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IMMUNE MEDIATED BLOOD DYSCRASIAS: THERAPEUTIC OPTIONS

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INTRODUCTION

Both immune mediated hemolytic anemia (IMHA, or AIHA if primary) and immune mediated thrombocytopenia (IMTP, or AITP if primary) are type 2 hypersensitivity responses in which the immune system tags the red blood cell or platelet, respectively, for destruction. Usually, destruction is mediated by mononuclear phagocytic activity. The ideal treatment would allow the host to recognize the tagged cells as “self” and therefore avoid ever targeting those cells for destruction. Unfortunately, except in the case where an underlying cause for secondary immune mediated destruction can be found and removed (e.g., drug administration, curable infection), this is seldom an option. The next best option would be to suppress only those aspects of immunity that cause the destruction of the tagged blood cells, leaving the other immune responses intact. This goal has not yet been achieved in either human or veterinary medicine. In most instances, these diseases are still treated with immunosuppressive drugs that suppress beneficial as well as harmful responses. Very few immunosuppressive therapies have been scientifically evaluated to demonstrate an ideal dosage or even simple efficacy. In addition to immune suppression, treatment of both IMHA and IMTP must include supportive care and attempted prevention of common complications.

AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA)

Immunosuppressive Drug Therapies

Unfortunately, although glucocorticoids and several other immunosuppressive drugs have been used to treat dogs with AIHA there have been few prospective studies to prove or refute the utility of these therapies. The standard of care remains high dose, intermediate acting glucocorticoid drugs. Typically, 2 to 4 mg/kg/day prednisolone divided BID is recommended for dogs. Due to relative steroid resistance, cats may require twice that dose, and prednisolone is often chosen for cats rather than prednisone. Some clinicians recommend initiating immunosuppressive therapy with dexamethasone rather than prednisolone, but evidence for an advantage of one drug over the other at equivalent dosages is lacking. The author uses dexamethasone injection (0.2-0.75 mg/kg/day) only when oral drug administration is difficult due to vomiting or the animal’s temperament. The animal is switched to oral prednisolone as soon as practically possible. No matter the steroid preparation chosen, the initial induction dosage should be tapered as allowed by the animal’s response to therapy. Typically, the first dose reduction occurs 1 to 3 weeks after PCV has normalized or when both the patient and RBC counts are stable (often PCV >30 at this point). Further dose reductions are made only after confirming that the PCV has remained stable after the initial dose reduction. Ideally, once disease is controlled, the use of steroids can be slowly tapered until it is discontinued altogether. There is no single ideal way to conduct this tapering, but certain principles apply. First, the clinical condition should be monitored closely. Worsening soon after a dose reduction would suggest that the taper is too rapid. Second, the severity of the condition holds implications as to the rapidity of the taper. Glucocorticoids used to treat life-threatening disease including AIHA or AITP should be tapered very slowly. Often, the taper will require anywhere from 4 to 9 months. For animals that have had a disease relapse, or in which the initial disease was particularly severe, a low, alternate-day dose of steroid may be continued indefinitely. Third, consolidated dosing with prolonged dosing intervals might spare hypothalamic-pituitary axis suppression while maintaining desirable biologic drug effect. For example, if administering 5 mg prednisone twice daily, it may be better to first switch to 10 mg once a day rather than simply decreasing the twice daily dose. The once daily dose can then be decreased incrementally. When the once daily total dose is in a minimal antiinflammatory dose range (~0.5 mg/kg for dogs), the dose interval can be changed to an every-other-day (EOD) basis. Once on an EOD schedule, the “on day” dose can be incrementally decreased while monitoring for recurrence of disease. Occasionally, an animal’s condition can be controlled only with high steroid dosages. In this situation, the addition of another immunosuppressive agent (e.g., azathioprine) might allow the required dose of steroid to be reduced. Combination therapy is particularly useful in animals with unacceptable adverse steroid effects.

While glucocorticoids remain the only drug with proven efficacy for the treatment of AIHA, high disease mortality demonstrates that alternative or additional therapies should be investigated. Although anecdotal testimonials support each of the following therapies, none has a scientifically demonstrated benefit. The additional or alternative immunosuppressive therapies described carry a risk of adverse effects, and some carry a hefty price as well. The author reserves these therapies for animals that have failed to respond to 5-7 days of glucocorticoids, or in the face of autoagglutination or intravascular hemolysis (processes associated with a worse prognosis). Some veterinary internists routinely employ an additional immunosuppressive therapy along with corticosteroids from the time of disease recognition.

Cyclophosphamide is an alkylating agent that impairs both resting and proliferating B and T lymphocytes, and has some antiinflammatory activity. This is one of the few immunosuppressive drugs that has undergone prospective, placebo controlled trial. Mason et al. compared the response of dogs with AIHA to treatment with prednisone alone or prednisone plus cyclophosphamide. No benefit was demonstrated, and in fact dogs receiving cyclophosphamide displayed delayed regenerative responses and a nonsignificant increase in mortality over dogs receiving prednisone alone. The author does not recommend this therapy. Similar to cyclophosphamide, the alkylating agent chlorambucil has also been advocated for the treatment of IMHA. No studies have evaluated the efficacy of this drug, but due to its many similarities to cyclophosphamide, the author does not recommend its use.

Azathioprine is another very popular choice as an additional immunosuppressive drug for dogs, but not cats, with AIHA. A purine analog, it affects only proliferating lymphocytes and is therefore less potent than cyclophosphamide. In addition to immune suppressive actions, the drug also has antiinflammatory actions. Because anywhere from 2-6 weeks are required for response to azathioprine, it is often begun at the time of diagnosis.
Often the regenerative response if blunted in dogs on azathioprine, with PCV stabilizing in the low 30’s. While adverse affects are uncommon, they can be quite severe. There are no published prospective studies evaluating the effectiveness of azathioprine in the treatment of immune mediated dyscrasias.

Cyclosporine, originally used to avoid transplant rejection, has gained popularity for treatment of autoimmunity in pet animals. Cyclosporine is an immunosuppressive agent that acts by selectively inhibiting the activation of T helper (Th) lymphocytes. Cyclosporine inhibits interleukin-2 (IL-2) transcription after binding to intracellular receptors. Interleukin 2 is crucial for the proliferation of activated T helper and T cytotoxic lymphocytes. Two different types of cyclosporin are available, an oil emulsion (e.g., Sandimmune) and a better absorbed microemulsified drug (e.g., Neoral). The dosage of both types of cyclosporine is adjusted based on trough concentrations of drug. The drug dose required to achieve therapeutic concentrations can be lowered by co-administration of ketoconazole to inhibit hepatic microsomal metabolism. Adverse effects are uncommon, but cost remains prohibitive in many situations. Currently, the utility of cyclosporine for the treatment of AIHA is undergoing prospective, placebo-controlled trial but preliminary results do not support benefit.

Newer drugs used to suppress immunity in people in the face of transplant rejection and rheumatic disease have been used anecdotally to treat dogs with AIHA. Leflunomide is an expensive inhibitor of pyrimidine biosynthesis that has been used anecdotally to treat dogs with AIHA. Leflunomide is an immunosuppressant that acts by inhibiting the enzyme required for purine synthesis. Since lymphocytes lack a salvage pathway for purine synthesis, there is inhibition of both B and T-lymphocyte responses. Like leflunomide, this drug is FDA approved for the prevention of allograft rejection but has also found use in the treatment of other immunologic and rheumatic diseases. It has been anecdotally reported to be effective in the treatment of a variety of immune mediated diseases of dogs, including myasthenia gravis. Thus far, there are no published accounts of its efficacy in the treatment of canine or feline AIHA.

Commercial intravenous immunoglobulins (IVIG) are derived from human beings and are used to treat people with immunodeficiency conditions. They are also used to treat immune mediated diseases including AIHA, and are the treatment of choice for children with AITP. Human IVIG are postulated to exert their immediate effect by blockade of the Fc receptors on mononuclear phagocytes, but multiple other mechanisms, including inhibition of autoantibody production, are likely responsible for their beneficial effect. The use of hIVIG has been reported in the treatment of dogs with refractory AIHA. These uncontrolled reports suggest that IVIG may have some utility in the short-term response of disease. Since the product is of human origin, it is expected that dogs (or cats) will mount an antibody response to the product, and repeat infusions may be associated with hypersensitivity reactions. The author has used IVIG with some perceived success in severe AIHA not responsive to more traditional therapies.

**Additional Immunologic Therapies**

Splenectomy has long been utilized to treat both humans and dogs with poorly controlled AIHA. Because the spleen is the major site of mononuclear phagocytic removal of IgG tagged RBC and platelets, and because the spleen is an important site for the production of antibody, removal of the spleen may ameliorate signs of class III AIHA and/or AITP. It is worth noting that splenectomy would not be expected to be useful in the treatment of the less common IgM mediated forms of hemolysis, as the liver is more important to the pathophysiology of those types of dyscrasias. Traditionally, splenectomy was reserved for animals with AIHA or AITP that were difficult to wean from initial high dosages of steroids, or in which only partial remission was achieved on steroid therapy. Recently, an abstract described the use of splenectomy to treat dogs with an acute presentation of severe AIHA. Dogs received glucocorticoids plus azathioprine, or those same drugs plus splenectomy within 48 hours of presentation. Survival was higher and time to recovery was shorter in dogs receiving immediate splenectomy. This means of therapy warrants further investigation.

Plasmapheresis is the removal of formed antibodies, including autoantibodies, from circulation. Whereas plasmapheresis is routinely used in human medicine, lack of equipment and expertise makes it difficult to perform on dogs and cats. A simplified form of plasmapheresis involves removing a unit of blood at a time, separating the cells from the plasma, and re-infusing the cells while disposing of the plasma. Obviously, this can be difficult to safely accomplish in anemic patients. Because the RBC (or platelets) are already coated with autoantibody, the removal of non-adhered antibody may be of limited utility in treatment of disease.

**Supportive Care**

Supportive care for animals with AIHA is crucial. Among the foremost concerns is the provision of adequate oxygen carrying capacity via transfusion of RBC or Oxyglobin®. There is no “number” for PCV or RBC count that should signal the need for transfusion. Instead, the decision should be based on clinical assessment of the patient. In general, cats tolerate lower RBC counts than do dogs. In both species, the rapidity of onset of anemia may have more to do with clinical signs than the absolute degree of anemia. Tachycardia, tachypnea, weakness, and bounding pulse are relative indicators that oxygen carrying support is required. In the past, practitioners were often reluctant to transfuse patients with AIHA for fear that transplanted cells may blunt the drive to a regenerative response, or for fear that RBC breakdown products may damage the renal tubules. There seems to be little merit to the former concern, and the latter is outweighed by the need to avoid hypoxemia. In the best case scenario, if immunologically mediated destruction is slowed, RBC transfusion should provide prolonged oxygen carrying support in comparison to Oxyglobin. Studies comparing the utility of packed RBC or whole RBC transfusion to Oxyglobin have produced conflicting results. For now, availability and cost are reasonable criteria by which to make a choice between the two.

**Prevention of Disease Complications**

Interestingly, dogs with IMHA seldom die from anemia. Instead, death is more often due to euthanasia or to
complications such as pulmonary thromboembolism (PTE) or disseminated intravascular coagulation (DIC). Although euthanasia was a common cause of death in many retrospective studies examining the outcome of AIHA, the reasons for euthanasia were not enumerated. It is likely that a poor prognosis, failure to respond to therapy, development of complications, and cost of treatment all played some role. Several studies have demonstrated that dogs with AIHA commonly develop thrombosis, and PTE is the most important form of thrombosis. In fact, PTE has been detected in up to 80% of dogs with AIHA that received post-mortem examinations. The reasons for development of thrombosis remain speculative, but risk factors have included the use of central catheters, hyperbilirubinemia (>5 mg/dL), increased ALP, hypoalbuminemia, thrombocytopenia, and transfusion. Another frequent complication of AIHA is DIC. Although the true prevalence of DIC in dogs with AIHA remains unknown because of a lack of complete hemostatic evaluation, up to 58% of dogs with such testing have been found to have DIC.

Prophylactic therapy for PTE and DIC includes the use of heparin. Once again, the ideal form of therapy or even the utility of such therapy is unknown. It is the author’s practice to routinely implement heparin therapy on diagnosis of AIHA. The author typically begins with 100-150 units/kg unfractionated heparin SQ TID to QID. Ideally, aPTT is then monitored daily and treatment adjusted to elevated PTT to approximately twice the starting value. The development of monitoring tools (assays of factor Xa inhibition, bedside PTT assays) and the use of other types of anticoagulant therapy (e.g., low molecular weight heparin, platelet aggregation inhibitors) may allow for better prophylaxis of PTE and DIC in the future. Several clinical studies of these treatments are in the initial stages.

Although not a direct complication of AIHA, GI ulceration can be a complication of high dose steroid use, especially when dexamethasone is used or when steroids are combined with NSAID drugs. There are no studies demonstrating the need for or adequacy of any particular GI protectant in the treatment of AIHA. Nonetheless, the author routinely administers such drugs. The author routinely administers famotidine, an H2 blocker with less potential for drug interaction than cimetadine or ranitidine. Proton pump inhibitors would be a reasonable alternative. These drugs are less expensive and readily available as compared to the synthetic prostaglandin misoprostol.

Vincristine is used to treat dogs with AITP but not AIHA. While the vinca alkaloid antineoplastic agent does possess immunosuppressive activity, the increased platelet counts that follow its use are not entirely the result of these actions. Vincristine is thought to increase platelet counts in patients with AITP by several mechanisms. In addition to a reduction in the degree of phagocytosis of platelets, interference with antiplatelet antibody formation, and interference with the binding of antiplatelet antibodies to platelets, vincristine also accelerates megakaryocytic breakdown and stimulates thrombopoiesis. In essence, in animals with narrow megakaryocytosis it can provide a temporary bump in peripheral platelet numbers by simply fragmenting narrow megakaryocytes into platelets more quickly. These new platelets will still be susceptible to phagocytic destruction, and may be somewhat less aggregable than other platelets. Nonetheless, prospective studies have indicated that animals with AITP treated with a single injection of vincristine had a more rapid recovery of platelet count and a shorter duration of hospitalization. It is the author’s routine practice to administer a single injection of vincristine in combination with standard prednisolone therapy to all dogs diagnosed with AITP. An alternative form of vincristine therapy involves pre-treating platelet rich plasma with the drug. The theory is that the vincristine-loaded platelets will be engulfed by mononuclear phagocytes which will then result in the death of the engulfing cells. This form of therapy is more expensive and has not been demonstrated to result in an improved outcome as compared to a simple IV injection of vincristine.

**Additional Immunologic Therapies**

Splenectomy and plasmapheresis have been used in the treatment of AITP in much the same way as for AIHA. The mechanism of AITP is largely IgG mediated mononuclear phagocytosis in the spleen. Although splenectomy has shown promise, prospective studies proving efficacy are lacking. Platelet counts should be greater than 80,000 at the time of surgery to optimize chance of recovery.

**Supportive Care**

Because the major complication of AITP is bleeding, provision of oxygen carrying support is the most crucial aspect of supportive care. Oxyglobin, packed RBCs, or whole blood transfusions are each capable of providing this support. While it seems intuitive that whole blood (WB) may have the advantage of supplying platelets, in fact this makes very little difference. Even when fresh (<6 hours from collection) WB is transfused, the platelet count is not expected to increase in much (~ 10 ml/kg WB will increase platelet count ~10,000/ul). Unlike transfused RBC which supply oxygen and are then re-used over and over by "refilling" with oxygen in the lungs, platelets are single use cells. Once adhered, they are out of circulation permanently. Rapid use and rapid destruction of transfused platelets means that essentially all transfused platelets are gone in minutes to hours after transfusion.

Although none are ideal, there are ways in which a more concentrated platelet product can be administered. These include platelet rich plasma (PRP), platelet concentrate (PC), and cryopreserved platelets. Somewhat laborious to produce, PRP is derived by using slow spins to remove plasma from RBC within hours of blood collection. Because some 20% of the platelets in fresh WB are lost in preparation, the advantage over WB is not number of platelets but....
conservation of RBC and the need to transfuse a smaller volume. These advantages are enhanced even further with the use of platelet concentrate, which is prepared by removing even more plasma volume from the platelet pellet. Both PRP and PC must be used within days of preparation. Cryopreserved platelets in DMSO are similar to PC but frozen, and have a storage life of up to 6 months (Midwest Animal Blood Services, Inc 877-517-MABS).

Supportive care also includes measures to minimize the risk of bleeding. Gentle handling is a must. Soft canned diets are suggested to minimize gingival trauma. Drugs that might affect platelet function should be avoided whenever possible (e.g., NSAIDs, penicillin derivatives, cimetidine, certain anesthetics and sedatives). Avoid IM injections and jugular venipuncture, and apply gentle pressure bandages after any venipuncture. Avoid cystocentesis. Although GI protectants are often used in much the same way they are used to treat AIHA, there is little evidence that they decrease GI hemorrhage, potentially one of the most severe complications of AITP. While there are no controlled studies to support its use, I have had very good success curbing the intensity of severe GI bleeding by the oral administration of barium to dogs that are not vomiting. The dose is empiric, but generally 10-20 ml PO TID. Because one of the few reasonable differential diagnoses for severe thrombocytopenia in the absence of evidence of marrow disease, vasculitis, or sepsis / DIC is rickettsial disease, it is common practice to begin doxycycline therapy while appropriate infectious disease titers are pending.

**Prevention of Disease Complications**

Neither PTE nor DIC have been reported as common consequences of isolated AITP, although either may occur in dogs with Evan’s syndrome. The most important complications of AITP are directly related to bleeding. Anemia and a lack of oxygen carrying capacity have been described, and are often attributable to severe GI bleeding. The other life-threatening complication of AITP relates to the site of bleeding. Bleeding into the CNS is often fatal in dogs with AITP. Unfortunately, there is little in the way of preventative measures to decrease the likelihood of CNS bleeding other than therapy aimed at increasing platelet count as rapidly as possible. Any evidence of CNS disease would warrant immediate transfusion of platelet products, even realizing that those platelets will be rapidly consumed. Although not life threatening, another complication of AITP is intracocular bleeding. Daily ocular exams are important, as topical therapy including steroids should be instituted as soon as possible to minimize risk of permanent vision loss.

*Previously presented at the 22nd Annual ACVIM Forum, Minneapolis, MN, June 2004.*

**REFERENCES**


Other suggested readings available by request.
Table 1.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Starting dose</th>
<th>~ Cost/week for 12 kg dog</th>
<th>Important/common adverse effects</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisone</td>
<td>2 to 4 mg/kg, ÷ BID or once daily</td>
<td>$6</td>
<td>PU/PD, polyphagia, panting, altered behavior</td>
<td>Initial dose is tapered slowly as allowed by clinical condition</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>2 mg/kg/day PO</td>
<td>$12</td>
<td>GI upset, myelotoxicity, hepatopathy</td>
<td>Initial dose is halved in 7-10 days, slowly tapered thereafter.</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>-oil-based -10 to 25 mg/kg + BID -micro emulsified 5 to 10 mg/kg +BID</td>
<td>$90 (either formula)</td>
<td>GI upset, gingival hyperplasia, rarely nephrotoxicity</td>
<td>Trough levels must be monitored to adjust dosage</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>50 mg/m² EOD PO or 200 mg/m² IV once/week</td>
<td>$20 PO, $53 IV</td>
<td>GI upset, myelotoxicity, sterile hemorrhagic cystitis</td>
<td>Not recommended by author</td>
</tr>
<tr>
<td>Chlorambucil</td>
<td>0.1 to 0.2 mg/kg daily</td>
<td>$33</td>
<td>GI upset, myelotoxicity</td>
<td>Not recommended by author</td>
</tr>
<tr>
<td>Leflunomide</td>
<td>4mg/kg daily</td>
<td>$259</td>
<td>GI upset, anemia, lymphopenia</td>
<td>Trough levels monitored to adjust dosage</td>
</tr>
<tr>
<td>Danazol</td>
<td>5 mg/kg BID to TID</td>
<td>$33</td>
<td>Virilization, hepatotoxicity</td>
<td>Not recommended by author</td>
</tr>
<tr>
<td>Mycophenolate mofetil</td>
<td>20-40 mg/kg +BID to TID</td>
<td>$48</td>
<td>GI upset</td>
<td>Little veterinary experience</td>
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<tr>
<td>IVIG</td>
<td>0.5 to 1.5 g/kg infused 6-12 hrs</td>
<td>$1,880</td>
<td>Hypersensitivity reaction</td>
<td>Single-use treatment in crisis</td>
</tr>
<tr>
<td>Vincristine</td>
<td>0.02 mg/kg IV once</td>
<td>$22</td>
<td>GI upset, causticity, peripheral neuropathy</td>
<td>Generally single use</td>
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