

EMERGENCY AND LONG-TERM MANAGEMENT OF CANINE IMMUNE-MEDIATED HEMOLYTIC ANEMIA

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Introduction

Immune-mediated destruction of red blood cells can be primary/idiopathic, in which there is no known etiology or inciting trigger and is the cause of IMHA. In 70-80% of dogs. Secondary IMHA occurs when the immune system develops IgM and/or IgG antibodies following stimulation by infection (*Babesia spp.*, *Anaplasma spp.*, *Mycoplasma spp.*, *Ehrlichia spp.*), exposure to drugs (sulfonamide, cephalosporin, carprofen), vaccination and neoplasia. An ACVIM Consensus statement published in 2019 recommended to screen for infectious diseases including tick-borne infection and heartworm by serology and PCR when an animal lives in or has travelled to an endemic area. In some geographic locations, IMHA appears to have a seasonal increase during warm weather when exposure to infectious agents and vector-borne diseases is possible. While screening tests with an in-house SNAP 4Dx tests for *Ehrlichia canis* and *ewingii*, *Anaplasma phagocytophilum* and *platys*, *Borrelia burgdorferi* and *Dirofilaria immitis*, the report has found low evidence for IMHA triggered by *Ehrlichia canis*, *Leishmania spp.*, *Neospora* and Hemotropic mycoplasma. Another report published in 2019 found IMHA developed in almost 15% of dogs naturally infected with *Ehrlichia ewingii*. Despite low evidence to support a cause and effect of *Anaplasma spp.*, *Bartonella spp.*, *Leishmania spp.* and *Ehrlichia spp.* and the development of IMHA, screening for these pathogens is strongly recommended. The Vector Borne Disease Laboratory at North Carolina State University has a comprehensive canine PCR and serology panel that screens for the presence of antibodies as well as infectious pathogen proteins.

Clinical Signs

Dogs with IMHA are often presented to their primary care veterinarian with a history of general malaise, exercise intolerance, inappetence, vomiting, diarrhea or collapse. Discolored red or orange urine may be present with hemoglobin- or significant bilirubinuria. Icteric mucous membranes or sclera may also be noted. At the time of presentation, physical examination can reveal signs of tachypnea, tachycardia, bounding femoral pulses, possibly a heart murmur, hepatosplenomegaly, fever, icterus, pallor, and sometimes petechiae in animals with concurrent thrombocytopenia. If there is suspicion of IMHA in an anemic patient, making a diagnosis starts with taking a thorough history that includes travel, vaccination status and when administered, exposure to fleas and ticks and compliance with preventative medications, heartworm testing and prevention and recent administration of any medications including antibiotics should be asked.

Diagnostic Testing

Diagnostic testing should be directed to determine whether the patient has anemia secondary to blood loss, destruction or lack of production. If petechiae are present, blood samples (whole blood and serum separator) should be obtained from peripheral venipuncture until thrombocytopenia has been ruled out. The ACVIM Consensus statement on IMHA has determined that there is a lack of a gold standard to make a definitive diagnosis of IMHA, and testing should be performed based on the index of suspicion and potential for secondary triggers of hemolysis on an individual patient basis.

Sufficient samples should be obtained to submit a complete blood count to an outside laboratory and have a pathologist review the slide. As it takes 4 to 5 days prior to the development of a regenerative response and adequate reticulocytosis, approximately 30% of dogs will present with a nonregenerative anemia. If the anemia remains nonregenerative after that time, a bone marrow core biopsy should be performed to determine whether pure red cell aplasia or maturation arrest or neoplasia is present. The CBC and blood smear evaluation can also detect the presence of leukocytosis as well as thrombocytopenia. Varying degrees of thrombocytopenia are common in dogs with IMHA, with 40% of dogs having platelet counts of < 100,000/uL and 25% less than 50,000/uL. Consumption of platelets secondary to disseminated intravascular coagulation rather than immune-mediated destruction is also possible.

Spherocytosis is found in 67-94% of dogs with IMHA, however is not pathognomonic for the disease and can be observed with coral snake envenomation and heredity disorders such as pyruvate kinase deficiency. Spherocytes are not usually characteristic in non-regenerative immune-mediated anemias or when there are antibodies directed at red blood cell precursors. Stored red blood cell products, for example, can contain high numbers of spherocytes. In addition to spherocytosis, ghost cells

and siderocytes may be present with IMHA, whereas the presence of Heinz bodies can suggest an oxidative injury to the red blood cells.

IgM cross-linking of red blood cells can cause autoagglutination. This must be differentiated macro- and microscopically from rouleaux formation that occurs with nonspecific inflammation. A Coomb's test is useful at detecting circulating anti-erythrocyte antibodies, however, is only 50-60% sensitive, depending on the laboratory and methodology used. Positive Coomb's testing can be observed in the absence of IMHA in dogs infected with *Leishmania*, *Dirofilaria immitis* and *Bartonella spp.* As the end result of Coomb's testing is agglutination, the test is not useful in patients with autoagglutination.

Basic diagnostic testing such as a PCV and a urinalysis can aid in determining the degree of anemia, but also investigate whether intravascular hemolysis is present. When the plasma appears pink tinged to port wine colored in appearance, the presence of red urine that does not clear with centrifugation further supports a diagnosis of intravascular hemolysis. Particularly in animals with intravascular hemolysis, hemoglobinuria, spherocytes and other fragmented red blood cells on a peripheral blood smear, a minimum of one abdominal radiograph should be performed to rule out metallic foreign bodies that could contain zinc.

Hyperbilirubinemia and bilirubinuria is also common in dogs with IMHA, although this finding may be equivocal in dogs with chronic low-grade destruction of red blood cells. The ACVIM Consensus statement concluded that only one of the following criteria is needed to be characteristic of an elevated bilirubin – icterus, total serum or plasma bilirubin above the high end of the normal reference range, or greater than 2+ bilirubinuria.

Thoracic radiographs and an abdominal ultrasound also should be performed to investigate for the presence of neoplastic processes that could trigger a secondary immune-mediated destruction of red blood cells. If there are any abnormal findings on thoracic radiographs or abdominal ultrasound, coagulation testing (APTT and PT) should be performed prior to performing fine needle aspirates to investigate for the presence of neoplasia, in particular hemophagocytic histiocytic sarcoma.

Therapy

The standard of care for treatment of IMHA is stopping the immune-mediated destruction of red blood cells, eliminating any inciting factors (when possible), and providing supportive care for treatment and prevention of hypoxemia, nausea, infection and thromboembolic complications. The cornerstone of immunosuppressive therapy is administration of glucocorticoids. At this time, there are no studies to support combination therapy of other immunosuppressive agents with glucocorticoids versus administration of glucocorticoids alone. As many dogs develop sometimes intolerable or potentially life-threatening side-effects of glucocorticoid therapy, administration of other therapies in addition to the glucocorticoid will often allow eventually tapering and discontinuation of the glucocorticoid over time. Glucocorticoid therapy is provided in two phases – first to put the patient into remission then second to have a chronic maintenance phase that is tapered over time. Prednisolone at a dose of 1 to 2 mg/kg PO Q24 hours or for larger dogs 50 mg/m² PO Q24h should be initiated. Once the patient's packed cell volume has normalized, the dose of steroid is dropped by 20 – 25% every 3-4 weeks until a dose of 0.5 mg/kg Q24h is achieved. Once this dose is achieved, the dose is gradually dropped to every other day, then discontinued over 4 to 9 months. A common cause of relapse is when drugs are tapered too quickly. Recovery is defined as a PCV greater than 36% and absence of spherocytes or autoagglutination.

Additional immunosuppressive agents should be considered in severe cases that require multiple transfusions, are not responsive to glucocorticoid therapy alone or if there is evidence of autoagglutination. Cyclosporine (5-10 mg/kg PO Q12h) has a rapid onset of action to suppress T cell function and release of inflammatory cytokines. Potential side effects of cyclosporine administration includes the development of opportunistic infections including dermatophytosis, gingival hyperplasia, and hepato- or nephrotoxicity. Mycophenolate mofetil (10 mg/kg IV, PO Q12h) is an immunosuppressive drug that acts to block T and B cell proliferation and the production of B-cell antibodies. Mycophenolate is well-absorbed, but has variable bioavailability, which appears to decrease with administration with food. Potential adverse side-effects include vomiting, diarrhea, lethargy, lymphopenia and skin infection. Leflunomide (2-4 mg/kg PO Q24h) is a newer immunosuppressive agent that blocks both T and B cell function. Like other immunosuppressive drugs, the most common side effects are vomiting and diarrhea. Bone marrow suppression with leukopenia and thrombocytopenia can also occur. Azathioprine is a purine antagonist that is used to suppress T cell function. It may take 1 to 2 weeks prior to allowing significant immunosuppression. Side effects can include vomiting, diarrhea, myelosuppression and pancreatitis. The recommended dose is 2 mg/kg PO Q24h for 5 to 7 days, then 2 mg/kg PO every other

day. A retrospective cohort study of azathioprine with prednisolone showed no benefit to its use over administration of prednisolone alone, with a similar 1 year survival, making its use somewhat equivocal as a standard therapy for IMHA.

Intravenous Immunoglobulin (IVIG) (0.25 – 2.2 g/kg IV over 6-12 hours) is a product that contains highly purified human IgG obtained from pooled plasma from healthy donors. While the immunomodulatory activity of the product is not well-known, it is thought that the IgG binds to the Fc receptors on activated neutrophils, macrophages, B cells, natural killer cells and platelets. The benefit of the product is that it is rapidly acting, however, there have not been any studies to determine whether its use improves long-term survival. Potential complications of IVIG include acute anaphylaxis, hypotension and thromboembolism.

Like a blood transfusion, IVIG can be infused through a peripheral venous catheter at an initially slower rate (0.1 ml/kg/min) while monitoring vital signs. The rate can slowly be increased to a maximum of 0.8 ml/kg/min until the infusion is complete. Because of the possibility of inducing an immune-response to a foreign protein, repeated doses over months to years is not recommended.

Other therapies which have been described but are not common include splenectomy and therapeutic plasma exchange. A small retrospective study of splenectomy in addition to standard immunosuppressive therapy in dogs with refractory IMHA described no relapse within 30 days of surgery with high post-splenectomy PCV and reduced need for post-operative transfusion requirements compared with medical management alone. As this study very small and retrospective, this therapy is not considered standard of care or routinely recommended at this time. Therapeutic plasma exchange can potentially remove circulating immunoglobulin in dogs with refractory IMHA. The availability of plasmapheresis however is limited to a few centers around the United States, making this option not widely feasible for the majority of patients.

Many animals with IMHA are inappetent and require oral administration of medications with common nausea-inducing side effects. Glucocorticoids also can induce gastrointestinal ulceration. For this reason, many clinicians advocate empiric use of antiemetic drugs (Maropitant 1 mg/kg IV or SQ or 2 mg/kg PO Q24h; ondansetron 0.1 mg/kg PO Q12-24h, 0.5-1 mg/kg IV Q6-12h) along with acid reduction (famotidine 0.5 - 1 mg/kg IV, SQ, PO Q12h; pantoprazole 1 mg/kg IV Q12h or omeprazole 1 mg/kg PO Q12h) and sometimes sucralfate (0.25 – 1 g PO Q8h) if hematemesis or melena is present.

Many animals with IMHA are normovolemic but show signs of decrease oxygen delivery due to low hemoglobin concentrations. Maintenance intravenous crystalloid fluids are sometimes required to maintain hydration in inappetent animals who are not drinking or are vomiting. In animals with significant hyperbilirubinemia or intravascular hemolysis, intravenous crystalloid fluids may be required to decrease risk of the nephrotoxic effects of bilirubin and hemoglobin pigment on the renal tubules.

Blood transfusions are another mainstay of therapy in the patient with IMHA and should be administered when the animal becomes clinical for the anemia. While it is tempting to administer a transfusion based on a specific target PCV, a concern is that the infused donor cells can also be under immune attack. For this reason, this author transfused when an animal is lethargic, inappetent, tachycardic or tachypneic or when the PCV drops below 12%. Studies have documented impaired oxygen delivery when with a PCV less than 10% in dogs. Multiple transfusions may be required over days to weeks before sufficient immunosuppression occurs to maintain a stable PCV.

Dogs do not possess innate alloantibodies to donor red blood cells prior to being transfused. For this reason, it is safe to transfuse a naïve dog with one red blood cell transfusion from an unmatched donor, when absolutely necessary in a life-saving situation when an animal may be dying before your eyes. Within 4 to 5 days of a transfusion, however, the recipient will develop antibodies, and must be cross-matched prior to subsequent transfusions. As Cornell Veterinary Specialists stocks only universal (DEA 1.1 negative) dog blood, we do not routinely perform blood types in all dogs who require a transfusion. If a dog has received a prior transfusion after 4 days, a cross match is performed to decrease the risk of transfusion reaction. As a large population of dogs with IMHA have autoagglutination, manual cross-match methods cannot be performed to test for compatibility. For this reason, a commercially available cross match kit (Rapid Vet H, DMA Laboratories, Inc.) is available that provides easy interpretation even in the presence of autoagglutination.

If an infectious pathogen known to contribute to the development of secondary IMHA is identified, treatment of the infection should be initiated ideally prior to immunosuppressive therapy. Given that the test is not cage -side and can take a week or more prior to finalization of test results, therapy targeted at empirically treating for pathogenic organisms is often initiated in the form of Doxycycline (5-10 mg/kg PO

Q12-24h) with other therapies such as immunosuppression during an acute hemolytic crisis. Immunosuppression itself carries the inherent risk of predisposing a patient to opportunistic infections such as bacterial cystitis, pyelonephritis and pyoderma. If an infection develops during immunosuppressive therapy, the infection should be treated based on bacterial culture and susceptibility testing whenever possible.

At the time of presentation and during therapy, most dogs with IMHA have evidence of hypercoagulability based on thromboelastography testing. While this is an interesting finding and can allow targeted therapy to prevent venous thrombosis, the specific pathogenesis of thromboembolism in dogs with IMHA is not well-understood. Studies have documented increased platelet activation, complement activation, increased tissue factor expression, and elevations in procoagulant microparticles and neutrophil extracellular traps in dogs with IMHA. An estimated 50 to 80% of dogs with IMHA develop life-threatening thromboembolic complications. Earlier therapies have advocated use of low-dose aspirin or unfractionated heparin to help prevent thromboembolic complications. More recent evidence is that low-dose aspirin at the previously recommended dose (0.5 mg/kg PO once daily) is ineffective at decreasing platelet activity in healthy dogs. Unfractionated heparin also has variable efficacy unless the dose is adjusted to an individual patient based on frequent monitoring of anti-Xa activity. This is time-consuming and costly, and therefore not a recommended therapy.

More recently, the use of oral anticoagulants with more reliable bioavailability and are advantageous over injectable therapies in that no therapeutic monitoring is required. Clopidogrel reduces platelet activity by irreversible binding to receptors on the platelet membrane. An initial loading dose of 10 mg/kg PO is recommended, followed by 2 mg/kg/day. Reduction of platelet function is observed within 24 hours of administration. While the therapy appears to be well-tolerated, survival rates in dogs with IMHA treated with clopidogrel are similar to those treated with combined therapy with aspirin or aspirin alone.

Rivaroxaban is a direct inhibitor of Factor Xa and has high and predictable bioavailability in healthy dogs. In a prospective study of dogs with IMHA treated with rivaroxaban (median dose 0.89 mg/kg/day PO) versus oral clopidogrel appeared to be well-tolerated.

Low molecular weight (fractionated) heparins are smaller and have more predictable pharmacokinetics compared with unfractionated heparin compounds. Enoxaparin (0.8 mg/kg SQ Q6-8h) has been shown to maintain anti-Xa activity in healthy dogs without adverse consequences of hemorrhage. Dalteparin (150 U/kg SQ Q12h) also can be used if available. While the drugs appear to be well-tolerated in dogs with IMHA, expense of therapy can be cost-prohibitive for some dogs and the frequent need of parenteral administration may not be possible for some clients.

Irrespective of the anticoagulant regimen chosen, a recent Consensus Statement on the rational use of antithrombotics in veterinary critical care documented a relatively small knowledge base and comparison of therapies to be able to recommend one therapeutic strategy over another at this time.

Monitoring varies from patient to patient and also may be based on clinician preference. More frequent monitoring every 7-14 days may be recommended as a patient is initially discharged from the hospital, followed by recheck examination every 2 – 4 weeks, particularly during any adjustment in medications, tapering, or relapse. One of the most important decisions to make after achieving remission in patients with IMHA is how and when to start tapering the drugs. As a general rule of thumb, one must consider whether the patient is experiencing side effects associated with any of the drugs and also consider the cost of each individual therapy. Tapering the drug that is the most expensive and/or causing the most side effects should be considered, then monitor the patient's PCV every 1 to 2 weeks after each dose reduction. The weaning should occur over a period of 3 to 6 months, to achieve administration of the lowest therapeutic dose possible. Some dogs can be weaned completely from medications over time. Relapse can occur at any point of therapy during the taper, after discontinuation of all medications or even years later. Relapse is defined as a decrease in hematocrit after initial improvement or normalization. One retrospective study showed that up to 10-12% of dogs will relapse within 5 years of the initial diagnosis of IMHA.

Prognosis for dogs with IMHA is initially considered guarded, as even with aggressive therapy mortality rates still range from 26 to 70%, depending on an individual's response to therapy. Many patients succumb to thromboembolic disease (pulmonary thromboembolism, mesentery, spleen), kidney or liver failure. In the acute phase of treatment, animals usually succumb to a lack of response to therapy, whereas long-term, death is usually secondary to the adverse complications of therapy or relapse. Negative prognostic indicators include the presence of autoagglutination, hyperbilirubinemia, thrombocytopenia, hypoalbuminemia, elevated alkaline phosphatase and the degree of leukocytosis.