CONSENSUS STATEMENT

Journal of Veterinary Internal Medicine

ACVIM consensus statement on the treatment of immune-mediated hemolytic anemia in dogs

James W. Swann[1] | Oliver A. Garden[2] | Claire L. Fellman[3] |
Barbara Glanemann[4] | Robert Goggs[5] | Dana N. LeVine[6] |
Andrew J. Mackin[7] | Nathaniel T. Whitley[8]

[1]Kennedy Institute of Rheumatology, University of Oxford, Oxford, United Kingdom

[2]School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

[3]Cummings School of Veterinary Medicine, Tufts University, North Grafton, Massachusetts

[4]Royal Veterinary College, University of London, London, United Kingdom

[5]College of Veterinary Medicine, Cornell University, Ithaca, New York

[6]College of Veterinary Medicine, Iowa State University, Ames, Iowa

[7]College of Veterinary Medicine, Mississippi State University, Mississippi State, Mississippi

[8]Davies Veterinary Specialists, Manor Farm Business Park, Huntingdon, United Kingdom

Correspondence
Oliver A. Garden, Department of Clinical Sciences and Advanced Medicine, Matthew J Ryan Veterinary Hospital, School of Veterinary Medicine, University of Pennsylvania, 3900 Delancey Street, Philadelphia, PA 19104.
Email: ogarden@upenn.edu

Immune-mediated hemolytic anemia (IMHA) causes severe anemia in dogs and is associated with considerable morbidity and mortality. Treatment with various immunosuppressive and antithrombotic drugs has been described anecdotally and in previous studies, but little consensus exists among veterinarians as to the optimal regimen to employ and maintain after diagnosis of the disease. To address this inconsistency and provide evidence-based guidelines for treatment of IMHA in dogs, we identified and extracted data from studies published in the veterinary literature. We developed a novel tool for evaluation of evidence quality, using it to assess study design, diagnostic criteria, explanation of treatment regimens, and validity of statistical methods. In combination with our clinical experience and comparable guidelines for humans afflicted with autoimmune hemolytic anemia, we used the conclusions of this process to make a set of clinical recommendations regarding treatment of IMHA in dogs, which we refined subsequently by conducting several iterations of Delphi review. Additionally, we considered emerging treatments for IMHA in dogs and highlighted areas deserving of future research. Comments were solicited from several professional bodies to maximize clinical applicability before the recommendations were submitted for publication. The resulting document is intended to provide clinical guidelines for management of IMHA in dogs. These guidelines should be implemented pragmatically, with consideration of animal, owner, and veterinary factors that may vary among cases.

1 | INTRODUCTION

Despite recognition as a disease of dogs in 1957, immune-mediated hemolytic anemia (IMHA) continues to claim the lives of up to half of affected dogs.[1-3] It is a prominent problem in a number of popular breeds, including Cocker and Springer Spaniels, Old English Sheepdogs, Bichon Frises, Bearded and Rough-coated Collies, Poodles, and Flat-coated Retrievers.[4] Treatment relies on nonspecific immune suppression by glucocorticoids to control autoimmune responses targeting red blood cell (RBC) antigens.[3,5] Immunosuppressive treatment is associated with a number of adverse effects that contribute to patient morbidity, both by virtue of the drugs used and the prolonged duration of treatment. In an endeavor to augment immunosuppression, and allow more rapid initial disease control and tapering of the glucocorticoid, a number of second-line drugs have made their way into routine treatment of dogs with IMHA, including azathioprine, cyclosporine, and mycophenolate mofetil.[2,5-7] Little consensus exists on either the specific drug or combination of drugs to use, or their dosages in individual patients.

A second component of treatment of IMHA in dogs is inhibition of thrombosis, in particular pulmonary thromboembolism, which is a prominent cause of morbidity and mortality.[8-10] Various thromboprophylactic regimens have been recommended, but overall consensus on the optimal drug, or drugs, to administer in these cases has remained elusive.[7,11] Furthermore, a number of supportive treatment strategies, such as blood transfusion, gastroprotectants, and antimicrobial drugs, are administered on a case-by-case basis, often without clear consensus on when and how they should be used. Emerging modalities offer promise for more targeted, rapid, or durable treatment responses for IMHA in dogs in the future, but there is an immediate need to provide therapeutic guidelines for currently available drugs.

The objective of this Consensus Statement therefore is to present recommendations for the treatment of IMHA in dogs, considering all of the available evidence as well as expert opinion. It focuses on immunosuppressive and thromboprophylactic drugs, but also considers supportive and emerging treatment modalities, during both the initial stabilization of patients and their long-term management. It also provides recommendations for future research in this area.

2 | MATERIALS AND METHODS

An extended methods section is available in Supporting Information S1.

2.1 | Scope of the work

Consensus statements are intended to produce clinical guidelines on contentious topics, particularly when a paucity of published data is available to inform clinical decisions. Such guidelines are produced when a panel of qualified individuals reaches agreement using whatever resources are available to them. In approaching this work, we chose to conduct a systematic review of epidemiological evidence investigating associations between therapeutic interventions and outcome in dogs with IMHA but, where published data were lacking, we also decided to take advantage of clinical experience, guidelines used in human medicine, and experimental studies. Our recommendations are intended to act as guidance for clinicians managing dogs with IMHA and should be implemented with consideration of patient, client, and veterinary factors that may vary among cases.

2.2 | Literature review

We searched 3 scientific databases (MEDLINE/PubMed, ISI Web of Science [Core Collection and BIOSIS Citation Index], and CAB Abstracts) in December 2017 using search strategies shown in Supporting Information S1. We excluded all references published before 1980, but included those published in languages other than English. All references were copied to reference management software (EndNote X8; Clarivate Analytics, Philadelphia).

2.3 | Curation of records

We removed duplicate records and then scanned the remaining abstracts to remove references that did not provide information on IMHA, did not contain primary data, reported data from a species other than the dog, described data from <5 dogs, or were duplicated in a later study. We obtained the full text of the remaining references and excluded those not providing information on therapeutic interventions and at least 1 direct outcome measure for dogs with primary IMHA. A total of 46 papers remained after this process, to which a newly published paper was added in March 2018. Of these 47 references, 7 were translated into English (from German, n = 6; and French, n = 1). The process used to curate references is illustrated in Figure 1.[12]

2.4 | Quality assessment

We designed a novel quality assessment and data extraction tool for those studies investigating the effect of ≥2 therapeutic interventions on outcome in dogs with IMHA; studies describing a single intervention were categorized as "descriptive association only." The quality assessment tool included 4 domains intended to assess the rigor of study design, diagnostic criteria for IMHA, explanation of treatment protocols, and validity of statistical methods. An additional domain related to masking, randomization, and intention-to-treat analysis was included for randomized controlled trials (RCTs), and the fragility index was calculated if sufficient data were available and for studies reporting

Identification

Records identified through database searching (n=3063)

Additional records identified through other sources (n=1)

Screening

Records after duplicates removed (n=1172)

Records screened (n=1172) → Records excluded (n=1013)

Eligibility

Full-text articles assessed for eligibility (n=159) → Full-text articles excluded (n=112)

Included

Studies included in qualitative synthesis (n=47)

FIGURE 1 Flow diagram indicating flow of information used in the systematic review. Diagram produced using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[12]

a significant difference in a binary outcome (ClinCalc Fragility Index Calculator. 2019. https://clincalc.com/Stats/FragilityIndex.aspx. Accessed July 2, 2018).[13] The treatment protocols, major outcome measures, and results of any statistical tests also were recorded for each paper. The tool was tested by all members of the panel using the same 2 papers, from which feedback informed the final version (shown in Supporting Information S2).

The papers for review were assigned randomly to members of the panel, such that each paper was reviewed by 2 different panel members; no panel member reviewed a paper to which they had contributed. Where differences were identified between the 2 reviewers of a paper, these were resolved by consensus before all of the results were pooled into a single spreadsheet (Supporting Information S3). Variation in statistical methods and outcome measures precluded quantitative meta-analysis.

The quality scores for individual questions within each domain were summated to produce scores for this domain for each paper; results of these assessments are shown in Figures 2, 4, 5, and 8, with associated summaries in Tables 1, 3–5.

2.5 | Delphi process

Each panel member drafted initial recommendations, with reference to the results of the quality appraisal; these were assembled to produce a working template for the Consensus Statement. The draft recommendations then were subjected to 4 rounds of Delphi review in their entirety, using an anonymized online questionnaire (Survey Monkey, San Mateo, California). At the conclusion of each round, suggestions were incorporated into the working template by 1 facilitator, and a transcript of all written comments was provided to each panel member. After 3 rounds of review, several outstanding differences were resolved in a meeting of all panel members before the recommendations were presented at the American College of Veterinary Internal Medicine (ACVIM) Forum on June 14, 2018 in Seattle, Washington.

2.6 | Determination of the strength of recommendations

The template outlined in the Grading of Recommendations Assessment, Development and Evaluation (GRADE) guidelines[28,29] (shown in Supporting Information S1) was used to classify the strength of each recommendation as either "weak" or "strong." To achieve this objective, every panel member assessed each recommendation according to 4 domains: balance of expected beneficial and harmful effects, strength of evidence, expected stakeholder values and preferences, and expected cost. Recommendations were considered "strong" if ≥5 panel members reached

this decision; all other recommendations were considered "weak." The decisions of panel members are shown in Supporting Information S1.

All "strong" recommendations were phrased with the conjugated verb "we recommend," whereas "weak" recommendations were phrased with "we suggest."

2.7 | Production of the Consensus Statement

The working document was further edited to incorporate comments generated during oral presentation, before submission to ACVIM for review by all members. The draft Consensus Statement also was submitted to the European College of Veterinary Internal Medicine, American College of Veterinary Emergency and Critical Care, and European College of Veterinary Emergency and Critical Care for solicitation of comments from members. Feedback from these specialist colleges was used by the panel members to modify the Consensus Statement, producing a final version that was submitted to the *Journal of Veterinary Internal Medicine.*

In the text of the Consensus Statement, we make specific recommendations, followed by the strength of recommendation and our rationale. We have applied the principles of evidence-based medicine, but in all cases expert opinion, coupled with inferences from parallel data from human medicine (when available[30]), was an integral part of the process.

3 | RESULTS AND RECOMMENDATIONS

3.1 | Timing of treatment

1. We recommend introducing immunosuppressive interventions after all diagnostic samples have been collected, provided doing so does not unduly delay institution of treatment.

Strength of recommendation: Strong

Rationale

There is limited evidence to suggest that treatment with prednisone for variable periods of time will not affect the result of a direct antiglobulin test,[31] but the effect of other immunosuppressive drugs on this and other tests has not been investigated in dogs. Administration of some drugs, particularly glucocorticoids, may limit the ability to detect underlying diseases, such as lymphoma. Therefore, diagnostic evaluation should be expedited in cases in which underlying cancer or infectious diseases are suspected. Further guidance is available in the Consensus Statement on Diagnosis of Immune-Mediated Hemolytic Anemia in Dogs and Cats.[32]

3.2 | Blood typing and cross-matching

An expanded version of this section, with further commentary and discussion of underlying evidence, is available in Supporting Information Appendix A1.

2. We recommend that clinicians consider the effect of autoagglutination on the results of cross matching and blood typing, and seek guidance from manufacturers of test kits.

Strength of recommendation: Strong

3.3 | Blood transfusion and blood products

3. We recommend administering packed red blood cells (pRBC) when dogs with IMHA display clinical features attributable to decreased tissue oxygen delivery. If pRBC are not available, administration of whole blood is a reasonable alternative.

Strength of recommendation: Strong

Rationale

Review of the literature did not identify any studies reporting an association between the number or volume of pRBC or whole blood transfusions with mortality in dogs with IMHA. The decision to transfuse should depend on factors specific to the individual patient, including severity of clinical signs, blood lactate concentration at rest,[33] normal PCV/hematocrit (Hct) expected in the breed, speed of onset of anemia, and availability of patient monitoring and supportive care. The PCV to trigger transfusion for a sighthound with acute onset disease therefore would be higher than for a Labrador Retriever with insidious onset anemia, and transfusion would be considered earlier for a patient with progressive anemia that cannot be hospitalized for intensive monitoring. In the absence of clinical signs, some (2/8) panel members recommended transfusing dogs with a PCV/Hct <12%, whereas the remainder of the panel did not have a numerical trigger. Dogs with IMHA typically are euvolemic, making pRBC preferable to whole blood because the plasma provides no added benefit, increases the risk of volume overload, and may increase the risk of transfusion reaction.

4. Fresh pRBC, ideally no older than 7-10 days, are recommended for use in dogs with IMHA. If these are not available, older units may be used but may be associated with a greater risk of complications and increased mortality.

Strength of recommendation: Strong

Rationale

Increasing age of pRBC was associated with increased risk of mortality in dogs with hemolysis, of which 90% were reported to be dogs with IMHA.[34] The conclusion of this retrospective study was limited by the absence of information on diagnosis of IMHA and a lack of methodologic detail on statistical analysis. A more recent retrospective study also reported an increased risk of hemolytic transfusion reactions with increasing age of the transfused pRBC.[35]

5. We recommend administering pRBC or whole blood in preference to bovine hemoglobin solutions (BHS).

Strength of recommendation: Strong

Rationale

Administration of products containing intact erythrocytes represents a more physiologic solution for improving oxygen-carrying capacity in dogs for several reasons. First, BHS scavenge nitric oxide, potentially activating platelets and causing vasoconstriction, which increases risk of hypertension.[36] Second, BHS exert a greater colloid osmotic (oncotic) pressure than do RBCs, increasing the risk of intravascular volume expansion and hypertension.[37] Transfused RBCs also are likely to have a longer circulating half-life than BHS, although the half-life of transfused RBCs is difficult to estimate in dogs with active hemolysis.[38]

Furthermore, in 1 study, administration of a BHS (HB-200) was associated with an increased relative risk of mortality.[18] Whether this increased risk was because of the solution itself or confounding factors, such as variation in disease severity between treatment groups, was unclear. Only a single direct comparison of BHS and pRBC has been undertaken in dogs during an RCT comparing administration of the 2 products for stabilization of dogs with babesiosis. No difference in recovery of acid/base and perfusion variables was found, but those dogs receiving pRBC had a faster clinical recovery.[39]

In conclusion, BHS could be administered if pRBC or whole blood are not available. However, BHS currently is unavailable in many countries, including the United States.

6. We do not recommend administering fresh frozen plasma routinely to dogs with IMHA.

Strength of recommendation: Strong

Rationale

Features of disseminated intravascular coagulation (DIC) have been described in up to 45% of dogs with primary IMHA; DIC therefore has been suggested as a risk factor for thromboembolic disease.[8,40–42] Based on guidelines in human medicine, there is little indication for plasma transfusions in nonhemorrhagic DIC patients not requiring invasive procedures.[43] Similarly, as summarized below and in Table 1 and Figure 2, clinical data do not support routine administration of plasma to dogs with IMHA.

In addition to standard treatment (including other blood products, heparin, and immunosuppressive drugs), administration of a single dose of fresh frozen plasma to dogs with IMHA did not decrease mortality or occurrence of thromboembolic events, or produce any improvement in plasma antithrombin concentration.[15] The conclusion of this study is subject to bias because it compared cohorts that were separated in time. A further study reported that the volume of plasma administered to IMHA non-survivors was greater than that administered to survivors at 14 days after diagnosis.[14] The authors concluded that administration of plasma was unlikely to be the cause of death because this association probably was confounded by other factors, including coagulation status of the dogs and prevalence of DIC.

TABLE 1 Summary of studies investigating the use of blood products in dogs with immune-mediated hemolytic anemia (IMHA)

Reference	Study design	Number of dogs with IMHA	Treatment groups	N	Outcomes	Method of statistical comparison	Statistical measure	Confidence interval	P value
Griebsch et al (2010)[14]	Retrospective case control	37			Plasma volume administered	Mann-Whitney U test for plasma volume	a	-	≤.001
			1: Survived to 14 days after diagnosis	4	Median 9 mL/kg				
			2: Died within 14 days of diagnosis	8	Median 15 mL/kg				
Thompson et al (2004)[15]	Retrospective cohort study	26			Mortality at discharge or shortly after / Mortality at 12 months	Chi-squared test for mortality at 12 months	a	-	.23
			1: Prednisone (2 mg/kg PO q12h), UFH (100 U/kg SC q6h), ± azathioprine (2 mg/kg PO q24h)	13	31% / 46%				
			2: As above, with fresh frozen plasma (10 mL/kg IV over 2 hours)	13[b]	38% / 77%				

Abbreviation: UFH, unfractionated heparin.
aNot stated.
bThis was a historical control population.

1146 | Journal of Veterinary Internal Medicine

ACVIM
Open Access
American College of
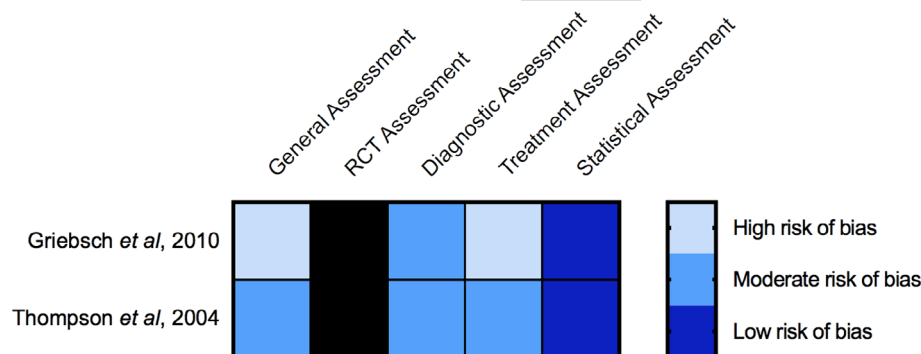Veterinary Internal Medicine

SWANN ET AL.

FIGURE 2 Results of evidence quality assessment for studies investigating the use of blood products in dogs with immune-mediated hemolytic anemia (IMHA). RCT, randomized controlled trial

3.4 | Immunosuppressive treatment

Our recommended approach is summarized in Figure 3. Failure to respond to treatment as expected should prompt consideration of whether criteria for diagnosis of nonassociative (primary) IMHA have been fulfilled, as outlined in the Consensus Statement on Diagnosis of Immune-Mediated Hemolytic Anemia in Dogs and Cats.[32]

7. We recommend that prednisolone or prednisone at an initial PO dosage of 2-3 mg/kg/day, or 50-60 mg/m^2/day for dogs >25 kg, be introduced after a diagnosis of IMHA has been reached. The drug may be administered as a single daily dose or divided into 2 daily doses. Dexamethasone (0.2-0.4 mg/kg/day) may be administered IV on a temporary basis if the patient initially will not tolerate PO drug treatment.

Strength of recommendation: Strong

Rationale

The dosage range for prednisone or prednisolone is based on review of published literature and clinical experience, with some panel members (2/8) recommending that the initial dosage of prednisone or prednisolone not exceed 2 mg/kg/day, or 40 mg/m^2/day for dogs >25 kg, owing to the risk of more severe adverse effects with higher dosages. Review of published literature, including a study population treated with prednisolone alone, indicated an initial response rate of approximately 80%, but did not find any evidence to suggest that IV dexamethasone is superior to PO prednisone or prednisolone.[2,5,18-21,24,25,44-46] Studies supporting previous statements that prednisolone may be more effective[47] or less irritating to the gastrointestinal tract[48] if given twice daily currently are lacking. Human medical literature suggests that once daily administration may be associated with fewer adverse mineralocorticoid effects.[49]

8. If the starting dosage of prednisone or prednisolone is >2 mg/kg/day, we recommend that it be decreased to ≤2 mg/kg/day within the first 1-2 weeks of treatment, provided the dog is responding to treatment, as demonstrated by a stable or increasing PCV/Hct.

Strength of recommendation: Strong

Rationale

This recommendation is based on clinical experience, suggesting that more severe adverse effects are observed if high dosages of glucocorticoids are used for prolonged periods.

9. We suggest that a second immunosuppressive drug may be introduced in any dog from the outset of treatment in an effort to decrease the dosage of glucocorticoid required. In particular, we suggest treating with 2 immunosuppressive drugs in the following situations:

○ The dog has clinical features at presentation consistent with severe or immediately life-threatening disease.
○ The PCV/Hct does not remain stable, with an absolute decrease of ≥5% within 24 hours, during the first 7 days of treatment with a glucocorticoid drug as described in #7 above.
○ The dog has continued to be dependent on blood transfusions after 7 days of treatment as described in #7 above.
○ The dog develops or, based on previous treatment, is expected to develop severe adverse effects related to the use of glucocorticoids at any time during its treatment. This is of particular relevance for dogs >25 kg in body weight.

Strength of recommendation: Weak

Rationale

These recommendations are largely based on clinical experience of the panel. The decrease of 5 percentage points in PCV/Hct (eg, from 25% to 20%) is intended to account for variations in these variables because of changes in hydration status or measurement error. Definitions of "steroid failure" are modified from several studies suggesting a poor response if an unstable PCV/Hct or persistent agglutination remain after 7-14 days.[8,15,26,27,50] We recommend introducing 2 drugs in dogs with severe disease from the outset because some dogs may show an inadequate response to a single agent. Introducing 2 drugs simultaneously increases the chance that an individual dog with life-threatening disease will receive a drug to which it will show a favorable response in the critical early phase of treatment. Numerous studies have evaluated possible prognostic factors for dogs with IMHA, either by exploring associations between survival and a single candidate variable, or by constructing a multivariable model to account for possible confounding factors. Among the latter models, increased serum bilirubin concentration (or clinical icterus) and increased serum urea or BUN concentration were identified
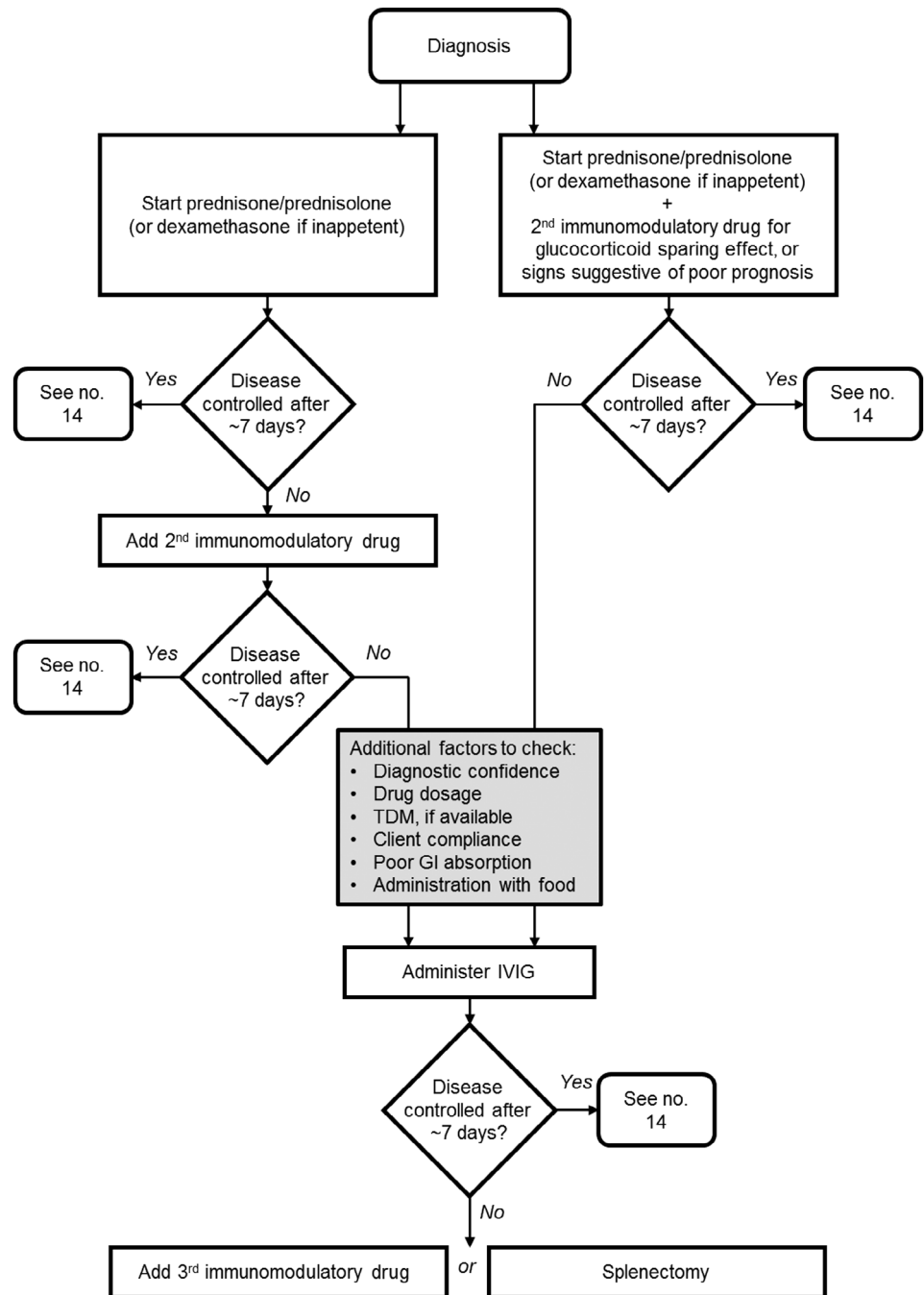
FIGURE 3 Flow diagram showing recommended approach for initial treatment of dogs with immune-mediated hemolytic anemia (IMHA). IVIG, intravenous immunoglobulin; TDM, therapeutic drug monitoring. Various outcomes make reference to Consensus Summary Statement #14, to which the reader is referred for further information

as independent predictors of mortality in 2 studies of different patient populations.[51] Both prognostic factors subsequently were identified by a further study of dogs with IMHA in the United Kingdom.[52] We suggest therefore that evaluation of serum bilirubin and urea concentrations may be of greatest utility in predicting outcome for dogs with IMHA (published values shown in Table 2), although several other factors have been identified as single predictors.[53]

10. Where a second drug is administered for treatment of IMHA in dogs, we suggest 1 of the following options (listed in alphabetical order):

- ○ Azathioprine: 2 mg/kg or 50 mg/m^2 PO q24h. After 2-3 weeks, the dosing interval may be increased to every other day until treatment is discontinued.
- ○ Cyclosporine: 5 mg/kg PO q12h. Adjustment of this dosage may be guided by therapeutic drug monitoring (TDM) (see recommendation #28).
- ○ Mycophenolate mofetil: 8-12 mg/kg PO q12h

   If these drugs are not available or tolerated, the following drug may be used, but its use is supported by less evidence than those listed above:

TABLE 2 Summary of studies reporting serum bilirubin and urea/blood urea nitrogen concentrations as prognostic factors for mortality in dogs with immune-mediated hemolytic anemia (IMHA)

Reference	Number of dogs with IMHA	Number of dogs included in analysis	Outcome measure	Analysis method	Prognostic factor	Hazard/odds ratio	95% confidence interval	P value	Median in survivors (IQR[d])	Median in non-survivors (IQR[d])
Goggs et al (2015)[52]	276	a	Mortality at discharge (all-cause mortality)	Multivariable logistic regression	Bilirubin[b]	1.010	1.003-1.017	<.01	0.76 mg/dL (0.44-1.87)[e]	5.09 mg/dL (1.58-23.88)[e]
					Urea[b]	3.046	1.592-5.830	<.01	41.4 mg/dL (30.0-58.9)[e]	59.2 mg/dL (43.2-94.3)[e]
					ASA status ≥3	2.709	1.068-6.870	.04	3 (3-3)[e]	3 (3-3)[e]
	276	a	Mortality at 30 days (all-cause mortality)	Multivariable logistic regression	Bilirubin[b]	1.009	1.002-1.016	.01	0.85 mg/dL (0.46-1.87)[e]	3.60 mg/dL (0.80-14.80)[e]
					Creatinine[b]	8.996	1.537-52.672	.02	0.72 mg/dL (0.61-0.86)[e]	0.80 mg/dL (0.59-1.07)[e]
					ASA status ≥3	2.173	1.020-4.629	.04	3 (2-3)[e]	3 (3-3)[e]
Piek et al (2011)[2]	222	164	Survival time (death because of IMHA)	Cox proportional hazards analysis	Icterus	2.94	1.60-5.42	.0005	a	a
					Urea (>56 mg/dL)	2.56	1.729-3.789	.0001	a	a
					Spherocytosis	0.38	0.20-0.72	.002	a	a
Reimer et al, (1999)[20]	70	a	Survival time (all-cause mortality)	Cox proportional hazards analysis	Bilirubin	a	a	.0003	a	a
					ALP[c] activity	a	a	.02	a	a
Swann and Skelly (2011)[5]	42	34	Survival time (all-cause mortality)	Cox proportional hazards analysis	Bilirubin	1.014	1.003-1.024	.010	0.71 mg/dL (SD 2.80)[f]	2.13 mg/dL (SD 3.03)[f]
					Urea	1.211	1.073-1.367	.002	36.3 mg/dL (SD 25.9)[f]	61.0 mg/dL (SD 38.4)[f]

Abbreivation: ASA, American Society of Anaesthesiologists.
[a]Not stated.
[b]Indexed to upper reference limit for each center.
[c]Alkaline phosphatase (ALP).
[d]Interquartile range.
[e]Calculated from Data S1.
[f]For dogs that did and did not survive to discharge; interquartile ranges (IQRs) not reported.

SWANN ET AL.

Journal of Veterinary Internal Medicine ACVIM

Open Access

American College of
Veterinary Internal Medicine

1149

○ Leflunomide: 2 mg/kg PO q24h. Adjustment of this dosage may be guided by TDM (see recommendation #28).

Strength of recommendation: Weak

Rationale

The benefit of adding a second drug has not been established. Case selection bias probably exists in the available (primarily retrospective) studies, with more severely affected patients often receiving additional immunosuppressive treatment. Prospective studies to establish definitively the superiority of any of the second-line immunosuppressive treatments are lacking. A summary of our review of studies comparing ≥2 treatment protocols for dogs with IMHA is shown in Table 3, with appraisal of the evidence quality of these articles in Figure 4.

Azathioprine Two previous studies suggested that administration of azathioprine may be associated with a more favorable outcome.[16,17] In the first, a retrospective cohort study, inclusion of azathioprine in the treatment regimen alongside prednisone and cyclophosphamide was associated with improved survival using Kaplan-Meier analysis with log rank test.[16] However, this conclusion was limited by incomplete information on the treatment protocols (Figure 4). In the second, a case series of dogs with IMHA and severe thrombocytopenia, dogs that received azathioprine (in combination with other drugs) were 4.27 times more likely to survive to 30 days after initiation of treatment compared to dogs that did not receive the drug, although the authors speculated that administration was more likely to have been a marker of survival (because dogs survived long enough to receive a second agent) than its cause.[17] This study was limited by incomplete presentation of survival information for each group (Table 3). Two further studies have suggested a possible favorable effect of azathioprine on survival, but associations between drug and outcome were limited in both analyses by the small numbers of patients (Table 3).[5,20] A final study reported no difference in outcome between dogs treated with azathioprine and prednisolone compared to a historical control population that received prednisolone alone.[2] Although the appraisal of this study was relatively favorable (Figure 4), the conclusion may be biased because the 2 cohorts of dogs were separated in time and differed with respect to other variables that the authors previously had identified as markers of a poor prognosis.[54]

Cyclosporine Administration of cyclosporine to dogs with IMHA is reported in numerous studies.[5,7,18,26,27,52,55] In 1 retrospective cohort study, the use of cyclosporine (in combination with other drugs) did not change the relative risk of death compared to all other treatment protocols that excluded the drug, although this study was limited by incomplete description of the treatment regimens (Figure 4).[18] A further retrospective study found no difference in mortality at 1 month or 1 year after diagnosis in dogs treated with prednisolone alone and those treated with prednisolone and cyclosporine, although this study also was limited by incomplete description of treatment regimens and less rigorous inclusion criteria (Figure 4).[5] A double-masked

randomized clinical trial comparing prednisone alone to prednisone and cyclosporine for treatment of dogs with IMHA was reported in abstract form only, but found no difference in survival between groups.[56]

Mycophenolate mofetil Use of mycophenolate has been reported in several recent studies.[6,23,27,50,52,55] One study documented similar efficacy of a protocol combining mycophenolate with prednisone compared to prednisone combined with another immunosuppressive drug (azathioprine or cyclosporine) for treating IMHA (Table 3), although this was a retrospective cohort study, with no randomization of dogs to either treatment regimen and with undetermined statistical power for comparison of outcome between treatment groups (Figure 4).[6]

Leflunomide Leflunomide is reported for the treatment of various refractory immune-mediated diseases in dogs, including IMHA,[57] and as a second-line drug for the treatment of IMHA.[27,55,58] No prospective or retrospective studies have compared its efficacy to other, more commonly used, second drugs, or to prednisone or prednisolone alone.

11. We recommend that cyclophosphamide not be administered to dogs with IMHA.

Strength of recommendation: Strong

Rationale

Review of published literature found evidence that cyclophosphamide treatment offered no benefit over treatment with glucocorticoids alone, and even could be detrimental to long-term prognosis.[18–20] However, 1 study was limited by the absence of a sample size calculation and by failure to report any statistical comparison of survival in the 2 treatment groups (Table 3, Figure 4).[19] A retrospective cohort study also was limited by lack of information on possible variability in enrolled cases and by incomplete information relating to treatment regimens (Figure 4).[18]

12. Administration of IV immunoglobulin (IVIG) at a dosage of 0.5-1 g/kg as a single infusion may be considered as a salvage measure in dogs not responding to treatment with 2 immunosuppressive drugs, but we do not recommend it for routine treatment.

Strength of recommendation: Strong

Rationale

Administration of IVIG may appear to be an attractive prospect because it is suggested to act quickly, but several studies showed no effect of this treatment on survival when compared to other immunosuppressive regimens in dogs with IMHA (Table 4).[18,21–25,59] The design and risk of bias varied among these studies, but all supported the same conclusion (Figure 5). Studies have indicated that administration of IVIG may be associated with more rapid recovery of a

TABLE 3 (Continued)

Reference	Study design	Number of dogs with IMHA	Treatment groups	N	Outcomes	Method of statistical comparison	Statistical measure	Confidence interval	P value
			1: Prednisone (1.9 mg/kg/d)	26		b	-	-	-
			2: Azathioprine (2.11 mg/kg/d) ±other drugs	27			0.731	0.44-1.21	>.05
			3: Cyclophosphamide (90 mg/m² PO q24h, n = 22 or 75 mg/m² PO q24h, n = 11) ±other drugs	33			1.59	1.07-2.36	≤0.05
			4: Cyclosporine (0.24 mg/kg/h IV CRI, n = 3, or 9.4 mg/kg PO q24h, n = 17, or 6.15 mg/kg PO q12h, n = 4) ±other drugs	24			1.22	0.79-1.87	>.05
			5: Danazol (mean 7.2 mg/kg PO q12h) ±other drugs	16			0.97	0.57-1.67	>.05
Mason et al (2003)[19]	Randomized controlled trial	18			Mortality at 8 days after presentation	f			
			1: Prednisone (1-2 mg/kg PO q12h)	10	20%		a	a	a
			2: Prednisone (1-2 mg/kg PO q12h) and cyclophosphamide (50 mg/m² PO or IV q24 for 4 days per week for 4 weeks)	8	38%		a	a	a
Piek et al (2011)[2]	Retrospective cohort study	222			Median survival time / Mortality at 6 months / Mortality at 1 year	Cox proportional hazards analysis	Hazard ratio		
			1: Prednisolone (2 mg/kg/d starting dose)	73	Not reached / 34% / 36%	Group 2 compared to 1	1.12	0.679-1.86	.65
			2: Prednisolone (2 mg/kg/d starting dose) and azathioprine (2 mg/kg/d, with maximum doses for large dogs)[b]	149	Not reached / 28% / 31%	g	-	-	-

(Continues)

TABLE 3 (Continued)

Reference	Study design	Number of dogs with IMHA	Treatment groups	N	Outcomes: Median survival time	Mean survival time	Mortality at discharge	Method of statistical comparison	Statistical measure	Confidence interval	P value
Reimer et al (1999)[20]	Retrospective cohort study	70	1: Prednisone only (average for all dogs 1.9 mg/kg q24 or q12h)	16	57 days	67.2 days		Kaplan-Meier[h]	a	a	P = .0001 for overall difference between groups[i]
			2: Prednisone and cyclophosphamide (average for all treated dogs 2.4 mg/kg/d)	28	28 days	215.4 days					
			3: Prednisone and azathioprine (average for all treated dogs 1.7 mg/kg/d)	5	974 days	931 days					
			4: Prednisone, cyclophosphamide, and azathioprine	16	15 days	779.3 days					
Swann and Skelly (2011)[5]	Retrospective cohort study	42	1: Prednisolone (mean 1.65 mg/kg/d)	11	452 days	620 days	9%	Chi-squared test for mortality at discharge, 1 month and 1 year			P = .03 for overall difference between treatment groups at discharge[j]. P values not stated for other comparisons
			2: Prednisolone (mean 2.57 mg/kg/d), azathioprine (1.8 mg/kg/d), and IVIG (n = 1)	9	194 days	360 days	0				
			3: Prednisolone (mean 2.35 mg/kg/d), cyclosporine (5 mg/kg q12h), cyclophosphamide (n = 2), and IVIG (n = 3)	17	9 days	158 days	41%				

(Continues)

TABLE 3 (Continued)

Reference	Study design	Number of dogs with IMHA	Treatment groups	N	Outcomes	Mortality at discharge	Mortality at 1 month	Mortality at 2 months	Method of statistical comparison	Statistical measure	Confidence interval	P value
Wang et al (2013)[6]	Retrospective cohort study	52			Mortality at discharge			Mortality at 2 months	Chi squared or Fisher's exact test for mortality at discharge, 1 month, and 2 months			P = .27 at discharge, P = .52 at 1 month, P = 1.000 at 2 months
			1: Prednisone (2.9 mg/kg/d or dexamethasone 0.4 mg/kg/d) and cyclosporine (10.6 mg/kg/d, n = 15) or (azathioprine 1.9 mg/kg/d, n = 6), or IVIG (0.5 g/kg, n = 1)	22	23%		30%	33%				
			2: Prednisone (2.6 mg/kg/d or dexamethasone 0.3 mg/kg/d) and mycophenolate mofetil (20.5 mg/kg/d)	30	9%		14%	33%				

Abbreviation: CRI, constant rate infusion; IVIG, intravenous immunoglobulin.

[a]Not stated.
[b]This was a historical control population.
[c]Incorrectly described by the authors as a "retrospective cross-sectional study."
[d]No outcome measures reported for each treatment group.
[e]Group 1 is presumed to be the reference group for other comparisons.
[f]No statistical comparison is reported in the paper; the fragility index was not calculated.
[g]Reference group.
[h]Statistical test used not stated.
[i]Insufficient cases to compare individual groups.
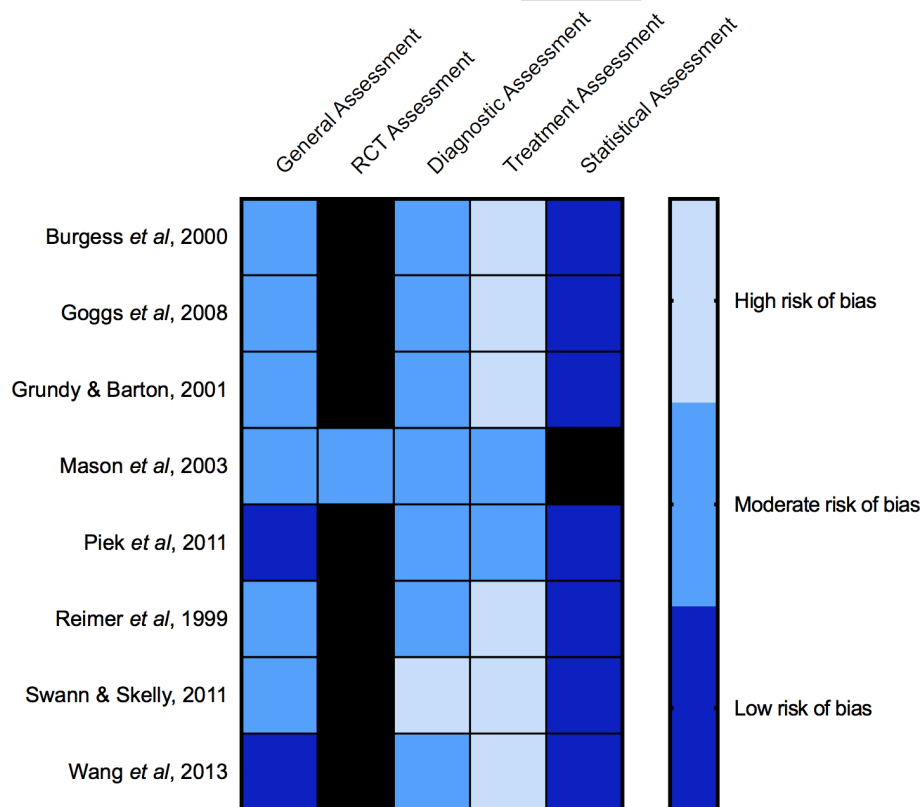[j]Insufficient cases to compare individual groups.

FIGURE 4 Results of evidence quality assessment for studies investigating the use of immunosuppressive drugs in dogs with immune-mediated hemolytic anemia (IMHA). RCT, randomized controlled trial

normal PCV,[22,60] or a lesser requirement for transfusions,[61] but 2 of these studies did not include a control group,[60,61] and the other contained a number of statistical anomalies[22] (Figure 5). Nevertheless, this evidence base may provide a rationale for use of IVIG as a salvage option in cases unresponsive to the treatments described in recommendations #7-10. In people, the recommended dosage of IVIG is 0.4-0.5 g/kg/day administered for 4-5 days,[30] but the efficacy and safety of treatment beyond 3 days has not been assessed in dogs. Availability of IVIG is limited in many countries, and it is expensive. Our recommended approach to management of refractory patients is shown in Figure 3.

13. We suggest that the use of ≥3 immunosuppressive drugs at the same time should be avoided.

Strength of recommendation: Weak

Rationale

This recommendation is based on clinical experience suggesting that combinations of ≥3 drugs rarely are required for management of IMHA and on published data indicating that some combinations are associated with more severe adverse effects, including an increased risk of opportunistic infections.[62,63] Also, no published evidence is available to demonstrate that combinations of multiple immunosuppressive drugs are associated with more effective control of disease. Our recommended approach to management of refractory patients is shown in Figure 3. If ≥3 drugs are administered simultaneously under this scheme, we recommend using drugs that target different immune

pathways, which would preclude concurrent administration of azathioprine and mycophenolate mofetil.

14. When the PCV/Hct has remained stable and >30% for 2 weeks after commencing treatment, with improvement in the majority of measures of disease activity (including spherocytosis, agglutination, serum bilirubin concentration, and reticulocyte count), we recommend decreasing the dosage of prednisone or prednisolone by 25%.

If a second drug has been introduced with the aim of limiting glucocorticoid-related adverse effects, the dosage of this drug should not be changed, but a greater reduction in the dose of prednisone or prednisolone (of 25%-50%) may be possible if the dog shows an adequate response to treatment.

Strength of recommendation: Strong

Rationale

This recommendation is based on clinical experience of the panel. One of the 8 panel members suggested that the serum bilirubin concentration should normalize before any dose reduction is made. Alternatively, if a dog is receiving prednisone or prednisolone in combination with another immunosuppressive drug, the dosage of the non-glucocorticoid drug could be decreased in increments before changing the dosage of prednisone or prednisolone if the non-glucocorticoid drug appears to be causing more adverse effects or is associated with unsustainable cost. However, in general, we recommend decreasing the dosage of prednisone or prednisolone first because glucocorticoids are associated with more prevalent and dose-related adverse effects (see recommendation #18 below).

TABLE 4 Summary of studies investigating the use of intravenous immunoglobulin (IVIG) in dogs with immune-mediated hemolytic anemia (IMHA)

Reference	Study design	Number of dogs with IMHA	Treatment groups	N	Outcomes	Method of statistical Comparison	Statistical measure	Confidence interval	P value
Gerber et al (2002)[21]	Retrospective cohort study	22			Mortality at discharge	Chi-squared test for mortality at discharge	[a]	[a]	NS
			1: Prednisolone (2-4 mg/kg/d)	13	38%				
			2: Prednisolone (2-4 mg/kg/d)[c] and IVIG (0.5-1 g/kg IV, maximum 12 g, at clinician discretion)	9	44%				
Grundy and Barton (2001)[18]	Retrospective cohort study[k]	88			[d]	Relative risk of mortality	Relative risk[h]		
			1: Prednisone (1.9 mg/kg/d)	26		[h]	-	-	-
			2: IVIG (mean 1 g/kg IV over 4-24 hours, unknown indication) ±other drugs	7			0.54	0.16-1.77	>.05
Kellerman and Bruyette (1997)[22]	Retrospective cohort study	37			PCV response during hospitalization[f]	Comparison of survival[i]	[a]	[a]	>.05
			1: Combination of prednisone, azathioprine, and cyclophosphamide	13	Mortality at discharge	Contingency table analysis for response to treatment[i]	Odds ratio 12.0 for a PCV response during hospitalization with IVIG[f]	[a]	.04
					23%				
			2: As above, with IVIG (0.5 g/kg IV over 4 hours), based on clinician preference	24	40%[g]				
					89%				
					8%[e]				
Oggier et al (2018)[23]	Retrospective case series[b]	21			Mortality at 1 year	No statistical comparison reported			
			1: Prednisolone (1.4-4.5 mg/kg/d) or dexamethasone (0.2-1.3 mg/kg/d) and mycophenolate mofetil (10-20 mg/kg/d)	6	33%				
			2: As above, with IVIG (0.26-1.6 g/kg IV) if finances permitted	15	27%				
Park et al (2016)[24]	Retrospective cohort study	37			Median survival time	Kaplan-Meier with log rank test	[a]	[a]	.65
					Mortality at 1 month				
					Mortality at 1 year				

(Continues)

TABLE 4 (Continued)

Reference	Study design	Number of dogs with IMHA	Treatment groups	N	Outcomes			Method of statistical Comparison	Statistical measure	Confidence interval	P value
			1: Prednisolone (2 mg/kg SC then PO q12h)	19	373 days	26%	47%	Chi squared for comparison of mortality[j]	Chi squared 2.961	a	.08
			2: As above, with IVIG (0.5 g/kg over 6 hours), unknown indication	18	225 days	17%	61%				
Whelan et al (2009)[25]	Randomized controlled trial	28			Mortality at discharge	Mortality at 14 days	Mortality at 5 months	Chi-squared test for comparison of mortality	a	a	NS
			1: Dexamethasone (0.3 mg/kg IV once) then prednisone (3 mg/kg PO q24h)	14	14%	21%	21%				
			2: As above, with IVIG (0.5 g/kg IV q24h for 3 days)	14	7%	7%	43%				

aNot stated.
bTwo cohorts were identified but not compared with respect to survival, hence the description of a retrospective case series.
cSome dogs received prednisolone sodium succinate or dexamethasone; see Supporting Information S3 for full details.
dNo outcome measures reported for each treatment group.
eAssuming "responders" were discharged.
fIn the subgroup of dogs that had not responded to prednisone alone by 7 days.
gThis group was reported to contain 4 dogs, so this percentage is not mathematically possible.
hGroup 1 is presumed to be the reference group for other comparisons.
iStatistical test used not stated.
jThe manuscript stated that mortality was compared at discharge, 1 month, 1 year, and 2 years, but only a single statistical value was reported, and it is unclear which this referred to.
kIncorrectly described by the authors as a retrospective cross-sectional study.

FIGURE 5 Results of evidence quality assessment for studies investigating the use of intravenous immunoglobulin in dogs with immune-mediated hemolytic anemia (IMHA). RCT, randomized controlled trial
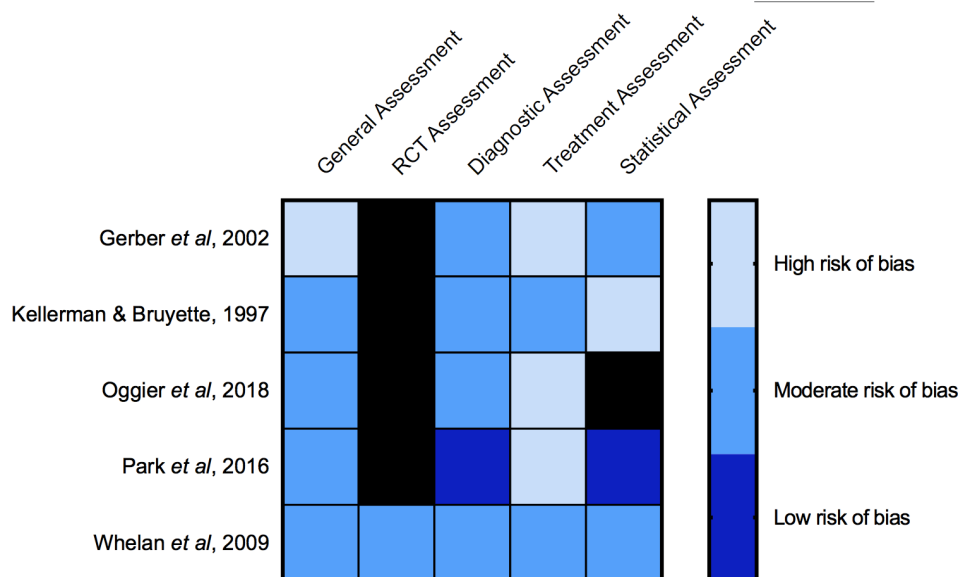
15. Provided the PCV/Hct remains stable and >30%, with improvement in the majority of measures of disease activity (including spherocytosis, agglutination, serum bilirubin concentration, and reticulocyte count), we recommend decreasing the dose of prednisone or prednisolone by 25% every 3 weeks.

   If a second drug has been introduced with the aim of limiting glucocorticoid-related adverse effects, the dosage of this drug should not be changed, but a greater reduction in the dose of prednisone or prednisolone (of 25%-33%), or a shorter interval between reductions (2 weeks), may be possible if the dog shows a good response to treatment.

   A typical duration of 3-6 months of treatment is expected for prednisone or prednisolone in the majority of cases, with an expected duration of 4-8 months for all immunosuppressive treatment.

Strength of recommendation: Strong

Rationale

This recommendation is based on clinical experience of the panel. One of the 8 panel members suggested that the serum bilirubin concentration should normalize before further dose reductions are made.

16. After stopping prednisone or prednisolone, we suggest 1 of the following options in dogs also receiving another immunosuppressive drug:

   - Continue to administer the other immunosuppressive drug at the same dosage for 4-8 weeks, then stop without tapering.
   - Taper the dosage of the other immunosuppressive drug in the same way as for the prednisone or prednisolone, as described in #15 above.

Strength of recommendation: Weak

Rationale

This recommendation is based on the clinical experience of the panel, with no published evidence to indicate which approach will result in a better outcome. Among the panel, 4/8 members currently adopt the first approach described above, 3/8 adopt the second approach, and 1/8 stops administering the second immunomodulatory drug at the same time as the prednisone or prednisolone is stopped, without tapering.

17. At a minimum, we recommend assessing the PCV/Hct before any dose reduction to ensure continued response to treatment. These variables should be assessed every 1-3 weeks during treatment. Additional tests to evaluate disease activity, including assessment for spherocytes, agglutination, and increased serum bilirubin concentration, also are recommended periodically to ensure continued response to treatment.

Strength of recommendation: Strong

Rationale

This recommendation is based on clinical experience of the panel.

3.5 | Monitoring for adverse effects associated with immunosuppressive treatment

18. *Glucocorticoids*: We recommend careful physical examination and review of owner observations in dogs receiving glucocorticoids to assess the severity of adverse effects. Urinalysis, with or without bacterial culture, may be considered every 8-12 weeks and is recommended if clinical features suggest urinary tract infection. We recommend obtaining baseline values for biochemical variables that may be affected by glucocorticoids before starting treatment with these drugs or any potentially hepatotoxic drugs.

Strength of recommendation: Strong

Rationale

The high frequency of adverse effects associated with initial immuno-suppressive dosages of glucocorticoids in dogs with IMHA, and their potential impact on owner willingness to pursue treatment, should not be underestimated. However, remarkably little information regarding glucocorticoid adverse effects is available in studies of dogs with IMHA, with polyuria and polydipsia reported in 29% of patients receiving glucocorticoids in 1 investigation.[5]

In 1 recent prospective study of dogs with inflammatory bowel disease, the following adverse effects were noted in the group of dogs receiving prednisone (at 1 mg/kg PO q12h): polydipsia (80% of treated dogs), polyuria (70%), excessive panting (55%), and lethargy (30%), with muscle weakness or atrophy, urinary incontinence, and temperament change reported less commonly.[64] Adverse effects were rated as moderate to severe in 30% of prednisone-treated dogs.[64] In a different study of dogs with immune-mediated polyarthritis, the following adverse effects were observed in dogs receiving prednisone after 45 days of therapy, after starting treatment at 1 mg/kg twice daily and with doses tapered by 25% every 2-3 weeks when in remission: polydipsia (100% of treated dogs), polyphagia (100%), polyuria (91%), and panting (81%), with an approximately 50% reduction in the prevalence of most of these adverse effects by 3 months.[65]

Other long-term adverse effects include alopecia; thin skin; calcinosis cutis; susceptibility to bruising; muscle atrophy; fat redistribution to the abdomen with a resulting pot-bellied appearance; hepatomegaly; predisposition to pyoderma, demodicosis, and urinary tract infections; and exacerbation of congestive heart failure.[66] Predisposition to diabetes mellitus and pancreatitis also is suspected to be associated with chronic glucocorticoid treatment.[67–69] Commonly observed clinicopathologic abnormalities include a stress leukogram and increased serum alkaline phosphatase (ALP) activity, with occasional polycythemia, thrombocytosis, hyperlipidemia, and hyperglycemia. Similar adverse effects are observed in human patients treated with glucocorticoids.[70]

Subclinical bacteriuria is common in dogs on long-term glucocorticoid treatment, but the clinical relevance of this finding is uncertain,[71,72] with no published data available to determine whether treating, or not treating, affected dogs will result in a more favorable outcome. Guidelines for treatment of urinary tract infection in dogs suggest antimicrobials could be considered in immunocompromised patients with subclinical bacteriuria because of the risk of ascending or systemic infection,[73] although comparable guidelines for human patients refrain from making any recommendation on this topic because of a lack of clinical evidence.[74] We therefore suggest periodic urinalysis in dogs treated with glucocorticoids, with or without bacterial culture. If clinical signs are observed or an active sediment associated with bacterial growth is found, we recommend appropriate antimicrobial treatment. If bacterial growth without active sediment is found in the absence of clinical signs, we do not make any recommendation regarding treatment, but, if treatment is not implemented, we recommend more frequent review of clinical signs and urinalysis results owing to the risk of ascending infection.

19. *Azathioprine*: We recommend that CBCs and relevant serum biochemical variables (especially alanine aminotransferase [ALT] activity) be monitored every 2 weeks during the first 2 months of treatment, and then every 1-2 months until treatment is discontinued.

Strength of the recommendation: Strong

Rationale

The standard starting dosage of azathioprine (2 mg/kg/day) in dogs usually is well tolerated. Gastrointestinal adverse effects such as nausea, anorexia, vomiting, and diarrhea occasionally are reported but typically are mild and self-limiting. Azathioprine can, however, also cause severe hepatotoxicity or marked myelosuppression in some dogs.[75,76]

Hepatotoxicity (typically characterized by a reversible increase in serum ALT activity in the absence of clinical signs) occurs in approximately 15% of German Shepherd dogs[75] and may be more common in German Shepherds. Hepatotoxicity usually develops in the first few weeks of treatment; if the drug is well tolerated for the first 2 to 4 weeks, it tends to be well-tolerated in the long-term.[75] Glucocorticoid treatment causes increases in serum ALT and ALP activity; the effects of azathioprine therefore may be difficult to distinguish from the effects of glucocorticoids. In general, an increase in ALT activity that approaches or exceeds the magnitude of increase of ALP should prompt concern.

Although marked myelosuppression is uncommon, chronic azathioprine usage sometimes causes mild to moderate poorly regenerative anemia.[77] Myelosuppression, in contrast to hepatotoxicity, can be delayed and occur months into treatment.[75,77] Marked myelosuppression and hepatotoxicity appear to be idiosyncratic non-dose-dependent reactions and typically are reversible if the problem is recognized early enough, and azathioprine is discontinued. Several individual case reports also have described pancreatitis in dogs receiving azathioprine, but cause and effect have not been established.[78,79] Deficiencies in a key enzyme involved in azathioprine metabolism, thiopurine methyltransferase (TPMT), can cause azathioprine toxicity in cats and people. Although TPMT expression in dogs is variable, TPMT deficiency does not appear to be associated with the severe toxicities sometimes seen in dogs.[76,80]

20. *Cyclosporine*: We recommend that dogs receiving cyclosporine be monitored for gastrointestinal adverse effects and gingival overgrowth. Relevant biochemical variables should be evaluated every 2-3 months owing to the risk of hepatotoxicity in a small proportion of dogs.

Strength of recommendation: Strong

Rationale

Adverse effects are uncommon with cyclosporine treatment in dogs, with the exception of gastrointestinal signs such as vomiting, diarrhea, anorexia, and nausea.[81] Administering the medication frozen or with food can decrease gastrointestinal adverse effects,[82] although administration with food carries a risk of altering drug absorption profiles.

Uncommonly, cyclosporine may cause idiosyncratic hepatotoxicity, which does not seem to be dose-dependent; gingival hyperplasia and hypertrichosis also have been reported occasionally.[83–87] Nephrotoxicity is a potential problem in people receiving cyclosporine, but clinically relevant renal damage has not been documented in dogs at standard dosages.[87,88]

An advantage of cyclosporine as an immunosuppressive drug is that it is not myelosuppressive. However, susceptibility to opportunistic infections and subclinical bacteriuria have been reported, particularly in combination with glucocorticoids and other immunosuppressive drugs.[72,89,90] Cyclosporine is metabolized by the hepatic cytochrome P-450 system; inadvertent drug overdosage therefore is possible if cyclosporine is given concurrently with drugs that inhibit P-450 enzymes. Ketoconazole has been used to decrease PO cyclosporine dosages in dogs by as much as 75%, although individual responses vary.[91,92] Experimentally, PO cyclosporine increases some markers of platelet activation in normal dogs, which may be a concern in dogs with IMHA, in which hypercoagulability and thromboembolism may be major contributors to patient mortality.[93] However, the clinical relevance of this phenomenon remains unclear.

21. *Mycophenolate mofetil*: We suggest that dogs should be monitored for gastrointestinal adverse effects. We also suggest that CBCs should be monitored every 2-3 weeks for the first month of treatment with mycophenolate mofetil, then every 2-3 months until treatment is discontinued.

Strength of recommendation: Weak

Rationale

Although the most commonly used form of mycophenolate (mycophenolate mofetil) is generally well tolerated in dogs, gastrointestinal adverse effects can sometimes limit the use of the drug.[6,50,94–98] In some cases, ulcerative colitis may develop that warrants discontinuation of the drug. Recommended starting dosages for mycophenolate mofetil in dogs have varied from 10 to 20 mg/kg PO q12h, but gastrointestinal signs at the higher end of the dosage range often will necessitate dose reductions. Reported prevalence of diarrhea can be ≥20%, and may be delayed for 1-2 weeks after starting treatment.[6,50,94–98] Generally, starting dosages of 7-10 mg/kg PO q12h (14-20 mg/kg total daily dose) are associated with a much lower prevalence of diarrhea.[99,100] In stable patients, therefore, a starting dosage of 8-12 mg/kg q12h is recommended. If diarrhea occurs, it often is responsive to dose reduction.[96,98]

In people, gastrointestinal signs, hepatotoxicity, and, less commonly, marked myelosuppression as well as a rare and fatal progressive multifocal leukoencephalopathy have been reported.[101,102] Unlike in humans, mycophenolate has not been reported to cause hepatotoxicity in dogs, and thus the benefit of monitoring biochemical profiles is questionable. However, mycophenolate potentially is myelosuppressive, hence the recommendation to monitor CBCs.

22. *Leflunomide*: We suggest that CBCs and relevant serum biochemical variables (especially ALT and ALP activities) be monitored every 2 weeks for the first 2 months in dogs receiving leflunomide, and then every 1-2 months until treatment is discontinued.

Strength of recommendation: Weak

Rationale

Leflunomide appears to be well tolerated in dogs, but its use has been limited in veterinary medicine compared to other drugs.[57,103] Occasional more serious adverse effects such as cutaneous drug reactions, hepatotoxicity, and pulmonary lesions (as found in human patients) may be recognized in dogs in the future. The most common adverse effects reported with leflunomide administration in dogs are occasional inappetence, lethargy, vomiting, and diarrhea.[57,58,104] However, a recent study also reported increased liver enzyme activity in approximately half of treated dogs (although it was difficult to separate this finding from the effect of concurrent glucocorticoid administration) and several instances of severe myelosuppression.[104] Although recommended oral dosages for leflunomide in dogs ranged from 3 to 4 mg/kg/day in the earlier literature,[103,105] a starting dosage of 2 mg/kg PO q24h appears to be efficacious, with possible lower risk of adverse effects.[58]

3.6 | Management of drug-associated myelosuppression

An expanded version of this section, with further commentary and discussion of underlying evidence, is available in Supporting Information Appendix A2.

23. As soon as myelosuppression is documented, we recommend that the causative drug be discontinued.

Strength of recommendation: Strong

24. For management of asymptomatic neutropenic patients, we recommend that, if the neutrophil count is between 1000 and 3000 cells/μL, antibiotics not be administered unless other independent risk factors for infection are present. When the neutrophil count is <1000 cells/μL, prophylactic antibiotics are indicated. We recommend close observation for any change in vital signs, demeanor, or development of new gastrointestinal signs, which could signal the onset of sepsis.

Strength of recommendation: Strong

25. We make the following recommendations for management of symptomatic neutropenic patients:

• Identify a source of infection, if possible.
• Institute parenteral, 4-quadrant antibiotic coverage, with reference to any known organ dysfunction. Antibiotics may be required at the high end of the recommended dosage range to offset decreased organ perfusion in sepsis.
• Institute IV fluid therapy and hemodynamic monitoring.

- Recombinant granulocyte colony stimulating factor could be considered for use in patients that have received an inadvertent overdose with a myelosuppressive drug or when profound neutropenia persists for >1 week.

Strength of recommendation: Strong

26. Once the patient has recovered from the neutropenic episode, and if further immunosuppressive treatment is required, we suggest a different drug be used than the drug that caused the neutropenia.

Strength of recommendation: Weak

3.7 | Management of infections in dogs receiving immunosuppressive drugs

An expanded version of this section, with further commentary and discussion of underlying evidence, is available in Supporting Information Appendix A3.

27. We suggest that the regimen of immunosuppressive drugs may need to be modified if infections are detected, but doing so depends on multiple factors, including the severity and nature of the infection, the severity and stage of treatment of the IMHA, and the immunosuppressive regimen employed. Clinicians also should consider that emerging infections may represent the underlying cause of the immune-mediated disease.

Strength of recommendation: Weak

3.7.1 | Therapeutic drug monitoring

28. If available, we suggest that appropriate TDM be considered in all cases, particularly in cases with actual or anticipated problems, including:

  ○ poor response to treatment,
  ○ possible interaction between drugs, and
  ○ emerging secondary infection.

Strength of recommendation: Weak

Rationale

Pharmacokinetic monitoring is available for cyclosporine and leflunomide, and pharmacodynamic monitoring (measuring suppression of T cell IL-2 synthesis) is available for cyclosporine. A list of centers currently offering TDM is shown in Supporting Information S4.

3.8 | Approach to relapse

Our recommended approach to investigation and management of relapses is summarized in Figure 6.

29. We recommend confirming the relapse using standard criteria for the diagnosis of IMHA (see Consensus Statement on Diagnosis of

Immune-Mediated Hemolytic Anemia in Dogs and Cats[32]) to ensure that there is no alternative cause of anemia.

Strength of recommendation: Strong

Rationale

The results of some tests may be affected by previous administration of immunosuppressive drugs. The clinician should be satisfied that sufficient abnormalities exist to diagnose a relapse, rather than another cause of anemia, such as gastrointestinal bleeding. Objective data on frequency of relapse in IMHA are scarce. Retrospective studies with long-term follow-up suggest a relapse rate of 11%-15%.[2,7] The following guidelines for management of relapsing IMHA are based on clinical reasoning and experience of the panel.

30. We recommend assessing the patient for any trigger factors that could derange immune homeostasis, with particular emphasis on emerging infection if the relapse occurred while the patient was still receiving immunosuppressive drugs.

Strength of recommendation: Strong

Rationale

Immune homeostasis may be affected by administration of drugs or vaccines, onset of inflammatory disease, emerging infection (particularly involving vector-borne agents), and neoplasia.

31. If the relapse occurred before any attempted dose reduction of the initial prednisone or prednisolone treatment, we suggest introducing an additional immunosuppressive drug (as described in recommendation #10). If a dog is already receiving 2 immunosuppressive drugs, we suggest performing TDM to ensure adequate drug dosage, as in recommendation #28.

Strength of recommendation: Weak

32. If the relapse occurred during tapering of immunosuppressive drugs (as described in recommendations #14-16), we suggest that the dosage of immunosuppressive drugs be increased. If the relapse manifested as fulminant disease, the initial (previously successful) induction protocol should be recommenced. If the relapse manifested as mild disease, the immunosuppressive drug dose should be increased back to the last dose that the patient was receiving before the most recent dose reduction.

Strength of recommendation: Weak

33. Once remission has been reestablished, we suggest commencing the tapering process more gradually than on the previous occasion, as outlined in recommendations #14-16. We suggest that the time from remission to the first dose reduction be doubled, and that the time interval between subsequent dose reductions be doubled.

Strength of recommendation: Weak
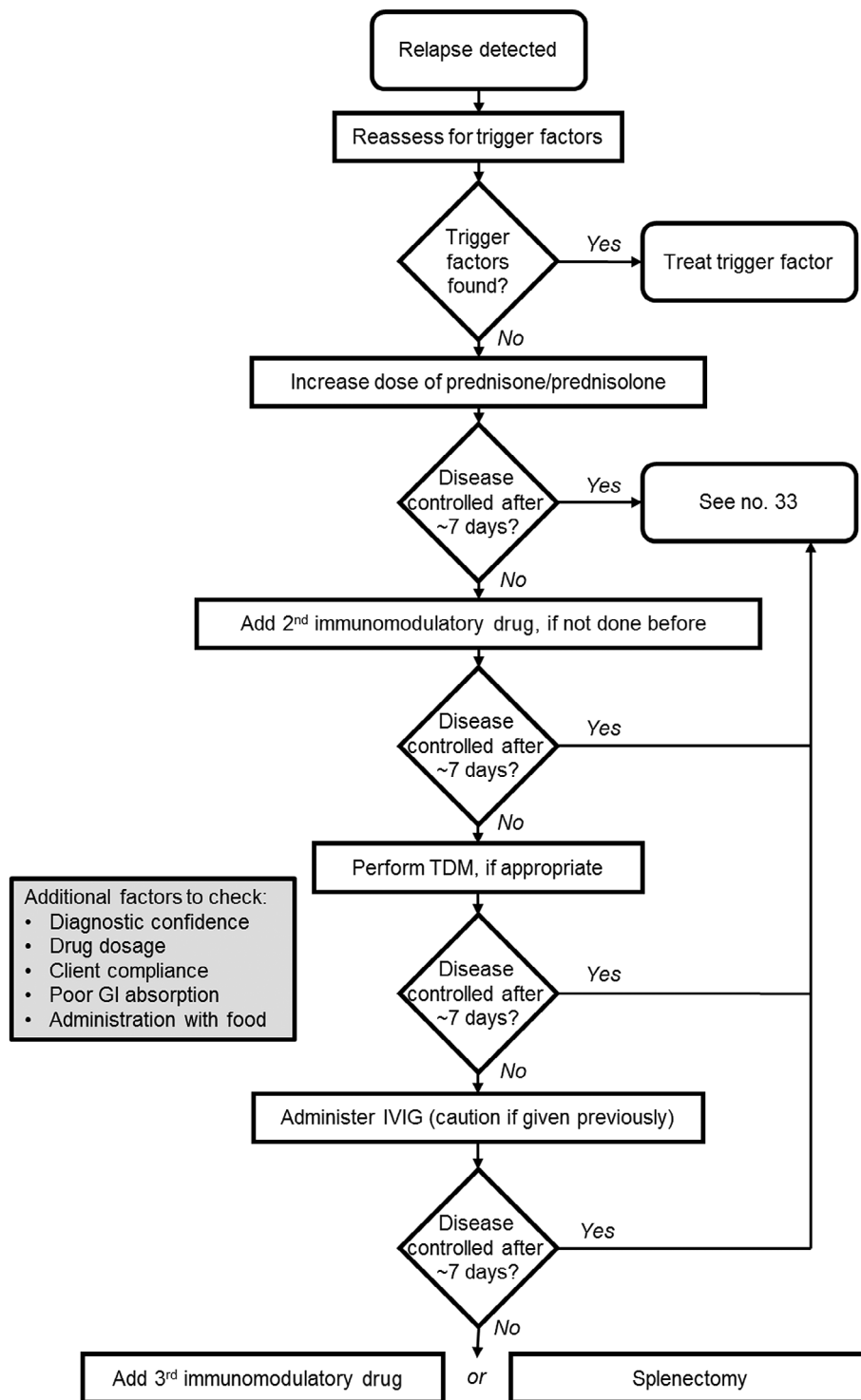
FIGURE 6 Flow diagram showing recommended approach for management of relapse in dogs with immune-mediated hemolytic anemia (IMHA). IVIG, intravenous immunoglobulin; TDM, therapeutic drug monitoring. Various outcomes make reference to Consensus Summary Statement #33, to which the reader is referred for further information

34. If recurrent relapses occur despite the measures described above, we suggest that lifelong immunosuppressive treatment may be required, aiming to maintain remission using the lowest possible dosage of immunosuppressive drug.

Strength of recommendation: Weak

35. In dogs requiring continuous immunosuppressive treatment, or suffering repeated relapses, we suggest that splenectomy be considered, provided that infection with vector-borne agents has been exhaustively excluded.

Strength of recommendation: Weak

Rationale

Splenectomy has been evaluated in 2 retrospective case series for relapsing or refractory IMHA. One study of 6 dogs showed 100% survival at 12 months postsplenectomy, with decreased or absent medication

requirement after surgery compared to before.[106] In another study of 10 dogs, 9 were alive 30 days postsplenectomy and the authors reported decreased transfusion requirements and increased PCV after surgery compared to before.[107] However, neither study enrolled a control group that did not undergo splenectomy, so it is impossible to determine whether splenectomy improved outcome.

Vector-borne agents may be latent in dogs with IMHA or acquired through blood transfusion. We are not aware of publications documenting infection in IMHA patients postsplenectomy, but this may reflect infrequent occurrence of splenectomy, short follow-up times, or both. Splenectomy per se is a procedure associated with a low risk of short-term complications,[106,107] but we recommend that immunosuppressive and antithrombotic medications be stopped or their dosage decreased to a minimum before surgery is undertaken. Further recommendations regarding screening for vector-borne agents are provided in the accompanying Consensus Statement on Diagnosis of Immune-Mediated Hemolytic Anemia in Dogs and Cats.[32]

36. If there appeared to be a temporal association between estrus cycle and relapse, we suggest that intact female dogs be spayed once they have been weaned off, or onto the lowest effective dose of, immunosuppressive drug(s). Similarly, if there appeared to be a temporal association between pregnancy and relapse, we suggest that the bitch not be bred again, or be spayed when weaned off, or onto the lowest effective dose of, immunosuppressive drug(s).

Strength of recommendation: Weak

Rationale

This recommendation is based on the clinical experience of the panel. Affected dogs also should not be receiving thromboprophylactic drugs at the time of surgery.

3.9 | Emerging immunomodulatory treatments for dogs with IMHA

An expanded version of this section, with further commentary and discussion of underlying evidence, is available in Supporting Information Appendix A4.

37. The use of a number of other treatments, including therapeutic plasmapheresis, liposomal clodronate, hyperbaric oxygen therapy, and melatonin, has been reported, scientifically, anecdotally, or in a small number of dogs with IMHA, but we suggest further investigation is required to determine whether these treatments are effective, and to establish how their use should be integrated with other treatments.

Strength of recommendation: Weak

3.10 | Antithrombotic treatment

The terms "thromboprophylaxis" and "antithrombotics" encompass both antiplatelet drugs, designed to inhibit platelet function (primary hemostasis), and anticoagulant drugs, designed to inhibit the activity of clotting factors (secondary hemostasis). We draw specific distinctions between

antiplatelet and anticoagulant drugs. An outline of our recommendations relating to antithrombotic treatment is shown in Figure 7. Additional guidance on the use of antithrombotic drugs in dogs is provided in the CURATIVE guidelines.[108]

38. We recommend that thromboprophylaxis be provided for all dogs with IMHA, except those with severe thrombocytopenia (platelet count <30 000/μL).

Strength of recommendation: Strong

Rationale

A substantial body of evidence indicates that IMHA in dogs is associated with an increased risk of thrombosis,[9,15,109–112] and that thrombotic disease is a leading cause of morbidity and mortality in dogs with IMHA.[10,113,114] The pathophysiology of thrombosis in IMHA is complex,[8,42] involving endothelial activation,[42] intravascular tissue factor expression,[115] procoagulant microparticle generation,[116] platelet activation,[117] and an imbalance of pro- and anticoagulant factors.[118–120] Previous studies have suggested that thromboprophylaxis may be particularly important in dogs with intravascular hemolysis, autoagglutination, marked leukocytosis, and increased liver enzyme activities.[9,10,53,121] Dogs with IMHA receiving high dosages of glucocorticoids,[122,123] and those receiving IVIG,[59,60,124] also may be at increased risk of thrombosis.

We have specified an arbitrary platelet concentration of 30 000/μL, below which we would not recommend that antithrombotics be administered, particularly if an antiplatelet drug is selected, because we considered that the risk of spontaneous hemorrhage is increased below this threshold. In the context of IMHA, some authors have classified severe thrombocytopenia as <50 000/μL,[17] whereas others have used <15 000/μL.[125] We recommend that antithrombotic drugs only be
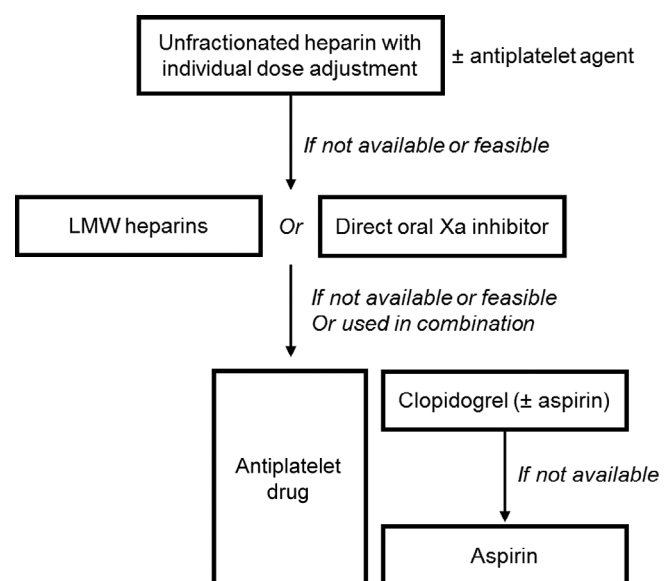

FIGURE 7 Algorithm showing recommended approach to selection of antithrombotic drugs for dogs with immune-mediated hemolytic anemia (IMHA). LMW, low molecular weight

administered to dogs with platelet counts >30 000/μL, because in these patients the thrombocytopenia is most likely consumptive.[126,127]

39. We suggest that thromboprophylaxis be initiated at the time of diagnosis and continued until the patient is in remission and no longer receiving prednisone or prednisolone.

Strength of recommendation: Weak

Rationale

The maximum risk period for mortality associated with IMHA appears to be the first 2 weeks after initiation of treatment.[54] This period represents the phase when pathophysiologic risk factors for thrombosis are maximal, likelihood of transfusion is highest, and any prothrombotic effect of immunosuppressive medications is greatest, if related to dosage. The risk of hemorrhage from recommended antithrombotic dosages is likely to be small.[128] As the disease process responds to immunosuppression, the likelihood of thrombosis may diminish, but the continued use of immunosuppressive medications may generate ongoing thrombotic risk.[129,130] Some dogs may require lifelong low doses of glucocorticoids to maintain remission. We suggest that thromboprophylaxis be discontinued if such dogs have been in remission for 6 months and no other risk factors exist.

40. Based on the pathophysiology of venous thromboembolism commonly encountered in dogs with IMHA, we suggest that a regimen incorporating anticoagulants may be preferred for thromboprophylaxis, particularly during the first 2 weeks after diagnosis. The available anticoagulants may be used alone or combined with antiplatelet drugs. If treatment with an anticoagulant, and its associated monitoring, is not available or feasible, we suggest administration of antiplatelet drugs in preference to no antithrombotic drug.

Strength of recommendation: Weak

Rationale

Thrombosis in IMHA predominantly affects the venous system, where thrombi form under low-shear conditions.[131] Such thrombi typically are rich in fibrin, and their formation is less dependent upon platelet number or function, providing a rationale for administration of anticoagulant drugs.[15,121,132] Although platelet activation can be detected in dogs with IMHA, this phenomenon probably occurs secondary to pathologic tissue factor-mediated thrombin generation, rather than as a primary event.[42,133,134] Nevertheless, the cell-based model of hemostasis posits that platelets are integral to hemostasis in vivo.[135,136] There is thus a rationale for the use of antiplatelet drugs in venous thrombosis.[132,137–140] Experimental data support this proposition to some degree,[141] and evidence in humans suggests that antiplatelet drugs do decrease the risk of venous thrombosis.[142–145] However, this should be viewed in the context of the substantial body of evidence in humans supporting administration of anticoagulants as first line prophylactic drugs for venous thrombosis.[146–148]

41. We suggest the administration of unfractionated heparin (UFH) with individual dose adjustment (using an anti-Xa assay) in preference to other drugs. This drug should not be used without individual dose adjustment. If this is not available or feasible, we suggest administering injectable low-molecular-weight heparins or direct PO Xa inhibitors. When using injectable low-molecular-weight heparins, we suggest individual dose adjustment (using an anti-Xa assay) may be useful to achieve a therapeutic dose. Suggested starting dosages for these drugs are:

   ○ Unfractionated heparin (IV): 100 U/kg bolus, then 900 U/kg/24 h
   ○ Unfractionated heparin (SC): 150-300 U/kg q6h
   ○ Dalteparin (SC): 150-175 U/kg q8h
   ○ Enoxaparin (SC): 0.8-1.0 mg/kg q6-8h
   ○ Rivaroxaban (PO): 1-2 mg/kg q24h

Strength of recommendation: Weak

Rationale

Insufficient evidence is available to make strong recommendations on the choice of anticoagulant in IMHA (Table 5 and Figure 8). The strongest evidence supports the use of individually dose-adjusted UFH.[26] Other anticoagulants including enoxaparin and rivaroxaban appear to be safe and may be efficacious,[27,55] but RCTs are lacking. In a prospective study of UFH use in dogs with IMHA, dosages of 300 U/kg SC q6h generated anti-Xa activities below the target ranges in more than half of the dogs.[149] A subsequent RCT of dogs with IMHA compared UFH treatment administered at constant dose with individually dose-adjusted UFH treatment based on anti-Xa monitoring.[26] This study demonstrated significantly longer survival in dogs given individually adjusted doses, with only 1/8 non-survivors compared to 6/7 non-survivors in the constant dose group (Table 5). Dosages of UFH of 150-566 U/kg q6h were required to achieve anti-Xa activities between 0.35 and 0.7 U/mL.[26] However, this study enrolled a small number of dogs and provided incomplete information on masking and randomization (Figure 8). Indeed, the study has a fragility index of only 2,[13] meaning that the outcome of only 2 cases would need to change to lose statistical significance, and suggesting that these findings warrant confirmation. One further RCT found no difference in survival in dogs treated with rivaroxaban compared with dogs treated with clopidogrel and ultra-low-dose aspirin (Table 5), but this study was underpowered and does not demonstrate equivalency of interventions.[27]

Various publications have determined pharmacokinetics of low-molecular-weight heparins in dogs.[150–152] Considerable variation in anti-Xa activities has been documented and some uncertainty remains about the efficacy of enoxaparin in some breeds of dog.[55,152,153] Dalteparin does appear efficacious in dogs for venous and arterial thromboprophylaxis.[154,155] The level of anti-Xa activity that confers thromboprophylaxis remains uncertain, but, given the variation in pharmacokinetics and that it is efficacy, monitoring anti-Xa activity may be justifiable. We believe it is reasonable to target 0.5-1.0 U/mL anti-Xa activity for both enoxaparin and dalteparin.[156]

Anti-Xa monitoring is not widely available to veterinarians. If an anti-Xa assay is not available, then it is reasonable to consider the

TABLE 5 Summary of studies investigating the use of antithrombotic drugs in dogs with immune-mediated hemolytic anemia (IMHA)

Reference	Study design	Number of dogs with IMHA	Treatment groups	N	Outcomes				Method of statistical comparison	Statistical measure	Confidence interval	P value
Helmond et al (2010)[26]	Randomized controlled trial	15			Median survival time	Mortality at 6 months						
			1: Constant dose of UFH (150 U/kg SC q6h on days 1-7 then q8h on days 7-28)	7	68 days	83%			Kaplan-Meier with log rank test	a	a	<.01
			2: UFH (150 U/kg SC q6h on days 1-3 then dose adjusted by nomogram to maintain anti-Xa levels of 0.35-0.7 U/mL)	8	>180 days	13%			Chi-squared test for mortality at 6 months	a	a	.03
Mellett et al (2011)[11]	Randomized controlled trial	24			Mortality at discharge	Mortality at 3 months	Transfusion volume					
			1: Clopidogrel (10 mg/kg on day 1 then 2-3 mg/kg/d PO q24h for 3 months)	8	13%	25%	Median 13 mL/kg		ANOVA for survival to discharge	a	a	.81
			2: Aspirin (0.5 mg/kg PO q24h)	8	13%	25%	Median 17 mL/kg		ANOVA for survival to 3 months	a	a	.56
			3: Both Aspirin and Clopidogrel	8	13%	13%	Median 14 mL/kg		ANOVA for transfusion volume	a	a	.29
Morassi et al (2016)[27]	Randomized controlled trial	24			Mortality at discharge	Mortality at 1 month	Mortality at 3 months					
			1: Clopidogrel (2-3 mg/kg PO q24h) and aspirin (1 mg/kg PO q24h)	12	17%	17%	25%		Fisher's exact test for mortality at discharge	a	a	.79
			2: Rivaroxaban (0.5-1 mg/kg PO q24h)	12	8%	17%	17%		Fisher's exact test for mortality at 3 months	a	a	.68
Weinkle et al (2005)[7]	Retrospective cohort study	151			Median survival time[c]	Mortality at 1 month	Mortality at 12 months					
			1: No antithrombotic treatment	27	~10-20 days	43%	55%		Kaplan-Meier with Gehan-Wilcoxon test and/or log rank test[b]	-	-	-
				76	~1500 days	18%	31%		Group 2 compared to 1	a	a	<.001

(Continues)

TABLE 5 (Continued)

Reference	Study design	Number of dogs with IMHA	Treatment groups	N	Outcomes	Method of statistical comparison	Statistical measure	Confidence interval	P value
			2: Aspirin (0.5 mg/kg PO q24h)						
			3: Heparin (25-75 U/kg SC q6-8h while hospitalized)	13	4 days / 83% / 83%	Group 3 compared to 1	a	a	.001
			4: Aspirin and heparin	27	~1400-1500 days / 33% / 36%	Group 4 compared to 3	a	a	.001

Abbreviation: UFH, unfractionated heparin.

[a]Not stated.

[b]Not stated which test was used to derive the stated P values.

[c]Estimated from Kaplan-Meier curves (Figures 2 and 3).

activated clotting time, activated partial thromboplastin time (aPTT), thrombin generation, or viscoelastic tests to monitor anticoagulant treatment.[153,156–164] Nomograms for adjustment of UFH treatment using aPTT and thromboelastographic assays have been proposed, but are currently only available in abstract form.[165,166] The original derivation of UFH aPTT prolongation targets was performed using thrombotic models in dogs.[167] Subsequently, these aPTT targets were demonstrated to correlate with recommended 0.35-0.70 U/mL anti-Xa activity targets in humans.[168,169] These activity targets protect against thrombosis in people,[146,170] and are the basis for the currently recommended targets for anti-Xa activity in dogs. However, we recognize that there is limited evidence of efficacy for these targets against patient-centered outcomes (eg, prevention of documented thrombosis, mortality) in dogs with clinical disease. Higher dosages than the initial starting dosages listed above may be necessary to attain these anti-Xa activity targets in dogs with diseases that predispose to thrombosis.[26,156] Initiating antithrombotic treatment at these dosages and increasing the dose incrementally based on individual monitoring may provide a margin of safety for patients against hemorrhagic complications. In our experience, many patients will require such dose escalation.

42. If antiplatelet drugs are administered, we suggest that clopidogrel be used in preference to aspirin. We suggest that clopidogrel be administered at a dosage of 1.1-4.0 mg/kg PO q24h. A single PO loading dose (eg, double the maintenance dosage or up to 10 mg/kg) may be useful for obtaining therapeutic plasma concentrations rapidly. If aspirin is selected as an antiplatelet drug, it should be administered at a dosage of 1-2 mg/kg q24h and could be combined with clopidogrel.

Strength of recommendation: Weak

Rationale

Clopidogrel may be efficacious for arterial thromboprophylaxis in dogs.[171–175] However, insufficient evidence is available to judge the efficacy of clopidogrel for prevention of venous thrombosis in dogs. Aspirin is a safe and effective drug for prevention of arterial thrombosis in dogs,[176] but insufficient evidence is available to judge the efficacy of aspirin for the prevention of venous thrombosis in dogs. Thirty percent or more of healthy dogs fail to respond to low dose aspirin,[177,178] and a minimum dosage of 2.0-5.0 mg/kg PO q12-24h is required for reliable platelet inhibition in responders.[179–181] Studies have demonstrated the failure of 1.0 and 3.5 mg/kg PO q12h of aspirin to reliably inhibit canine platelets.[182,183] Although aspirin at a dosage of 0.5 mg/kg q24h in combination with glucocorticoids appears safe,[184] aspirin dosages ≥2 mg/kg administered to dogs receiving concurrent prednisolone may be associated with increased gastrointestinal bleeding.[185]

One retrospective study of dogs with IMHA suggested those receiving ultra-low-dose aspirin had a survival benefit over those dogs receiving heparin (Table 5).[7] However, this study is limited by less rigorous inclusion criteria and is confounded by lack of control
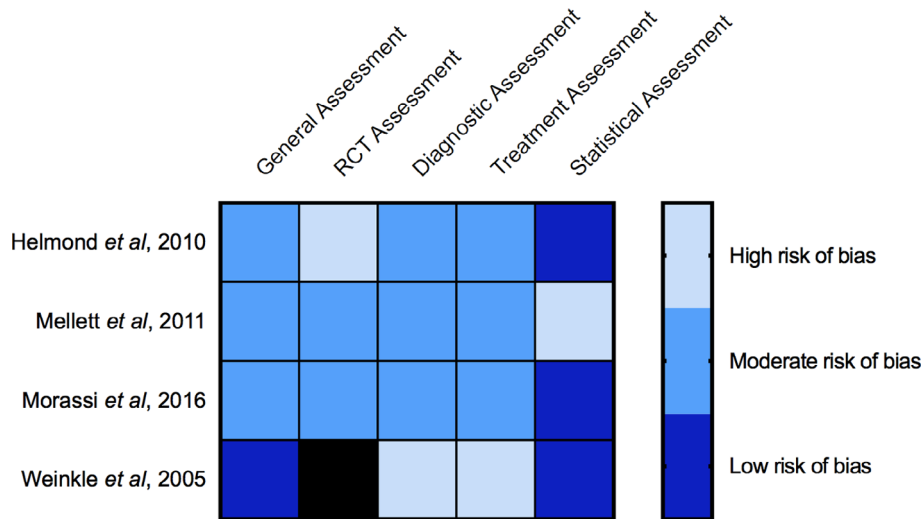
FIGURE 8   Results of evidence quality assessment for studies investigating the use of antithrombotic drugs in dogs with immune-mediated hemolytic anemia (IMHA). RCT, randomized controlled trial

for illness severity (Figure 8). Other retrospective data (published only as an abstract[186]) suggest that individualized heparin dosing may provide superior thromboprophylaxis compared to aspirin. Among the antiplatelet drugs, 1 RCT detected no difference in mortality in dogs treated with ultra-low-dose aspirin, clopidogrel, or both (Table 5).[11] However, this study appears to have been underpowered and may have used an inappropriate statistical test for comparison of survival between groups (Figure 8).

3.11 | Supportive care and antimicrobial treatment for dogs with IMHA

An expanded version of this section, with further commentary and discussion of underlying evidence, is available in Supporting Information Appendix A5.

43. We suggest that gastroprotectant treatment is administered only to dogs with IMHA with either:

    ○ ongoing evidence of gastrointestinal ulceration (eg, melena), or
    ○ known or potential risk factors for development of ulcers and gastrointestinal bleeding.

Strength of recommendation: Weak

44. Where gastroprotectant treatment is indicated, we recommend administration of a proton pump inhibitor for a defined period of time, with discontinuation as soon as risk factors for ulcer development or bleeding abate.

Strength of recommendation: Strong

45. We recommend assessing the risk of infection with hemotropic or vector-borne pathogens in individual dogs presenting with IMHA, depending on their geographic location, lifestyle, and travel or importation history. In dogs considered to have a high risk of infection, we recommend empirical administration of appropriate antimicrobial drugs while awaiting the results of

definitive diagnostic tests for these pathogens. Such definitive diagnostic tests should be the basis of longer term antimicrobial treatment recommendations.

Strength of recommendation: Strong

3.12 | Monitoring of dogs in remission from IMHA

46. When a dog has recovered fully from IMHA and has stopped receiving all treatment, we suggest continued monitoring of CBCs or other hematologic measurements for 4 weeks to confirm treatment-free remission. Beyond this period of time, we suggest that regular CBCs or other hematologic measurements are not required. Nevertheless, owners should be encouraged to monitor for clinical signs associated with anemia and to contact a veterinarian immediately if these are observed.

Strength of recommendation: Weak

Rationale

Monitoring of dogs with IMHA after cessation of treatment is poorly described in the literature. However, in our experience, relapses tend to occur acutely, so routine CBCs would seem to be of little or no use in predicting them once the dog has recovered fully.

3.13 | Recommendations for future research

Further investigation of the pathogenesis and heterogeneity of IMHA in dogs is likely to identify novel biomarkers of disease activity and prognosis, as well as avenues for more specific and targeted treatment. Such studies also could explore the prevalence and extent of thrombosis in dogs with IMHA. Prospective, randomized, multicenter clinical trials are required to address a number of questions, including whether the addition of a second drug improves outcome in all, or only a subset of, patients; whether 1 drug is superior to another as an adjunct to glucocorticoids; and, whether 1 thromboprophylactic regimen is superior

to another. Intrinsic to successful treatment is the effective monitoring of disease outcomes. Therapeutic drug monitoring is recognized as a useful tool to optimize drug dosage, but evidence-based reference targets for pharmacokinetic and pharmacodynamic assays currently are lacking. A number of new and emerging treatments promise to deliver more rapid targeted control of aggressive autoimmune responses, but further work is required in this area before firm recommendations can be made. For example, the impact of therapeutic plasmapheresis on patient outcome, and how it should be used in individual patients, remains to be established.

Comparison of results derived from different studies will be facilitated by consistent reporting of diagnostic criteria, treatment regimens, and outcome measures. We therefore have developed a set of reporting guidelines for future studies of dogs with IMHA to improve consistency among reports (Supporting Information Appendix A6).

CONFLICT OF INTEREST DECLARATION

Andrew Mackin is associated with the Mississippi State University Pharmacodynamic Laboratory, which offers therapeutic drug monitoring of cyclosporine as a commercial assay. All other authors had no conflicts of interest to declare.

OFF-LABEL ANTIMICROBIAL DECLARATION

A number of the drugs described in this consensus statement are not licensed for veterinary use.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

James W. Swann https://orcid.org/0000-0001-7988-9997
Oliver A. Garden https://orcid.org/0000-0002-4133-9487
Claire L. Fellman https://orcid.org/0000-0003-4799-3372
Barbara Glanemann https://orcid.org/0000-0003-4830-7610
Robert Goggs https://orcid.org/0000-0001-7446-6987
Dana N. LeVine https://orcid.org/0000-0002-1089-5640
Andrew J. Mackin https://orcid.org/0000-0001-8465-0198

REFERENCES

1. Lewis RM, Schwartz RS, Gilmore CE. Autoimmune diseases in domestic animals. *Ann N Y Acad Sci*. 1965;124:178-200.
2. Piek CJ, van Spil WE, Junius G, Dekker A. Lack of evidence of a beneficial effect of azathioprine in dogs treated with prednisolone for idiopathic immune-mediated hemolytic anemia: a retrospective cohort study. *BMC Vet Res*. 2011;7:15.
3. Swann JW, Skelly BJ. Systematic review of evidence relating to the treatment of immune-mediated hemolytic anemia in dogs. *J Vet Intern Med*. 2013;27:1-9.
4. Miller SA, Hohenhaus AE, Hale AS. Case-control study of blood type, breed, sex, and bacteremia in dogs with immune-mediated hemolytic anemia. *J Am Vet Med Assoc*. 2004;224:232-235.
5. Swann JW, Skelly BJ. Evaluation of immunosuppressive regimens for immune-mediated haemolytic anaemia: a retrospective study of 42 dogs. *J Small Anim Pract*. 2011;52:353-358.
6. Wang A, Smith JR, Creevy KE. Treatment of canine idiopathic immune-mediated haemolytic anaemia with mycophenolate mofetil and glucocorticoids: 30 cases (2007 to 2011). *J Small Anim Pract*. 2013;54:399-404.
7. Weinkle TK, Center SA, Randolph JF, Warner KL, Barr SC, Erb HN. Evaluation of prognostic factors, survival rates, and treatment protocols for immune-mediated hemolytic anemia in dogs: 151 cases (1993-2002). *J Am Vet Med Assoc*. 2005;226:1869-1880.
8. Scott-Moncrieff JC, Treadwell NG, McCullough SM, Brooks MB. Hemostatic abnormalities in dogs with primary immune-mediated hemolytic anemia. *J Am Anim Hosp Assoc*. 2001;37:220-227.
9. Carr AP, Panciera DL, Kidd L. Prognostic factors for mortality and thromboembolism in canine immune-mediated hemolytic anemia: a retrospective study of 72 dogs. *J Vet Intern Med*. 2002;16:504-509.
10. Klein MK, Dow SW, Rosychuk RA. Pulmonary thromboembolism associated with immune-mediated hemolytic anemia in dogs: ten cases (1982-1987). *J Am Vet Med Assoc*. 1989;195:246-250.
11. Mellett AM, Nakamura RK, Bianco D. A prospective study of clopidogrel therapy in dogs with primary immune-mediated hemolytic anemia. *J Vet Intern Med*. 2011;25:71-75.
12. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med*. 2009;151:264-269. W264.
13. Ridgeon EE, Young PJ, Bellomo R, Mucchetti M, Lembo R, Landoni G. The fragility index in multicenter randomized controlled critical care trials. *Crit Care Med*. 2016;44:1278-1284.
14. Griebsch C, Arndt G, Kohn B. Evaluation of different prognostic markers in dogs with primary immune-mediated hemolytic anemia. *Berl Munch Tierarztl Wochenschr*. 2010;123:160-168.
15. Thompson MF, Scott-Moncrieff JC, Brooks MB. Effect of a single plasma transfusion on thromboembolism in 13 dogs with primary immune-mediated hemolytic anemia. *J Am Anim Hosp Assoc*. 2004; 40:446-454.
16. Burgess K, Moore A, Rand W, Cotter SM. Treatment of immune-mediated hemolytic anemia in dogs with cyclophosphamide. *J Vet Intern Med*. 2000;14:456-462.
17. Goggs R, Boag AK, Chan DL. Concurrent immune-mediated haemolytic anaemia and severe thrombocytopenia in 21 dogs. *Vet Rec*. 2008;163:323-327.
18. Grundy SA, Barton C. Influence of drug treatment on survival of dogs with immune-mediated hemolytic anemia: 88 cases (1989–1999). *J Am Vet Med Assoc*. 2001;218:543-546.
19. Mason N, Duval D, Shofer FS, Giger U. Cyclophosphamide exerts no beneficial effect over prednisone alone in the initial treatment of

1168 | Journal of Veterinary Internal Medicine

ACVIM
Open Access
American College of
Veterinary Internal Medicine

SWANN ET AL.

acute immune-mediated hemolytic anemia in dogs: a randomized controlled clinical trial. *J Vet Intern Med*. 2003;17:206-212.

20. Reimer ME, Troy GC, Warnick LD. Immune-mediated hemolytic anemia: 70 cases (1988-1996). *J Am Anim Hosp Assoc*. 1999;35:384-391.

21. Gerber B, Steger A, Hassig M, et al. Use of human intravenous immunoglobulin in dogs with primary immune mediated hemolytic anemia. *Schweiz Arch Tierh*. 2002;144:180-185.

22. Kellerman DL, Bruyette DS. Intravenous human immunoglobulin for the treatment of immune-mediated hemolytic anemia in 13 dogs. *J Vet Intern Med*. 1997;11:327-332.

23. Oggier D, Tomsa K, Mevissen M, Glaus T. Efficacy of the combination of glucocorticoids, mycophenolate-mofetil and human immunoglobulin for the therapy of immune mediated haemolytic anaemia in dogs. *Schweiz Arch Tierh*. 2018;160:171-178.

24. Park S, Kim H, Kang B, et al. Prognostic factors and efficacy of human intravenous immunoglobulin G in dogs with idiopathic immune-mediated hemolytic anemia: a retrospective study. *Korean J Vet Res*. 2016;56:139-145.

25. Whelan MF, O'Toole TE, Chan DL, et al. Use of human immunoglobulin in addition to glucocorticoids for the initial treatment of dogs with immune-mediated hemolytic anemia. *J Vet Emerg Crit Care*. 2009;19:158-164.

26. Helmond SE, Polzin DJ, Armstrong PJ, Finke M, Smith SA. Treatment of immune-mediated hemolytic anemia with individually adjusted heparin dosing in dogs. *J Vet Intern Med*. 2010;24:597-605.

27. Morassi A, Bianco D, Park E, Nakamura RK, White GA. Evaluation of the safety and tolerability of rivaroxaban in dogs with presumed primary immune-mediated hemolytic anemia. *J Vet Emerg Crit Care*. 2016;26:488-494.

28. Brozek JL, Akl EA, Compalati E, et al. Grading quality of evidence and strength of recommendations in clinical practice guidelines part 3 of 3. The GRADE approach to developing recommendations. *Allergy*. 2011;66:588-595.

29. Guyatt GH, Oxman AD, Vist GE, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ*. 2008;336:924-926.

30. Hill QA, Stamps R, Massey E, et al. The diagnosis and management of primary autoimmune haemolytic anaemia. *Br J Haematol*. 2017; 176:395-411.

31. Caviezel LL, Raj K, Giger U. Comparison of 4 direct Coombs' test methods with polyclonal antiglobulins in anemic and nonanemic dogs for in-clinic or laboratory use. *J Vet Intern Med*. 2014;28: 583-591.

32. Garden OA, Kidd L, Mexas AM, et al. American College of Veterinary Internal Medicine Consensus Statement on the diagnosis of immune-mediated hemolytic anemia in dogs and cats. *J Vet Intern Med*. 2019; In Press.

33. Holahan ML, Brown AJ, Drobatz KJ. The association of blood lactate concentration with outcome in dogs with idiopathic immune-mediated hemolytic anemia: 173 cases (2003-2006). *J Vet Emerg Crit Care*. 2010; 20:413-420.

34. Hann L, Brown DC, King LG, Callan MB. Effect of duration of packed red blood cell storage on morbidity and mortality in dogs after transfusion: 3,095 cases (2001-2010). *J Vet Intern Med*. 2014;28:1830-1837.

35. Maglaras CH, Koenig A, Bedard DL, Brainard BM. Retrospective evaluation of the effect of red blood cell product age on occurrence of acute transfusion-related complications in dogs: 210 cases (2010-2012). *J Vet Emerg Crit Care*. 2017;27:108-120.

36. Pawson P, Dowell FJ. Investigation of the effects of a polymerised bovine haemoglobin solution on tension in isolated canine saphenous artery. *J Small Anim Pract*. 2014;55:301-308.

37. Muir WW, Wellman ML. Hemoglobin solutions and tissue oxygenation. *J Vet Intern Med*. 2003;17:127-135.

38. Hughes GS Jr, Antal EJ, Locker PK, et al. Physiology and pharmacokinetics of a novel hemoglobin-based oxygen carrier in humans. *Crit Care Med*. 1996;24:756-764.

39. Zambelli AB, Leisewitz AL. A prospective, randomized comparison of Oxyglobin (HB-200) and packed red blood cell transfusion for canine babesiosis. *J Vet Emerg Crit Care*. 2009;19:102-112.

40. Klag AR, Giger U, Shofer FS. Idiopathic immune-mediated hemolytic anemia in dogs: 42 cases (1986-1990). *J Am Vet Med Assoc*. 1993; 202:783-788.

41. Feldman BF. Demographics of canine immune-mediated haemolytic anaemia in the southeastern United States. *Comp Haematol Int*. 1996;6:42-45.

42. Kidd L, Mackman N. Prothrombotic mechanisms and anticoagulant therapy in dogs with immune-mediated hemolytic anemia. *J Vet Emerg Crit Care*. 2013;23:3-13.

43. Squizzato A, Hunt BJ, Kinasewitz GT, et al. Supportive management strategies for disseminated intravascular coagulation. An international consensus. *Thromb Haemost*. 2016;115:896-904.

44. Bennett D, Finnett SL, Nash AS, Kirkham D. Primary autoimmune haemolytic anaemia in the dog. *Vet Rec*. 1981;109:150-153.

45. Day MJ. Serial monitoring of clinical, haematological and immunological parameters in canine autoimmune haemolytic anaemia. *J Small Anim Pract*. 1996;37:523-534.

46. Schwendenwein I. The autoimmune hemolytic anemia (AIHA) in dogs – a survey of the clinical picture, diagnosis, pathogenesis and therapy of 8 cases. *Wien Tierarztl Monatsschr*. 1988;75:121-127.

47. Behrend EN, Greco DS. Clinical applications of glucocorticoid therapy in nonendocrine diseases. In: Bonagura JD, Kirk RW, eds. *Current Veterinary Therapy XII: Small Animal Practice*. Philadelphia, PA: W.B. Saunders; 1995:406-413.

48. Cohn L. Glucocorticoid therapy. In: Ettinger SJ, Feldman E, eds. *Textbook of Veterinary Internal Medicine*. St. Louis, MO: W.B. Saunders; 2005:503-508.

49. Czock D, Keller F, Rasche FM, Häussler U. Pharmacokinetics and pharmacodynamics of systemically administered glucocorticoids. *Clin Pharmacokinet*. 2005;44:61-98.

50. West LD, Hart JR. Treatment of idiopathic immune-mediated hemolytic anemia with mycophenolate mofetil in five dogs. *J Vet Emerg Crit Care*. 2014;24:226-231.

51. Swann JW, Skelly BJ. Systematic review of prognostic factors for mortality in dogs with immune-mediated hemolytic anemia. *J Vet Intern Med*. 2015;29:7-13.

52. Goggs R, Dennis SG, Di Bella A, et al. Predicting outcome in dogs with primary immune-mediated hemolytic anemia: results of a multi-center case registry. *J Vet Intern Med*. 2015;29:1603-1610.

53. Piek CJ. Canine idiopathic immune-mediated haemolytic anaemia: a review with recommendations for future research. *Vet Q*. 2011;31: 129-141.

54. Piek CJ, Junius G, Dekker A, Schrauwen E, Slappendel RJ, Teske E. Idiopathic immune-mediated hemolytic anemia: treatment outcome and prognostic factors in 149 dogs. *J Vet Intern Med*. 2008;22: 366-373.

55. Panek CM, Nakamura RK, Bianco D. Use of enoxaparin in dogs with primary immune-mediated hemolytic anemia: 21 cases. *J Vet Emerg Crit Care*. 2015;25:273-277.

56. Husbands B, Polzin D, Armstrong PJ, et al. Prednisone and cyclosporine vs. prednisone alone for treatment of canine immune mediated hemolytic anemia (IMHA). *J Vet Intern Med*. 2004;18:389.

57. Gregory CR, Stewart A, Sturges B, et al. Leflunomide effectively treats naturally occurring immune-mediated and inflammatory diseases of dogs that are unresponsive to conventional therapy. *Transplant Proc*. 1998;30:4143-4148.

58. Sato M, Veir JK, Legare M, Lappin MR. A retrospective study on the safety and efficacy of leflunomide in dogs. *J Vet Intern Med*. 2017; 31:1502-1507.

59. Spurlock NK, Prittie JE. A review of current indications, adverse effects, and administration recommendations for intravenous immunoglobulin. *J Vet Emerg Crit Care*. 2011;21:471-483.

60. Scott-Moncrieff JC, Reagan WJ, Snyder PW, Glickman LT. Intravenous administration of human immune globulin in dogs with immune-mediated hemolytic anemia. *J Am Vet Med Assoc*. 1997;210:1623-1627.

61. Link M, Dorsch R. Therapy of immune-mediated haemolytic anaemia in the dog using human immunoglobulin. *Tierarztl Prax Ausg K Kleintiere Heimtiere*. 2001;29:229-233.

62. Gregory CR, Kyles AE, Bernsteen L, et al. Results of clinical renal transplantation in 15 dogs using triple drug immunosuppressive therapy. *Vet Surg*. 2006;35:105-112.

63. Hopper K, Mehl ML, Kass PH, Kyles A, Gregory CR. Outcome after renal transplantation in 26 dogs. *Vet Surg*. 2012;41:316-327.

64. Dye TL, Diehl KJ, Wheeler SL, Westfall DS. Randomized, controlled trial of budesonide and prednisone for the treatment of idiopathic inflammatory bowel disease in dogs. *J Vet Intern Med*. 2013;27:1385-1391.

65. Rhoades AC, Vernau W, Kass PH, Herrera MA, Sykes JE. Comparison of the efficacy of prednisone and cyclosporine for treatment of dogs with primary immune-mediated polyarthritis. *J Am Vet Med Assoc*. 2016;248:395-404.

66. Masters AK, Berger DJ, Ware WA, et al. Effects of short-term anti-inflammatory glucocorticoid treatment on clinicopathologic, echocardiographic, and hemodynamic variables in systemically healthy dogs. *Am J Vet Res*. 2018;79:411-423.

67. Campbell KL, Latimer KS. Transient diabetes mellitus associated with prednisone therapy in a dog. *J Am Vet Med Assoc*. 1984;185:299-301.

68. Jeffers JG, Shanley KJ, Schick RO. Diabetes mellitus induced in a dog after administration of corticosteroids and methylprednisolone pulse therapy. *J Am Vet Med Assoc*. 1991;199:77-80.

69. Ohta H, Morita T, Yokoyama N, et al. Serial measurement of pancreatic lipase immunoreactivity concentration in dogs with immune-mediated disease treated with prednisolone. *J Small Anim Pract*. 2017;58:342-347.

70. McDonough AK, Curtis JR, Saag KG. The epidemiology of glucocorticoid-associated adverse events. *Curr Opin Rheumatol*. 2008;20:131-137.

71. Torres SM, Diaz SF, Nogueira SA, et al. Frequency of urinary tract infection among dogs with pruritic disorders receiving long-term glucocorticoid treatment. *J Am Vet Med Assoc*. 2005;227:239-243.

72. Peterson AL, Torres SM, Rendahl A, et al. Frequency of urinary tract infection in dogs with inflammatory skin disorders treated with ciclosporin alone or in combination with glucocorticoid therapy: a retrospective study. *Vet Derm*. 2012;23:201-e243.

73. Weese JS, Blondeau JM, Boothe D, et al. Antimicrobial use guidelines for treatment of urinary tract disease in dogs and cats: antimicrobial guidelines working group of the international society for companion animal infectious diseases. *Vet Med Int*. 2011;2011:263768.

74. Nicolle LE, Bradley S, Colgan R, et al. Infectious Diseases Society of America guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. *Clin Infect Dis*. 2005;40:643-654.

75. Wallisch K, Trepanier LA. Incidence, timing, and risk factors of azathioprine hepatotoxicosis in dogs. *J Vet Intern Med*. 2015;29:513-518.

76. Rodriguez DB, Mackin A, Easley R, et al. Relationship between red blood cell thiopurine methyltransferase activity and myelotoxicity in dogs receiving azathioprine. *J Vet Intern Med*. 2004;18:339-345.

77. Kaneko N, Une S, Itamoto K, et al. A case of long-term inhibition of erythropoiesis by azathioprine in a dog. *Yamaguchi J Vet Med*. 2004;31:37-40.

78. Moriello KA, Bowen D, Meyer DJ. Acute pancreatitis in two dogs given azathioprine and prednisone. *J Am Vet Med Assoc*. 1987;191:695-696.

79. Houston DM, Taylor JA. Acute pancreatitis and bone marrow suppression in a dog given azathioprine. *Can Vet J*. 1991;32:496-497.

80. Kidd LB, Salavaggione OE, Szumlanski CL, Miller JL, Weinshilboum RM, Trepanier L. Thiopurine methyltransferase activity in red blood cells of dogs. *J Vet Intern Med*. 2004;18:214-218.

81. Steffan J, Parks C, Seewald W, North American Veterinary Dermatology Cyclosporine Study Group. Clinical trial evaluating the efficacy and safety of cyclosporine in dogs with atopic dermatitis. *J Am Vet Med Assoc*. 2005;226:1855-1863.

82. Steffan J, Strehlau G, Maurer M, Rohlfs A. Cyclosporin A pharmacokinetics and efficacy in the treatment of atopic dermatitis in dogs. *J Vet Pharmacol Ther*. 2004;27:231-238.

83. Diesel A, Moriello K. Medical Management of cyclosporine-induced gingival overgrowth using oral azithromycin in six dogs. *Vet Sci*. 2015;2:13-22.

84. Namikawa K, Maruo T, Honda M, Hirata H, Lynch J, Madarame H. Gingival overgrowth in a dog that received long-term cyclosporine for immune-mediated hemolytic anemia. *Can Vet J*. 2012;53:67-70.

85. Nam HS, McAnulty JF, Kwak HH, et al. Gingival overgrowth in dogs associated with clinically relevant cyclosporine blood levels: observations in a canine renal transplantation model. *Vet Surg*. 2008;37:247-253.

86. Adamo PF, Rylander H, Adams WM. Ciclosporin use in multi-drug therapy for meningoencephalomyelitis of unknown aetiology in dogs. *J Small Anim Pract*. 2007;48:486-496.

87. Calne RY, White DJ, Pentlow BD, et al. Cyclosporin A: preliminary observations in dogs with pancreatic duodenal allografts and patients with cadaveric renal transplants. *Transplant Proc*. 1979;11:860-864.

88. Vaden SL, Breitschwerdt EB, Armstrong PJ, et al. The effects of cyclosporine versus standard care in dogs with naturally occurring glomerulonephritis. *J Vet Intern Med*. 1995;9:259-266.

89. McAtee BB, Cummings KJ, Cook AK, et al. Opportunistic invasive cutaneous fungal infections associated with administration of cyclosporine to dogs with immune-mediated disease. *J Vet Intern Med*. 2017;31:1724-1729.

90. Dowling SR, Webb J, Foster JD, Ginn J, Foy DS, Trepanier LA. Opportunistic fungal infections in dogs treated with ciclosporin and glucocorticoids: eight cases. *J Small Anim Pract*. 2016;57:105-109.

91. Patricelli AJ, Hardie RJ, McAnulty JE. Cyclosporine and ketoconazole for the treatment of perianal fistulas in dogs. *J Am Vet Med Assoc*. 2002;220:1009-1016.

92. Dahlinger J, Gregory C, Bea J. Effect of ketoconazole on cyclosporine dose in healthy dogs. *Vet Surg*. 1998;27:64-68.

93. Thomason J, Lunsford K, Stokes J, et al. The effects of cyclosporine on platelet function and cyclooxygenase expression in normal dogs. *J Vet Intern Med*. 2012;26:1389-1401.

94. Ackermann AL, May ER, Frank LA. Use of mycophenolate mofetil to treat immune-mediated skin disease in 14 dogs - a retrospective evaluation. *Vet Dermatol*. 2017;28:195-e144.

95. Barnoon I, Shamir MH, Aroch I, et al. Retrospective evaluation of combined mycophenolate mofetil and prednisone treatment for meningoencephalomyelitis of unknown etiology in dogs: 25 cases (2005-2011). *J Vet Emerg Crit Care*. 2016;26:116-124.

96. Dewey CW, Cerda-Gonzalez S, Fletcher DJ, et al. Mycophenolate mofetil treatment in dogs with serologically diagnosed acquired myasthenia gravis: 27 cases (1999-2008). *J Am Vet Med Assoc*. 2010;236:664-668.

97. Yau VK, Bianco D. Treatment of five haemodynamically stable dogs with immune-mediated thrombocytopenia using mycophenolate mofetil as single agent. *J Small Anim Pract*. 2014;55:330-333.

98. Young WM, Oh A, Williams JG, et al. Clinical therapeutic efficacy of mycophenolate mofetil in the treatment of SARDS in dogs – a prospective open-label pilot study. *Vet Ophthalmol*. 2018;21:565-576.

99. Cummings FO, Rizzo SA. Treatment of presumptive primary immune-mediated thrombocytopenia with mycophenolate mofetil versus cyclosporine in dogs. *J Small Anim Pract*. 2017;58:96-102.

100. Woolcock AD, Wang A, Haley A, Kent M, Creevy KE, Platt SR. Treatment of canine meningoencephalomyelitis of unknown aetiology with mycophenolate mofetil and corticosteroids: 25 cases (2007-2012). *Vet Med Sci*. 2016;2:125-135.

101. Wang K, Zhang H, Li Y, et al. Safety of mycophenolate mofetil versus azathioprine in renal transplantation: a systematic review. *Transplant Proc*. 2004;36:2068-2070.

102. Neff RT, Hurst FP, Falta EM, et al. Progressive multifocal leukoencephalopathy and use of mycophenolate mofetil after kidney transplantation. *Transplantation*. 2008;86:1474-1478.

103. Colopy SA, Baker TA, Muir P. Efficacy of leflunomide for treatment of immune-mediated polyarthritis in dogs: 14 cases (2006-2008). *J Am Vet Med Assoc*. 2010;236:312-318.

104. Fukushima K, Eguchi N, Ohno K, et al. Efficacy of leflunomide for treatment of refractory inflammatory colorectal polyps in 15 Miniature Dachshunds. *J Vet Med Sci*. 2016;78:265-269.

105. Gregory CR. Immunosuppressive approaches to the prevention of graft vascular disease. *Transplant Proc*. 1998;30:878-880.

106. Feldman BF, Handagama P, Lubberink AA. Splenectomy as adjunctive therapy for immune-mediated thrombocytopenia and hemolytic anemia in the dog. *J Am Vet Med Assoc*. 1985;187:617-619.

107. Horgan JE, Roberts BK, Schermerhorn T. Splenectomy as an adjunctive treatment for dogs with immune-mediated hemolytic anemia: ten cases (2003-2006). *J Vet Emerg Crit Care*. 2009;19:254-261.

108. Goggs R, Blais MC, Brainard BM, et al. American College of Veterinary Emergency and Critical Care (ACVECC) Consensus on the Rational Use of Antithrombotics in Veterinary Critical Care (CURATIVE) guidelines: small animal. *J Vet Emerg Crit Care*. 2019;29:12-36.

109. de Laforcade A. Diseases associated with thrombosis. *Top Companion Anim Med*. 2012;27:59-64.

110. Laurenson MP, Hopper K, Herrera MA, Johnson EG. Concurrent diseases and conditions in dogs with splenic vein thrombosis. *J Vet Intern Med*. 2010;24:1298-1304.

111. Respess M, O'Toole TE, Taeymans O, Rogers CL, Johnston A, Webster CRL. Portal vein thrombosis in 33 dogs: 1998-2011. *J Vet Intern Med*. 2012;26:230-237.

112. Vanwinkle TJ, Bruce E. Thrombosis of the portal-vein in 11 dogs. *Vet Pathol*. 1993;30:28-35.

113. Bunch SE, Metcalf MR, Crane SW, Cullen JM. Idiopathic pleural effusion and pulmonary thromboembolism in a dog with autoimmune hemolytic anemia. *J Am Vet Med Assoc*. 1989;195:1748-1753.

114. Johnson LR, Lappin MR, Baker DC. Pulmonary thromboembolism in 29 dogs: 1985-1995. *J Vet Intern Med*. 1999;13:338-345.

115. Piek CJ, Brinkhof B, Teske E, et al. High intravascular tissue factor expression in dogs with idiopathic immune-mediated haemolytic anaemia. *Vet Immunol Immunopathol*. 2011;144:346-354.

116. Kidd L, Geddings J, Hisada Y, et al. Procoagulant microparticles in dogs with immune-mediated hemolytic anemia. *J Vet Intern Med*. 2015;29:908-916.

117. Weiss DJ, Brazzell JL. Detection of activated platelets in dogs with primary immune-mediated hemolytic anemia. *J Vet Intern Med*. 2006;20:682-686.

118. Fenty RK, Delaforcade AM, Shaw SE, et al. Identification of hypercoagulability in dogs with primary immune-mediated hemolytic anemia by means of thromboelastography. *J Am Vet Med Assoc*. 2011; 238:463-467.

119. Goggs R, Wiinberg B, Kjelgaard-Hansen M, Chan DL. Serial assessment of the coagulation status of dogs with immune-mediated haemolytic anaemia using thromboelastography. *Vet J*. 2012;191:347-353.

120. Bauer N, Moritz A. Characterisation of changes in the haemostasis system in dogs with thrombosis. *J Small Anim Pract*. 2013;54: 129-136.

121. McManus PM, Craig LE. Correlation between leukocytosis and necropsy findings in dogs with immune-mediated hemolytic anemia: 34 cases (1994-1999). *J Am Vet Med Assoc*. 2001;218:1308-1313.

122. Flint SK, Abrams-Ogg ACG, Kruth SA, Bersenas AM, Wood RD. Independent and combined effects of prednisone and acetylsalicylic acid on thromboelastography variables in healthy dogs. *Am J Vet Res*. 2011;72:1325-1332.

123. Flint S, Abrams-Ogg A, Kruth S, et al. Thromboelastography in dogs with immune-mediated hemolytic anemia treated with prednisone, azathioprine and low-dose aspirin. *J Vet Intern Med*. 2010;24:681.

124. Tsuchiya R, Akutsu Y, Ikegami A, et al. Prothrombotic and inflammatory effects of intravenous administration of human immunoglobulin G in dogs. *J Vet Intern Med*. 2009;23:1164-1169.

125. Orcutt ES, Lee JA, Bianco D. Immune-mediated hemolytic anemia and severe thrombocytopenia in dogs: 12 cases (2001-2008). *J Vet Emerg Crit Care*. 2010;20:338-345.

126. Bateman SW, Mathews KA, Abrams-Ogg AC, et al. Diagnosis of disseminated intravascular coagulation in dogs admitted to an intensive care unit. *J Am Vet Med Assoc*. 1999;215:798-804.

127. Wiinberg B, Jensen AL, Johansson PI, Rozanski E, Tranholm M, Kristensen AT. Thromboelastographic evaluation of hemostatic function in dogs with disseminated intravascular coagulation. *J Vet Intern Med*. 2008;22:357-365.

128. Palareti G. Direct oral anticoagulants and bleeding risk (in comparison to vitamin K antagonists and heparins), and the treatment of bleeding. *Semin Hematol*. 2014;51:102-111.

129. Kol A, Nelson RW, Gosselin RC, Borjesson DL. Characterization of thrombelastography over time in dogs with hyperadrenocorticism. *Vet J*. 2013;197:675-681.

130. Burns MG, Kelly AB, Hornof WJ, et al. Pulmonary-artery thrombosis in 3 dogs with hyperadrenocorticism. *J Am Vet Med Assoc*. 1981; 178:388-393.

131. Aird WC. Vascular bed-specific thrombosis. *J Thromb Haemost*. 2007;5:283-291.

132. Mackman N. New insights into the mechanisms of venous thrombosis. *J Clin Invest*. 2012;122:2331-2336.

133. Ridyard AE, Shaw DJ, Milne EM. Evaluation of platelet activation in canine immune-mediated haemolytic anaemia. *J Small Anim Pract*. 2010;51:296-304.

134. Zoia A, Gerou-Ferriani M, Drigo M, Caldin M. Case-control study of plasma mean platelet component concentration and survival analysis for dogs with immune-mediated hemolytic anemia. *J Am Vet Med Assoc*. 2018;252:1384-1392.

135. Hoffman M, Monroe DM. A cell-based model of hemostasis. *Thromb Haemost*. 2001;85:958-965.

136. Smith SA. The cell-based model of coagulation. *J Vet Emerg Crit Care*. 2009;19:3-10.

137. Lowe GD. Common risk factors for both arterial and venous thrombosis. *Br J Haematol*. 2008;140:488-495.

138. Agnelli G, Becattini C. Venous thromboembolism and atherosclerosis: common denominators or different diseases? *J Thromb Haemost*. 2006;4:1886-1890.

139. Lowe GD. Arterial disease and venous thrombosis: are they related, and if so, what should we do about it? *J Thromb Haemost*. 2006;4: 1882-1885.

140. Sobieszczyk P, Fishbein MC, Goldhaber SZ. Acute pulmonary embolism: don't ignore the platelet. *Circulation*. 2002;106:1748-1749.

141. Cooley BC, Herrera AJ. Cross-modulatory effects of clopidogrel and heparin on platelet and fibrin incorporation in thrombosis. *Blood Coagul Fibrinolysis*. 2013;24:593-598.

142. Group PEPPtC. Prevention of pulmonary embolism and deep vein thrombosis with low dose aspirin: Pulmonary Embolism Prevention (PEP) trial. *Lancet*. 2000;355:1295-1302.

143. Brighton TA, Eikelboom JW, Mann K, et al. Low-dose aspirin for preventing recurrent venous thromboembolism. *N Engl J Med*. 2012; 367:1979-1987.

144. Larocca A, Cavallo F, Bringhen S, et al. Aspirin or enoxaparin thromboprophylaxis for patients with newly diagnosed multiple myeloma treated with lenalidomide. *Blood*. 2012;119:933-939.

145. Matsuo H, Matsumura M, Nakajima Y, et al. Frequency of deep vein thrombosis among hospitalized non-surgical Japanese patients with congestive heart failure. *J Cardiol*. 2014;64:430-434.

146. Guyatt GH, Akl EA, Crowther M, Gutterman DD, Schünemann HJ, American College of Chest Physicians Antithrombotic Therapy and Prevention of Thrombosis Panel. Executive summary: antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest*. 2012;141:7S-47S.

147. Kearon C, Akl EA, Ornelas J, et al. Antithrombotic therapy for VTE disease: CHEST guideline and expert panel report. *Chest*. 2016;149: 315-352.

148. Afshari A, Ageno W, Ahmed A, et al. European guidelines on perioperative venous thromboembolism prophylaxis: executive summary. *Eur J Anaesthesiol*. 2018;35:77-83.

149. Breuhl EL, Moore G, Brooks MB, Scott-Moncrieff JC. A prospective study of unfractionated heparin therapy in dogs with primary immune-mediated hemolytic anemia. *J Am Anim Hosp Assoc*. 2009;45:125-133.

150. Brainard BM, Koenig A, Babski DM, Blong AE, Scherk JR. Viscoelastic pharmacodynamics after dalteparin administration to healthy dogs. *Am J Vet Res*. 2012;73:1577-1582.

151. Grebe S, Jacobs C, Kietzmann M, et al. Pharmacokinetics of the low molecular weight heparin Fragmin (R) D in dogs. *Berl Munch Tierarztl Wochenschr*. 2000;113:103-107.

152. Lunsford KV, Mackin AJ, Langston VC, Brooks M. Pharmacokinetics of subcutaneous low molecular weight heparin (enoxaparin) in dogs. *J Am Anim Hosp Assoc*. 2009;45:261-267.

153. Pouzot-Nevoret C, Barthelemy A, Cluzel M, et al. Enoxaparin has no significant anticoagulation activity in healthy Beagles at a dose of 0.8 mg/kg four times daily. *Vet J*. 2016;210:98-100.

154. Mestre M, Clairefond P, Mardiguian J, Trillou M, le Fur G, Uzan A. Comparative effects of heparin and PK 10169, a low molecular weight fraction, in a canine model of arterial thrombosis. *Thromb Res*. 1985;38:389-399.

155. Morris TA, Marsh JJ, Konopka R, Pedersen CA, Chiles PG. Antithrombotic efficacies of enoxaparin, dalteparin, and unfractionated heparin in venous thrombo-embolism. *Thromb Res*. 2000; 100:185-194.

156. Lynch AM, deLaforcade AM, Sharp CR. Clinical experience of anti-Xa monitoring in critically ill dogs receiving dalteparin. *J Vet Emerg Crit Care*. 2014;24:421-428.

157. Green RA. Activated coagulation time in monitoring heparinized dogs. *Am J Vet Res*. 1980;41:1793-1797.

158. Hellebrekers LJ, Slappendel RJ, van den Brom WE. Effect of sodium heparin and antithrombin III concentration on activated partial thromboplastin time in the dog. *Am J Vet Res*. 1985;46:1460-1462.

159. Mischke R. Heparin in vitro sensitivity of the activated partial thromboplastin time in canine plasma depends on reagent. *J Vet Diagn Invest*. 2003;15:588-591.

160. Babski DM, Brainard BM, Ralph AG, Pittman JR, Koenig A. Sonoclot (R) evaluation of single- and multiple-dose subcutaneous unfractionated heparin therapy in healthy adult dogs. *J Vet Intern Med*. 2012; 26:631-638.

161. Jessen LR, Wiinberg B, Jensen AL, et al. In vitro heparinization of canine whole blood with low molecular weight heparin (dalteparin) significantly and dose-dependently prolongs heparinase-modified tissue factor-activated thromboelastography parameters and prothrombinase-induced clotting time. *Vet Clin Pathol*. 2008;37: 363-372.

162. McLaughlin CM, Marks SL, Dorman DC, et al. Thromboelastographic monitoring of the effect of unfractionated heparin in healthy dogs. *J Vet Emerg Crit Care*. 2017;27:71-81.

163. Allegret V, Dunn M, Bedard C. Monitoring unfractionated heparin therapy in dogs by measuring thrombin generation. *Vet Clin Pathol*. 2011;40:24-31.

164. Gara-Boivin C, Del Castillo JRE, Dunn ME, et al. Effect of dalteparin administration on thrombin generation kinetics in healthy dogs. *Vet Clin Pathol*. 2017;46:269-277.

165. Hanel RM. Heparin Monitoring in Critically Ill Dogs, Proceedings of the 2017 ACVIM Forum, June 2017, Washington, D.C.

166. Hanel RM, Birkenheuer AJ, Hansen B, et al. Thromboelastography or activated partial thromboplastin time for heparin anticoagulation to prevent thrombosis: the TOP HATT trial. *J Vet Emerg Crit Care*. 2017;27(S1):S4.

167. Wessler S, Morris LE. Studies in intravascular coagulation. IV. The effect of heparin and dicumarol on serum-induced venous thrombosis. *Circulation*. 1955;12:553-556.

168. Basu D, Gallus A, Hirsh J, Cade J. A prospective study of the value of monitoring heparin treatment with the activated partial thromboplastin time. *N Engl J Med*. 1972;287:324-327.

169. Chiu HM, Hirsh J, Yung WL, Regoeczi E, Gent M. Relationship between the anticoagulant and antithrombotic effects of heparin in experimental venous thrombosis. *Blood*. 1977;49:171-184.

170. Kearon C, Akl EA, Comerota AJ, et al. Antithrombotic therapy for VTE disease antithrombotic therapy and prevention of thrombosis, 9th ed: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest*. 2012;141:e419S-e496S.

171. Brainard BM, Kleine SA, Papich MG, Budsberg SC. Pharmacodynamic and pharmacokinetic evaluation of clopidogrel and the carboxylic acid metabolite SR 26334 in healthy dogs. *Am J Vet Res*. 2010;71:822-830.

172. Borgarelli M, Lanz O, Pavlisko N, et al. Mitral valve repair in dogs using an ePTFE chordal implantation device: a pilot study. *J Vet Cardiol*. 2017;19:256-267.

173. Hasa AA, Schmaier AH, Warnock M, et al. Thrombostatin inhibits cyclic flow variations in stenosed canine coronary arteries. *Thromb Haemost*. 2001;86:1296-1304.

174. van Giezen JJ, Berntsson P, Zachrisson H, et al. Comparison of ticagrelor and thienopyridine P2Y(12) binding characteristics and antithrombotic and bleeding effects in rat and dog models of thrombosis/hemostasis. *Thromb Res*. 2009;124:565-571.

175. Bjorkman JA, Zachrisson H, Forsberg GB, et al. High-dose aspirin in dogs increases vascular resistance with limited additional anti-platelet effect when combined with potent P2Y12 inhibition. *Thromb Res*. 2013;131:313-319.

176. Dyken ML, Campbell RL, Muller J, et al. Effect of aspirin on experimentally induced arterial thrombosis during the healing phase. *Stroke*. 1973;4:387-389.

177. Dudley A, Thomason J, Fritz S, et al. Cyclooxygenase expression and platelet function in healthy dogs receiving low-dose aspirin. *J Vet Intern Med*. 2013;27:141-149.

178. Sharpe KS, Center SA, Randolph JF, et al. Influence of treatment with ultralow-dose aspirin on platelet aggregation as measured by whole blood impedance aggregometry and platelet P-selectin expression in clinically normal dogs. *Am J Vet Res*. 2010;71:1294-1304.

179. Brainard BM, Meredith CP, Callan MB, et al. Changes in platelet function, hemostasis, and prostaglandin expression after treatment with nonsteroidal anti-inflammatory drugs with various cyclooxygenase selectivities in dogs. *Am J Vet Res*. 2007;68:251-257.

180. Nielsen LA, Zois NE, Pedersen HD, et al. Platelet function in dogs: breed differences and effect of acetylsalicylic acid administration. *Vet Clin Pathol*. 2007;36:267-273.

181. McLewee N, Archer T, Wills R, et al. Effects of aspirin dose escalation on platelet function and urinary thromboxane and prostacyclin levels in normal dogs. *J Vet Pharmacol Ther*. 2017;41:60-67.

182. Hoh CM, Smith SA, McMichael MA, et al. Evaluation of effects of low-dose aspirin administration on urinary thromboxane metabolites in healthy dogs. *Am J Vet Res*. 2011;72:1038-1045.

183. Grauer GF, Rose BJ, Toolan L, Thrall MA, Colgan SP. Effects of low-dose aspirin and specific thromboxane synthetase inhibition on whole blood platelet aggregation and adenosine triphosphate secretion in healthy dogs. *Am J Vet Res*. 1992;53: 1631-1635.

184. Heather Graham A, Leib MS. Effects of prednisone alone or prednisone with ultralow-dose aspirin on the gastroduodenal mucosa of healthy dogs. *J Vet Intern Med*. 2009;23:482-487.

185. Whittemore J, Mooney A, Mawby D, Thomason J. Platelet function and endoscopic changes after clopidogrel, aspirin, prednisone, or combination therapy in dogs. *J Vet Intern Med*. 2017;31: 1282.

186. Orcutt E, Armstrong P, Helmond S, Smith S. Comparison of individually monitored unfractionated heparin versus low-dose aspirin on survival of dogs with immune mediated hemolytic anemia. *J Vet Intern Med*. 2009;23:693.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.