibody, complement, or both. An indirect way to detect spherocytosis is the osmotic red cell fragility test.[5] Neither presence of spherocytes, positive Coombs' test, nor increase of the osmotic red cell fragility indicates whether the etiology of the immune-mediated hemolysis is primary or secondary.

Previous studies have reported mortality rates up to 70% during the 1st 3 weeks of treatment.[3,4,6] The presence of autoagglutination,[3] the degree of reticulocytosis,[7] severity of anemia,[7] thrombocytopenia,[8] severe leukocytosis,[6,9] increase in bands,[9] serum bilirubin concentration,[3,7,8,10] and increase in prothrombin time (PT)[6,9] all were associated with poor outcome. Clinical decision-making in cases of idiopathic IMHA might be easier when the clinician is provided with factors that predict outcome. The objective of this study was to identify the prognostic variables that determine the outcome in dogs with idiopathic IMHA treated with prednisolone and azathioprine following a standard protocol. These factors might define areas of future research that will hopefully result in a better prognosis for these patients.

Materials and Methods

Patients

The dogs that were included were referred to the Utrecht University Clinic of Companion Animals (UUCCA) between January

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1, 1994, and December 31, 2000. Inclusion criteria were hematocrit $<30\%$ and either a positive Coombs' test or presence of spherocytes in a blood smear. In addition, these dogs had been treated according to a standard immunosuppressive protocol, and a complete medical record had to be present. Dogs were excluded if they had evidence of diseases that could induce IMHA, such as neoplasia, medications, and infectious diseases. As a result, dogs that had visited areas where ehrlichiosis and babesiosis are endemic were excluded unless serologic examination for *Ehrlichia canis* and *Babesia canis* or *B. gibsonii* was negative. Dogs with a travel history to countries in which the above infectious diseases are endemic within 3 weeks before diagnosis of idiopathic IMHA also were excluded. Also, dogs that received immunosuppressive treatment for longer than 14 days before referral to the UUCCA were excluded.

Complete history and physical examination were recorded, including age at time of 1st diagnosis of idiopathic IMHA, occurrence of generalized weakness, anorexia, vomiting, diarrhea, and dark red urine as well as the presence of an increased rectal body temperature, pale mucous membranes, icterus, petechiae, lymphadenopathy, and cranial abdominal organomegaly. Additional diagnostics were performed when judged necessary by the attending clinician.

Laboratory Tests

All tests were performed at the UUCCA. The following laboratory tests were performed on admission, and during return visits, approximately 4 and 10 weeks after starting the therapeutic protocol: complete blood count, reticulocyte count, presence of spherocytes, Coombs' test and osmotic red cell fragility testing, and plasma urea and creatinine concentrations. Results of PT, activated partial thromboplastin time (APTT), and fibrinogen concentration were recorded when performed. PT and APTT were considered prolonged when they were increased at least 10% above results obtained with normal pooled canine citrated plasma.

A monovalent direct Coombs' antiglobulin test was performed with anti-dog IgG[a] and IgM[b] antibodies and an anti-dog complement antibody[c] for agglutination of the patients' red cells as described before.[5]

The osmotic fragility of the erythrocytes was determined as described previously.[5] Plasma creatinine concentrations were corrected for body weight as described previously.[11]

Therapy

Blood transfusions alone or in combination with IV fluid therapy were given when judged necessary by the attending clinician. The number of blood transfusions was noted. All clinic-owned donor dogs used in the study are dog erythrocyte antigens 1.1 and 1.2 negative. Whenever a client-owned donor dog was used or when a 2nd transfusion was given, cross matches were performed.

A standard protocol of immunosuppressive treatment consisting of a combination of prednisolone and azathioprine was instituted in all dogs. The *outcome category* was assessed at least once daily during the hospitalization period and during return visits that were scheduled 4 and 10 weeks after the start of therapy.

As long as the outcome category was *no effect*, prednisolone[d] was given in a dosage of 2 mg/kg/day PO. Dogs that were not able to take oral medication were treated with dexamethasone (0.5–1 mg/kg/day) IV or SC. As soon as the outcome was assessed as *improvement*, prednisolone therapy was started at a dosage of 2 mg/kg/day PO for 3 days, followed by 1.5 mg/kg/day PO for 7 days, 1 mg/kg/day PO for 10 days, 0.5 mg/kg/day PO for 14 days, after which the same dose was given on alternate days for 14 days, and subsequently tapered down to 0.25 mg/kg/day PO for 21 days. Azathioprine[e] was started at a dosage of 2 mg/kg/day PO for dogs weighing <20 kg. The daily azathioprine dose in dogs of 25, 30, 40, and 50 kg was maximized at 45, 50, 60, and 70 mg, respectively. Azathioprine treatment was stopped 10 days after prednisolone treatment. If the outcome was assessed as *complete recovery* at the 4- or 10-week return visit the prednisolone therapy protocol as described above was followed. If a *relapse* was diagnosed at any time during this treatment, the prednisolone therapy protocol was started from the beginning. If at the 4- or 10-week return visit the outcome was assessed as *improvement* and not yet as *complete recovery*, the duration of the interval at which prednisolone was tapered, as described above, was doubled.

Outcome

The response to therapy and its adverse effects were recorded from the medical records. The response was assessed at the 1st and 2nd return visit. At both times, the findings were compared with the last visit.

We defined 4 outcome categories. *Complete recovery* was defined as an increase in hematocrit to $>36\%$, a negative Coombs' test, and an osmotic red cell fragility within normal reference range. The category *improvement* included dogs that experienced lesser increases in hematocrit as well as dogs that had hematocrits $>36\%$ that still had a positive Coombs' test or an increased osmotic red cell fragility. *No effect* of therapy was defined as no increase in hematocrit. A *relapse* was defined as a decrease in hematocrit after an initial *improvement* or *complete recovery*, in combination with recurrence of a positive Coombs' test or increased red cell fragility. The time at which a *relapse* occurred was recorded from the medical records.

The hematocrit, the corrected reticulocyte percentage, the platelet count, results of the Coombs' test, and the osmotic fragility of the erythrocytes were recorded from the medical records at the times of the 1st and 2nd return visits and when a relapse occurred. Vomiting and diarrhea were divided into 3 categories: absent, <2 days, or >2 days.

Survival was determined at the last date of contact with the owner by the attending clinician or by one of the investigators (G.J.) at the time of the study. The outcome was divided into 4 categories: death caused by IMHA, death by another cause, alive but still on immunosuppressive treatment, and alive without treatment.

Data Analysis

Statistical analysis of the data was performed by S-plus statistical package.[f] The data set was split into a group that was in the study for ≤ 14 days and a group that was in the study >14 days. All variables that were measured at the time of diagnosis were compared between groups. Comparison between groups for categorical variables was performed with the Fisher's exact test. Continuous variables were compared between groups by the Wilcoxon rank-sum test. $P < .05$ was considered significant.

Survival analysis was performed for both groups as well as for the entire data set. The endpoint of the study was death caused by IMHA. Dogs that were alive at the end of the study and dogs that died by a cause other than IMHA were censored. Survival curves were drawn with the Kaplan-Meier method.

All variables recorded at the date of 1st diagnosis were evaluated as possible prognostic indicators in a univariate Cox proportional hazard model, except the variable breed. Variables significant at the $P < .15$ level in the univariate model were analyzed in a multivariate model allowing for interaction among variables. Multivariate analysis was performed by forward stepwise selection with a $P < .05$ in the likelihood ratio test as a criterion for inclusion. Compliance with the proportional hazard assumption was tested graphically by plotting the Schoenfeld residuals against time.

Results

Patients

One hundred ninety-seven dogs were selected from the clinicopathologic database of the UUCCA. Seventy-nine of these dogs had both a positive Coombs' test and spherocytes, 109 dogs only a positive Coombs' test, and 9 dogs only spherocytes. Forty-three dogs were excluded because they were positive for at least one of the exclusion criteria.

As a result, 149 dogs were included in the study. Twenty-six dogs were crossbreeds, 11 were English Cocker Spaniels, 9 old English Sheepdogs, 8 Dachshunds, and 7 were Labrador Retrievers. Each of the following breeds was represented by 5 dogs: Bouvier des Flandres, German Shepherd dog, Jack Russell Terrier, and Maltese. The remaining 68 dogs were of other breeds. Sixty-one of the 149 dogs were males (46 intact, 15 castrated) and 88 were female dogs (51 intact, 37 neutered). Median age at the time of 1st diagnosis of idiopathic IMHA was 5 years (range, 13 weeks–13 years).

The median duration of clinical signs preceding the 1st diagnosis of idiopathic IMHA was 6 days (range, 1–131 days). One hundred forty-four dogs had a history of weakness, 119 dogs were anorectic, in 66 dogs dark red urine was noticed, 44 dogs had vomited, 23 dogs had a period of diarrhea, 16 dogs were dyspneic, and in 15 dogs signs of hemorrhagic diathesis were seen. In 5 dogs, their history suggested the occurrence of syncopes. Physical examination disclosed pale mucous membranes in 146 dogs, 69 dogs had an increased rectal body temperature, 57 dogs had icterus, 51 dogs had cranial abdominal organomegaly, 16 dogs had generalized lymphadenopathy, and in 8 dogs petechiae were seen.

Laboratory Tests

The laboratory examination results of the 149 dogs at the time of diagnosis are listed in Table 1. Six dogs had leukopenia, 23 had normal leukocyte counts, and 119 dogs had leukocytosis (missing data = 1). A left shift with $>0.3 \times 10^9$/L bands was seen in 117 dogs (missing data = 1).

Thirty-seven dogs had a platelet count $<50 \times 10^9$/L, 82 dogs had a platelet count $<150 \times 10^9$/L, 9 dogs had a normal platelet count, and 12 dogs had a platelet count $>400 \times 10^9$/L (missing data = 9). Spherocytes were seen in 77 dogs.

The PT and APTT were determined in 98 dogs. The PT was prolonged in 45 dogs and the APTT in 66 dogs, respectively. Fibrinogen concentration was determined in 96 dogs. Seventeen dogs had hypofibrinogenemia, 46 dogs had normofibrinogenemia, and 33 dogs had a fibrinogen concentration >5 g/L. The Coombs' test was positive in 143 dogs. Fourteen dogs were positive for IgM, 27 dogs for IgG, 15 dogs for the combination of IgG and IgM, 4 dogs for IgM and complement, 27 dogs for IgG and complement, and 55 dogs for the combination of IgG, IgM, and complement. The osmotic red cell fragility test was positive in 117 of the 142 dogs in which it was performed. Spherocytes were present in all 6 dogs in which the Coombs' test was negative or not performed.

Therapy

Blood transfusions were given to 98 dogs: only once in 78 dogs, twice in 18 dogs, 3 times in 1 dog, and 4 times in another dog. The median hematocrit in dogs that received 1 blood transfusion was 12% (range, 4–21%). The median hematocrit in dogs that did not receive a blood transfusion was 18% (range, 10–27%).

All dogs were treated with prednisolone and azathioprine according to the protocol described above. Dogs that were not able to take oral medication were treated with dexamethasone IV or SC for 1 or 2 days before the protocol was started. The median duration of treatment in the whole data set was 59 days (range, 0–622 days; n = 92) for prednisolone and 53 days (range, 0–622 days; n = 84) for azathioprine, respectively. The median duration of prednisolone therapy and azathioprine in dogs that survived for more than 14 days was 69 days (range, 2–622; n = 96) and 83 days (range, 2–622), respectively. Figure 2 shows duration of prednisolone therapy in dogs that survived IMHA.

Table 1. Laboratory results at the time of diagnosis in 149 dogs with immune-mediated hemolytic anemia (IMHA).

	Median	Range	n	Reference Values
Ht (%)	13	4–27	149	42–57
Corrected reticulocyte (%)	2.7	0.01–19	147	<2
Leukocytes ($\times 10^9$/L)	27.9	2.1–130	148	5.9–13.8
Bands ($\times 10^9$/L)	1.4	0–22.1	148	0–0.3
Thrombocytes ($\times 10^9$/L)	122	0–958	140	150–400
Urea (mmol/L)	7.6	2.9–69.5	123	3.0–12.5
Creatinine corrected for body weight (μmol/L)	42	0–652	112	<50
PT (seconds)	8	6–12	98	7 ± 1
PT patient minus PT control (seconds)	0	0–5	98	
APTT (seconds)	16	11–98	98	14 ± 1
APTT patient minus APTT control (seconds)	3	0–83	98	
Fibrinogen (g/L)	3.9	0.6–13.8	96	2–5
Osmotic red cell fragility (mOsm/L)	238	120–317	139	<162

PT, prothrombin time; APTT, activated partial thromboplastin time.

Table 2. Laboratory results in 93 dogs with immune-mediated hemolytic anemia (IMHA) that were reexamined during treatment.

	First Control Visit[a]			Second Control Visit[a]		
	Median	Range	n	Median	Range	n
Ht (%)	35	6–50	93	40	11–54	66
Reticulo-cytes (%)	0.9	0.1–28	87	0.8	0.1–12	60
Thrombocytes ($\times 10^9$/L)	402	8–986	88	278	3–834	63
		# positive	n		# positive	n
Coombs' test		16	85		7	60
Osmotic red cell fragility		62	82		30	58

[a]First control visit took place after a median of 25 days (range, 2–83 days) and the 2nd control visit after a median of 77 days (range, 21–399 days) after diagnosis.

Treatment Outcome

Details of the response and occurrence of adverse effects are presented only for the dogs that were re-examined during return visits at the UUCCA and not for dogs for which follow-up was performed by the referring veterinarians. The 1st return visit after the diagnosis of IMHA occurred after a median duration of 25 days (range, 2–83 days) in 93 dogs, and 66 of these dogs had a 2nd return visit after a median duration of 77 days (range, 21–399 days). Results for hematocrit, reticulocyte percentage, platelet count, Coombs' test, and osmotic red cell fragility testing at the time of the 1st and 2nd return visits are presented in Table 2. Therapy had no effect in 3 dogs, 87 dogs improved, and 5 dogs completely recovered by the 1st control visit. Of the 66 dogs that were examined for a 2nd time, 4 dogs had a relapse, 42 dogs were still considered improved, and an additional 20 dogs were completely recovered.

A relapse was diagnosed in 18 dogs after a median of 112 days (range, 32–1,757 days). The median hematocrit at the time of the relapse was 28% (range, 6–44%). The median reticulocyte percentage was 6.1% (range, 0.1–22%). The median platelet count was 132×10^9/L (range, 0–382 $\times 10^9$/L). The Coombs' test was positive in 10 of 14 dogs in which it was performed. The osmotic red cell fragility was increased in 11 of 12 dogs in which this test was performed.

The presence of adverse effects was recorded during the whole period that treatment with prednisolone and azathioprine was continued. Vomiting was absent in 75 dogs, present for <2 days in 5 dogs, and present for >2 days in 7 dogs. Diarrhea was absent in 72 dogs, present for <2 days in 5 dogs, and present for >2 days in 9 dogs. Twelve dogs had leukopenia ($<5 \times 10^9$/L) and 12 dogs had thrombocytopenia ($<100 \times 10^9$/L).

Survival data are presented for all 149 dogs in the study. The last date of contact with the owner occurred after a median of 46 days (range, 0–2,026 days). The estimated survival rate for the 1st 14 days of the study was 78.5% (95% confidence interval [CI]: 71.9–85.6%). At this time, 30 deaths because of IMHA had occurred and 23 dogs were censored because they were lost for follow-up. The estimated half-year survival for the whole group was 72.6% (95% CI: 64.9–81.3%) (Fig 1). The estimated half-year survival rate for the 96 dogs that survived the 1st 14 days was 92.5% (95% CI: 86–99.3%).

Data Analysis

The age at the time of diagnosis and the time that clinical signs started were significantly ($P = .0168$ and .0174,

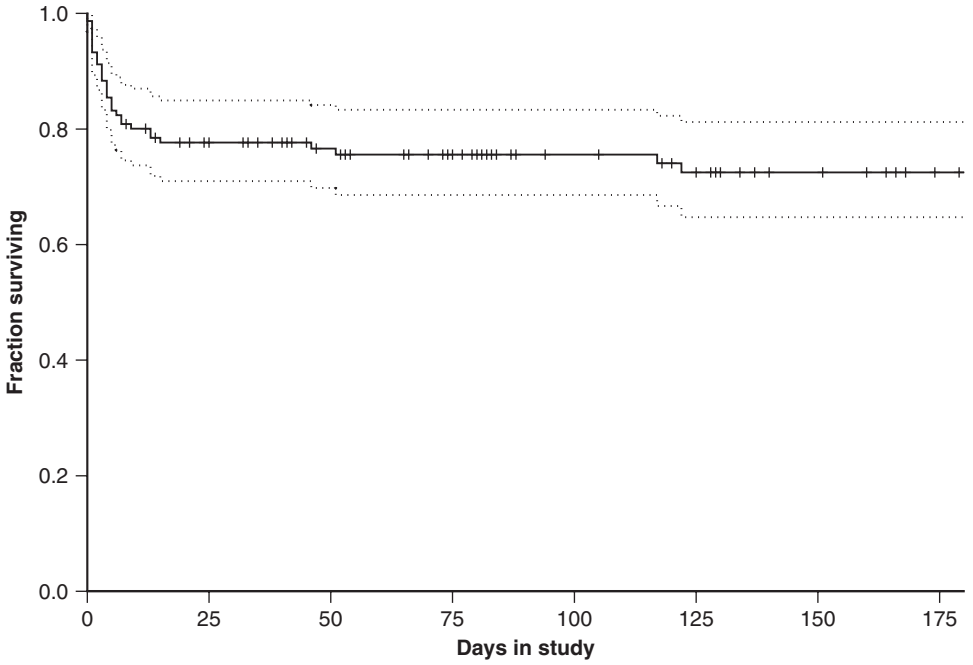

Fig 1. Estimated Kaplan-Meier half-year survival times for 149 dogs with idiopathic immune-mediated hemolytic anemia (IMHA) treated according to a standard protocol of prednisolone and azathioprine.

Table 3. Univariate Cox proportional hazards results for risk of death in 149 dogs with idiopathic immune-mediated hemolytic anemia (IMHA) that were included in the multivariate analysis.

	HR	n[a]	95% CI	*P*
Fibrinogen (g/L)	0.774	96	0.647–0.925	.00146
Urea (20 mmol/L)	2.11	123	1.41–3.15	.00285
Creatinine corrected for body weight (50 μmol/L)	1.27	112	1.12–1.43	.00466
PT patient minus PT control (seconds)	1.44	98	1.14–1.82	.00559
thrombocytes (50×10^9/L)	1.23	140	1.03–1.45	.00671
Osmotic red cell fragility (mOsm/L)	0.365	142	0.177–0.75	.0107
APTT patient minus APTT control (seconds)	1.03	98	1.01–1.06	.0235
Spherocytes	0.476	149	0.246–0.923	.0247
Icterus	2	149	1.07–3.77	.0321
IgM (titer of highest positive dilution)	1.05	148	1.0–1.11	.0405
Petechiae	2.51	149	0.887–7.13	.124

CI, confidence interval; HR, hazard ratio; PT, prothrombin time; APTT, activated partial thromboplastin time; IgM, immunoglobulin M.
[a]Number of dogs in which the parameter was determined for which the Cox proportional hazards model calculated the HR.

respectively) lower in the group surviving >14 days (median, 5.5 years; range, 19 weeks–12 years) than in the group surviving ≤ 14 days (median, 7 years; range, 14 weeks–13 years). Plasma urea concentration was significantly ($P = .0229$) lower in the group surviving >14 days (median, 7.1 mmol/L; range, 2.9–61 mmol/L) than in the group surviving ≤14 days (median, 8.9 mmol/L; range, 3.8–70.0 mmol/L). Increased osmotic red cell fragility was noted significantly ($P = .0126$) more often in the group that survived >14 days (79/89) than in the group that survived <14 days (38/53).

Compliance with the proportional hazard assumption was valid for all variables that had a $P < .15$ in the univariate analysis. Results for the univariate analysis of the whole data set are shown in Table 3. Receiving a blood transfusion and the number of transfusions received both were significant negative predictors for survival in the univariate analysis. Duration of treatment in the group that survived the 1st 14 days did not significantly affect survival (hazard ratio [HR] 1, 95% CI: 0.995–1.01; $P = .92$). Because blood transfusions and duration of treatment are not prognostic criteria at the time of diagnosis, they were not put into the multivariate model.

Multivariate analysis of the entire data set indicated that urea (HR = 2.849, 95% CI: 1.689–4.805), bands (HR 1.105, 95% CI: 1.018–1.199), petechiae (HR 4.011, 95% CI: 1.188–13.538), and platelet count (HR 0.712, 95% CI: 0.555–0.913) were positive predictors of death. For the dogs surviving ≤ 14 days, the presence of icterus (HR 4.61, 95% CI: 1.53–13.89), petechiae (HR 11.31, 95% CI: 2.56–49.99), and urea (HR 2.51, 95% CI: 1.51–4.17) were all positive predictors of death. For dogs surviving >14 days, an increase in leukocyte count (HR 0.373, 95% CI: 0.184–0.759) was associated with a decreased risk of death and the presence of fever (HR 11.555, 95% CI: 1.353–98.66) was associated with an increased risk of death (Table 4).

Discussion

In this study, dogs with idiopathic IMHA that had been treated according to a protocol of prednisolone and azathioprine were investigated with the objective to determine the treatment outcome and to identify prognostic variables in order to define areas of future research. It is difficult to compare study outcomes of canine IMHA with respect to survival, outcome, and efficacy of therapy because study populations are small, composition of study groups differs among clinics, and usually more than 1 treatment protocol is used within a

Table 4. Multivariate Cox proportional hazards results for risk of death in short- and long-term survivor dogs with idiopathic immune-mediated hemolytic anemia (IMHA).

	HR	95% CI	*P*
Entire data set (n = 115, 34 observations deleted because of missing values)			
Urea (20 mmol/L)	2.849	1.689–4.805	.0038
Thrombocytes (50×10^9/L)	0.712	0.555–0.913	.0005
Bands ($\times10^9$/L)	1.105	1.018–1.199	.0007
Petechiae	4.011	1.188–13.538	.046
Dogs surviving <14 days (n = 47, 6 observations deleted because of missing values)			
Urea (20 mmol/L)	2.51	1.51–4.17	.0005
Icterus	4.61	1.53–13.89	.0024
Petechiae	11.31	2.56–49.99	.0027
Dogs surviving >14 days (n = 96)			
Fever	11.555	1.353–98.66	.0042
Leukocytes (10×10^9/L)	0.373	0.184–0.759	.0018

CI, confidence interval; HR, hazard ratio.

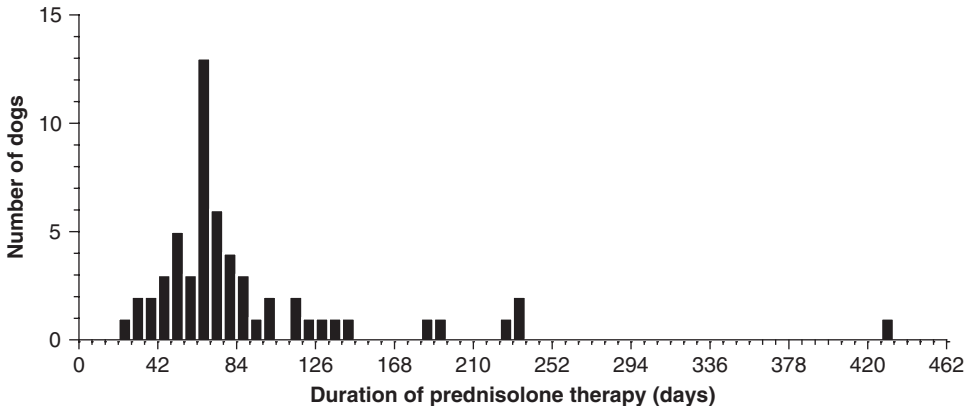

Fig 2. Duration of prednisolone therapy in the 96 dogs in this study that were treated according to a standard protocol of prednisolone and azathioprine and did not die because of immune-mediated hemolytic anemia (IMHA).

study.[6,7,9,10,12,13] We present here a large cohort of dogs with IMHA that were uniformly treated. This provides statistical power to the analysis as well as the opportunity to report on the duration of immunosuppressive therapy that was necessary to maintain clinical remission of IMHA.

The estimated Kaplan-Meier half-year survival time for all 149 dogs in our study was 72.5%. Judging from the accompanying 95% CI (65–81%), this survival time is comparable to those reported by others.[9] Most reports describe only the immunosuppressive protocol that was used in the 1st weeks of therapy. Clinical studies that report on effectiveness of long-term treatment are lacking.[14] Our protocol incorporates guidelines to adjust the therapy to clinical outcome. As a result, duration of therapy differed among dogs (Fig 2), but did not significantly influence survival in the dogs that survived the 1st 14 days. The estimated half-year survival time in this subgroup of the data set was 92.5% (95% CI: 86–99.3%), and 87 of 95 dogs that were examined at the 1st control visit 25 days after the diagnosis were categorized as *improved* (Table 2). As can be seen in Figure 2, most dogs were treated for 3 months, and only in a small number of dogs was prednisolone therapy necessary >6 months. Because adverse effects of prednisolone occurred in virtually every dog, only adverse effects of azathioprine were monitored. The thrombocytopenia that was seen in 12 dogs during treatment could have been a result of idiopathic IMHA but was more likely caused by bone marrow toxicity of azathioprine therapy because concurrent leukopenia also was present.[2,15] We can conclude from these findings that the described combination protocol of prednisolone and azathioprine was effective with regard to survival and clinical outcome, and a duration of therapy of approximately 3 months was sufficient in successfully treated dogs.

Multivariate analysis (Table 4) indicated an increase in urea concentration, an increase in bands, a decrease in thrombocytes, and the presence of petechiae at the time of diagnosis as the major individual negative prognostic indicators. With the exception of an increase in plasma urea concentration and petechiae that to our knowledge have not been reported before, these variables overlap with prognostic factors found by others.[8,9] To appreciate the effect of an individual variable on survival, the HRs have been given for clinically relevant changes (Table 4). The presence of more than one of these prognostic variables increases the death risk by the product of their respective HRs. For example, the presence of petechiae in a patient increases the death risk to die because of IMHA by a factor 4.011, but when present in combination with a increase of plasma urea concentration of 20 mmol/L and a decrease in thrombocytes of 50×10^9 L/L, it confers an estimated death risk that is 16 times higher as in a patient that has no increase in plasma urea concentration, no petechiae, and normal thrombocytes.

The mortality associated with idiopathic IMHA ranges between 29 and 70%.[3,7,8,10,13] As has been reported by others, in this study, a large part of the overall mortality because of IMHA was concentrated in the 1st 2 weeks after diagnosis.[7,10,12] The fact that the proportional hazard assumption was valid for all variables that were introduced into the multivariate analysis does not exclude the possibility that the influence of these variables on the outcome of treatment is different over time. For this reason, the data set was split into a group surviving ≤ 14 days and dogs that survived >14 days. The initial steep descent of the Kaplan-Meier curve in the 1st 14 days in this and in other studies[3,6,16] suggested this time span as the logical cut-off.

The multivariate model that best predicted death in the dogs that survived <14 days had an increased plasma urea concentration and the presence of icterus and petechiae in it as main determinants (Table 4). Petechiae has the highest HR, both in the whole data set, and in the group that survived <14 days. Petechiae can be the result of immune-mediated destruction of platelets, which in combination with IMHA has been associated with a poorer outcome than immune-mediated thrombocytopenia or IMHA alone.[4] The fact that both thrombocytopenia and petechiae were major independent variables in the multivariate analysis suggests that they are the result of independent pathophysiologic pathways. Severe thrombocytopenia[8,17,18] and hyperbilirubinemia[8] have been shown to be risk factors for the presence of thromboembolism in dogs with idiopathic IMHA. Abnormalities in

PT and APTT and other coagulation parameters may be indicative of diffuse intravascular coagulation (DIC) or result from thromboembolism and are often found in dogs with idiopathic IMHA.[6,8,18–20] Postmortem examination of dogs with IMHA revealed pulmonary thromboembolism in 10–80% of cases as well as thromboembolisms in other organs.[18,21] Evidence for the presence of prothrombotic tendencies also has been reported in prospective studies that indicate the presence of hemostatic abnormalities suggestive of DIC[20,22] and activation of platelets in dogs with IMHA.[23] For these reasons, we hypothesize that prothrombotic tendencies in IMHA play an important role in the development of both petechiae and thrombocytopenia.

Icterus in IMHA can be explained by increased bilirubin production and a moderate decrease in hepatic bilirubin clearance, most likely because of impaired liver function resulting from centrolobular necrosis caused by hypoxia.[24] Increased plasma urea concentration in IMHA most likely results from hypoxia-induced renal injury, concurrent thromboembolic renal disease, and prerenal factors.[25] The severity of anemia has been suggested as a negative prognositic indicator[7]; however, it was not identified in other studies[6,9,26] and was also not found in the univariate or multivariate analysis in this study. Therefore, we hypothesize that activation of coagulation during IMHA is an important pathway that contributes to the development of an increased plasma urea concentration and icterus. Future studies should focus on examining the pathogenesis of thrombotic tendencies in dogs with IMHA. This approach will help to develop diagnostic markers as well as more effective treatment protocols.

Leukocytosis, including a left shift, is a common laboratory finding associated with IMHA[3,6,8,10] (Table 1). The presence of leukocytosis with a left shift has been associated with increasingly severe histopathological lesions.[26] One study reported leukocytosis as a negative prognostic indicator, without having evaluated the contribution of a left shift.[10] In our study, a left shift predicted death in the entire data set (Table 4). However, once dogs survived the 1st 14 days, an increase in leukocytes (Table 4) at the time of diagnosis was associated with a decreased death risk. From this observation, it can be concluded that the left shift most likely is associated with increased risk of death rather than the magnitude of the leukocyte response. In an inflammatory response, extravasation of leukocytes is followed in time first by increased release and then by increased production of leukocytes by the bone marrow.[27] The left shift that precedes a leukocytosis reflects both the magnitude and acuteness of onset of the inflammatory response.[27] Fever at the time of diagnosis increased the risk of death in long-term survivors (Table 4). Cytokines such as tumor necrosis factor-α, interleukin-1, and interleukin–6, released by phagocytes, act as endogenous pyrogens and mobilize neutrophils, which results in the left shift and leukocytosis as explained above.[27] Fever and leukocytes are the major independent variables in the multivariate model that describes survival in dogs that survived the 1st 14 days and are not part of the univariate and multivariate models that describe the whole data set containing plasma urea concentration, thrombocytes, left shift and petechiae, nor of the model for the subset of dogs that survived <14 days that contains plasma urea concentration, icterus, and petechiae. This suggests that the influence of thrombotic tendencies on survival mainly affects the 1st 2 weeks and the consequences of the inflammatory response still exert their influence if dogs survive these 2 weeks.

The presence of spherocytes was associated with increased survival, both in this study and in an independent data set of 143 dogs with idiopathic IMHA (Slappendel and Teske, unpublished data). A positive IgM titer was associated with a negative effect on survival in this study (Table 3). Hemolysis in IMHA results from either IgM-mediated intravascular complement activation or IgG-mediated erythrophagocytosis that leads to spherocyte formation and decreased osmotic red cell fragility.[1] The opposite survival effect of having spherocytes versus an IgM titer might be explained by the following factors. First, IgM-mediated hemolysis has been reported to be more severe than IgG-mediated hemolysis.[1] Second, glucocorticoid therapy is more effective in reducing the clearance of IgG-coated cells.[1] Because spherocytes are mainly the result of partial phagocytosis of IgG-sensitized red cells, the fact that hemolysis is less severe and the therapy protocol is more effective might explain the more favorable outcome in these dogs.

From this retrospective cohort study, it can be concluded that the main predictors of mortality in dogs with idiopathic IMHA are the presence of increased plasma urea concentration, bands, thrombocytopenia, and petechiae at the time of diagnosis. The estimated Kaplan-Meier half-year survival was 72.6% (95% CI: 64.9–81.3%). Mortality occurred mostly within the 1st 2 weeks. Cox proportional hazards analysis revealed increased plasma urea concentration, icterus, and petechiae as major independent predictors of mortality in the 1st 2 weeks. In most dogs, a 3-month protocol of azathioprine with prednisolone maintained clinical remission. The estimated half-year survival for dogs that survived the 1st 2 weeks was 92.5% (95% CI: 86–99.3%). Future research should be directed at determining if thrombotic tendency in dogs with IMHA is the main contributor to the development of increased plasma urea concentration, icterus, thrombocytopenia, and petechiae.

Footnotes

[a] Central Blood Laboratory, Amsterdam, The Netherlands
[b] Nordic, Tilburg, The Netherlands
[c] Nordic
[d] Prednisolone, Alfasan International BV, Woerden, The Netherlands
[e] Imuran, Glaxo-Wellcome, Zeist, The Netherlands
[f] S-PLUS 6.1, Insightful Corporation, Seattle, WA

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