

# Canine autoimmune hemolytic anemia: management challenges

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**Abstract:** Immune-mediated hemolytic anemia is one of the most common manifestations of canine immune-mediated disease, yet treatment regimens remain nonstandardized and, in some cases, controversial. The main reason for this, as for most diseases in veterinary medicine, is the lack of large-scale placebo-controlled trials so that the efficacy of one treatment over another can be established. Most of the evidence used for treatment comes from retrospective studies and from personal preference and experience, and because of this, treatment regimens tend to vary among institutions and individual clinicians. Management of immune-mediated hemolytic anemia includes immunosuppression, thromboprophylaxis, and supportive care measures to help prevent and treat concurrent conditions.

**Keywords:** IMHA, canine immune-mediated disease, management regimens

## Introduction

Immune-mediated hemolytic anemia (IMHA) is considered to be the most common autoimmune disease in dogs<sup>1</sup> and is encountered in first opinion and specialist practices in North America and Europe. Clinical features of the disease are the result of a spontaneous autoimmune response directed against normal glycoprotein molecules on the surface of the erythrocyte.<sup>2</sup> Production of autoreactive antibodies during this response results in destruction of erythrocytes, either by complement-mediated lysis (so-called intravascular hemolysis) or due to phagocytosis by cells of the monocyte–phagocyte system in the liver and spleen (extravascular hemolysis). These processes result in anemia and, in some cases, prehepatic icterus due to accumulation of unconjugated bilirubin.

Following diagnosis, many dogs with IMHA receive blood products that are intended to achieve cardiovascular stability by improving the delivery of oxygen to peripheral tissues. A number of clinical parameters, grouped together as the Anemic Dog Clinical Assessment Score, have been evaluated as guides for administration of transfusions<sup>3</sup>; however, there is currently no consensus regarding the optimal trigger for this procedure in dogs. The availability of blood products varies according to geographical location and type of veterinary practice, with some institutions maintaining colonies of donor animals and others relying on client-owned dogs or on charitable blood donation services for their supply.<sup>4</sup>

Glucocorticoid products are commonly used for treatment of IMHA in dogs; in a recent systematic review of studies produced at tertiary referral institutions, we found that these drugs had been administered to all of the dogs included in the review.<sup>5</sup>

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Several other immunosuppressive drugs also have been used to treat the disease, including azathioprine,<sup>6,7</sup> leflunomide,<sup>8</sup> ciclosporin,<sup>9,10</sup> and mycophenolate mofetil.<sup>11,12</sup> There is currently no consensus among veterinarians regarding the optimal immunosuppressive regimen that should be used after a dog has been diagnosed with IMHA, and there has been considerable variation in the choice, dosage, duration, and rate of reduction of drugs used in studies published in the past 20 years.<sup>5</sup>

IMHA is considered to have a poor prognosis in dogs, with mortality rates of 50%–70% reported in older studies,<sup>6,13</sup> and rates of ~30%–40% in more recent studies,<sup>14</sup> presumably reflecting improvements in awareness of disease, speed of diagnosis, and availability of supportive care and blood products. A number of previous studies have investigated the associations between survival and the biochemical, hematologic, or clinical parameters that can be measured at presentation to determine whether these might have prognostic value. Review of the studies that assessed prognostic factors using a multivariable statistical model revealed that serum bilirubin and urea concentrations had each been significantly associated with survival in more than one previous study,<sup>15</sup> suggesting that these variables might be helpful in guiding clinical decisions in future.

## Predicting disease outcome using scoring systems

Because clinical presentation and response to treatment are variable in dogs with IMHA, a number of scoring systems have been developed to estimate the severity of disease and prognosis for dogs when they are diagnosed. These scores have been generated by investigating associations between individual clinicopathological variables and outcome, measured as survival time after diagnosis or percentage mortality at a defined time point. Summaries of two different prognostic scores are presented in Table 1. In a recent study, Goggs et al<sup>14</sup> found that a combination of the American Society of Anesthesiologists' clinical grade and serum concentrations of bilirubin and urea or creatinine predicted mortality with greater accuracy than either of the scores described by Whelan et al<sup>16</sup> or Ishihara et al<sup>17</sup> in a sample of 276 dogs in the British Isles, but the authors unfortunately did not present their own model in the same form of a user-friendly score. An additional study revealed that the score developed in Japan did not predict survival in dogs with IMHA in Australia,<sup>18</sup> highlighting the importance of using prognostic tools in a population for which they have been validated.

**Table 1** Summary of two different prognostic scores developed for use in dogs with immune-mediated hemolytic anemia

Score	Parameter	Definition	Score
CHAOS (Whelan et al) <sup>16</sup>	Age	≥7 years	2
	Rectal temperature	≥38.9°C	1
	Persistent agglutination after dilution in saline	Positive	1
	Serum albumin concentration	<30 g/L	1
	Serum bilirubin concentration	≥85.5 μmol/L	2
	Total possible score		7
Ishihara et al <sup>17</sup>	Sex	Male	1
	Season	Warm	1
	Packed cell volume	<20%	1
	Platelet count	<200×10 <sup>3</sup> /μL	1
	Serum total protein concentration	<6 g/dL	1
	Total possible score		5

**Abbreviation:** CHAOS, canine haemolytic anaemia objective score.

Development of “intravascular hemolysis”, a term used inconsistently to describe some combination of hemoglobinuria, hemolysed plasma, and presence of persistent agglutination of red blood cells, has traditionally been considered an indicator of a poor prognosis in dogs with IMHA. However, this perception is not supported by the majority of clinical studies of factors associated with survival: only two studies indicated using univariate analyses that autoagglutination was associated with decreased survival. One of these studies subsequently showed no difference between cases that had or did not have undefined “intravascular hemolysis”,<sup>19</sup> while the other demonstrated that agglutination was associated with survival to discharge but not to 1 year after diagnosis.<sup>20</sup> In contrast, serum total bilirubin concentration, which is an indicator of the severity of either intravascular or extravascular hemolysis, has been associated consistently with survival in several previous studies that utilized either univariate or multivariate analyses, albeit with considerable variation in the apparent size of the effect.<sup>10,13–15,19,21–24</sup> Consequently, we believe that this variable is likely to be the most useful for the clinician in guiding therapy and estimating prognosis, particularly because it is easily measured in the majority of primary care and specialist practices.

## Complications of conventional treatment

### The use of glucocorticoids and their side effects

Corticosteroids, mainly the glucocorticoid – prednisolone, have been the mainstay of treatment for many years. No

protocols are described that do not include corticosteroids, probably because many would consider it unethical to conduct a treatment trial that withholds this class of drugs.<sup>25</sup> When other drugs are assessed for their usefulness in IMHA, they are given in conjunction with corticosteroids and compared to steroids alone. There have been no known multiple-patient published trials that have shown that any other immunosuppressive drug can be used alone in the management of IMHA, although anecdotal reports have suggested that this may be possible and ciclosporin has been used for this purpose by these authors in circumstances when owners have refused to use steroid therapy due to the side effects encountered.

Inflammatory responses are regulated by creating a balance between signals that stimulate and those that suppress. Endogenous glucocorticoids have a physiological role in suppression/modulation of inflammatory responses. Chronic autoimmune and inflammatory diseases develop when the stimuli to perpetuate the immune response outweigh the ability of endogenous glucocorticoids to suppress the response. This results in the hyperactivation of the immune system characteristic of these diseases. Treatment with synthetic glucocorticoids is used to control immune-mediated disease, taking advantage of the fact that endogenous glucocorticoids regulate immune responses.

Although steroids are undeniably effective at treating IMHA, they do so with a number of potentially severe side effects that have an impact on an owner's willingness to continue with treatment. Steroids cause polydipsia and polyuria through their interference with antidiuretic hormone function, polyphagia, panting, restlessness, muscle weakness and wasting, thromboembolic disease, and proteinuria. These signs of iatrogenic hyperadrenocorticism can be mild to severe and sometimes life threatening. In people, steroid use is much more restricted by side effects, and hypertension and osteoporosis are commonly seen. For this reason, it is rare that immune-mediated diseases in humans are managed with steroids alone, and other drugs, splenectomy, and monoclonal antibody treatments are used both for their efficacy and for their steroid-sparing effects.<sup>26</sup>

Steroids have the ability to worsen the clinical signs in an animal that already has signs of disease in another body system. An example of this would be the ability of steroid therapy to induce or worsen congestive heart failure in a dog with preexisting heart disease (eg, mitral valve endocardiosis or occult cardiomyopathy). In human medicine, the long-accepted recommendations are to avoid glucocorticoid administration in patients with heart failure due to the sodium and water retention they induce. More recently, it has been

suggested that glucocorticoids may actually act synergistically with diuretics in some patients to improve diuresis and therefore clinical well-being. This area has not been explored in dogs and, as such, specific recommendations are difficult to justify.<sup>27</sup>

The drugs and their dosing regimens commonly used for the management of IMHA along with other management options are summarized in Table 2.

## Opportunistic infections

Veterinarians managing dogs with IMHA may be concerned about the possibility of opportunistic infections when administering immunosuppressive drugs at high doses or in combinations, and a number of different infectious complications have been reported with the use of ciclosporin, alone or in combination with glucocorticoids. These include fungal infections,<sup>30</sup> bacterial urinary tract infections,<sup>34</sup> disseminated nocardiosis,<sup>35–37</sup> and papillomavirus-induced lesions,<sup>38</sup> but there is little evidence available in dogs to indicate how often these complications arise.

In people, opportunistic infections do occur in patients receiving immunosuppressive therapy for a number of different autoimmune diseases and following transplantation.<sup>39,40</sup> These appear to affect the respiratory tract most commonly, resulting in pneumonia with or without the involvement of the opportunistic organism *Pneumocystis jirovecii*. The latter organism could present a theoretical risk to dogs receiving immunosuppressive therapy but has been reported only in dogs with immunoglobulin deficiency, with or without B-cell hypoplasia.<sup>41–43</sup> Immunocompromised people are also predisposed to development of active infections with *Toxoplasma gondii*, and a number of case reports indicate that similar complications could occur in dogs treated with immunosuppressive drugs due to reactivation of latent *T. gondii* and *Neospora caninum* infections.<sup>44–46</sup>

## Management of complex cases

### Failure to control the disease/relapses

This section is illustrated by two case examples presented below.

### Case example I

#### Signalment

Six-year-old male whippet; weight: 16 kg.

#### History

This dog had a history of immune-mediated thrombocytopenia that had been previously treated with prednisolone

**Table 2** Management options for IMHA

Drug or management method	Dose	Mechanism of action	Adverse effects	Cost and practicalities of use
<b>Steroids</b>				
Glucocorticoid	Prednisolone: 2 mg/kg/d reducing to 1 mg/kg/d after 2–4 weeks; dexamethasone: 0.2–0.3 mg/kg/d until oral medication can be started	Alters gene transcription; downregulates Fc receptor expression, decreases antigen processing, and suppresses T-cell function	PU/PD, polyphagia, panting, muscle wasting, weight gain, opportunistic infections, GI ulceration, diabetes mellitus, osteoporosis, thromboembolic disease	Inexpensive; 1 mg, 5 mg, and 25 mg tablets available; injectable dexamethasone convenient if vomiting present
Anabolic steroid	Nandrolone: 1–5 mg/kg intramuscularly/ subcutaneously, maximum dose 40–50 mg per dog; repeated every 21 days	Thought to stimulate erythropoiesis in nonregenerative anemia; mechanism unproven	Sodium, calcium, potassium, chloride, phosphate retention; water retention; hepatotoxicity, behavioral changes, reproductive abnormalities (oligospermia, estrus suppression)	Parenteral form only; easy to administer; lack of information about efficacy means anabolic steroids are rarely recommended
<b>Steroid-sparing drugs</b>				
Azathioprine	2 mg/kg/d po for no more than 14 days; then decrease to 1 mg/kg po every day or 2 mg/kg EOD depending on ease of dosing; or, 50 mg/m <sup>2</sup> every other day	Thiopurine analog	Hepatotoxicity, <sup>28</sup> bone marrow suppression, pancreatitis; most side effects follow use of 2 mg/kg daily dosing for >2 weeks though side effects can occur in dogs that have pre-existing disease in these organs or disease susceptibility	Inexpensive (25 mg and 50 mg tablets); other doses have to be reformulated; do not split tablets; not licensed for use in dogs in the UK
Ciclosporin	2.5–5 mg/kg, po, q12h	Calcineurin inhibitor	GI signs (vomiting, diarrhea, anorexia), gingival hyperplasia, <sup>29</sup> opportunistic infections; <sup>30</sup> ciclosporin is not cytotoxic and is not myelosuppressive as it is specific for lymphocytes	Expensive; capsules available in 10 mg, 25 mg, 50 mg, and 100 mg formulations; liquid formulation available; not licensed for use for this condition but licensed for use in dogs in the UK
Mycophenolate mofetil	10 mg/kg, po, q12h	Purine synthesis inhibitor	GI upset, lethargy, hepatotoxicity, bone marrow suppression	250 mg and 500 mg capsules; available as Cellcept and also in generic form; not licensed for use in dogs in the UK
Leflunomide	1–4 mg/kg, po, q24h	Pyrimidine synthesis inhibitor	GI upset, bone marrow suppression, hepatotoxicity	10 and 20mg tablets Not licensed for use in dogs in the UK
Lithium carbonate	10–11 mg/kg orally, bid	Stimulates erythropoiesis	Diarrhea, hypersalivation, seizures, increased liver enzymes, PU/PD	Little information about use in dogs; serum levels should be measured; <sup>31,32</sup> not licensed for use in dogs in the UK
<b>Rescue therapies</b>				
hIVIg	0.5–1.0 g/kg, iv, over 6–12 hours	Fc receptor blockade	Thromboembolic disease, volume overload, anaphylaxis	Expensive; do not repeat infusion; not licensed for use in dogs in the UK
Plasmapheresis	N/A	Microporous membrane separation of plasma from other blood components before immunoglobulins are filtered from plasma to limit immune response	Hypocalcemia due to anticoagulants in circuit	Requires specialist equipment/expertise; limited experience in dogs <sup>33</sup>
Splenectomy	N/A	Underlying etiology of IMHA involves antibody-mediated red cell destruction by the spleen; removal of spleen removes site of destruction	Increased susceptibility to infectious agents, risks associated with anesthesia and surgery in a dog with IMHA (thromboembolic disease etc)	One-off surgical and aftercare costs; patient needs to be stabilized for anesthesia – usually requires transfusion

**Abbreviations:** bid, twice daily; GI, gastrointestinal; hIVIg, human intravenous immunoglobulin; IMHA, immune-mediated hemolytic anemia; N/A, not applicable; po, per os; q12h, every 12 hours; q24h, every 24 hours; EOD, every other day; PU/PD, polyuria/polydipsia; iv, intravenous.

and azathioprine. The treatment had been tapered off and had stopped completely 9 months prior to the dog becoming unwell again.

The dog had a 3-week history of lethargy and inappetence. He was identified as having a regenerative anemia at his primary practice (packed cell volume [PCV]: 25%) and was put on treatment using prednisolone at a dose rate of 1 mg/kg/d initially, which was increased to 2 mg/kg/d 12 days before referral because the initial response to treatment was poor. Although the response to the dose increase was positive at first (PCV increased to 42%), the dog again deteriorated, the PCV dropped, and he was referred to the Queen's Veterinary School Hospital.

This was a client-owned animal. Owner consent was obtained plus consent for the use of drugs not licensed in this species. This was in no way an experimental procedure but was an attempt to rescue a clinical case from dramatic deterioration using licensed and non-licensed drugs that are reported to be useful in these cases.

### Clinical examination

The dog was lethargic but reasonably bright. He had normal temperature and respiratory rate, and a heart rate of 80 bpm. His mucous membranes were pale and icteric. His temperature was 38.9°C.

### Investigation

Blood work showed moderate anemia (PCV: 25%), with evidence of regeneration and spherocytosis. There was mild thrombocytopenia (platelet count:  $109 \times 10^9/L$ ; range:  $175\text{--}500 \times 10^9/L$ ). Total protein was 74 g/L (60–80 g/L). Bilirubin was mildly increased (56 mmol/L; range: 0–12 mmol/L). Coombs test was negative. In-saline agglutination test was positive.

Survey radiographs of thorax and abdomen revealed no abnormalities, and abdominal ultrasonography was unremarkable.

### Diagnosis

The history, clinical examination, and laboratory findings were compatible with a diagnosis of immune-mediated hemolytic anemia (IMHA).

### Management

The dog continued to be on prednisolone (2 mg/kg/d) and azathioprine was added (2 mg/kg/d). Over the next 24 hours, the dog's condition deteriorated significantly and he became pyrexia, lethargic, pale, and tachypneic. His PCV was found

to have dropped to 11%. He was given one unit of packed red cells, and his posttransfusion PCV was 25%. He also received 10 g of human intravenous immunoglobulin (hIVIg) as a constant rate infusion over 4 hours.

The dog was sent home with PCV stable at 20% and receiving prednisolone (2 mg/kg/d), azathioprine (2 mg/kg/d), ciclosporin (3 mg/kg, bid), and clopidogrel (56.25 mg/d).

The dog returned to the hospital for reassessment after 3 days, by which time his PCV was 25% with a strongly regenerative response. Ciclosporin caused persistent diarrhea in this dog and was tapered and discontinued over 2 weeks. After 2 weeks, the prednisolone dose was decreased to 1 mg/kg/d. A recheck after 1 month showed that the PCV had increased to 44%, and the dog was doing well.

Medication was maintained at immunosuppressive levels for 3 months, then a tapering regimen began.

### Discussion

This case shows that in dogs with a predisposition for immune-mediated disease, different diseases can present either at the same time or, as in this case, serially in a dog's lifetime. When therapy with prednisolone was started to treat IMHA, it began at the fairly modest dose of 1 mg/kg/d. Although this was increased after 7 days, response was transient. The dog then deteriorated rapidly, having been relatively stable for a period of about 3 weeks. Treatment intensity was increased using azathioprine, but this drug is thought not to have rapid onset of action and probably did little to prevent the hemolytic crisis that the dog entered. hIVIg was chosen at this point because of its perceived rapid onset of action. This patient responded well both clinically and hematologically and was discharged from the hospital in a stable condition.

## Case example 2

### Signalment

Eight-year-old male, entire, Irish setter dog; weight 42 kg.

### History

This Irish setter had a history of reduced exercise tolerance, disorientation and collapse on exercise, and tachypnea. He was hypothyroid and had been receiving thyroid supplementation.

This was a client-owned animal. Owner consent was obtained.

### Clinical examination

The dog was bright and appeared normal in attitude. He had a normal temperature; respiratory rate was 56 bpm and heart



rate was 150 bpm. His mucous membranes were pale, with a normal capillary refill time. His abdomen appeared to be pendulous, with suspected cranial abdominal organomegaly.

### Investigation

Blood work showed marked anemia (PCV: 14%), with no evidence of regeneration. There was marked leukocytosis along with neutrophilia ( $31.56 \times 10^9/L$ ; range:  $3-11.5 \times 10^9/L$ ). There was marked thrombocytosis (platelet count:  $2,275 \times 10^9/L$ ; range:  $175-500 \times 10^9/L$ ). Total protein was 70 g/L (range: 60–80 g/L). No spherocytosis or in-saline agglutination was seen. Bilirubin was normal (3.1 mmol/L; range: 0–12 mmol/L). Coombs test was negative.

Survey radiographs of thorax and abdomen revealed no abnormalities, and abdominal ultrasonography was unremarkable.

Bone marrow aspiration and core biopsy were suggestive of pure red cell aplasia (PRCA) as there were few to no red cell precursors identified. The majority of cells were myeloid, with evidence of maturation to the segmented neutrophil stage.

### Diagnosis

The history, clinical examination, and laboratory findings were compatible with a diagnosis of PRCA of presumed immune-mediated etiology in the absence of any other pathological changes.

### Management

The dog was treated with prednisolone (2 mg/kg/d) and ciclosporin (2.5 mg/kg, bid). He remained nonregenerative and was transfusion dependent for the following 3 weeks. At this point, lithium carbonate (10 mg/kg, bid) was added to his dosage regimen. Three weeks later, when again the dog required transfusion, ciclosporin was withdrawn and mycophenolate mofetil was added (10 mg/kg, bid).

### Outcome

No improvement was seen in the red cell parameters, and no indication of regeneration was seen. In addition, the dog developed a skin lesion on the bridge of his nose, which cytologically was found to contain fungal hyphae. Lithium carbonate dose was decreased to 10 mg/kg/d following some adverse effects. The dog continued to do badly, however, made no response to mycophenolate, and was euthanized due to both absence of response to treatment and the development of hemorrhagic gastroenteric signs.

### Discussion

This dog illustrates some of the frustrations encountered with bone marrow-centered disease. There were few indi-

cations of the etiology of the anemia in this Irish setter and the disease was presumed to be immune mediated in the absence of any other drug-related, toxic, neoplastic, or inflammatory disease. The dog was supported with transfusions and was immunosuppressed. When there was no response, other additional therapies were used, including lithium carbonate and then mycophenolate mofetil. Lithium has a small body of literature to support its use and was not helpful in this case (Table 1). Mycophenolate has been reported in small numbers of canine IMHA cases and was reported to provoke few side effects in canine patients when used in conjunction with steroids. It may prove useful as an alternative agent in the management of this disease.<sup>11</sup> Unfortunately, it also was not helpful in this case, and the dog was euthanized.

Survival curves constructed from data obtained from dogs with IMHA show curves with similar slopes reflecting the fact that most deaths occur in the first 2 weeks after diagnosis.<sup>13,24,47</sup> Problems arise when immunosuppressive regimens are instituted and there is either no control of the hemolytic process or no regenerative state is achieved. In these circumstances, clinicians often look to increase the breadth of the immunosuppression by adding other agents. Most dogs achieve initial control using prednisolone  $\pm$  another agent, usually azathioprine or ciclosporin. When there is a less-than-optimal response to treatment, other agents are added, including azathioprine and ciclosporin, to increase to two immunosuppressive agents or three depending on whether the dog was on mono or dual therapy to begin with. If this does not alter the disease process, there are a number of options for clinicians, none of which is the accepted and evidence-proven route.

Several studies have looked at the role of human intravenous immunoglobulin (hIVIg) for this purpose. As far back as 1997, Kellerman and Bruyette suggested that hIVIg would be a good option for dogs that did not respond to prednisolone therapy within 7 days.<sup>48</sup> This was reiterated by a later study,<sup>49</sup> but better long-term survival was not proven.<sup>50,51</sup> In one published prospective study, again hIVIg did not alter response to treatment or survival,<sup>23</sup> although the survival data compared favorably to those in other studies (24/28 survived to 2 weeks, 86%). The question that has not been answered by these studies is whether hIVIg is able to alter prognosis and survival in a dog that would not otherwise have responded to standard medication, ie, one that would be expected to have a poor prognosis based on our current understanding of prognostic markers. For this to be answered, clinicians would have to have at their disposal a robust prognostic scoring system that could reliably pick out dogs that may benefit from more intensive drug regimens.

In the literature on human medicine, a strong case is made for the role of splenectomy in IMHA patients, with survival rates encouragingly high.<sup>52</sup> The idea that splenectomy may be helpful is decades old in veterinary medicine too, with reports dating back to 1985 reporting 100% survival in three dogs with IMHA.<sup>53</sup> More recently, a retrospective study evaluated ten dogs that were splenectomized as part of their treatment for IMHA.<sup>54</sup> This study showed 90% survival at 30 days, with packed cell volumes (PCVs) rising significantly 3 days after the procedure and reduced requirement for transfusion. These studies appear to suggest that IMHA could be managed successfully by splenectomy and that this technique warrants further investigation through a larger-scale prospective study.

Disease relapse also poses problems for IMHA cases. Recommended treatment times vary anecdotally between 3 months and 6 months and are rarely standardized, though some authors<sup>55</sup> have found it helpful to use standard protocols within their institution, with the result that retrospective studies become more meaningful and data collection is easier. The treatment regimen described at Utrecht, the Netherlands, follows a 69-day gradually tapering course. Relapses prompt a return to the beginning of the regimen. Too rapid tapering has been associated with increased incidence of relapse, but strong evidence for this is lacking in the literature.

## Nonregenerative immune-mediated anemia

Classically, IMHA is diagnosed when an animal has a regenerative anemia associated with autoagglutination, Coombs test positivity, and spherocytosis in the absence of any recognizable underlying disease pathology. The regenerative response does not happen immediately, however, and 3–5 days are required before an adequate reticulocyte response is recognized after acute hemolysis. If there is no appropriate reticulocyte response after 5 days, then it is still possible that immune-mediated disease is the cause, and animals may have either pure red cell aplasia (PRCA) or nonregenerative IMHA.<sup>56</sup> PRCA is diagnosed when erythroid aplasia results from presumed immune-mediated destruction of precursor cells within the bone marrow.<sup>56–58</sup> Nonregenerative IMHA appears to be a related disorder in that immune-mediated red cell destruction takes place within the marrow, and maturation arrest of the erythroid lineage at different stages of maturation can be recognized. In addition, some forms of nonregenerative IMHA are associated with bone marrow erythroid hyperplasia<sup>59</sup> and other pathological changes such as dysmyelopoiesis, myelonecrosis, myelofibrosis, and hemophagocytic syndrome. Dogs that have similar

hematological changes peripherally can therefore have very different bone marrow profiles varying from PRCA to erythroid hyperplasia.

Although the names PRCA and nonregenerative IMHA have been given to these conditions based on the cytological evaluation of the bone marrow, from a purely clinical perspective, it would appear that PRCA is just one end of a continuum of immune-mediated disease, which begins as destruction of early precursors and ends with destruction of mature circulating cells. These diseases all have the same feature of immune-mediated cell lysis and should all respond to immunosuppression, albeit to different degrees. Giving a specific disease name, such as PRCA, suggests it is a separate disease entity and somehow is unrelated to peripheral IMHA. We would argue that these diseases are closely related and are only separated by the stage of maturation of the cell type that is the specific target for autoantibody production. As such, management regimens are similar in all cases.

The prognosis for dogs with PRCA is reported to be fair within the available literature, with survival rates of 72%–79%.<sup>56,59</sup> However, numbers are low and the authors' perception is that these diseases are more difficult to manage. In one study,<sup>59</sup> dogs with nonregenerative IMHA and erythroid hyperplasia were shown to have statistically significant lower 60-day survival rate (56%) when compared with dogs showing maturation arrest or PRCA. Again, low numbers make this information open to interpretation. Many clinicians would choose a more aggressive multidrug form of therapy from the outset for these cases or would ramp up therapy after treatment with steroid alone fails to resolve the disease within a reasonable timeframe (10–14 days). The evidence for such choices is lacking, however, and because much of the literature is  $\geq 8$  years old, it does not include information about newer drug choices such as mycophenolate or even ciclosporin.

## Thromboembolic disease

Formation of thrombi is one of the major complications suffered by dogs with IMHA,<sup>47,60</sup> and the pathophysiology of this process has been reviewed elsewhere.<sup>61</sup> Pulmonary thromboembolism (PTE) is reported most commonly, but vessels in other locations, including the brain, spleen, portal vasculature, and kidneys, may be affected.<sup>22,24,62</sup> One of the major challenges involved in managing PTE in dogs with IMHA is the difficulty in diagnosing this complication ante mortem. Formation of a thrombus does not result in reliable changes in the radiographic appearance of the lungs,<sup>63</sup> nor does it consistently cause hypoxemia in all cases.<sup>64</sup> A recent

study indicated that computed tomography with pulmonary angiography, which is considered the gold standard imaging modality for diagnosis of PTE in people, might produce greater diagnostic accuracy,<sup>65</sup> but this form of imaging is expensive and not readily available in clinical practice. As a result, PTE is often suspected if there are consistent clinical and laboratory findings, such as acutely increased respiratory rate and effort, hypoxemia, and increased alveolar-to-arterial oxygen gradient,<sup>66</sup> but it may be difficult to establish whether these changes could have a different etiology, such as secondary bacterial pneumonia, acute respiratory distress syndrome, or increased respiratory drive due to cerebral hypoxia.

There has been frequent speculation that development of thromboembolic disease is predictive of poor outcome in dogs with IMHA,<sup>22,62</sup> but definitive proof of this hypothesis is lacking, partly due to the difficulty in confirming the existence of the problem in live animals. Paradoxically, greater maximal amplitude values obtained by thromboelastography in dogs with IMHA were associated with improved survival,<sup>67,68</sup> probably because development of consumptive coagulopathy is associated with poor prognosis.

Due to its importance as a possible cause of additional morbidity and mortality, there has been extensive discussion of the use of anticoagulant therapy, either for symptomatic animals or as prophylaxis for those recently diagnosed with the disease.<sup>61</sup> These discussions are somewhat hampered by an incomplete understanding of the processes that result in a systemic hypercoagulable state and increased risk of clinically detectable thromboembolic disease, as well as by the lack of concrete evidence to support an association between development of thromboembolic disease and increased risk of death.

Among the drugs evaluated in clinical practice, some inhibit platelet activation (aspirin and clopidogrel) and some limit the activity of coagulation factors (unfractionated and low-molecular-weight heparin). As with immunosuppressive treatment, there is no consensus among veterinarians as to the preferred form of anticoagulant therapy,<sup>5</sup> and it is likely that requirements will differ widely between individual patients according to the severity of their disease.

A large retrospective study investigated the outcome associated with several different anticoagulant regimens that were administered to dogs with IMHA shortly after diagnosis and alongside a standardized immunosuppressive protocol that included prednisone and azathioprine.<sup>60</sup> This study demonstrated that administration of aspirin at an “ultra-low” dose of 0.5 mg/kg twice daily resulted in improved survival, compared to dogs that received no anticoagulant drug, heparin alone,

or a combination of aspirin and heparin. A smaller prospective and randomized study evaluated the use of clopidogrel alone, ultra-low-dose aspirin alone, or a combination of the two drugs, but this did not reveal any significant difference in survival between the three regimens.<sup>69</sup> While apparently not supporting the use of clopidogrel in dogs with IMHA, this study is likely to have been underpowered, recruiting just six cases per group. There is suspicion that a proportion of dogs with IMHA will also have platelets that are relatively resistant to the action of aspirin, as has been reported in people. In this situation, it may be of benefit to consider the use of an alternative antiplatelet drug if platelet function testing becomes more affordable and accessible in veterinary practice in future.

Use of heparin for management of thrombi has several theoretical advantages because venous thromboembolic disease is classically associated with inappropriate activation of the coagulation cascade, rather than abnormal platelet activity.<sup>61</sup> This class of drugs is also used widely in people who have similar diseases, including deep vein thrombosis and PTE related to recent surgery or cardiac disease.<sup>70</sup> Both unfractionated and low-molecular-weight heparin have been administered to dogs with IMHA in an attempt to reduce the frequency and severity of thromboembolic disease, but the regimens and the studies used to evaluate their effects have varied widely.<sup>5</sup>

Helmond et al<sup>71</sup> performed a randomized controlled trial to compare the effects of administering unfractionated heparin at a conventional and fixed dose from the point of diagnosis with those in dogs in whom the dose of heparin was adjusted on a daily basis according to the results of repeated factor X assays. The latter group of dogs had significantly improved survival and required a wider variety of different heparin doses to achieve the desired reduction in factor X concentration. This study highlights the importance of close monitoring of heparin therapy if it is to be instituted; previous reports in people also indicate that different individuals require different doses of unfractionated heparin, limiting its use outside of the hospital environment.<sup>72</sup> While possibly representing a valuable addition to therapy in dogs in whom PTE or similar disease is suspected, some forms of heparin are expensive, and access to factor X assays is limited to a small number of veterinary institutions. Similar assays are available at many human hospitals, so monitoring may be feasible if a good working relationship exists with a local center.

## Bilirubin encephalopathy

There have been two case reports of dogs suffering neurological signs that appeared to be related to increased serum bilirubin concentrations,<sup>73</sup> one in a dog with primary IMHA.<sup>74</sup>



This phenomenon, properly termed bilirubin encephalopathy and occasionally kernicterus, has been described most commonly in human neonates and rarely in adults with deficient activity of the hepatic enzyme uridine diphosphate glucuronyl transferase, which is responsible for conjugation of bilirubin.

Bilirubin encephalopathy is suspected in people with excessive total serum bilirubin concentrations (usually >500 mmol/L) who begin to show neurological signs. In the dog with IMHA in which it was suspected, magnetic resonance imaging of the brain revealed increased intensity in T2-weighted images of the basal ganglia and other central nuclei,<sup>74</sup> and post mortem examination of both adult dogs diagnosed with the disease showed yellow pigmentation of the thalamus.

In clinical practice, the majority of dogs do not develop hyperbilirubinemia that is so severe as to result in bilirubin encephalopathy, but we believe that this condition should be considered in icteric animals that develop neurological signs with no other apparent etiology.

## Distal renal tubular acidosis

A case series published in 2009 described distal renal tubular acidosis in three dogs with IMHA.<sup>75</sup> In all three cases, the diagnosis was based on the presence of metabolic acidosis with normal anion gap and inappropriately high urine anion gap and bicarbonate excretion.

The acidemia was treated in all three dogs with infusions of sodium bicarbonate, but all were ultimately euthanized. Immune-mediated disease is reported to be the most common cause of acquired distal renal tubular acidosis in people,<sup>76</sup> particularly in association with systemic lupus erythematosus, rheumatoid arthritis, and Sjogren's syndrome, affecting up to one quarter of patients.<sup>77–79</sup> It has been suggested that the phenomenon develops due to production of autoantibodies that interfere with the action of ion transporters in the distal tubule.<sup>80</sup>

It is not known how frequently distal renal tubular acidosis develops in dogs with IMHA or other autoimmune diseases, or whether it has any impact on the outcome in affected dogs. In people, acidosis is usually treated with potassium citrate rather than sodium bicarbonate, as this will treat both acidosis and hypokalemia, which are frequently observed concurrently.<sup>81</sup>

## Hemoglobin-related acute kidney injury

Experience gained from treating human injuries during World War II suggested that excessive circulating concentrations of myoglobin and free hemoglobin could cause acute kidney injury, and the same syndrome was produced experimentally by injecting hemoglobin into dogs in 1947.<sup>82</sup> More recent studies suggest that heme proteins cause damage to the

kidneys in several different ways, including oxidant damage to cellular molecules and changes in blood flow due to sequestration of nitric oxide.<sup>83</sup>

It would appear that azotemic acute kidney injury is not common in dogs with IMHA as it has not been reported frequently in dogs with this disease, although increased serum concentrations of both urea and creatinine have been associated with decreased survival in affected dogs.<sup>7,10,14,50</sup>

Interestingly, a study of dogs at high risk of hemoglobin-induced nephropathy (including dogs with IMHA, dogs infected with *Babesia canis rossi*, and dogs that had been injected with large amounts of hemoglobin) reported serum creatinine concentrations within normal limits in all groups.<sup>84</sup>

Glomerular filtration rate was also measured in all individuals and was reported to be “normal”, but it is not clear whether these values were standardized to breed and age group reference intervals.

In conclusion, while release of large amounts of free hemoglobin into the circulation could increase the risk of acute kidney injury, this phenomenon has not been reported frequently in the veterinary literature. Furthermore, in animal patients with greatly reduced blood oxygen-carrying capacity and systemic inflammation, it may be difficult to establish whether azotemia has developed due to the effect of heme moieties in the kidneys.<sup>85</sup>

## Association between vaccination and IMHA

There have been anecdotal reports that recent vaccination could be associated with development of IMHA in dogs, and a study conducted in the US suggested that dogs with IMHA were much more likely to have been vaccinated in the month prior to onset of clinical signs than a group of control dogs presented for management of other diseases.<sup>19</sup> The results of this study were not duplicated by another, larger investigation, which found no relationship between timing of vaccination and onset of clinical signs.<sup>22</sup> While there does appear to be a consistent relationship between some vaccines and development of immune thrombocytopenic purpura in people,<sup>86</sup> there are only sporadic reports of development of acute autoimmune hemolytic anemia after vaccination.<sup>87</sup>

A topic often raised by owners and veterinarians is whether continued vaccination induces relapse of IMHA, but, to our knowledge, there have been no published studies reporting the frequency of this phenomenon in dogs with IMHA. While there is no evidence to suggest that vaccination will cause relapses, it is possible for serum antibody titers against canine distemper virus, parainfluenza virus, and adenovirus 1 to be measured

to guide the decision to vaccinate. Protective antibody titers against *Leptospira* organisms are unlikely to persist beyond 1 year, so we usually counsel owners and veterinarians that the decision to administer this vaccine should be based on a consideration of risks and benefits for the individual animal. For example, there may be little indication to vaccinate a dog that lives mostly indoors in an area with a low prevalence of *Lep-tospira* infections, whereas vaccination may well be indicated in working dogs or in dogs that are exposed to waterways.<sup>88</sup>

## Conclusion: future directions and comparative aspects

IMHA throws up many challenges for the small animal physician, and it remains a disease that may respond unpredictably or poorly to medication and may still be fatal in a significant number of cases. Treatment of canine patients relies almost solely on immunosuppression, with splenectomy not universally used as a reasonable alternative. The situation is similar in human medicine wherein steroids remain in use as first-line agents with or without cyclophosphamide.<sup>89</sup> In people, there has been an attempted movement away from immunosuppression toward the use of therapeutic monoclonal antibodies, though these remain rather nonspecific and therefore of mixed efficacy.<sup>89</sup> In children particularly, treatments with agents such as rituximab, a monoclonal antibody against the cluster of differentiation (CD)-20 molecule which causes B-cell depletion, are used to avoid high doses of steroids or splenectomy, both of which cause significant and severe side effects.<sup>90</sup> The search for more specific ways to treat autoimmune hemolytic anemia in people continues and relies on the need to define the origin of the stimulus to mount an immune response against red blood cells, so that the stimulus can be removed and the immune dysregulation corrected. However, this approach may still be some way in the future for human and veterinary patients.

## Disclosure

The authors report no conflicts of interest in this work.

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