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Review

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Systematic Review of Prognostic Factors for Mortality in Dogs with Immune-mediated Hemolytic Anemia

J.W. Swann and B.J. Skelly

Background: Treatment of dogs with primary immune-mediated hemolytic anemia (IMHA) is difficult and frequently unrewarding. Prognostic factors have been evaluated in a number of previous studies, and identification of such factors would be beneficial to enable selection of appropriate therapeutic regimens and supportive care.

Objectives: The aim of the current study was to undertake a critical appraisal of the risk of bias in evidence relating to prognostic indicators for mortality in dogs with IMHA.

Animals: Three hundred and eighty client-owned dogs with spontaneous primary idiopathic IMHA reported in 6 previous studies.

Methods: A systematic review was conducted to evaluate evidence relating to prognostic factors for mortality in dogs with primary IMHA. Search tools were employed to identify articles and a validated appraisal tool was used to assess the quality of individual studies by considering inclusion and exclusion criteria, measurement of prognostic, outcome and confounding variables, and statistical methods.

Results: Few studies evaluated prognostic indicators for IMHA in dogs, and all of these suffered from methodologic flaws in at least 1 major area. Fifteen different variables were identified as prognostic indicators, with 2 variables identified by >1 study.

Conclusions and Clinical Importance: There are few pieces of high-quality evidence available to enable estimation of prognosis for dogs presenting with primary IMHA.

Key words: AIHA; IMHA; Prognosis; Systematic review.

Primary immune-mediated hemolytic anemia (IMHA) is the result of a spontaneous autoimmune response directed against antigens expressed on the surface of erythrocytes. Production of autoreactive antibodies is the defining event in this type 2 autoimmune response. Antibodies may facilitate direct intravascular lysis of red blood cells or phagocytosis and extravascular destruction by cells of the monocyte-phagocyte system in the liver and spleen. Immune-mediated hemolytic anemia is reported to be the most common immune-mediated disease of dogs, and the majority of cases are idiopathic.[1]

Management of IMHA is challenging, and affected animals frequently require blood transfusions and other forms of advanced supportive care when they are presented acutely.[2] Numerous immunosuppressive and antithrombotic medications have been studied for treatment of the disease, but there is no consensus regarding the optimal regimen that should be employed.[3]

Abbreviations:

IL-18	interleukin 18
IMHA	immune-mediated hemolytic anemia
MCP-1	macrophage chemoattractant protein 1
QUIPS	quality in prognosis studies

Several studies have sought to identify simple prognostic factors that can be measured at the point of presentation to guide clinicians in the provision of appropriate care. Because there appears to be a wide spectrum of disease severity in dogs with IMHA, establishment of valid prognostic indicators may enable concentration of health resources on those patients that appear to be severely affected, while avoiding unnecessary adverse effects in patients that are mildly affected.[4,5] Recognition of heterogeneity in the population of dogs with primary IMHA using similar prognostic indicators also is likely to be important in future studies assessing the efficacy of therapeutic interventions.[2]

To facilitate systematic evaluation of the risk of bias in studies of prognostic factors, Hayden et al[6] developed the Quality in Prognosis Studies (QUIPS) tool, which contains 30 questions arranged into 6 domains to provide a comprehensive assessment of the quality of a study. As validation, this tool has been used in more than 80 reviews of prognostic studies in various areas of human medicine, and a recent review of studies that used the QUIPS tool demonstrated good agreement among reviewers and showed that reviewers found the tool simple to use.[7]

The aim of the current study was to systematically evaluate the current evidence relating to identification of prognostic factors for mortality in dogs with primary IMHA by using the QUIPS tool.

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Materials and Method

Search Strategy

The online databases of PubMed, ISI Web of Science, and CAB Abstracts were searched from 1980 to October 2013 using the following search terms: (dog OR dogs OR canis OR canine OR canidae) AND (IMHA OR AIHA OR hemolytic anemia OR hemolysis OR immune-mediated hemolytic anemia). Variants of the search terms also were used to account for possible differences in spelling of the major keywords, and all searches were conducted on 31st October 2013. The records of articles identified were transferred to a bibliographic software package,[a] and duplicates were removed. The titles and abstracts of articles were scanned by the primary author to identify those of relevance, and the full text of these studies was obtained. Articles that were not published in English were translated to enable assessment. Selection of articles was not performed in a blinded manner because the primary author is familiar with literature pertaining to IMHA in dogs.

Inclusion and Exclusion Criteria

For inclusion in the review, studies were required to fulfill the following 4 inclusion criteria:

- Study presented primary data from client-owned dogs with spontaneous disease
- Study was published as a complete report in a peer-reviewed journal
- Study evaluated prognostic indicators for mortality using data collected from dogs with primary IMHA
- Study used multivariable analysis to assess potential prognostic factors and exclude confounding factors

Where data from the same group of animals were used to produce >1 article, only the study reporting data from the largest number of animals was included. Studies also were excluded if the investigation of prognostic factors was not related to outcome measures of mortality.

Critical Appraisal

Studies were evaluated independently by each of the authors using the QUIPS tool developed by Hayden et al.[6,7] This tool consists of 30 questions divided into 6 key domains, and several of the questions were modified by the authors for this study. The complete tool used for assessment is shown in Table S1. The authors completed the assessment for each study and assigned a grade of low, moderate or high risk of bias for each domain. Where differences were identified between the authors of this paper, these were resolved by consensus.

A κ score was calculated to determine the degree of interobserver variability for initial scoring[8] by comparing the number of domains scored as low or moderate risk of bias in each study. A commercially available software package was used to conduct this analysis.[b]

Reporting of Results

Variation in specific outcome measures and definitions of prognostic factors among studies precluded quantitative synthesis of results. Relevant information from each study was abstracted into tabular format and major conclusions were described. The principal summary measure was the final multivariable model with the hazard or odds ratio and confidence intervals for each variable. Additional information collected included numbers of animals included in the multivariable analysis, years of data collection, demographic characteristics of the study population, statistical methods employed, and definition of the outcome measure(s) relating to mortality. The review was presented according to the PRISMA template[9] for reporting of systematic reviews.

Results

Search techniques identified 1,640 records, of which 6 (0.4%) were selected for inclusion in the review.[10–16] Reasons for exclusion of the remaining studies are shown in Figure 1.

Study Characteristics

The articles included in the study used data from 380 dogs with primary IMHA to produce models investigating prognostic factors for mortality, with a median sample size of 56 (range, 20–222). The studies reported data from dogs that were presented for treatment of IMHA between 1988 and 2010. Two studies collected data prospectively,[15,16] whereas 4 studies were retrospective cohort studies. Study populations were based in the Netherlands (n = 2), United Kingdom (2), United States (1), and Japan (1).

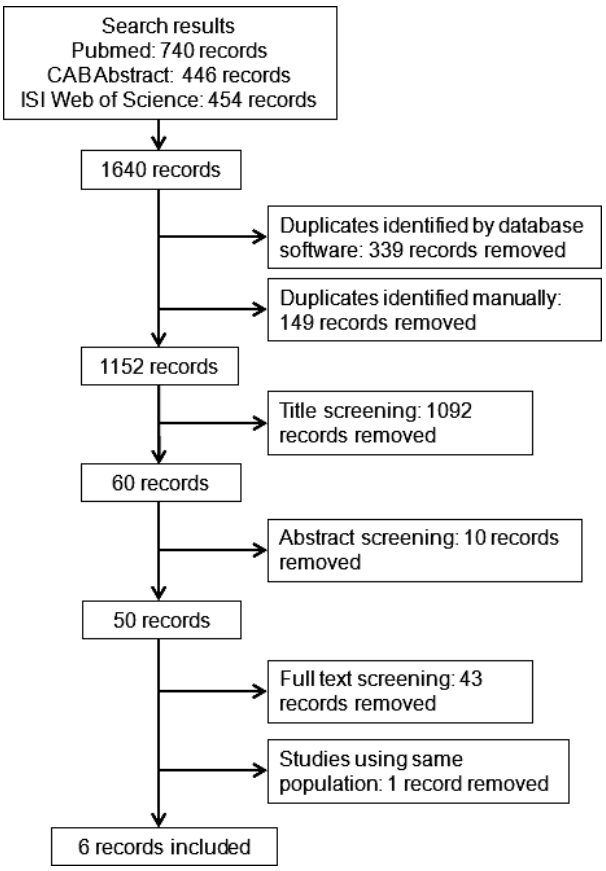


Fig 1. Summary of recruitment process for studies.

The characteristics of the study populations described in each article are shown in Table 1. Two studies provided incomplete demographic data relating to the animals that were recruited.[14,15]

Assessment of Quality

The results of systematic review of evidence quality are shown in Table 2. None of the studies had a high risk of bias in domains assessing measurement of prognostic factors or outcome variables, but high risks of bias were observed in all other domains for at least 1 study. Two studies had a low risk of bias in ≥1 domains,[10,15] whereas each of the others had a high risk of bias in at least 1 domain. The κ score for interobserver agreement was 0.7 (standard error, 0.3).

Prognostic Factors

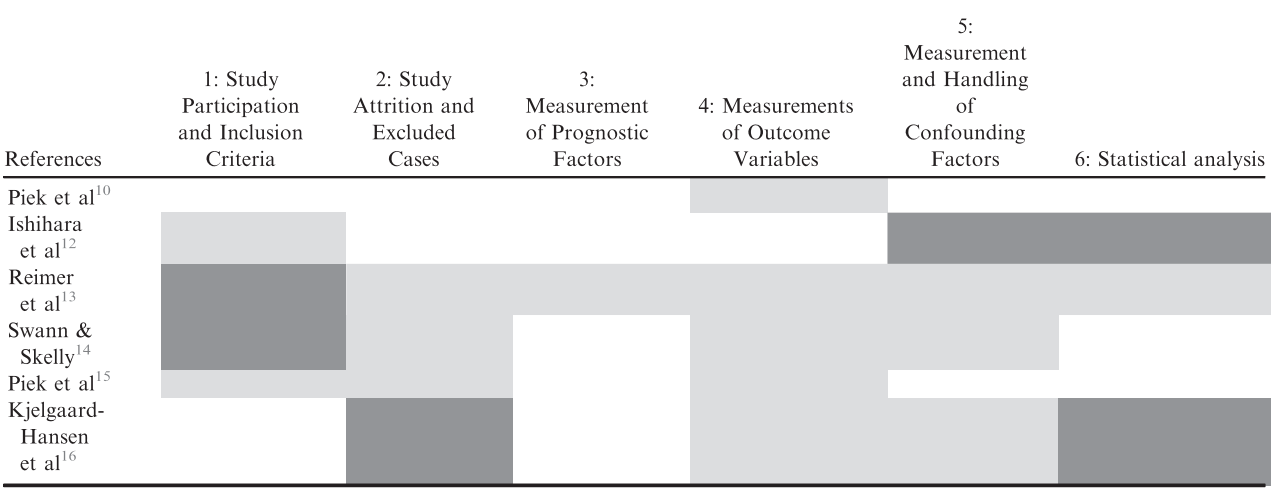
A summary of the major findings of each study is shown in Table 3. Fifteen different prognostic factors were identified, with 2 factors (serum bilirubin and urea or blood urea nitrogen concentrations) each identified by 2 different studies. Cox proportional hazards analysis was employed in 5 studies using actual survival times, and multivariable logistic regression with an endpoint of mortality at 30 days after presentation was used in the remaining study.

Table 1. Demographic data abstracted from articles included in review.

References	N		Time Period	Setting	Country	Age (years)	Sex	Breed
	With Primary IMHA	Included in Multivariable Analysis (%)						
Piek et al[10]	222	164 (73.9)	Jan 1994–Dec 2000 and Jan 2002–Dec 2005	Tertiary referral hospital	Netherlands	Separate data presented for 2 treatment groups: 1: (n = 149) median: 5.7, range: 0.3–13.9; 2: (n = 73) median: 4.6, range: 0.4–12.7	68 ME 20 MN 73 FE 61 FN	43 breeds, Most common: Cross-breed (n = 26), cocker spaniel (11), old English sheepdog (9)
Ishihara et al[12]	71	*	Apr 1997–Mar 2006	Tertiary referral hospital	Japan	Mean 6.2, median 6.4, range: 0.5–14.2	24 ME 9 MN 27 FE 11 FN	23 breeds. Most common: shih tzu (n = 17), cocker spaniel (7), Welsh corgi (6)
Reimer et al[13]	70	*	Jan 1988–Feb 1996	Tertiary referral hospital	United States	Median 6, range: 1–13	49 M 21 F	Most common: cross-breed (n = 12), cocker spaniel (11), poodle (7)
Swann & Skelly[14]	42	34 (81.0)	2002–2010	Tertiary referral hospital	United Kingdom	*	*	23 breeds. Most common: cocker spaniel (n = 8), Labrador retriever (6)
Piek et al[15]	24	21 (87.5)	Sep 2007–Oct 2008	Tertiary referral hospital	Netherlands	*	9 ME 2 MN 3 FE 10 FN	*
Kjelgaard-Hansen et al[16]	20	*	Oct 2008–Oct 2009	Tertiary referral hospital	United Kingdom	Mean 7.2, SD ± 2.9	2 ME 1 MN 2 FE 15 FN	11 breeds. Most common: English springer spaniel (n = 6), cocker spaniel (3), Labrador retriever (2)

*Not stated. M(E/N): male (entire/neutered), F(E/N): female (entire/neutered).

Table 2. Results of quality assessment of studies included in review.



Dark gray: high risk of bias; light gray: moderate risk of bias; white: low risk of bias.

Table 3. Prognostic factors for mortality identified by studies included in review.

References	Study Design	Outcome Measure	Statistical Method	Prognostic Factors Identified		
				Factor	OR/HR	95% CI
Piek et al[10]	Retrospective cohort	Survival time (death because of IMHA)	Cox proportional hazards analysis	Serum [urea] (>56 mg/dL)	2.56	1.729–3.789
				Icterus	2.94	1.60–5.42
				Spherocytosis	0.38	0.20–0.72
Ishihara et al[12]	Retrospective cohort	Survival time (death because of IMHA)	Cox proportional hazards analysis	Sex (male)	1.59	*
				Season (warm)	1.68	*
				PCV (<20%)	1.56	*
				Platelet count (<200,000/μL)	1.63	*
				Total protein (<6 g/dL)	1.78	*
Reimer et al[13]	Retrospective cohort	Survival time (all-cause mortality)	Cox proportional hazards analysis	Serum [bilirubin]	*	*
				Serum ALP activity	*	*
Swann & Skelly[14]	Retrospective cohort	Survival time (all-cause mortality)	Cox proportional hazards analysis	Serum [urea]	1.211	1.073–1.367
				Serum [bilirubin]	1.014	1.003–1.024
Piek et al[15]	Prospective cohort	Survival time (death because of IMHA)	Cox proportional hazards analysis	Serum [creatinine] (>0.23 mg/dL)	1.15	1.00–1.35
				Monocyte count (>100/μL)	2.32	1.34–6.05
				APTT	1.12	1.03–1.26
Kjelgaard-Hansen[16]	Prospective cohort	Mortality at 30 days (all-cause mortality)	Multivariable logistic regression	IL-18	*	*
				MCP-1	*	*

OR, odds ratio; HR, hazard ratio; CI, confidence interval; APTT, activated partial thromboplastin time; PCV, packed cell volume; ALP, alkaline phosphatase; IL-18, interleukin 18; MCP-1, monocyte chemoattractant protein 1.
*Not stated.

Discussion

The aim of this review was to assess the risk of bias in studies investigating prognostic factors for mortality in dogs with primary IMHA. A small number of studies have evaluated prognostic factors for mortality in dogs with primary IMHA using appropriate methods to exclude potential confounding factors, and a high or moderate risk of bias was identified in at least 1 area of each of these studies

using a validated quality assessment tool. Several different prognostic factors were identified in different study populations, with 2 factors identified by >1 investigation.

Of the large numbers of studies evaluated for inclusion in this review, only 6 ultimately were selected. The small number of studies available for review reflects a paucity of published evidence relating to the natural history of canine IMHA in particular and diseases of dogs in general.[17]

In common with published scientific literature relating to the treatment of IMHA,[3] studies evaluating prognostic factors for mortality were subject to high or moderate risks of bias in at least 1 domain. This apparent lack of quality did not relate to basic study design, however, because retrospective cohort studies represent an effective way to evaluate prognostic factors.[18] Instead, the risk of bias related chiefly to failure to report important characteristics of the study population, failure to report methods used to exclude potential confounding factors, and incomplete description of prognostic models.

Study Population

Four studies[12–15] reported inclusion criteria that were not considered reliable for diagnosis of primary IMHA, increasing the risk of bias associated with case selection. In particular, 3 studies[12,13,15] failed to report procedures that were used to exclude underlying causes of IMHA in dogs presenting with evidence of hemolytic anemia, such as thoracic and abdominal imaging and appropriate tests for infectious agents. In 2 instances,[13,14] the diagnosis of IMHA could have been based on clinical evidence of hemolysis, whereas we consider tests that identify the presence of antibodies specific for erythrocyte antigens (eg, saline agglutination test, observation of spherocytes on a blood smear, Coombs' test) essential for a confident diagnosis of the disease.[1,2]

Excluded Cases

Only 1 study[10] reported details of the group of animals that were not included and the reasons for exclusion. Evaluation of this group of animals is important to ensure that cases with certain characteristics that may have prognostic relevance, such as more severe anemia, have not been excluded from the analysis. Information regarding cases that have not been included rarely is presented in retrospective studies in the veterinary literature, but this information also would be helpful to determine whether studies have reported data from representative samples of animals.

Prognostic Factors

Prognostic factors generally were well-described and appropriate for dogs with IMHA, but these differed widely among studies, and methods of measurement were not always stated explicitly. Most variables considered were laboratory parameters that can be measured consistently across multiple centers, although 1 study included the presence of icterus,[10] which is a subjective judgment that may differ among individuals. The majority of studies reported prognostic factors that are widely measured in general practice, but 1 study evaluated the cytokines IL-18 and MCP-1,[16] which are unlikely to be measured outside of a research environment.

One study[12] included season of presentation as a prognostic factor. Previous studies have reported conflicting results regarding the seasonal incidence of IMHA, with some reporting a higher incidence in warmer months[19,20] and others showing no association.[14,21–23] Suggested reasons for the apparent association include the effect of environmental temperature on immune responses, greater risk of dehydration or respiratory distress in warmer months, and the potential effect of an undetected infectious agent, which raises concern that some of the cases reported by Ishihara and others[12] may have suffered from IMHA secondary to an infectious disease process.

Hazard and odds ratios were of small magnitude for most of the factors identified, and the clinical relevance of these factors therefore is questionable. Interestingly, ratios derived from studies that evaluated the same variables as single prognostic factors[19,24,25] often were of much greater magnitude, suggesting that confounding factors may have a considerable effect on variables such as serum bilirubin concentration. Because of the small number of cases included in many of the studies, confidence intervals also were wide, and the true clinical validity of each prognostic factor therefore is difficult to estimate.

Confounding Factors

Articles were excluded if they did not evaluate potential confounding factors in the context of a multivariable model, either by Cox proportional hazards analysis or multivariable logistic regression. Although many more studies have evaluated prognostic factors for mortality in IMHA, these typically only considered individual factors without accounting for the effects of multiple variables. These studies were considered unreliable because dogs with IMHA often are systemically ill and showing evidence of dysfunction in multiple organs, and variations in individual biochemical and hematologic variables may be spuriously associated with survival times.

The studies included in this review mainly considered hematologic, biochemical, and clinical variables as potential prognostic factors, but the exact factors evaluated in each model were not stated in a number of studies. One study selected potential confounding factors on the basis of previous evidence, but important factors, such as severity of anemia, were not included.[16] Several of the studies evaluated the effect of different treatment protocols on survival, but did not consider this variable in subsequent prognostic models.[12–16]

These observations highlight the importance of rational selection of variables for inclusion in multivariable models. Use of a small number may omit factors that have an important modifying or confounding effect, but inclusion of too many factors may increase the risk of spurious associations or correlations among similar variables, or the risk of overfitting multivariable models.[18]

Outcome Measures

As with studies of therapeutic regimens in dogs with IMHA,[3] outcome measures varied widely, and the duration of follow-up periods was not stated in any of the reports. Furthermore, only 3 studies[10,12,15] used death caused by IMHA for development of the prognostic model and the remainder used all-cause mortality. Conclusions drawn using each of these outcome measures will differ markedly in their clinical relevance. Losses to follow-up were described in 2 studies,[13,14] but the impact of these cases on model building was not considered in any of the investigations.

Statistical Methods

The purpose of this review was not to provide a detailed critique of statistical techniques employed when building prognostic models, because this process has been described elsewhere.[4,26] Nevertheless, there was considerable variation in the strategies used to construct these models, and numerous deficiencies were observed. Models frequently were constructed with large numbers of prognostic factors evaluated in samples that had low event rates. There are no concrete rules for building models, but it is often stated that 10 events should be available for every prognostic factor that is included so that the final model is not distorted by spurious associations.[27] Three studies did not report data necessary to evaluate the importance of an association, such as the hazard or odds ratio and its confidence interval.[12,13,16]

Although Cox proportional hazards analysis is an effective strategy for identification of prognostic factors, it relies on availability of complete survival data for a large proportion of the cases included, and final results may not be representative if largely based on right-censored data. Some of the studies reported here appeared to rely on automated model building algorithms provided by statistical software programs, whereas optimal strategies are likely to take account of many more factors, such as *a priori* importance of variables and changes in model parameters when single variables are added or removed. Only 2 of the studies reported the use of diagnostic tests to evaluate the adequacy-of-fit and predictive capabilities of the model produced,[10,15] and none subjected their model to the gold standard test: validation in an independent sample of dogs with IMHA.[28,29]

Limitations

This review included only studies that used multivariable models to evaluate prognostic factors for IMHA, which led to the exclusion of many studies that investigated single factors. This design may have excluded a large amount of information regarding prognostic factors for IMHA, but we considered this to be an important criterion for selection of studies with valid results. The review also excluded reports of studies that were not published in peer-reviewed journals because we believed abstracts did not provide sufficient methodologic detail to evaluate studies as compared to complete published reports. Despite attempts to appraise studies in an objective and consistent manner, use of the QUIPS tool involves subjective judgement in assigning a score for each of the 6 domains. Because specific criteria were provided for most of the 30 questions forming the appraisal tool, we consider this subjective component to be minimal, and calculation of interobserver κ value showed good consistency between the 2 evaluators.

Practical Applications

Identification of prognostic factors was used to produce a clinical score in 1 instance,[12] but this model included sex and season of presentation as factors, and we are unsure why these variables should be important prognostic factors. The approach used in this study, however, has the potential to be practically useful by allowing the clinician to calculate a simple score on presentation. Similar scores, including the survival prediction index (SPI) and acute patient physiologic and laboratory evaluation (APPLE) scores, have been developed in studies of animals admitted to veterinary intensive care units to guide clinical interventions and provide a global indicator of illness severity in research studies.[30,31]

Of the other studies considered here, 3 identified icterus or hyperbilirubinemia as a significant prognostic factor, and Piek et al[11] reported a considerable effect size for development of icterus. Hyperbilirubinemia may represent a simple, widely available indicator of prognosis if it performs well alongside more complete models, but previous work also suggests that there could be considerable overlap in serum bilirubin concentrations between dogs that died while hospitalized and those that were discharged.[14]

Conclusion

Measurement and evaluation of prognostic factors has the potential to improve the clinical management of cases of IMHA in dogs and to allow resources to be targeted appropriately. Although several prognostic factors were identified in the studies considered in this review, effect sizes generally were small when potential confounding factors were taken into consideration, and none of the prognostic models has undergone external validation. Variable methodology and reporting further

emphasize the need for standardized definitions and collaborative research in this field in the future.

Footnotes

[a] EndNote X5 (Thomson Reuters Philadelphia, PA)
[b] IBM Corp. Released 2011, IBM SPSS Statistics for Windows, Version 20.0.; IBM Corp, Armonk, NY

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Conflict of Interest Declaration: The authors wrote one of the papers reviewed in this article.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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Supporting Information

Additional Supporting Information may be found online in Supporting Information:

Table S1. Questions used to assess each study within 6 major domains. Modified from Hayden et al 2006.