FULL PAPER *Clinical Pathology*

Evaluation of Prognostic Factors and Establishment of a Prognostic Scoring System for Canine Primary Immune-Mediated Hemolytic Anemia

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ABSTRACT. Clinical courses of primary immune-mediated hemolytic anemia (pIMHA) in dogs are highly variable, however, limited information is available to predict their accurate prognoses. To evaluate the prognostic significance of clinical factors and to propose a scoring system to predict prognoses, the medical records of seventy-one dogs with pIMHA were reviewed. Overall mortality rate of dogs with pIMHA was 39% and most of the dogs died within 3 months from diagnosis. Sex, body weight, seasonality, packed corpuscular volume (PCV), platelet count (PLT), total plasma protein (TP), blood urea nitrogen, albumin, total bilirubin, sodium ion, prothrombin time, and fibrin/fibrinogen degradation products before immunosuppressive treatment can influence on survival time in dogs with pIMHA. A prognostic scoring system using a combination of sex, seasonality, PCV, PLT and TP can be statistically significant for raising the accuracy of prognostic prediction. Using the scoring system for prognostication in dogs with pIMHA may enable veterinarians to predict a prognosis easily and accurately.

KEY WORDS: canine, mortality, outcome, primary IMHA, prognosis.

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Canine immune-mediated hemolytic anemia (IMHA) is a common cause of anemia in dogs. IMHA is distinguished between primary IMHA (pIMHA) and secondary IMHA (sIMHA) in the pathogenesis. The pIMHA is thought to be an idiopathic autoimmune disorder and the sIMHA is thought to be resulted from other diseases and conditions including neoplasia, infectious agents, drug exposure, other autoimmune diseases and vaccination [1, 4, 9, 14, 15, 17, 21].

In previous reports, the incidence of pIMHA was about 60 to 75% of canine IMHA [18] and the mortality was 26 to 70% (total mortality rate: 52%) [2, 3, 7, 12, 23, 25]. Fatal causes in canine pIMHA are due to progressive hemolysis and organ failure in association with deuteropathic disseminated intravascular coagulation (DIC) or thromboembolism. IMHA is highly associated with thromboembolism, which is a common cause of death [3, 8, 13, 25]. In fact, approximately 60% of dogs with pIMHA were suspected of accompanying DIC [3, 23], and pulmonary thromboembolism was confirmed at postmortem examination in 10 to 32% of dogs with IMHA in previous reports [8, 13].

Because of such high mortality, several studies reported prognostic factors associated with pIMHA in dogs. Some reports concluded that dogs presenting thrombocytopenia, hypoalbuminemia and hyperbilirubinemia at diagnosis had shorter survival time [3, 12, 26]. Other reports showed that leukocytosis could worsen prognosis [19, 26]. However, except for these factors, no consensus has been obtained with respect to the prognostic factor and there has been no report describing prognostication using multiple factors in dogs with pIMHA.

Therefore, the present study was carried out to reevaluate association of clinical parameters obtained before immunosuppressive treatment with influence on the prognosis, and to establish a scoring system using multiple factors for prognostication in dogs with pIMHA.

MATERIALS AND METHODS

Patients and diagnostic criteria: Medical records of dogs with pIMHA referred to the Veterinary Medical Center at the University of Tokyo (UT-VMC) from April 1997 to March 2006 were reviewed. Criteria for a diagnosis of pIMHA were presence of regenerative anemia (packed cell volume, PCV<37%), presence of either spherocytosis or spontaneous persistent agglutination of erythrocytes (i.e., agglutination that persisted after dilution with saline), or positive direct Coombs' test, and exclusion of underlying diseases causing sIMHA such as neoplasia, systemic infection including rickettsiae, bacteria and viruses, and other autoimmune diseases. Dogs presented with thrombocytopenia and vaccinated within a month before showing anemia (suspected concurrent immune-mediated thrombocytopenia and vaccination-induced IMHA) were excluded. In this study, the day when pIMHA was diagnosed and immunosuppressive drugs were administered for the first time was defined as day 1, and the medical data was reviewed for analysis.

Analyzed factors: Information extracted from medical

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records included signalment, history, clinical signs, urinalysis, complete blood count (CBC), blood chemistry profiles, examination of blood coagulation and fibrinolytic system on day 1. Signalment and history included age, sex, breed, body weight, vaccination history and seasonality at diagnosis. In terms of seasonality, a period from April to September was defined as the warm season and the other period was as the cold season in Japan. Clinical signs included fever, respiratory distress, pulse rate, hypophagia, vomiting, bleeding, color of visible mucous membrane, hepatomegaly and splenomegaly. PCV, white blood cell count (WBC), platelet count (PLT) and total plasma protein (TP) were contained in CBC. As blood chemical profiles, alkaline phosphatase (ALP), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), blood urea nitrogen (BUN), creatinine (Cre), calcium (Ca), inorganic phosphorus (IP), total bilirubin (T-Bil), albumin (Alb), sodium ion (Na), potassium ion (K), chloride ion (Cl) and C-reactive protein (CRP) were reviewed. Examination of blood coagulation and fibrinolytic system contained prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrin/fibrinogen degradation products (FDP).

Treatment: Dogs were treated with corticosteroids (prednisolone) alone or in combination with immunosuppressive agents (azathioprine, cyclosporine or intravenous human immunoglobulin). Anticoagulants (low-molecular weight heparin and ultralow-dose aspirin) were administered to prevent hypercoagulability following pIMHA. Moreover, as supportive care, oxygen inhalation, blood transfusion, fluid therapy and antibiotic treatment were performed if necessary.

Distinction between a survival and a dead group: Dogs died of severe anemia, hemorrhage and organ failure in association with pIMHA (i.e., deuteropathic DIC) were defined as a dead group. Dogs surviving at the end point of research (October 2007) or died of the unassociated causes with pIMHA were defined as a survival group.

Statistical analysis: Data were analyzed by use of a statistical software (JMP, version 4, The Statistical Discovery Software, SAS Campus Drive, Cary, NC, U.S.A.). Survival probabilities were constructed using Kaplan-Meier survival estimates. In analyzing prognostic factors, Log-rank tests were used to determine whether each factor and finding at diagnosis influenced on survival. To establish a prognostic scoring system, simultaneously significant predictors of survival were identified from combinations of significant prognostic factors by Cox's proportional hazards modeling. Those factors were scored individually to 0 or 1, and each score was summed. Then, Log-rank tests were performed to compare statistically with survival proportion of each score group. A value of $P<0.05$ was considered to be significant in all statistical tests.

RESULTS

Patient profiles: Seventy-one dogs with pIMHA were entered into this study. Age of the dogs at diagnosis ranged from 0.5 to 14.2 years (mean, 6.2 years; median, 6.4 years). There were 33 males including 9 castrated males and 38 females including 11 spayed females. There was no significant difference in prevalence of pIMHA between male and female dogs. Twenty-three canine breeds were represented; Shih Tzus (n=17, 24%), American Cocker Spaniels (n=7, 10%), Welsh Corgis (n=6, 8%), Miniature Duchshunds (n=6, 8%), Golden Retrievers (n=4, 6%), Miniature Schnauzers (n=3, 4%) and Mixed-breed dogs (n=8, 11%) were commonly observed. Other breeds were represented by one to two dogs. Moreover, Shih Tzus, American Cocker Spaniels and Welsh Corgis had significantly higher incidence of pIMHA than the other breeds in all dogs admitted to UT-VMC for the same period.

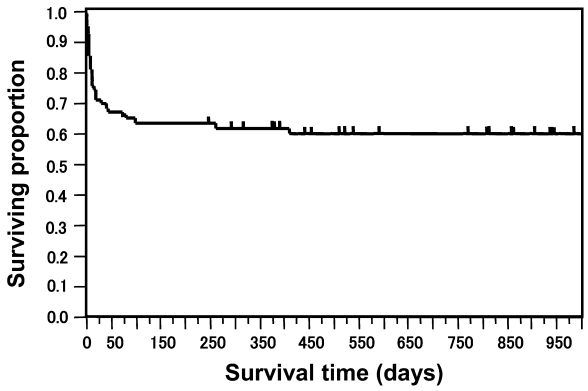


Fig. 1. The Kaplan-Meier survival curve for 71 dogs with pIMHA. Forty-three dogs (43/71, 61%) are contained in a survival group. Twenty-eight dogs (28/71, 39%) are contained in a dead group. Censored dogs are indicated by ticked marks.

Overall outcome: The survival group contained 43 dogs (43/71, 61%) and the dead group contained 28 dogs (28/71, 39%). Overall mortality rate in the population studied here was 39% (28/71) and most of the dead dogs (24/28, 86%) were deceased within three months from diagnosis (0 to 82 days; mean, 16.5 days; median, 9.0 days) due to severe progressive anemia, hemorrhage and organ failure in association with pIMHA (Fig. 1).

Prognostic factors: Twelve factors significantly influenced on survival time after immunosuppressive treatment in dogs with pIMHA. The mean values of body weight (12 kg) and PCV (20%) in the present study classified all dogs into two groups of a poor prognostic group and a good prognostic group, and the cut-off values of PLT, TP, BUN, Alb, Na, PT and FDP were referred to each reference range.

Male, body weight over 12 kg, seasonal incidence among the warm season from April to September, PCV<20%, PLT<200 × $10^3/\mu l$, TP<6 g/d*l*, BUN>25 mg/d*l*, Alb<2.6 g/d*l*, T-Bil>2 mg/d*l*, Na<140 mmol/*l*, PT>9 sec and FDP>5 μg/m*l* on day 1 gave dogs with pIMHA significant poor prognoses, respectively (Table 1). No statistical differences were observed between the other factors and prognoses of dogs with pIMHA in this study.

Table 1. Factors significantly influenced survival time of dogs with pIMHA

Factors	Risk factor (upside)	Number of dogs	Median survival days	*P*-value
Sex	Male	33	412	<0.04
(n=71)	Female	38	–	
BW	> 12 kg	23	45	<0.02
(n=71)	≤ 12 kg	48	–	
Seasonality	Warm season	39	263	<0.001
(n=71)	Cold season	32	–	
PCV	< 20%	35	263	<0.03
(n=71)	≥ 20%	36	–	
PLT	$< 200 \times 10^3/\mu l$	38	74	<0.007
(n=65)	$\geq 200 \times 10^3/\mu l$	27	–	
TP	< 6 g/d*l*	17	74	<0.006
(n=63)	≥ 6 g/d*l*	46	–	
BUN	> 25 mg/d*l*	24	21	<0.005
(n=65)	≤ 25 mg/d*l*	41	–	
Alb	< 2.6 g/d*l*	19	82	<0.04
(n=50)	≥ 2.6 g/d*l*	31	–	
T-Bil	> 2 mg/d*l*	15	13	<0.0009
(n=59)	≤ 2 mg/d*l*	44	–	
Na	< 140 mmol/*l*	5	18	<0.007
(n=47)	≥ 140 mmol/*l*	42	–	
PT	> 9 sec	7	6	<0.0006
(n=45)	≤ 9 sec	38	–	
FDP	≥ 10 μg/m*l*	8	13	<0.03
(n=40)	< 10 μg/m*l*	32	–	

BW, body weight; Warm season, April to September; Cold season, October to March; PCV, packed corpuscular volume; PLT, platelet count; TP, total plasma protein; BUN, blood urea nitrogen: Alb, albumin; T-Bil, total bilirubin; Na, sodium ion; PT, prothrombin time; FDP, fibrin/fibrinogen degradation products; –, more than half of the cases survived.

A prognostic scoring system: The simultaneously significant prognostic factors were determined from combinations of the twelve prognostic factors by Cox's proportional hazards modeling. In the present study, a combination of sex, seasonality, PCV, PLT and TP could be the most appropriate for prognostication (Table 2). Fifty-eight dogs in which these five factors were estimated on day 1 were scored as described on Table 3. Each score was summed and the dogs were classified into six score groups (score 0 to 5). The significant differences in survival time among individual score groups were observed by Log-rank tests (P<0.0001) (Fig. 2). Dogs with score 0 and 1 had the best prognosis since no dog in these groups died of pIMHA during the research period. Dogs with score 2 and 3 had intermediate prognoses. Dogs with score 4 and 5 had unfavorable prognoses since most of the dogs deceased within one month from initial treatment. Based on these outcomes, a simplified prognostic scoring system was also constructed to clarify the differences in survivals among individual risk groups and to apply more easily in clinics (Fig. 3). In the simplified scoring system, dogs with score 0 and 1 were defined as the low risk group, dogs with score 2 and 3 were as the intermediate

Table 2. The result of Cox's proportional hazards modeling in 58 dogs with pIMHA

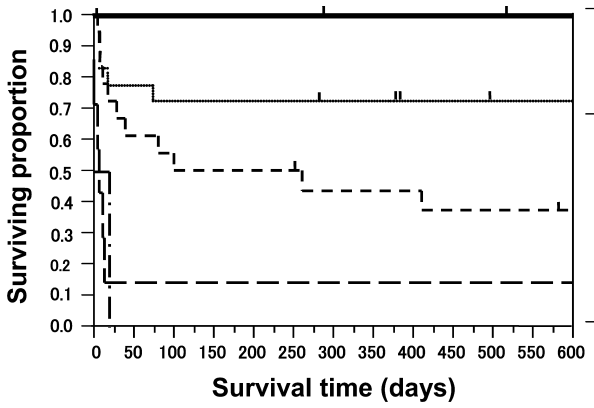
Risk factors	Odds Ratio	*P*-value
Sex; male	1.59	<0.03
Seasonality; warm season	1.68	<0.03
PCV; <20%	1.56	<0.04
PLT; $<200 \times 10^3/\mu l$	1.63	<0.04
TP; <6 g/d*l*	1.78	<0.01

Warm season, April to September; PCV, packed corpuscular volume; PLT, platelet count; TP, total plasma protein.

Table 3. The scoring system constructed in the present study

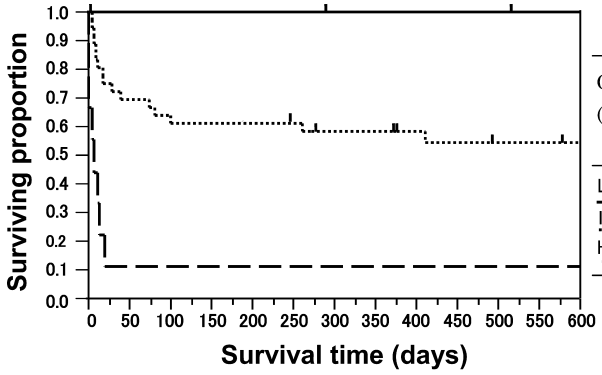
Prognostic factors	Score; 1	Score; 0
Sex	Male	Female
Seasonality	Warm season	Cold season
PCV	<20%	≥20%
PLT	$<200 \times 10^3/\mu l$	$\geq 200 \times 10^3/\mu l$
TP	<6 g/d*l*	≥6 g/d*l*

Warm season, April to September; Cold season, October to March; PCV, packed corpuscular volume; PLT, platelet count; TP, total plasma protein.



Score	Number of dogs	Median survival days
0	3	-
1	9	-
2	18	-
3	19	263
4	7	7
5	2	21

Fig. 2. Survival stratified by the prognostic score at diagnosis. Fifty-eight dogs are included in the prognostic scoring system. The differences in survival among the six groups are statistically significant (Log-rank tests, $P<0.0001$). Censored dogs are indicated by ticked marks. –, more than half of the cases survived.



Group (prognostic scores)	Number of dogs	Median survival days
Low risk (0-1)	12	-
Intermediate risk (2-3)	37	-
High risk (4-5)	9	7

Fig. 3. Survival stratified by the simplified prognostic score at diagnosis. Fifty-eight dogs are included in the simplified prognostic scoring system. In the system, dogs with score 0 and 1 are defined as the low risk group, dogs with score 2 and 3 are defined as the intermediate risk group, and dogs with score 4 and 5 are defined as the high risk group. The differences in survival among the three groups are statistically significant (Log-rank tests, $P<0.0001$). Censored dogs are indicated by ticked marks. –, more than half of the cases survived.

risk group, and dogs with score 4 and 5 were as the high risk group. The significant differences in survival time among the risk groups were also observed (Log-rank tests, $P<0.0001$).

DISCUSSION

The present study suggested that several factors could be useful in prognostication for canine pIMHA. As prognostic factors, sex, body weight, seasonality, PCV, PLT, TP, BUN, Alb, T-Bil, Na, PT and FDP before immunosuppressive therapy significantly affected outcome. Of these factors, especially, sex, body weight, seasonality, BUN, Na and FDP had not been reported as prognostic factors in dogs with pIMHA previously. Although some studies reported female dogs might have predisposition to pIMHA [3, 7, 18, 20, 23], there was no previous evidence reported that the sex distinction influenced on survival time in dogs with pIMHA. In an experimental study, the greater sensitivity of male rabbit platelets to *in vivo* collagen induced aggregation and the associated increase in thromboxane B_2 generation might influence on the greater thrombotic tendency of males [11]. Taking into consideration that most causes of death in this study were suspected to be severe hemorrhage and organ failure due to thromboembolism (DIC and pulmonary thromboembolism), the similar mechanism might have an effect inducing DIC or thromboembolism following pIMHA on male dogs, either intact or castrated.

The reasons why dogs with over 12 kg of body weight had poorer prognoses might be associated with high prevalence of thromboembolism in large-sized breed dogs such as Golden Retrievers [8]. Moreover, such large-sized breed dogs might be more frequently affected by high mercury-induced conditions due to relatively small surface area per body weight. It promoted dehydration and hyperpnea, and thus worsened hypoxemia resulted from anemia. The latter

mechanism could be applied to the result that onset during warm season gave dogs with pIMHA poorer prognoses significantly.

Increased BUN could be caused by renal or prerenal azotemia, increased catabolism of body tissues, or increased protein digestion [22]. The renal azotemia might not be assumed since Cre was not elevated in most cases. It was assumed that gastrointestinal bleeding which resulted in increased protein digestion could be frequently caused by blood coagulation disorder such as DIC following pIMHA. Therefore, dogs presented increased BUN could have a higher risk of death from pIMHA. Loss of Na promoted by sweat and hemorrhage due to blood coagulation disorder could lead to decreased circulating blood volume. This mechanism could also worsen hypoxemia which resulted from anemia.

Increased FDP concentration is one of diagnostic criteria of DIC as others are thrombocytopenia and prolonged PT or APTT [3]. Thrombocytopenia and prolonged PT or APTT have already reported as prognostic factors in dogs with pIMHA [3, 26]. PLT and PT also significantly influenced on survival time in the present study. One study reported 61% of the dogs with DIC had increased amounts of FDP [5]. Increased FDP concentration could also be a predictor of DIC.

Severe anemia (PCV<20%) before immunosuppressive treatments might reflect the severity of hemolysis due to progressive pIMHA [12] or hemorrhage resulted from blood coagulation disorders. Because of these causes, dogs presenting severe anemia at diagnosis would have poorer prognoses. Hypoalbminemia might be caused by impaired hepatic function which led to decreased production of Alb [3, 22]. Hypoproteinemia could occur following hypoalbuminemia or protein loss due to hemorrhage resulted from hypercoagulable states [3]. Dogs with high serum T-Bil concentrations on day 1 showed significantly higher mortality rate. This has been reported as a common finding in multiple literatures [3, 12, 26]. Acute hemolysis, hypoxic damages in the liver or thromboembolism leading to impaired hepatic biliary excretion might be responsible for hyperbilirubinemia [12].

Previous studies reported that female dogs, especially female spayed dogs, presented pIMHA more frequently than males [3, 7, 18, 20, 23]. Moreover, Cocker Spaniels, Doberman Pinschers, English Springer Spaniels, Irish Setters, Miniature Poodles, and Old English Sheepdogs had higher prevalence of pIMHA than other breeds [18]. However, the present study revealed no significant difference in prevalence of pIMHA between male and female dogs, and the highest prevalence in Shih Tzus. These results might be caused by geographical differences between this study and the previous studies.

Overall mortality rate in the population studied here was 39%, which could be relatively low rate compared with that of other previous reports (total mortality rate, 52%) [2, 3, 7, 12, 23, 25]. This result might reflect the achievement of proper treatment with immunosuppressive, anticoagulant and supportive therapies although all of the cases were referred to UT-VMC by private veterinary hospitals and many cases showed severe symptoms and anemia. In the present cases, cyclophosphamide was not used as an immunosuppressive agent because previous studies suggested that it exerted no beneficial effect, and further, increase of mortality [6, 16, 23]. Intravenous human immunoglobulin was administered as a rescue treatment for the cases with severe hemolysis [6, 10, 24]. Ultralow-dose aspirin was proactively administered as an anticoagulant treatment based on the previous study [26]. Above progresses of the treatment of pIMHA might achieve relatively low mortality. Since most of the dead dogs (24/28, 86%) were deceased within three months from diagnosis (0 to 82 days; mean, 16.5 days; median, 9.0 days) due to severe anemia, hemorrhage and organ failure in association with pIMHA in this study, initial treatment for pIMHA in dogs should be promptly and properly started as possible to recover anemia and hypercoagulable states.

To establish a prognostic scoring system, combined factors of sex, seasonal incidence, PCV, PLT and TP could be the most appropriate for prognostication. The combination of the five factors could predict prognoses most synthetically and accurately in dogs with pIMHA, and the factors could be evaluated routinely in clinical fields. The present study is the first report proposed the prognostic scoring system in dogs with pIMHA. The present scoring system can be concise in clinics and useful in informed consent for owners. The prospective study to verify the utilities of the system and clinical application are expected.

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