

Immune-Mediated Hematopoietic Diseases^a

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IMMUNE-MEDIATED HEMOLYTIC ANEMIA

General Features/Immunopathogenesis

Immune-mediated hemolytic anemia (IMHA) is a fairly common hematologic disorder in dogs but is relatively uncommon in cats. It is caused by immunoglobulin (IgG/IgM) and or complement-mediated red blood cell (RBC) destruction. It can be primary (idiopathic) or secondary to a wide variety of other conditions including parasites (*Ehrlichia*, *Babesia*), neoplasia, drugs, vaccines, viruses, and certain inflammatory diseases.¹

In dogs the majority of cases are considered to be idiopathic in nature. Because of the tendency for these cases to occur in clusters, a viral etiology is suspected. In one study 40% of cases were diagnosed between May and June.² The age range for dogs is 1 to 13 years with a mean age of 6.4 years. Females may be more commonly affected.

Clinical Features

The clinical signs associated with IMHA are usually acute or peracute; chronic cases are rare. The most common clinical signs are anorexia, lethargy, and exercise intolerance. Occasionally the owners report vomiting and small bowel diarrhea.

Physical examination reveals pallor, occasionally icterus, and depression; fever may or may not be present. Depending on the severity of the anemia, tachycardia and hyperpnea may be present. Hepatosplenomegaly may be detected on abdominal palpation. Dogs with IMHA and concurrent immune-mediated thrombocytopenia usually have evidence of petechiae and/or melena.

Hematologic Features

The hemogram reveals moderate to severe anemia. The mean packed cell volume (PCV) is approximately 15%; however most dogs have a PCV less than 20%. Hemolytic anemias are characterized by macrocytosis, hypochromia, and reticulocytosis. Reticulocytes are immature, anucleate RBCs and have more endoplasmic reticulum than a mature RBC. New methylene blue (NMB) precipitates and stains this endoplasmic reticulum. Reticulocyte counts can be performed in-house by mixing equal volumes (5 drops) of blood and NMB stain in a test tube and incubating the mixture for 10 minutes; 1000 RBCs are counted, and the number of reticulocytes is then expressed as a percentage. The reticulocyte index is calculated by multiplying the percentage of reticulocytes by the patient's PCV and dividing by 40. Regeneration is indicated when the reticulocyte index is above 2.5.

IMHA needs to be differentiated from other forms of hemolysis (see box on p. 100). It is very important to question the owner about recent vaccination, potential ingestion of a toxin or foreign body, and drug history. Of dogs with immune-mediated hemolytic anemia, 33% do not have evidence of regeneration at the time of presentation.² Potential explanations for this are that the immune response is directed at the level of the stem cell or that the disease was hyperacute and there is a lag period of 2 to 3 days during which there is no evident reticulocytosis.

Spherocytes are frequently detected on examination of the blood smear. Spherocytes are formed when there is sufficient antibody adherent to the red cell surface to induce some complement system activation but not enough to cause lysis of the RBC. The adherent antibody and complement proteins cause significant RBC membrane damage and induce decreased deformability of the RBC. The RBC becomes spherical and loses central pallor and discoid shape.

Extensive complement activation and erythrocyte lysis result in hemoglobinemia. Circulating hemoglobin is rapidly bound by serum haptoglobin, and the resulting complex is too large to be filtered through the glomerulus. The available haptoglobin will be saturated with severe hemolysis, and free hemoglobin will filter through the renal glomerulus resulting in hemoglobinuria.

Autoagglutination of the RBCs due to antibody bound to the red cell membrane surface may be detected when a drop of blood is placed on a slide. Autoagglutination should be differentiated from rouleaux

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CAUSES OF HEMOLYTIC ANEMIA IN DOGS

Immune mediated
 Drug induced
 Infectious
 Haemobartonella
 Babesia
 Cytauxzoon
 Ehrlichia
 Oxidative injury
 Onion ingestion
 Acetaminophen
 Benzocaine
 Inherited metabolic disorders
 Pyruvate kinase deficiency
 Phosphofructokinase deficiency
 Anemia/chondrodysplasia
 Nonspherocytic hemolytic anemia
 Microangiopathic hemolytic disease
 Zinc induced
 Hypophosphatemia

formation by adding a drop of saline to the slide. If agglutination is still present after the addition of saline, it is more likely autoagglutination.

Changes in white cells are common. A severe neutrophilic leukocytosis with a left shift and monocytosis are often present. The presence of thrombocytopenia may indicate concurrent immune-mediated thrombocytopenia or disseminated intravascular coagulation (DIC).

Diagnosis

The diagnosis is largely based on the clinical signs in association with the hematologic changes previously noted. The strongest evidence for a diagnosis of IMHA is the presence of autoagglutination or a positive direct Coombs' test. The direct Coombs' test quantitates the amount of antibodies or complement bound to the RBC membrane. The Coombs' reagent must be species-specific and recognize IgG, IgM, and complement C3b. False-positives can be the result of nonspecific antibody or complement that does not primarily involve the RBC present on the surface of the RBC. In addition, false-positives can be associated with previous blood transfusions (within the past 3 to 21 days).

It is important to remember that not all cases of IMHA have positive Coombs' tests. One report found 74% of dogs to have positive Coombs' tests; however, I have not found this to be true in our hospital population. Most dogs presented to our hospital in the last 4 years have had negative Coombs' tests. False-negatives may be associated with inadequate antiserum, previous

steroid administration, or excess/insufficient antibody.

Bone marrow biopsies are indicated in animals with acute, severe, nonregenerative anemia. A distinct maturation block at one stage anywhere from the rubriblast to the metarubricyte may be seen in animals with immune-mediated disease. The stages that are present are hyperplastic. In pure red cell aplasia, there is an absence of erythroid cells in the marrow, presumably due to the destruction of committed stem cells.

Therapy and Prognosis

The mainstay of therapy for IMHA is immunosuppressive doses of corticosteroids. Prednisone is most often used at a dose of 2 to 4 mg/kg once or twice a day. This dose is generally continued for 4 to 7 days and then slowly tapered over a period of 4 to 6 weeks, provided the animal is clinically and hematologically improved or stable. Corticosteroids suppress macrophages, decrease antibody binding to red cells, suppress immunoglobulin production, and impair neutrophil killing and cell-mediated immunity. Clinical responses are usually apparent within 48 to 72 hours.

Despite aggressive therapy with corticosteroids, some dogs continue to deteriorate. My clinical impression is that this usually occurs in dogs that are both hyperbilirubinemic and have evidence of autoagglutination. In these cases, cyclophosphamide^a at a one time dose of 250 mg/m² orally or intravenously can be administered in addition to prednisone.

Blood transfusions should be administered only to animals with severe clinical signs associated with their anemia. Dogs with IMHA may be prone to destroying the transfused cells, and transfusions may delay the regenerative response. There is no magical PCV value that necessitates a transfusion—the decision should be based solely on clinical impression.

Maintenance therapy of dogs with IMHA includes prednisone at a dosage of 1 mg/kg every other day and azathioprine^b at a dose of 50 mg/m² every other day. Efforts should be made to slowly taper the prednisone over a period of 4 to 6 weeks and to continue the azathioprine over 3 to 6 months. Close hematologic monitoring is necessary because of azathioprine's potential to cause myelosuppression.

Refractory IMHA may be treated with danazol^c at a dose of 5 mg/kg twice daily. Danazol is an androgenic steroid that has been shown to decrease expression of Fc receptors on macrophages. It is available in 50, 100, and 200 mg capsules. Unfortunately it is expensive. One 100 mg tablet costs approximately \$2.00.

^aCytoxan®—Bristol-Myers Squibb Oncology.

^bImuran®—Glaxo Wellcome.

^cDanocrine®—Sanofi Winthrop.

Recently, human γ -globulin (0.5 to 1.5 g/kg IV over 12 hours) was administered to a small number of dogs suspected of having IMHA nonresponsive to conventional therapy.³ Hematologic improvement was noted in the dogs studied; however, the cost of this therapy for a 20 kg dog is \$680.00.

Most dogs diagnosed with IMHA require lifelong immunosuppressive therapy and monitoring. There is a 30% initial mortality. Deaths are largely attributable to multiorgan thrombosis, pancreatitis, DIC, and euthanasia because of poor response to medical management.

IMMUNE-MEDIATED THROMBOCYTOPENIA

General Features/Immunopathogenesis

Immune-mediated thrombocytopenia (IMT) is relatively common in dogs and uncommon in cats. It is caused by increased antibody and/or complement-mediated platelet destruction within the reticuloendothelial system (spleen, bone marrow, liver) or decreased production of platelets secondary to an antibody or complement-mediated destruction of megakaryocytes within the bone marrow. IMT may be primary (idiopathic) or secondary. Most cases in dogs are idiopathic. IMT may occur alone or in combination with IMHA (Evans's syndrome) or systemic lupus erythematosus. Most animals are middle-aged, and females are overrepresented.

Clinical Features

Clinical signs are usually of acute onset and related to a primary hemostatic defect including petechiae, ecchymoses, and superficial bleeding, which are usually visualized on the gingival surfaces of the oral cavity, external genitalia, and inguinal surfaces. In addition, melena may be detected on rectal palpation.

Clinical signs associated with bleeding are not usually prevalent until the platelet count is below 30,000/ μ l; however, they may also be related to the rapidity of thrombocytopenia development, the stability of the capillary endothelial membrane, the incidence of traumatic events, and adequate function of the remaining platelets. If the thrombocytopenia is severe and the bleeding pronounced, acute collapse may occur. Fever and splenomegaly may also be detected on physical examination.

Diagnosis/Hematologic Features

One of the most common causes of "thrombocytopenia" is laboratory error; therefore a diagnosis of thrombocytopenia should always be confirmed by repeating a platelet count in animals without evidence of petechia. Once the diagnosis is confirmed, every effort should be made to try to determine the cause. There are many causes of thrombocytopenia in dogs

CAUSES OF THROMBOCYTOPENIA IN DOGS

Decreased Platelet Production

Immune mediated
Idiopathic bone marrow aplasia
Drug induced
Ehrlichia, Rocky Mountain spotted fever,
Haemobartonella
Adenovirus, parvovirus
Babesia
Histoplasmosis
Myelophthisis
Cyclic thrombocytopenia

Increased Platelet Destruction, Sequestration/Utilization

Immune mediated
Live viral vaccination
Drug induced
Microangiopathy
Disseminated intravascular coagulation
Hemolytic uremic syndrome
Vasculitis
Splenic torsion
Endotoxemia
Acute hepatic necrosis
Neoplasia

(see box above), and a diagnosis of IMT is usually made by exclusion of other causes of thrombocytopenia. It is important to question the owner regarding any medications the animal may be receiving. There are numerous drugs reported to cause thrombocytopenia; however, just because a certain drug is not mentioned in the literature does not exclude the possibility of an idiosyncratic drug reaction in that animal.⁴ Appropriate serum titers should be submitted to the laboratory to detect the presence of *Ehrlichia*, and the dog should be started on doxycycline (5 mg/kg twice daily).

The hemogram may or may not reveal the presence of anemia secondary to blood loss or concurrent IMHA. The blood smear should be evaluated for hemoparasites and RBC fragments. A leukocytosis with a left shift is typically present.

A bone marrow biopsy should be performed in animals with thrombocytopenia. Bone marrow biopsies of dogs with IMT usually reveal megakaryocytic hyperplasia; however, megakaryocytic hypoplasia may also be seen as a result of immune-mediated destruction of stem cells. Bone marrow biopsies are helpful in ruling out thrombocytopenia secondary to myelophthisis. There are no really good definitive tests for IMT; essentially the diagnosis is made by exclusion and response to therapy.

CAUSES OF NEUTROPENIA IN DOGS AND CATS^a

Decreased or Ineffective Production of Cells in the Proliferating Pool

Myelophthisis (neoplastic infiltration of the bone marrow; D,C)

Drug induced

Chemotherapeutic agents (D,C)

Chloramphenicol (C)

Griseofulvin (C)

Sulfa-trimethoprim (D,C)

Estrogen (D)

Phenylbutazone (D)

Phenobarbital (D)

Other

Toxins (inorganic solvents, benzene; D,C)

Infectious diseases

Parvovirus (D,C)

Retrovirus (feline leukemia virus, feline immunodeficiency virus; C)

Histoplasmosis (D,C)

Ehrlichia (D)

Toxoplasmosis (D,C)

Canine distemper virus infection (D)

Canine hepatitis virus infection (D)

Idiopathic bone marrow hypoplasia/aplasia (D,C)

Cyclic neutropenia of gray collies (D)

Acquired cyclic neutropenia (D,C)

Steroid-responsive neutropenia (D,C)

Sequestration of Neutrophils in Marginating Pool

Endotoxic shock (D,C)

Anaphylactic shock (D,C)

Anesthesia (D,C)

Sudden, Excessive Tissue Demand, Destruction, or Consumption

Infectious diseases (peracute, overwhelming bacterial infection; D,C)

Viral (D)

Drug induced (D,C)

Immune mediated (D,C)

Paraneoplastic (D)

D = dogs; C = cats.

^aModified from Couto CG: Leukopenia and leukocytosis, in Reinhardt RW (ed): *Essentials of Small Animal Internal Medicine*. St Louis, Mosby-Year Book, 1992, p 916.

Therapy and Prognosis

IMT is treated with immunosuppressive doses of corticosteroids.⁵ Prednisone is usually administered at a dose of 2 to 4 mg/kg once or twice a day; responses are usually seen within 48 to 72 hours. The dose of prednisone is then gradually tapered over a period of 4 to 6 weeks. Some authors suggest that dexamethasone at a

dose of 0.3 to 0.4 mg/kg orally once daily may be beneficial in cases refractory to prednisone; however, controlled clinical trials addressing this issue are lacking.

Most cases of IMT require lifelong therapy, and I therefore usually start dogs on azathioprine at a dose of 50 mg/m² concurrently with the initial dose of prednisone. Dogs receiving this drug should be monitored for hematologic toxicity as discussed above. Blood transfusions should be administered as needed to maintain adequate oxygen-carrying support.

I often administer vincristine^d to dogs that do not respond to corticosteroids within the first 48 to 72 hours and require multiple blood transfusions. Vincristine stimulates megakaryocyte endomitosis, and therefore platelets are released from the bone marrow earlier than normal.⁶ The effectiveness of vincristine relies on the presence of megakaryocytic hyperplasia prior to its administration.

Dogs with refractory IMT can be treated with danazol, as discussed above for the therapy of IMHA. Splenectomy has also been suggested for the treatment of refractory IMT, although there is much debate regarding the effectiveness of this procedure.

IMMUNE-MEDIATED NEUTROPENIA

Immune-mediated neutropenia (IMN) is relatively uncommon in both dogs and cats. It may be primary (idiopathic) or secondary to drugs, infection, and neoplasia or may be associated with other immune diseases. There are few reported cases in the literature.⁷ Clinical signs associated with neutropenia in dogs and cats are usually vague and nonspecific. Anorexia, lethargy, and pyrexia are the most common clinical features associated with neutropenia.

Hematologic features include the presence of a severe neutropenia, often associated with a monocytosis. Evaluation of neutropenic dogs and cats should include a detailed drug history, vaccination history, complete physical examination in search of a septic process, serology or virology for infectious diseases, and bone marrow cytology or histopathology. Sequential evaluation of the leukogram is helpful in excluding transient or cyclic neutropenia and/or hematopoiesis.

A definitive diagnosis of IMN requires the demonstration of antineutrophil antibodies by leucoagglutination or immunofluorescent techniques; however, these tests are not readily available, and therefore the diagnosis is usually based on exclusion of other causes of neutropenia (see the box at left) and response to therapy.

Therapeutic trials of immunosuppressive doses of corticosteroids (prednisone; 2 to 4 mg/kg once or ^dOncovin®—Eli Lilly and Company.

twice a day) can be instituted after the exclusion of other causes of neutropenia. Responses are usually noted within 48 to 72 hours. Treatment is continued as for dogs with IMHA.

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