The Hemolytic Anemias

Hemolytic anemia is characterized by shortened red cell lifespan caused by destruction of circulating red cells. Hemolytic anemias may be difficult to separate from acute blood loss because hemolytic anemias, like acute blood loss, are responsive anemias characterized by the typical features of red cell regeneration in the peripheral blood: anisocytosis reticulocytosis (polychromasia in routine smears), occasional Howell-Jolly bodies, and occasional nucleated red cells. However, in general hemolytic anemias are more responsive than blood loss anemias. While anemias with absolute reticulocyte counts between 100,000/µl and 250,000/µl could be the result of either blood loss or hemolysis, anemias with absolute reticulocyte counts of more than 250,000/µl are most likely hemolytic.

In addition to the degree of regeneration being useful as an indicator of blood loss versus hemolysis, the presence of hemoglobinemia and/or hemoglobinuria is an indicator of an intravascular hemolytic condition. However, hemoglobinemia and/or hemoglobinuria are only seen in extreme cases of intravascular hemolysis; many cases of intravascular hemolysis and all cases of extravascular hemolysis lack these findings.

Changes in red cell morphology may also be used to help differentiate hemolytic anemias from blood loss anemias and to identify specific causes of hemolysis. As for the other findings listed above, these morphologic red cell changes may not be present in every case of a given syndrome.

The paragraphs that follow describe the principal hemolytic syndromes we encounter clinically and outline the principal features that may be used to diagnose and differentiate them.

Immune-Mediated Hemolytic Anemia

Immune-mediated hemolytic anemia is caused by the presence of circulating anti-red cell antibodies, which bind to the surfaces of red cells and cause premature destruction.

The typical immune-mediated anemia is extremely regenerative, with marked polychromasia (reticulocytosis) and anisocytosis. In some cases autoagglutination (three dimensional clumping) of red cells is apparent on routinely-stained smears; this finding can be confirmed by evaluation of a saline-diluted wet preparation. Establishing that autoagglutination is present is extremely important diagnostically; demonstration of autoagglutination confirms that red cells are coated by antibodies and in cases of anemia, establishes the diagnosis of immune-mediated disease without further testing.

By far the most consistent and significant “morphologic footprint” of immune-mediated hemolysis on the peripheral blood smear is spherocytosis. Spherocytes by definition are small, round red cells that stain intensely and lack central pallor. Spherocytes are often present in large numbers in immune-mediated hemolytic anemia; however, they should not be regarded as a pathognomonic finding.

Whenever clinical signs and peripheral blood morphology suggest a possible immune-mediated hemolytic anemia, the diagnosis should be confirmed with the direct antiglobulin (Coomb’s) test (DAT). The direct antiglobulin test is performed by mixing washed red cells of the patient with Coomb’s reagent (species specific anti-IgG and anti-C3 antibody). When the Coomb’s reagent is present in proper concentration, and if IgG or C3 is on the surface of the washed red cells, then the Coomb’s reagent molecules serve as a bridge linking together affected red cells, forming an agglutinated lattice work.

Since the endpoint of the DAT is agglutination, the test is not needed where autoagglutination is present. In effect, autoagglutination is an in vivo DAT, and as mentioned earlier, confirms the presence of immune-mediated disease.

Isoimmune Hemolytic Anemia

Isoimmune hemolytic anemia results from the introduction of incompatible blood group antigens into a sensitive recipient. In domestic animals, isoimmune hemolytic anemia occurs as a consequence of transfusion reactions, cross-placental transfer during pregnancy in the horse, and vaccination with blood origin products.
Within a given species, certain antigens (blood group subtypes) are more important than others in determining risk for isoimmune hemolytic reactions following transfusion or other exposure. In dogs DEA1 (also known as CEA1), DEA2 (CEA2), and DEA3 (CEA3) are highly immunogenic and will sensitize recipients that are negative for these blood group antigens. Anti-DEA1 and anti-DEA2 antibodies are not naturally occurring and require prior sensitization in negative individuals before hemolysis will occur. Naturally occurring antibodies to DEA3 are found in DEA3 Θ dogs and can result in hemolysis on initial exposure. Anti-DEA5 and anti-DEA7 antibodies are naturally occurring in DEA5 Θ and DEA7 Θ individuals and will result in increased erythrocyte removal. However, they generally do not induce full-blown hemolytic reactions.

Type A and B antibodies occur naturally in cats. Type B ⊕ cats with naturally occurring anti-A antibodies may have life threatening hemolytic reactions when transfused with A ⊕ blood. Type A ⊕ cats with naturally occurring anti-B antibodies have early erythrocyte removal (but not full-blown hemolysis) when transfused with Type B ⊕ blood.

To avoid isoimmune transfusion reactions, antigen negative blood donors can be used. Alternatively, blood cross-matching can be used prior to transfusion to determine when blood may be safely transfused. The major cross-match tests erythrocytes from the donor against serum from the recipient and seeks to identify antibodies in the recipient. A positive test (incompatible cross match) is indicated by agglutination in dogs and cats. An incompatible major cross-match indicates that the transfusion should not be done because of the life-threatening systemic consequences of a major hemolytic event.

The minor cross-match tests serum of the donor against the red cells of the recipient. Incompatibilities here are less significant and usually not life threatening; the transfused antibody will be diluted in the recipient.

Ideally, transfusions are safest when both the major and minor cross-matches are negative. However, when absolutely necessary, blood with a minor blood cross-match incompatibility may be used for transfusion.

**Infectious Hemolytic Anemias**

Bacterial, protozoal, and rickettsial agents have all been established as causes of hemolytic anemias in animals. In dogs, the principal infectious hemolytic anemias are babesiosis and leptospirosis, in cats, hemobartonellosis (feline infectious anemia).

In the United States, canine babesiosis is primarily a disease of the Sunbelt (Southern California, Texas, Louisiana, Florida, etc.) but has also been reported in the more northern climes. The etiologic agents *Babesia canis* and *Babesia gibsoni* are tick-borne protozoa.

Canine babesiosis can range from a relatively mild, almost subclinical disease to a full-blown intravascular hemolytic anemia characterized by hemoglobinemia and hemoglobinuria. Morphologically, features on the peripheral blood film are those of a marked regenerative anemia. In addition, parasitized red cells can also be seen. *Babesia* are tear-drop-shaped protozoa that measure 2-4µ in length and are located within the red cell cytoplasm. There may be 1–4 organisms per parasitized cell. *Babesia gibsoni* is smaller than *Babesia canis*.

Canine leptospirosis is a relatively infrequently diagnosed disease but one of increasing frequency as vaccination for leptospirosis has decreased. Leptospira organisms do not attack red cells directly, but rather release a potent hemolysin into the systemic circulation. This results in an acute intravascular hemolytic anemia. Dogs with acute leptospirosis are often icteric; it is likely that this icterus has both prehepatic and primary hepatic components.

The principal infectious hemolytic anemia of cats is feline infectious anemia (hemobartonellosis/mycoplasmosis). Mycoplasmosis may be thought of as both a primary and secondary disease. In its primary form, mycoplasmosis is a typical hemolytic anemia in terms of clinical presentation and peripheral blood morphology. The organisms of *Mycoplasma haemofelis* are small (approximately 1µ in diameter) basophilic spherical bodies, which form chains around the margins or across the surfaces of infected red cells on the peripheral blood smear. Parasitemia is transient, so evaluation of blood films for organisms on 4 consecutive days is recommended if organisms are not initially seen. Unlike *Babesia sp., Mycoplasma sp.* does not cause intravascular hemolysis and hemoglobinemia/hemoglobinuria. Rather, infected red cells are sequestered in the spleen, recognized as abnormal by splenic macrophages, and removed by the process of phagocytosis (extravascular hemolytic anemia). As expected,
splenomegaly may be a presenting clinical feature. The primary form of mycoplasmosis responds favorably to antibiotic document (tetracycline) therapy, and the prognosis is fair to good.

Unfortunately, primary mycoplasmosis is a relatively uncommon disease; mycoplasmosis occurs much more commonly as an accompaniment to more severe, often immunosuppressive diseases such as feline leukemia viral infection and feline infectious peritonitis. Under these circumstances, mycoplasma organisms are often found in large numbers in the peripheral blood, but the anemia seen is usually nonresponsive because of the underlying primary disease. In these cases, treatment for mycoplasmosis may well remove the organisms but does little to alleviate the clinical syndrome. Because of the close association between Mycoplasma haemofelis infection and feline leukemia virus–related disease (FeLV), a FeLV test is indicated whenever the presence of Mycoplasma haemofelis is established.

Heinz Body Hemolytic Anemia
Heinz bodies are masses of precipitated hemoglobin that result from the oxidation of the globin moiety of the hemoglobin molecule. Normally, hemoglobin is dispersed in a fluid state in the cytoplasm of the red cell; when precipitated masses of hemoglobin are present, there is interference with normal cell flexibility. Since red cells measuring 7µ in diameter are forced to squeeze through sinusoidal openings measuring 3µ or less, flexibility is essential. When cells containing Heinz bodies squeeze through these openings, the portion of the cell containing the Heinz body is often trapped behind, and the cell lyses or is fragmented. This mechanism of cell destruction is intravascular hemolysis. Alternatively, cells containing large Heinz bodies may be too inflexible to pass through the sinusoidal openings at all. When this occurs the trapped cell is eventually phagocytized, a form of extravascular hemolysis.

The diagnosis of Heinz body hemolytic anemia is based on the presence of a highly responsive anemia (usually of acute clinical onset and occasionally with hemoglobinemia, hemoglobinuria) and the identification of Heinz bodies. On Wright’s stained smears, Heinz bodies are generally seen as small, irregular, nipple-like projections from the red cell surface, which stain like hemoglobin. The Heinz bodies often project from the red cell surface because when globin sulfhydryl groups are oxidized, so too are the sulfhydryl groups on the proteins of the red cell membrane. When oxidation of sulfhydryl groups in the membrane occurs adjacent to oxidation of sulfhydryl groups in hemoglobin, the two become fused by disulfide bonds.

If there is a question concerning the presence of Heinz bodies, vital stains (new methylene blue) may be used to more clearly demonstrate them. With new methylene blue Heinz bodies will stain a royal blue, easily distinguished from the precipitated reticulum of reticulocytes.

Hereditary Hemolytic Anemias
Several hereditary red cell enzyme deficiencies have been described in dogs and cats and present clinically as hemolytic syndromes. The most important of these are pyruvate kinase deficiency and phosphofructokinase deficiency. Both pyruvate kinase and phosphofructokinase are glycolytic enzymes. Deficiencies of either lead to hemolysis because these deficiencies interrupt normal glycolytic production of erythrocytic ATP. ATP is required to maintain erythrocyte membrane stability and red cell volume through the action of ATP-driven membrane pumps.

Pyruvate kinase deficiency has been described in basenji, beagle, chihuahua, dachshund, miniature poodle, pug, Cairn terrier, and West Highland white dogs and Abyssinian and Somali cats. The trait is incompletely inherited, so mildly affected animals are carriers. Resulting anemias in severe cases are described as non-spherocytic hemolytic anemias. Although no true spherocytes are seen on blood films, scattered spherochinocytes are often observed. In the early stages, these cases often present as compensated hemolysis. Hematocrits are normal or only slightly reduced, but reticulocytosis is marked. As time goes on, however, the marrow is unable to keep pace with the accelerated red cell turnover, and anemia becomes increasingly severe. There is no treatment for this condition, and the anemia is eventually fatal. Myelofibrosis is commonly seen at termination.

Phosphofructokinase deficiency has been described in families of English springer spaniels, cocker spaniels, and mixed-breed dogs. The deficiency produces hemolytic anemia with no distinctive morphologic characteristics but is seen most commonly with exertion or hyperventilation.
Fragmentation Hemolytic Anemias

Fragmentation hemolysis occurs when normal red cells are torn apart while circulating through an abnormal vasculature. Because of their pathogenesis, these anemias are also known as angiopathic or microangiopathic hemolytic anemias.

In addition to the usual responsive peripheral blood picture, these anemias are characterized by the presence of schistocytes on the peripheral blood smear. Schistocytes are irregularly shaped, often pointed poikilocytes, which represent the remnants of red cells that have been traumatically disrupted while circulating.

The causes of fragmentation hemolytic anemia are myriad. For example, altered blood flow in cardiac disease may cause shearing of red cells as a result of turbulence. Hemangiosarcoma, a neoplasm of vessels, often is accompanied by fragmentation hemolysis. Glomerulonephritis, a disease characterized by marked morphologic alteration of the glomerular capillary tufts, is frequently accompanied by fragmentation hemolysis. Finally, disseminated intravascular coagulopathy (DIC), secondary to any of a wide variety of underlying diseases, is characterized by fibrin deposition in the microvasculature, with resultant fragmentation hemolysis.