Hemolytic Anemias – Diagnosis & Management of Immune-mediated, Infectious, Toxic and Hereditary Hemolytic Anemias

Anemii hemolitice - Diagnosticul și managementul anemiilor hemolitice hemolitice, infecțioase, toxice și ereditare hemolitic mediate imun

Urs Giger, *Prof. Dr. med. vet. MS FVH*
Dipl. ACVIM & ECVIM-CA (Internal Medicine) & Dipl. ECVCP (Clinical Pathology)
School of Veterinary Medicine, University of Pennsylvania, Philadelphia, USA
giger@upenn.edu; www.vet.upenn.edu/penngen

Immune-mediated hemolytic anemia (IMHA) is a common hematological disorder in dogs, may be primary (idiopathic, autoimmune) or occur secondarily to underlying diseases and is often associated with life-threatening complications. The diagnostic approach and its management with immunosuppression and transfusion support will be discussed with emphasis on evidence and controversies.

Introduction

Immune-mediated hemolytic anemia (IMHA) is one of the most common and serious hemolytic anemias in dogs, but occurs rarely in other animal species. In IMHA an immune response, including anti-erythrocytic antibodies, complement and macrophages, targets directly or indirectly erythrocytes and a hemolytic anemia ensues. There are many triggers for IMHA such as infections, drugs and other agents, and cancer leading to secondary IMHA, but in many dogs no cause is identified (so-called idiopathic, autoimmune or primary IMHA) or a genetic predisposition has been proposed (Cocker spaniels). Furthermore, alloimmune hemolytic anemias, such as hemolytic transfusion reactions, both acute and delayed, and neonatal isoerythrolysis (only litters from transfused bitches), are caused by specific anti-erythrocytic alloantibodies. In contrast to other species, dogs with IMHA also develop an often overwhelming inflammatory response resulting in thrombosis and necrosis of various organs. And while the anemia can be corrected with transfusions, these complications in dogs are causing severe morbidity and mortality despite aggressive immunosuppression and antithrombotic interventions.

Immune Destruction of Erythrocytes

Regardless of the underlying cause, IMHA results from a breakdown in immune self-tolerance or from a deficit in the control mechanism that regulates B and T lymphocyte activity as well as macrophage reactivity. Immune destruction of erythrocytes is initiated by the binding of IgG or IgM antibodies to the surface of erythrocytes. Under most clinical circumstances, immune destruction is an extravascular process that depends on recognition of erythrocytes opsonized with IgG, IgM and/or complement by specific receptors on reticuloendothelial cells. Macrophages with engulfed erythrocytes may be noted on cytological examination of blood and tissue aspirates as erythrophagocytosis, but this is not definitive proof of an immune-mediated process.

Antibody-coated erythrocytes may also be lysed by complement fixation and the membrane attack complex, which is clinically noted as intravascular hemolysis.

A diagnosis of IMHA must demonstrate accelerated immune destruction of erythrocytes. Evidence of a hemolytic anemia is suggested clinically by icterus and a regenerative anemia with hyperbilirubinuria, and hemoglobinemia and hemoglobinuria refers to an intravascular process. However, the erythroid response in the bone marrow may be blunted by the immune and inflammatory process or the underlying disease thereby leading to non-regenerative anemias. Besides documenting a hemolytic anemia, one or more of the following three hallmarks must be present to support a diagnosis of immune-mediated hemolysis: persistent autoagglutination, marked spherocytosis and a positive direct Coombs' test result. As in human medicine, the Coombs' test should be considered the best test to definitively diagnose IMHA, although marked spherocytosis and persistent/true autoagglutination (after 3x washing of EDTA blood with saline) are other important parameters indicating immune-destruction of erythrocytes.

Autoagglutination

Anti-erythrocytic IgM and in large quantities IgG antibodies may cause direct erythrocyte autoagglutination. The autoagglutination may be seen by naked eye in an EDTA tube or on a glass slide or may become apparent as small clumps of erythrocytes on blood smears. For yet unexplained reasons, canine erythrocytes have a tendency to unspecifically agglutinate in the presence of plasma and colder temperatures as well as possibly with excessive EDTA anticoagulant. Mixing blood with one drop of saline may break up rouleaux formation but not other forms of unspecific red cell agglutination. It is, therefore, important to determine whether the agglutination persists after "saline washing", which has been coined persistent or true autoagglutination. This is accomplished by adding physiologic saline to the tube containing a small amount of EDTA-anticoagulated blood, mixing, centrifuging and removing the supernatant including the plasma and repeating this saline washing 3 times. True or persistent autoagglutination is indicative of an immune process, but precludes the performance of Coombs' test or blood typing and crossmatching procedures which are based upon an agglutination reaction as result. Those based upon chromatographic techniques do not seem to be affected by autoagglutination as free red cells can move along the strip. If the agglutination breaks up after washing, the Coombs' test is expected to be positive, if it is a case of IMHA. There is no evidence for washing away red cell bound antibodies in dogs.

Spherocytosis

If erythrocytes are only partially phagocytized or lysed by complement in circulation, erythrocytes with reduced surface area to volume ratio, known as spherocytes, are formed. They appear spherical and microcytic with no central pallor and are considered fragile. Note proper areas on the blood smear needs to be reviewed to find spherocytes in between single regular discoid red cells. Large numbers of spherocytes (>20/microscopic high power field) are nearly diagnostic for IMHA, whereas small numbers may be seen with other conditions including DIC, endotoxemia and zinc intoxication. In our experience all dogs with marked spherocytosis and suspected to have IMHA also had a positive Coombs' test. However, only 60-80% of dogs with a positive Coombs' test or clinically diagnosed with IMHA had marked spherocytosis. Hereditary spherocytosis due to genetic

membrane defects has rarely been seen in dogs, but should be considered as a differential diagnosis in dogs with negative Coombs' test results.

Because of the difficulties with the Coombs' test (see below), Slappendale had proposed to use the erythrocytic osmotic fragility test at specific saline concentrations as a mean to diagnose IMHA and this test is currently used in various clinics in Europe. However, there are many other reasons for increased fragility of erythrocytes beside IMHA including hereditary red cell defects. This test is not used in human medicine and has not been shown to be superior to determination of marked spherocytosis and a positive Coombs' test in dogs with IMHA. The osmotic fragility test is also a cumbersome and not well standardized technique.

Positive Direct Coombs' Test Result

The direct Coombs' test is also known as direct antiglobulin test (DAT) and is used to detect antibodies and complement on the surface of erythrocytes when the anti-erythrocyte antibody strength or concentration is too low to cause spontaneous agglutination (subagglutinating titer). Separate canine-specific IgG, IgM, and C3b antibodies as well as polyvalent antiglobulin reagents are available. They are added at various concentrations after washing the patient's erythrocytes free of plasma (3x as shown above) and mixtures are generally incubated at room temperature or 37°C (cold agglutinins appear to be rarely of clinical importance and rarely cause hemolysis). The strength of the Coombs' reaction does not necessarily predict the severity of hemolysis, but reaction changes are useful in monitoring the disease.

Typically tube or microtiter methods have been used exclusively in the reference or teaching laboratory setting, but a flow cytometric method has also been introduced in a couple of places. A standardized, sensitive, and simple gel column method was available by DiaMed (Switzerland), but unfortunately the company was sold to another company which decided to not pursue the veterinary market. A novel standardized antiglobulin test method has just been developed by Alvedia (France) similar to the immunochromatographic strip technique for blood typing of dogs and cats (see updates on blood typing and crossmathing). Although many commercial laboratories offer Coombs' testing for dogs, clinicians have questioned the tests sensitivity and specificity and often forgo the test and/or use response to therapy as a diagnostic. However, negative Coombs' test results may be seen because of technical reasons, insufficient quantities of bound antibodies, the presence of weakly bound antibodies, or the disease in remission. The Coombs' test stays positive for days to months after initiating treatment. A few days of immunosuppressive therapy will likely not reverse the Coombs' test result, as unlikely a transfusion would cause a positive Coombs' test result. Thus, dogs with negative Coombs' test results should be reevaluated for other causes of hemolytic anemia.

In a recent prospective study of anemic and non-anemic dogs we compared various direct Coombs' test methods including microtiter plate assays, gel column, capillary, and immunochromatographic techniques using polyvalent antiglobulins in a laboratory setting and found excellent correlations between tests and with spherocytosis and without noticeable interference by immunosuppressive or transfusion therapy in anemic dogs.

In conclusion, a diagnosis of IMHA requires the documentation of red blood cell destruction and an immune process. While regenerative anemia, icterus, and hyperbilirubinuria are suggesting a hemolytic anemia, evidence of true autoagglutination, spherocytosis, and/or a positive direct Coombs' test are required to document immune destruction. The authors also recommend monitoring IMHA patients for the disappearance of these immunological parameters to adjust and taper therapy.

Therapeutic Considerations

A diagnosis of IMHA requires the documentation of red blood cell destruction and an immune process. While regenerative anemia, icterus, and hyperbilirubinuria are indicating the presence of a hemolytic anemia, evidence of (1) true autoagglutination after washing, (2) marked spherocytosis, and/or (3) a positive direct Coombs' test are required to document immune destruction. The prognostic factors for IMHA are poorly defined unless IMHA is secondary to an underlying disease. Severe anemia, icterus, leukocytosis, hypoalbuminemia and thrombotic evidence are unfavorable findings. Because the severity of IMHA ranges from indolent to life-threatening disease and serious complications seen with IMHA, therapy has to be tailored for each patient and depends in part on whether the IMHA is primary or secondary in nature. Removal of the triggering agent or treatment of the underlying condition can bring the IMHA rapidly under control.

Fluids, Blood Transfusions, Oxygen and Oxyglobin in IMHA

Restoration and maintenance of tissue perfusion with crystalloid fluids is important, even when it results in further lowering of the hematocrit. When severe anemia and a dropping hematocrit lead to signs of tissue hypoxia, packed red blood cell transfusions appear beneficial. The increased oxygen-carrying capacity provided by the transfused red blood cells may be sufficient to maintain the animal's hematocrit for a few days, while other treatment modalities have time to become effective. The notion that transfusions pose an increased hazard to animals with IMHA has been overemphasized and is not supported by retrospective clinical studies. Fresher blood products are possibly an advantage. However, the common occurrence of autoagglutination may make blood typing and crossmatching of the patient impossible. In these cases DEA 1- blood should be transfused. Additional blood types are being recognized which may be also important.

If compatible blood is not available, the bovine hemoglobin solution Oxyglobin, a highly purified bovine hemoglobin solution, if available, may be administered and provides increased oxygen-carrying capacity and plasma expansion. The original FDA study documented the beneficial effects of Oxyglobin in dogs, whereas recent retrospective studies do not allow any conclusions. In contrast to blood and Oxyglobin, oxygen inhalation therapy is of little benefit, unless the animal with IMHA is suffering form pulmonary disease such as pulmonary thromboembolism. Thanks to adequate transfusion support, animals with IMHA rarely die because of anemia, but because of secondary complications such as thromboemboli and infections.

Immunosuppressive Therapy for IMHA

The insufficient understanding of the pathogenesis, the generally guarded prognosis, the lack of good therapeutic trials, the serious drug side effects, and the high costs of

intensive care greatly hamper the successful management of dogs with IMHA. The main goal of immunosuppressive therapy is to reduce (1) phagocytosis, (2) complement activation, and (3) anti-erythrocytic antibody production. Glucocorticoids are the initial treatment of choice for canine, feline and human IMHA. They interfere with both the expression and function of macrophage Fc receptors and thereby immediately impair the clearance of antibody-coated erythrocytes by the macrophage system. In addition, glucocorticoids reduce the degree of antibody binding and complement activation on erythrocytes, and only after weeks, diminish the production of autoantibodies. Thus, oral prednisolone at a dose of 1-2 mg/kg twice daily is the mainstay treatment. Alternatively, oral or parenteral dexamethasone at an equipotent dose of 0.6 mg/kg daily can be used, but is likely not more beneficial.

There is no evidence that other immunosuppressive agents are effective. They should not be used initially as they are associated with severe side effects. Additional immunosuppressive therapy is warranted when prednisone fails, only controls the disease at persistently high doses, or when it causes unacceptable side effects. They are generally used together with prednisolone, but may eventually be used independently. Historically, cytotoxic drugs such as cyclophosphamide were added, however a small randomized study and several retrospective surveys failed to show any beneficial effects, but may be associated with greater morbidity and mortality in the acute management of IMHA. Retrospective studies and anecdotal reports with azathioprine, cyclosporine, danazol, mycophenylate, and human intravenous immunoglobulin suggest some efficacy, but controlled prospective clinical trials that document their efficacy are lacking. For instance, there is no evidence that azathioprine is effective and from a mechanistic view point it only inhibits antibody production and as it is an antimetabolite, it is only effective after a few weeks. Furthermore, the side effects of acute pancreatitis and agranulocytosis to aplasia makes this in most cases unsuitable. Cyclosporine at 5-10 mg/kg is likely the best and safest second agent but blood drug levels have to be determined in order to avoid toxicity and underdosing. Highly immunosuppressive agents from transplantation medicine such as mycophenylate and leflunamide are other agents which have been tried but no definitive beneficial effects have been reported. Finally, human intravenous immunoglobulin at 2 x 1 g/kg may rescue a non-responding IMHA patient but relapses are common.

One other agent is melatonin which has been added as immunotherapy which is begin but it is unclear if this has any beneficial effects. Splenectomy may be considered particularly in refractory cases with large spleen, but even a normal spleen may excessively clear antibody-coated red blood cells. Furthermore, splenic histopathology, toxicology and infectious disease screens may offer a diagnosis of an underlying disease. Finally, because of the apparently severe agglutination and the inflammatory and necrotic process, plasma exchange therapy has been used in a few cases and appeared to be helpful in expediting response and avoiding serious complications.

It should be noted that an apparent therapeutic response to immunosuppressive therapy is insufficient evidence for the diagnosis of IMHA. Response to therapy may be indicated by a hematocrit that rises or stabilizes, an appropriate reticulocytosis, diminished autoagglutination, and fewer spherocytes; this response can be expected to be seen within days to weeks. The subsiding of autoagglutination would allow the

performance of a direct Coombs' test and thereby permit the direct documentation of anti-erythrocytic antibodies. As glucocorticosteroid therapy is associated with well-known side effects, the initial dose will be tapered by reducing the amount by one-third every 7-14 days and moving toward every other day therapy. In secondary IMHA with appropriate control of the underlying disease, the tapering can be accomplished more rapidly.

Thromboembolic and Other Complications with IMHA

Because of the potential of gastrointestinal ulceration by glucocorticosteroids and other immunosuppressives, gastrointestinal protectants such as sucralfate may be considered. Because dogs with IMHA suffer from an immune deregulation which may have been triggered by an infection and are treated with immunosuppressive agents, these patients are prone to experience infections; it is, therefore, prudent to administer preventative as well as therapeutic antibiotics to these dogs with IMHA on immunosuppressive therapy.

Thromboemboli and DIC are unique serious complications that greatly contribute to the morbidity and mortality of dogs with IMHA which are not typically seen in humans and cats with IMHA. Although the pathogenesis remains unknown, venipuncture, catheters, confinement, and glucocorticosteroids as well as other immunosuppressive agents may be contributing factors. Thus far, no study has definitively documented any successful prevention and/or management protocol for these life-threatening hemostatic problems in canine IMHA. Predisposing factors should, whenever possible, be limited, and adequate perfusion and tissue oxygenation should be provided with fluids and transfusions or Oxyglobin. Generally, anticoagulation therapy is instituted after there is some evidence or suspicion of thromboemboli. Unfractionated Heparin (dose of 50-300 IU/kg subcutaneously every 6 hours or by continuous intravenous infusion) or Low Molecular Weight Heparin (LMWH; Dalteperin 150 IU/kg sc every 12 hours) are the most commonly used drugs and is used. The replacement of coagulation factors and antithrombin III has not been proven to be beneficial. Antiplatelet agents may also be used and for instance an ultralow dose of aspirin (1 mg kg once daily) has been advocated by a couple of groups, but other studies question its efficacy. Other antithrombotic agents such as modern antithrombotic agents have been used occasionally, but their efficacy and safety remain also unproven.

In conclusion, the successful management of IMHA remains a challenge; immunosuppressive therapies beyond glucocorticosteroids have not been proven to be effective but can be associated with serious side effects. Furthermore, the tendency to inflammation, necrosis and thromboembolism of dogs with IMHA contributes greatly to the morbidity and mortality of dogs with IMHA and effective preventative and therapeutic interventions have not yet been established.

Author's studies were supported in part by grants from the National Institutes of Health (OD010939) and the AKC Canine Health and other Foundations. The author is scientific advisor to various companies and the director of the non-for-profit PennGen Laboratory offering genetic and hematological testing.