

Canine autoimmune hemolytic anaemia : management challenges

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“Canine autoimmune hemolytic anemia: management challenges”.

Veterinary Medicine: Research and Reports

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Abstract

Immune mediated haemolytic anaemia is one of the most common manifestations of canine immune mediated disease yet treatment regimens remain non-standardised 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 personal preference and experience and because of this treatment regimens tend to vary between institutions and between individual clinicians.

Management of IMHA includes immunosuppression, thromboprophylaxis, and supportive care measures to help prevent and treat concurrent conditions.

Keywords

IMHA, canine immune-mediated disease, management regimens

Conflict of interest:

The Authors do not have any conflicts of interest to disclose.

Immune-mediated hemolytic anemia (IMHA) is considered to be the most common autoimmune disease of dogs¹ 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.² 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, pre-hepatic icterus due to accumulation of unconjugated bilirubin.

Following diagnosis, many dogs with IMHA receive blood products, which 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 (ADCAS), have been evaluated as guides for administration of transfusions,³ but 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.⁴

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 review⁵. Several other immunosuppressive drugs also have been used to treat the disease, including azathioprine,^{6,7} leflunomide,⁸ ciclosporin^{9,10} and mycophenolate mofetil.^{11,12} There is currently no consensus among veterinarians regarding the optimal immunosuppressive regimen that should be employed 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 published studies of the past twenty years.⁵

Immune-mediated hemolytic anaemia is considered to have a poor prognosis in dogs, with mortality rates of 50-70% reported in older studies,^{6,13} and rates of approximately 30-40% in more recent studies,¹⁴ 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 associations between survival and biochemical, hematologic or clinical parameters that can be measured at presentation to determine if 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,¹⁵ suggesting that these variables might be helpful in guiding clinical decisions in future.

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Predicting disease outcome using scoring systems

Because clinical presentation and response treatment are so 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.,¹⁴ found that a combination of the American Society of Anesthesiologists (ASA) 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.,¹⁶ or Ishihara et al.,¹⁷ 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,¹⁸ highlighting the importance of using prognostic tools in a population for which they have been validated.

Development of ‘intravascular haemolysis’, a term used inconsistently to describe some combination of haemoglobinuria, haemolysed 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 that autoagglutination was associated with decreased survival using univariable analyses. One of these studies subsequently showed no difference between cases that did or did not have undefined 'intravascular haemolysis'¹⁹ while the other demonstrated that agglutination was associated with survival to discharge but not to one year after diagnosis.²⁰ In contrast, serum total bilirubin concentration, which is an indicator of the severity of either intravascular or extravascular haemolysis, has been associated consistently with survival in several previous studies that utilised either univariable or multivariable analyses, albeit with considerable variation in the apparent size of the effect.^{15, 19, 21, 13, 22, 10, 23, 24, 14} Consequently, we believe that this variable is likely to be the most useful for the clinician in guiding therapy and estimating prognosis, particularly since it is easily measured in the majority of primary care and specialist practices.

2. 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.²⁵ 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 though 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 auto-immune 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 hyper-activation 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 often have an impact on an owner's willingness to continue with treatment. Steroids cause polydipsia and polyuria through their interference with ADH 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.²⁶

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 pre-existing heart disease (e.g. mitral valve endocardiosis or occult cardiomyopathy). In human medicine the long-accepted recommendations were to avoid glucocorticoid administration in patients with heart failure due to the sodium and water retention they induce. More latterly, it has been suggested that glucocorticoids may actually act synergistically with diuretics in some patients to improve diuresis and therefore clinical wellbeing. This area has not been explored in dogs and as such, specific recommendations are difficult to justify.²⁷

The drugs and their dosing regimens commonly used for the management of IMHA along with other management options are summarised 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 including fungal infections,³⁰ bacterial urinary tract infections,³⁴ disseminated nocardiosis^{35,36,37} and papillomavirus-induced lesions,³⁸ 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.^{39,40} 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.^{41,42,43} 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.^{44,45,46}

3. Management of complex cases

Failure to control the disease/relapses:

(This section is illustrated by 2 case examples submitted as a separate file)

Survival curves constructed from data from dogs with IMHA show curves with similar slopes reflecting the fact that most deaths occur in the first two weeks after diagnosis.^{13,24,47} Problems arise when immunosuppressive regimens are instituted and there is either no control of the haemolytic

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 +/- 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 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.⁴⁸ This was reiterated by a later study⁴⁹ but better long term survival was not proven.^{50,51} In the one published prospective study, again hIVIg did not alter response to treatment or survival²³ although the survival data compared favourably to 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 i.e. 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 human literature a strong case is made for the role of splenectomy in IMHA patients with survival rates encouragingly high.⁵² The idea that splenectomy may be helpful is decades old in veterinary medicine too with reports dating back to 1985 reporting 100% survival in 3 dogs with IMHA.⁵³ More recently a retrospective study evaluated 10 dogs that were splenectomised as part of their treatment for IMHA.⁵⁴ This study showed a 90% survival at 30 days with PCVs rising significantly 3 days after the procedure and a 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 and 6 months and are rarely standardized, though some authors⁵⁵ 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 follows a 69 day gradually tapering course. Relapses prompt a return to the beginning of the regimen. Too rapid tapering has been associated with an increased incidence of relapse but strong evidence for this is lacking in the literature.

Non-regenerative immune-mediated anaemia

Classically, IMHA is diagnosed when an animal has a regenerative anaemia associated with autoagglutination, Coombs' test positivity and spherocytosis and in the absence of any recognisable underlying disease pathology. The regenerative response does not happen immediately however, and 3-5 days are required before an adequate reticulocyte response is recognised after acute haemolysis. 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 non-regenerative IMHA⁵⁶. PRCA is diagnosed when erythroid aplasia results from presumed immune-mediated destruction of precursor cells within the bone marrow.^{56,57,58} Non-regenerative 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 recognised. In addition, some forms of non-regenerative IMHA are associated with bone marrow erythroid hyperplasia⁵⁹ and other pathological changes such as dysmyelopoiesis, myelonecrosis, myelofibrosis and haemophagocytic syndrome. Dogs that have similar haematological changes peripherally can therefore have very different bone marrow profiles varying from PRCA to erythroid hyperplasia.

Although the names PRCA and non-regenerative 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 that 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 auto-antibody 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%.^{56,59} However, numbers are low and the authors' perception is that these diseases are more difficult to manage. In one study,⁵⁹ dogs with non-regenerative 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 multi-drug 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 as much of the literature is 8 or more 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^{47,60}, and the pathophysiology of this process has been reviewed elsewhere.⁶¹ Pulmonary thromboembolism (PTE) is reported most commonly, but vessels in other locations, including the brain, spleen, portal vasculature and kidneys may be affected.^{22,24,62} 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,⁶³ nor does it consistently cause hypoxemia in all cases.⁶⁴ 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,⁶⁵ 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, hypoxaemia and increased alveolar to arterial oxygen gradient,⁶⁶ but it may be difficult to establish whether these changes could have a different aetiology, such as secondary bacterial pneumonia, acute respiratory distress syndrome (ARDS) or increased respiratory drive due to cerebral hypoxia.

There has been frequent speculation that development of thromboembolic disease is predictive of a poor outcome in dogs with IMHA,^{22,62} 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,^{67,68} probably because development of consumptive coagulopathy is associated with a 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.⁶¹ 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, and 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, 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,⁵ 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.⁶⁰ 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 randomised study evaluated the use of clopidogrel alone, ultra-low dose aspirin alone or a combination of the two drugs, but did not reveal any significant difference in survival between the three regimens.⁶⁹ Whilst 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 anti-platelet 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.⁶¹ This class of drug is also used widely in people that have similar diseases, including deep vein thrombosis and PTE related to recent surgery or cardiac disease.⁷⁰ 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.⁵

Helmond and others⁷¹ performed a randomised controlled trial to compare the effects of administering unfractionated heparin at a conventional and fixed dose from the point of diagnosis, with those of in which 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.⁷² Whilst possibly representing a valuable addition to therapy in dogs in which 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,⁷³ one in a dog with primary IMHA.⁷⁴ 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 (UDP) glucuronyl transferase, which is responsible for conjugation of bilirubin.

Bilirubin encephalopathy is suspected in people with excessive total serum bilirubin concentrations (usually in excess of 500 $\mu\text{mol/l}$) that 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,⁷⁴ 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 will not develop hyperbilirubinaemia 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 aetiology.

Distal renal tubular acidosis: A case series published in 2009 described distal renal tubular acidosis in three dogs with IMHA.⁷⁵ 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,⁷⁶ particularly in association with systemic lupus erythematosus, rheumatoid arthritis, and Sjogren's syndrome, in which it may affect up to one quarter of patients.^{77,78,79} 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.⁸⁰

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, the acidosis is usually treated with potassium citrate rather than sodium bicarbonate, as this will treat the acidosis and hypokalaemia, which is frequently observed concurrently.⁸¹

Haemoglobin-related acute kidney injury: Experience gained from treating human injuries during the Second World War suggested that excessive circulating concentrations of myoglobin and free haemoglobin could cause acute kidney injury, and the same syndrome was produced experimentally by injecting haemoglobin into dogs in 1947.⁸² More recent studies suggest that haem 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.⁸³

It would appear that azotaemic 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.^{14,50,10,7} Interestingly, a study of dogs at high risk of haemoglobin-induced nephropathy (including dogs with IMHA, dogs infected with *Babesia canis rossi* and dogs that had been injected with large amounts of haemoglobin) reported serum creatinine concentrations within normal limits in all groups.⁸⁴ Glomerular filtration rate was also measured in all individuals and was reported to be 'normal', but it was not clear whether these values were standardised to breed and age group reference intervals.

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

4. Association between vaccination and immune-mediated haemolytic anaemia

There have been anecdotal reports that recent vaccination could be associated with development of IMHA in dogs, and a study produced in the United States suggested that dogs with IMHA were much 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.¹⁹ 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.²² While there does appear to be a consistent relationship between some vaccines and development of immune thrombocytopaenic purpura in people,⁸⁶ there are only sporadic reports of development of AIHA after vaccination.⁸⁷

A topic often raised by owners and veterinarians is whether continued vaccination will induce 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 titres against canine distemper virus, parainfluenza virus and adenovirus 1 to be measured to guide the decision to vaccinate. Protective antibody titres against *Leptospira* organisms are unlikely to persist beyond one 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 *Leptospira* infections, whereas vaccination may well be indicated in working dogs, or in dogs that have exposure to waterways.⁸⁸

5. 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 employed as a reasonable alternative. The situation is similar in human medicine where steroids remain in use as first line agents with or without cyclophosphamide.⁸⁹ In people, there has been an attempted movement away from immunosuppression towards the use of therapeutic monoclonal antibodies though these remain rather non-specific and therefore of mixed efficacy.⁸⁹ In children particularly, treatments such as rituximab, a monoclonal antibody against the CD20 molecule that causes B-cell depletion, are used to avoid high doses of steroids or splenectomy, both of which cause significant and severe side effects.⁹⁰ The search for more specific ways to treat autoimmune haemolytic anaemia 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 in human medicine as well as for veterinary patients.

Table 1 : Summary of two different prognostic scores developed for use in dogs with immune-mediated haemolytic anaemia

Score	Parameter	Definition	Score
CHAOS (after Whelan et al, 2006)	Age	≥7 years	2
	Rectal temperature	≥38.9C	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, 2010	Sex	Male	1
	Season	Warm	1
	Packed cell volume	<20%	1
	Platelet count	<200x10 ³ /μl	1
	Serum total protein concentration	<6 g/dl	1
	Total possible score		5

Drug or management method	Dose	Mechanism of action	Adverse effects	Cost and practicalities of use
STEROIDS				
Glucocorticoid	Prednisolone: 2 mg/kg/day reducing to 1mg/kg/day after 2-4 weeks. Dexamethasone 0.2–0.3 mg/kg/day until oral medication can be started.	Alters gene transcription. Down regulates Fc receptor expression, decrease antigen processing, suppress T cell function	PU/PD, polyphagia, panting, muscle wasting, weight gain, opportunistic infections, GI ulceration, diabetes mellitus, osteoporosis, thromboembolic disease	Inexpensive. 1, 5 and 25mg tablets available. Injectable dexamethasone convenient if vomiting.
Anabolic steroid	Nandrolone: 1-5mg/kg i.m., subcutaneously, maximum dose 40-50mg/dog. Repeat every 21 days.	Thought to stimulate erythropoiesis in non-regenerative anaemia. Mechanism unproven.	Sodium, calcium, potassium, chloride, phosphate retention; Water retention hepatotoxicity, behavioral changes, reproductive abnormalities (oligospermia, oestrus suppression).	Parenteral form only. Easy to administer. Lack of information about efficacy means anabolic steroids are rarely recommended.
STEROID-SPARING DRUGS				

Azathioprine	2 mg/kg/day PO for no more than 14 days; then decrease to 1mg /kg PO every day or 2mg/kg EOD depending on ease of dosing Or, 50mg/m ² every other day	Thiopurine analogue	Hepatotoxicity (28), bone marrow suppression, pancreatitis. Most side-effects follow using 2mg/kg daily dosing for > 2weeks unless in susceptible individuals	Inexpensive (25 and 50-mg tablet). Other doses have to be reformulated. Do not split tablets <i>Not licensed for use in dogs in UK</i>
Ciclosporin	2.5-5 mg/kg PO q 12 h	Calcineurin inhibitor	GI signs (vomiting, diarrhoea, anorexia), gingival hyperplasia, ²⁹ opportunistic infections. ³⁰ Ciclosporin is not cytotoxic and is not myelosuppressive as it is specific for lymphocytes.	Expensive. Capsules available in 10, 25, 50 and 100mg. Liquid formulation available. <i>Not licensed for use for this condition but licensed for use in dogs in UK.</i>
Mycophenolate mofetil	10 mg/kg PO q 12 h	Purine synthesis inhibitor	GI upset, lethargy, hepatotoxicity, bone marrow suppression	250mg and 500mg capsules. Available as Cellcept and also generic form. <i>Not licensed for use in dogs in UK</i>
Leflunomide	1–4 mg/kg PO q 24 h	Pyrimidine synthesis inhibitor	GI upset, bone marrow suppression, hepatotoxicity	10 and 20mg tablets <i>Not licensed for use in dogs in UK</i>

Lithium carbonate	10-11mg/kg orally, BID	Stimulates erythropoiesis	Diarrhoea, hypersalivation, seizures, increased liver enzymes, PU/PD	Little information about use in dogs. Serum levels should be measured. ^{31,32} <i>Not licensed for use in dogs in UK</i>
RESCUE THERAPIES				
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 <i>Not licensed for use in dogs in UK</i>
Plasma-pheresis	N/A	Microporous membrane separation of plasma from other blood components before immunoglobulins are filtered from plasma to limit immune response	Hypocalcaemia due to anticoagulants in circuit	Requires specialist equipment/expertise. Limited experience in dogs. ³³
Splenectomy	N/A	Underlying aetiology 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 anaesthesia and surgery in a dog with IMHA (thromboembolic disease etc)	One off surgical and aftercare costs. Patient needs to be stabilised for anaesthesia – usually requires transfusion.

Table 2: Management options for IMHA.

Case example 1:

Signalment: 6-yr-old, male whippet. Weight 16kg

History: This dog had a history of immune-mediated thrombocytopenia that had been previously treated with prednisolone 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 anaemia at his primary practice (PCV 25%) and began treatment using prednisolone at a dose rate of 1mg/kg/day initially that was increased to 2mg/kg/day 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.

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

Investigation: Blood work showed a moderate anaemia (PCV 25%) with evidence of regeneration and spherocytosis. There was a mild thrombocytopenia (platelet count $109 \times 10^9/l$, range 175-500 $\times 10^9/l$). Total protein was 74g/l (60-80g/l). Bilirubin was mildly increased (56 μ mol/l, range 0-12 μ mol/l). Coombs' test was negative. In saline agglutination test was positive.

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

Diagnosis: The history, clinical examination and laboratory findings were compatible with a diagnosis of IMHA.

Management: The dog continued on prednisolone (2mg/kg/day) and azathioprine was added (2mg/kg/day). Over the next 24 hours the dog's condition deteriorated significantly and he became pyrexia, lethargic, pale and tachypnoeic. His PCV was found to have dropped to 11%. He was given one unit of packed red cells and his post-transfusion PCV was 25%. He also received 10g of human intravenous immunoglobulin as a constant rate infusion over 4 hours.

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

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 diarrhoea in this dog and was tapered and discontinued over two weeks. After 2 weeks, the prednisolone dose was decreased to 1mg/kg/day. A recheck after one 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 1mg/kg/day. 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 a rapid onset in action and probably did little to prevent the hemolytic crisis that the dog entered. Human IVIg was chosen at this point because of its perceived rapid onset in action. This patient responded well both clinically and also haematologically and was discharged from the hospital in a stable condition.

Case example 2

Signalment: 8-year-old, male, entire, Irish setter dog, 42kg

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

Clinical examination: The dog was bright and appeared normal in attitude. He had a normal temperature; respiratory rate was 56 and heart rate 150bpm. 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 a marked anaemia (PCV 14%) with no evidence of regeneration. There was a marked leukocytosis with a neutrophilia ($31.56 \times 10^9/l$, range $3-11.5 \times 10^9/l$). There was a marked thrombocytosis (platelet count $2275 \times 10^9/l$, range $175-500 \times 10^9/l$). Total protein was 70g/l (60-80g/l). No spherocytosis or in saline agglutination was seen. Bilirubin was normal ($3.1 \mu\text{mol/l}$, range $0-12 \mu\text{mol/l}$). Coombs' test was negative.

Survey radiographs of thorax and abdomen revealed no abnormalities and abdominal ultrasound 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 aetiology in the absence of any other pathological changes.

Management: The dog was treated with prednisolone (2mg/kg/day) and ciclosporin (2.5mg/kg BID). He remained non-regenerative and was transfusion-dependent for the following 3 weeks. At this point

lithium carbonate (10mg/kg, BID) was added to his dosage regimen. Three weeks later, when again the dog required transfusion, ciclosporin was withdrawn and mycophenolate mofetil added (10mg/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 that cytologically was found to contain fungal hyphae. Lithium carbonate dose was decreased to 10mg/kg/day following some adverse effects. The dog continued to do badly however, made no response to mycophenolate and was euthanased due to there being no response to treatment and due to the development of haemorrhagic gastroenteric signs.

Discussion:

This dog illustrates some of the frustrations encountered with bone marrow-centered disease. There were few indications of the aetiology of the anaemia in this Irish setter and the disease was presumed to be immune-mediated in the absence of any other drug, 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 (see 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 (Wang et al., 2013). Unfortunately, it also was not helpful in this case and the dog was euthanased.

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