

Immune-Mediated Hemolytic Anemia: Current Perspectives

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Immune-mediated hemolytic anemia (IMHA) is one of the most common hematologic diseases seen in dogs with a reported mortality rate that ranges between 29% and 77% in the veterinary literature.¹⁻⁹ Hemolysis results from the binding of immunoglobulins to red blood cell surface antigens, causing those cells to be lysed by complement intravascularly or removed from circulation by mononuclear phagocytes. IMHA may be a particularly frustrating disease for both owners and clinicians because of its waxing and waning clinical course, the potential for sudden complications, and the expense associated with treatment.

Immune-mediated red blood cell destruction may be classified in a number of ways. Primary or idiopathic autoimmune hemolytic anemia (AIHA) refers to immune-mediated hemolysis in the absence of an identifiable trigger factor, whereas secondary IMHA results from an underlying process such as neoplasia, infectious disease, or drug reaction. IMHA may also be categorized based on whether it results in intravascular or extravascular hemolysis. Intravascular hemolysis results from the lysis of red blood cells by complement within the vasculature, and may be identified by the presence of free hemoglobin within the plasma and urine. Extravascular hemolysis results when there are insufficient antibodies present to cause complement fixation, and antibody-labeled red blood cells are removed by the reticuloendothelial system within the spleen and liver. Extravascular hemolysis tends to be a more gradual process and may be identified by the presence of bilirubin, rather than hemoglobin within the plasma and urine. IMHA may also be classified based on the presence or absence of autoagglutination. Autoagglutination is the spontaneous clumping of red blood cells and results from the cross-linking of erythrocytes by large numbers of antibodies. We have noted autoagglutination in approximately 70% of dogs treated for IMHA.

IMHA is typically a disease of middle-aged to older pets. As with other types of immune mediated disease, a female gender predisposition has been reported. At Michigan State University in the past eight years, approximately 2/3 of IMHA cases were seen in female dogs. Although any breed may develop IMHA, a number of breed predispositions have also been reported and include Cocker Spaniels, Poodles, Shih Tzus, Lhasas, Old English Sheepdogs, Border Collies, and Springer Spaniels. A seasonal predilection has also been suspected, as some studies have observed a larger number of cases presenting in spring and summer months. This may be a result of increased exposure to outdoor allergens or antigenic stimulation, or may simply reflect the overall increase in patient admissions seen during these months.

Clinical signs of IMHA may be acute or chronic, depending upon the rate of hemolysis. With chronic disease, symptoms such as lethargy, weakness, inappetance, vomiting, diarrhea, and pigmenturia are most commonly reported, whereas with more rapid hemolysis, acute collapse may be the first symptom noted. It is not uncommon for dogs to be brought in for "possible urinary tract infection" because the owners have noted discoloration of the urine with hemoglobin or bilirubin. Symptoms related to anemia, including tachycardia, tachypnea, and systolic ejection murmurs may also be noted on physical exam. Hepatosplenomegaly is not unusual as these organs are common sites for extramedullary hematopoiesis as well as clearance of antibody-labeled erythrocytes. Fevers are frequently seen as a result of release of endogenous pyrogens like IL-1 and IL-8. Reactive lymphadenopathies may also be seen.

Initial in-house diagnostics should include PCV/TS, blood smear, and slide agglutination test, as these are inexpensive, easy to perform, and will frequently provide a great deal of information about the cause of the anemia. The importance of interpreting the PCV in conjunction with the total solids (TS) cannot be overemphasized. If the PCV and TS are both low, blood loss (rather than hemolysis) should be suspected. In contrast, a low PCV with a normal TS would be consistent with hemolysis or decreased red blood cell production. To differentiate these two clinical entities, the plasma of the spun sample should be carefully evaluated for the presence of hemoglobin or bilirubin that may suggest hemolysis. Blood smears may also be useful in differentiating hemolysis from decreased production anemia, as the presence of significant polychromasia and anisocytosis indicates the presence of a regenerative response. Blood smears should also be evaluated for blood parasites and telltale alterations in red blood cell morphology. Spherocytes are small, round erythrocytes with loss of central pallor, that result when antibodies bound to red blood cell membranes lead to a portion of the membrane being phagocytized or "pinched off" by macrophages. Large numbers of these cells are typically seen in dogs with immune-mediated hemolysis. Ghost cells, which appear as "empty" cell membranes may be seen with intravascular hemolysis. Finally, a slide agglutination test should be performed when hemolysis is suspected. In this test, a drop of anticoagulated blood from a purple top tube or capillary tube is mixed with several drops of saline. Autoagglutination may be evidenced by the development of obvious flecks within the drop of blood. Autoagglutination is caused by cross-linking of antibodies bound to the erythrocyte membranes, and as such is diagnostic for an immune-mediated component to the hemolysis.

A number of other diagnostics should be considered in the evaluation of animals suspected to have IMHA. CBC, chemistry, and urinalysis should be run as part of a minimum database. The presence of hemoglobinemia/hemoglobinuria or

bilirubinemia/bilirubinuria may suggest intravascular or extravascular hemolysis, respectively. Leukocytosis is frequently noted on the CBC from patients with IMHA and may result from non-specific “gearing up” of the bone marrow, or from tissue damage secondary to hypoxia and thrombosis. White blood cell counts in excess of 45,000/ μ l have been associated with a more guarded prognosis.¹⁰ Platelet counts should also be evaluated. Moderate thrombocytopenias may suggest consumptive coagulopathy or tick-borne illness, while severe thrombocytopenias (<50,000/ μ l) should prompt consideration of a concurrent immune-mediated thrombocytopenia. Reticulocyte count should always be performed to assess regenerative response. Immune-mediated hemolytic anemias are typically strongly regenerative, though it may take three days for regenerative response to be noted. Non-regenerative anemias should prompt suspicion of red cell aplasia, precursor-directed immune-mediated anemia (PIMA), or other form of decreased production anemia. A Coombs test is indicated if hemolysis is suspected but autoagglutination is not present. The Coombs test, or direct antiglobulin test, is essentially a test for the presence of antibodies or complement bound to erythrocyte membranes. It is performed by adding anti-dog antibodies (immunoglobulins directed against canine IgG, IgM, or complement) to a sample of the patient’s red blood cells. If autoantibodies are present on the patient’s blood cells, the antiserum binds to them and cross-linking occurs. Because the end (positive) result of this test is agglutination, the Coombs test need not be run if the patient is already autoagglutinating.

A search should also be conducted for possible trigger factors. History taking should include questioning about recent vaccinations or medications. Recent vaccination (ie. within 4 weeks) has been associated with the development of IMHA.³ Sulfa drugs, penicillins, and cephalosporins may also cause IMHA by acting as haptens, substances that become adsorbed to erythrocyte membranes. If these haptens are targeted by the immune system, the entire red blood cell may be destroyed. Neoplasias such as hemangiosarcoma, lymphoma, myeloproliferative diseases, and hemophagic histiocytosis are another common trigger factor, and chest radiographs and abdominal ultrasound are frequently performed to rule out these entities. Testing should also be performed for tick-borne illnesses such as *Ehrlichiosis* and *Babesiosis*.

Treatment of IMHA consists of improving tissue oxygen delivery, suppressing the immune response, preventing some of the major complications of IMHA (such as thromboembolic disease), and hopefully preventing future recurrence. In the emergent patient, tissue oxygenation may be improved greatly by the administration of intravenous fluids. Although some clinicians worry about “diluting” an already anemic patient with IV fluids, in actuality, fluids will improve tissue oxygen delivery in the hypovolemic patient by maximizing cardiac output. However, the majority of dogs with IMHA will also require blood transfusion or oxyglobin during the course of their hospitalization, as immunosuppressive therapies are not rapidly effective in stopping the hemolytic process. The decision to transfuse is based on a number of factors, including hematocrit values, clinical signs, and the chronicity of the anemia. Clinical signs of anemia such as reluctance to eat, tachycardia unresponsive to fluids, tachypnea, dyspnea, lethargy, and altered mentation should prompt consideration for transfusion.

A number of drugs may be considered for the purpose of immunosuppression. Prednisone is the mainstay of therapy in dogs with IMHA, and at this time no other drug has been *proven* to work better than prednisone alone. Clinical experience suggests that 2 mg/kg/day in dogs provides adequate immunosuppression in the dog. Higher doses are not necessarily more immunosuppressive but may be associated with an increased risk of gastrointestinal complications. Prednisolone, rather than prednisone, should be used in cats as the bioavailability of prednisone is limited in this species.¹¹ Additionally, cats may require higher doses than dogs, and the author typically uses 4 mg/kg/day in cats. A growing number of retrospective studies have suggested that azathioprine may improve long-term survival, and that cyclophosphamide may be associated with a poorer outcome.^{2,4,9,12,13} Caution should be used in interpreting these studies as inherent bias may be present due to their retrospective or small scale nature. In our clinic, we frequently use azathioprine (2 mg/kg q24h for 7 days then q48h), cyclosporine (Atopica 5-10 mg/kg/day divided), or mycophenolate (10 mg/kg PO q12h) as adjunct therapies and to facilitate prednisone weaning later in the course of treatment. Intravenous immunoglobulin (IVIG) is also occasionally used in patients who are slow to respond to conventional therapy. IVIG is essentially purified IgG antibodies collected from the pooled plasma of over 2000 human donors. It is believed to act primarily by blocking macrophage Fc-receptors, thereby decreasing phagocytosis of red blood cells. Downregulation of antibody production, enhanced catabolism of antibodies, and suppression of cytokine release are other possible mechanisms of action. Although one small prospective study did not demonstrate more rapid response times in IMHA patients receiving IVIG at presentation, clinical experience and a number of retrospective studies have demonstrated its utility as a rescue therapy in individual patients.¹⁴⁻¹⁷ IVIG is typically dosed at 0.5-1 mg/kg given over 6 hours. Side effects include vomiting, fever, potential for anaphylaxis, and possible increased risk of thrombosis.

Thromboembolic disease (TE) is a frequent complication of IMHA. In studies of dogs with IMHA that underwent necropsy, TE was identified in 60-80% of cases.^{8-10, 18-20} Sites most commonly affected were the pulmonary and splenic vasculature. Although exact mechanisms for the prothrombotic state have not been elucidated, increased concentrations of procoagulant factors, decreased concentrations of anticoagulant and fibrinolytic factors, vasculitis, enhanced platelet reactivity, the presence of antiphospholipid antibodies, liberation of RBC stroma, blood transfusion, and administration of steroids have all been hypothesized to play a role in the development of TE. Changes in primary hemostasis are also thought to play a role in the development of a pro-thrombotic state. Weiss & Brazzell demonstrated increased platelet P-selectin expression in dogs with IMHA, supporting the hypothesis that platelets circulate in an activated state.²¹ Documentation of the pro-thrombotic state remains challenging in clinical cases and has traditionally

been based upon detection of increased fibrinolysis (increased fibrin degradation products (FDPs) and D-dimers) and decreased endogenous anticoagulants (antithrombin) rather than rate of clot formation. Recently, our group has documented hypercoagulability as assessed by thromboelastography in this patient population. 26/26 dogs with idiopathic IMHA enrolled in this study all had an MA (maximal amplitude; a reflection of clot strength) that was significantly greater than normal.

Though antemortem identification of thromboembolic events can be challenging, data from 110 dogs treated for IMHA at the Michigan State University Veterinary Teaching Hospital between 2004 and 2007 showed that 34% were suspected to have developed TE during their hospital stay. Of the dogs with suspected TE, 51% had pulmonary thromboembolism (PTE) alone, 8% had portal venous thrombosis (PVT) alone, and 41% had both PTE and PVT. The development of TE appears to significantly contribute to the morbidity and mortality of IMHA. In our data, survival to discharge in dogs with TE was significantly lower than in dogs without TE (49% vs 81%) and median duration of hospitalization was longer (7 days vs 4 days). Of note however, 7 of 7 dogs with PVT identified on ultrasound whose owners opted for aggressive therapy all survived, suggesting that early identification and management of this problem may improve outcome. We are currently evaluating CT angiography as a technique for definitive identification and monitoring of pulmonary and portal clots.

Because hemostatic abnormalities are common in dogs with IMHA, obtaining baseline coagulation testing at the time of admission is strongly recommended. In our critical care unit, dogs with IMHA are then treated with heparin sodium at a loading dose of 150 units/kg IV followed by a continuous infusion of 30-60 units/kg/hour. The heparin dose is adjusted daily to prolong the activated partial thromboplastin time (aPTT) to 1.5-2 times the baseline value. Twenty-six dogs prospectively enrolled in a coagulation study and heparinized based upon this protocol all survived to discharge and serial evaluation of thromboelastography showed normalization of parameters related to clot formation by 30 days, once hemolysis was no longer taking place. Low dose aspirin (0.5 mg/kg PO BID)²² may also be started during hospitalization, particularly in cases where there is failure to achieve a target aPTT. Plavix (2 mg/kg q24h) or aspirin (0.5 mg/kg q12h) are frequently started at the time of discharge to prevent rebound hypercoagulation associated with heparin withdrawal.

Gastrointestinal protectants, such as pepcid (0.5 mg/kg q24h) or sucralfate, are used by many clinicians in hopes of preventing GI ulceration. At this time there is no evidence to suggest that these medications are effective in preventing ulcers, and in our hospital, they are typically administered only once ulceration is suspected to have occurred. Gastric ulceration should be suspected if melena, vomiting, or reluctance to eat develop, or if serum total protein begins to fall in conjunction with the hematocrit. It is important to recognize the development of GI blood loss, because the resulting drop in hematocrit can otherwise be easily confused with treatment failure.

Dogs with idiopathic IMHA are at risk for recurrence of disease, and care should be taken not to wean the immunosuppressive drugs too quickly. Prednisone is typically maintained within the immunosuppressive range for at least one month following hospital discharge, and then may be decreased by approximately 20-25% each month, provided that the hematocrit remains stable. If azathioprine or other adjunctive agent is being administered in conjunction with the prednisone, it may be discontinued one month after discontinuing prednisone. In total, the weaning process should span at least 4-6 months. Labwork should be rechecked one week after each decrease in drug dosage to make sure that the change is tolerated. If relapse occurs during the weaning process, immunosuppressive dose prednisone should be reinstated, then gradually weaned back to the lowest effective dose. Following weaning, it is frequently recommended that vaccines be avoided, though the association between vaccines and IMHA development is still unproven. Splenectomy may be considered for dogs with recurrent or refractory disease.

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