REGENERATIVE AND NON-REGENERATIVE ANEMIA IN DOGS AND CATS

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SUMMARY

Anemia is defined as a decrease in red blood cell (RBC) mass. A decrease in packed cell volume (PCV) commonly is used as an indication of RBC mass in veterinary medicine because measurement of PCV is easy, accurate, and inexpensive. Automated instruments calculate hematocrit (HCT), which often is used interchangeably with PCV. However, there may be minor differences between the HCT calculated by automated instruments and the PCV determined by centrifugation. Decreases in PCV and HCT usually are accompanied by similar decreases in hemoglobin concentration and the number of circulating RBC. Anemia occurs when there is an imbalance between RBC production and RBC loss, destruction, or senescence. Assessing hydration status is important in the interpretation of the PCV or HCT because relative anemia can occur with over hydration and anemia may be masked by dehydration.

Anemia is a clinical finding that occurs in association with many diseases. Classification of anemia as regenerative or non-regenerative can aid in determining the underlying etiology. Classification schemes rely on the reticulocyte count, RBC indices, and pathogenesis. Regenerative anemia is associated with an increased reticulocyte count, whereas the reticulocyte count is normal in non-regenerative anemia. Most regenerative anemias have an increased mean corpuscular volume (MCV) and a decreased mean corpuscular hemoglobin concentration (MCHC). The macrocytic hypochromic RBCs are associated with increased numbers of polychromatophilic cells (reticulocytes), which are larger and have less hemoglobin than mature RBCs. The reticulocyte response to chronic external hemorrhage is variable, and if iron deficiency develops, the indices will indicate microcytosis and hypochromasia. Most non-regenerative anemias are normocytic, normochromic. The pathogenesis of regenerative anemia includes hemorrhage, which can be external or internal, and hemolysis, which can be extravascular or intravascular. The pathogenesis of non-regenerative anemia includes anemia of chronic disease, chronic kidney disease, or primary bone marrow disease. In reality, the classification schemes often are combined to determine the cause of the anemia.

Clinical findings

Clinical findings associated with anemia variable, and are related to the decreased oxygen carrying capacity of blood, as well as the underlying disease that is causing anemia. Dogs and cats with anemia often are presented for weakness, lethargy, exercise intolerance, hyperpnea, and exertional dyspnea. Astute owners may detect pale or icteric mucous membranes. There may be a functional murmur, splenomegaly, an abdominal mass, or various other clinical findings depending on the cause of the anemia. Duration and rapidity of progression of the clinical findings may be helpful in determining the cause of the anemia. Abrupt onset often indicates acute hemorrhage or hemolysis, which typically are associated with regenerative anemia,
whereas gradual onset is more typical with causes of non-regenerative anemia.

**Laboratory findings**

Using modifiers to describe the severity of anemia may be helpful in determining the cause of the anemia. For example, IMHA often is associated with marked anemia, whereas anemia of chronic disease typically causes only mild to moderate anemia. Evaluation of the PCV in tandem with the plasma protein also may be helpful in determining the cause of the anemia. External hemorrhage typically causes a decrease in the PCV and total protein (TP) concentration, whereas with internal hemorrhage and hemolysis, the PCV will be decreased but the TP may be normal or only slightly increased. Anemia of chronic disease may be associated with an increased TP concentration due to increased production of acute phase reactants and immunoglobulins.

Most laboratories report absolute reticulocyte counts. Newer automated instruments are more sensitive in detection of reticulocytes so the reference interval may be higher than previously used reference intervals. However, the reference interval for the laboratory or in-house instrument should be used to determine whether the anemia is regenerative. The reticulocyte count may be normal in acute hemorrhage or hemolysis. An increased reticulocyte count typically is evident within 2-3 days, and reaches a maximum response in 4-7 days. Newer instruments also generate information about reticulocyte size and hemoglobin content, which may be helpful in the detection of early iron deficiency anemia.

Depending on the cause of the anemia, animals with more severe anemia typically have a more intense reticulocyte response than those with mild anemia, hemolysis usually stimulates a greater reticulocyte response than hemorrhage, and the reticulocyte response often is greater in dogs than in cats. In most dogs and cats with non-regenerative anemia, the reticulocyte response is minimal or absent. However, adequacy of the regenerative response may be difficult to interpret. Absolute reticulocyte numbers and corrected reticulocyte indices can be used as guidelines to gauge the regenerative response, but sequential monitoring of RBC parameters may provide the most useful information. Other specific laboratory findings will be discussed below.

**Hemorrhage (Regenerative, non-regenerative, or in between)**

Hemorrhage can be internal or external. Hemorrhage into joints and the abdominal cavity are examples of internal hemorrhage. Hemorrhage from lacerations, loss from the gastrointestinal or urinary tract, or external or internal parasites are examples of external hemorrhage. Hemorrhage also can be acute or chronic. The primary concern with acute blood loss is decreased circulatory volume and tissue perfusion. Healthy animals can survive loss of 25-30% of blood volume without replacement therapy. Acute loss of 30-40% of blood volume can result in hypovolemic shock, and loss of > 50% of the blood volume results in death. If there is external hemorrhage, both PCV and plasma proteins decrease, whereas internal hemorrhage is associated with normal plasma proteins and decreased PCV.

The regenerative response to hemorrhage is variable. In some cases, reticulocytes are
increased, but are not above the reference interval. RBC indices also are variable, in part depending on the regenerative response, the duration of the hemorrhage, and whether there is internal or external hemorrhage. Chronic external blood loss may progress to iron deficiency anemia, which is characterized by microcytic, hypochromic RBCs. Newer instruments can measure reticulocyte indices, similar to RBC indices. The presence of decreased reticulocyte hemoglobin concentration (CHCMr), decreased reticulocyte hemoglobin content (Chr), and increased percentages of reticulocytes with low CHCMr and Low Chr may be helpful in detecting iron deficiency earlier than MCV and MCHC.

Examination of the blood smear may be helpful. Iron deficiency due to chronic external hemorrhage is characterized by hypochromic, microcytic RBCs and poikilocytosis (keratocytes, blister cells, apple stem cells, schistocytes or fragments, and target cells). There also may be increased numbers of nRBCs, RBC fragments, and acanthocytes in dogs with splenic hemangiosarcoma. Additional diagnostic tests to consider for cases of suspected hemorrhage include fecal examination for parasites and occult blood, urinalysis for hematuria, a platelet count and a coagulation panel, and thoracic and abdominal radiography or ultrasound for effusions or masses. Gastrointestinal hemorrhage may be associated with increased blood urea nitrogen with a normal creatinine so a biochemical profile may be helpful. Thrombocytosis or thrombocytopenia may be present. Iron deficiency typically is characterized thrombocytosis, whereas immune-mediated thrombocytopenia is associated with decreased numbers of platelets. An iron panel may be helpful. Iron deficiency is characterized by decreased serum iron, normal or increased total iron binding capacity, decreased transferrin saturation, and decreased serum ferritin.

**Hemolysis (usually regenerative)**

Hemolytic anemia usually has a more marked regenerative response than anemia from hemorrhage because iron and protein are readily available. Hemolysis can be either extravascular or intravascular, although sometimes both occur together. Extravascular hemolysis occurs when RBC destruction occurs “outside” the blood vessel, and refers to RBC phagocytosis by macrophages in the spleen, liver, and lymph nodes. Extravascular hemolysis usually has a more gradual onset than intravascular hemolysis and occurs more commonly than intravascular hemolysis in dogs and cats. Intravascular hemolysis occurs when there is RBC destruction within the blood vessel, usually due to antibody or complement binding, although there are other causes of intravascular hemolysis. Hemoglobin is released into the plasma in intravascular hemolysis and imparts a red discoloration to the plasma, and may cause an increased MCHC and hemoglobinuria.

Evaluation of the blood smear may be particularly helpful in patients with regenerative anemia, especially if the cause is hemolysis. Polychromasia and anisocytosis usually are present. Nucleated RBCs can be part of a regenerative response or may indicate splenic dysfunction. Howell-Jolly bodies are nuclear remnants, which also may increase with regeneration. Basophilic stippling may be seen occasionally as an indication of a regenerative response in dogs and cats, but also may increase in lead toxicity in dogs. Additional morphologic findings will be described below in association with IMHA and oxidative damage, which are examples of causes of hemolytic anemia in dogs and cats.
**IMHA**

Immune-mediated hemolytic anemia (IMHA) is the most common cause of hemolytic anemia in dogs. IMHA causes RBC destruction due to membrane bound antibody, complement, or both. IMHA may be from antibody directed against the animal’s own RBC antigens or from an immune response against infectious agents or drugs. Predisposing factors include hemotropic parasites, drugs, bacterial or viral infections, hormones, genetic influences, stress, and perhaps vaccination, but the cause of IMHA often is unknown. IMHA occurs most frequently in middle-aged, female dogs and is the most common cause of hemolytic anemia in dogs. There is an increased incidence in some breeds.

Clinical findings include acute onset of signs associated with anemia, fever due to hemolysis, icterus, and hemoglobinemia and hemoglobinuria if there is intravascular hemolysis. There may be splenomegaly, petechiae, and hemorrhage if there also is immune-mediated thrombocytopenia, and clinical signs related to thrombosis may occur. Extravascular hemolysis is more common than intravascular hemolysis. Macrophages phagocytize RBCs or part of the RBC membrane, resulting in formation of spherocytes, which in addition to RBC agglutination, are a hallmark of IMHA. There frequently is moderate to marked leukocytosis due to neutrophilia with a left shift and monocytosis, likely due to cytokine stimulation of myelopoiesis. About 33% of dogs with IMHA also have antibodies directed against platelets and prothrombotic tendencies may contribute to thrombocytopenia. The diagnosis of IMHA can be supported by a positive Coombs’ test. Some dogs and cats may have antibodies directed against erythroid precursors in the bone marrow, in which case the anemia may be non-regenerative.

**Oxidative damage**

Hemolytic anemia associated with oxidative damage to RBCs can occur in dogs and cats. Causes of oxidative damage include onions, leeks, garlic, chives, acetaminophen, benzocaine, propylene glycol, zinc, and numerous other drugs and toxins, so a history of potential exposure is important. Oxidative damage to RBCs may cause Heinz body or eccentricocyte formation, so recognition of these RBC abnormalities from evaluation of the blood smear may be very helpful in the diagnosis of hemolytic anemia. Methemoglobinemia can occur due to oxidative damage to the iron moiety of hemoglobin. Oxidative damage typically is associated with extravascular hemolysis, but some causes are associated with intravascular hemolysis. In many cases, anemia resolves with removal of the oxidative compound.

**Other causes of hemolytic anemia**

Hemolytic anemia can occur with some infectious agents, including hemotropic parasites and some leptospira serovars. Although the diagnosis of hemotropic parasites sometimes can be made by blood smear evaluation, more sensitive serologic and DNA-based assays are available. Other less common causes of hemolytic anemia include microangiopathic disease, marked hypophosphatemia, and rarely, intrinsic RBC defects such as enzyme deficiencies and cytoskeletal abnormalities.
**Chronic renal disease (non-regenerative)**

The primary pathogenesis of non-regenerative anemia associate with chronic renal disease is decreased production of erythropoietin. There also may be decreased bone marrow response to erythropoietin due to hypocalcemia. Other contributing factors include inhibition of erythropoiesis by parathyroid hormone, decreased RBC survival, and chronic blood loss due to platelet dysfunction. Laboratory findings include moderate to marked normocytic, normochromic non-regenerative anemia; azotemia, hyperphosphatemia, hypo- or hypercalcemia; and decreased urine specific gravity. Treatment with recombinant human erythropoietin may induce increased erythropoiesis, but most dogs and cats eventually develop antibodies to recombinant human erythropoietin, resulting in recurrence of anemia.

**Anemia of chronic disease (non-regenerative)**

Anemia of chronic disease is a relatively common cause of mild to moderate non-regenerative anemia in dogs and cats. Chronic disease may be from inflammation associated with infectious or immune-mediated causes, or may be from neoplasia. RBC indices usually are normocytic, normochromic, but long-standing cases may become mildly microcytic, hypochromic. The anemia develops over 1-3 weeks and the PCV usually is not less than 20%, but cats with anemia of chronic disease tend to develop anemia more rapidly than dogs and the PCV may be more severely decreased. The pathogenesis is related to increased concentration of inflammatory cytokines such as interleukin-1, interleukin-6, tumor necrosis factor alpha, and gamma interferon. These cytokines may suppress erythropoietin production and impair response to erythropoietin by erythroid precursors in the bone marrow. Increased production of a protein called hepcidin by hepatocytes causes decreased iron absorption and increases iron uptake and retention by macrophages. There may be decreased serum iron concentration, decreased transferrin saturation, and increased ferritin concentration. Additional laboratory findings may include an inflammatory leukogram and polyclonal hypergammaglobulinemia. Other clinical findings, such as evidence of neoplasia, may be present. Anemia of chronic disease resolves with alleviation of the primary disease.

**Non-regenerative anemia associated with primary bone marrow disease (non-regenerative)**

Anemia associated with primary bone marrow disease often is severe. Primary bone marrow disease often affects more than one cell lineage, so other cytopenias may be present. Primary bone marrow disease may be from aplastic anemia, myelodysplasia, myeloproliferative or lymphoproliferative diseases, infectious diseases, drug or chemical toxicity, and immune-mediated diseases.

Aplastic anemia causes pancytopenia and is characterized by a hypoplastic bone marrow. Diagnosis often requires a bone marrow biopsy. Aplastic anemia may be secondary to immune-mediated mechanisms; known exposure to a drug, chemical, radiation, or infectious agent; or may be idiopathic. Myelodysplasia likely is a stem cell disorder and is characterized by peripheral cytopenias and a normocellular to hypercellular bone marrow with <20-30% blasts. Myelodysplasia may progress to hematopoietic neoplasia.
Myeloproliferative diseases most often are due to neoplastic proliferation of myeloid precursors and are associated with circulating neoplastic hematopoietic cells. These include acute and chronic myeloid leukemia. Lymphoproliferative diseases most often are due to neoplastic proliferation of lymphoid precursors and also are associated with circulating neoplastic hematopoietic cells. These include acute and chronic lymphoid leukemia and lymphoma. A bone marrow aspirate, cytochemical stains, immunophenotyping by flow cytometry, and DNA-based testing may be helpful in the diagnosis of myeloproliferative and lymphoproliferative disorders.

Non-regenerative anemia due to primary bone marrow disease may be caused by some infectious agents such as *Ehrlichia canis*, feline leukemia virus, *Histoplasmosis capsulatum*, and *Leishmania spp*. Anemia may be mild, moderate, or severe, and there may be other CBC abnormalities, depending on the infectious agent. There may be marked infiltration of the bone marrow with inflammatory cells.

Many drugs and chemicals have been associated with erythrosuppression, resulting in non-regenerative anemia. In some dogs and cats with non-regenerative anemia, there may be immune-mediated suppression of erythropoiesis that may be responsive to immunosuppressive therapy. Hypothyroidism and hypoadrenocorticism may be associated with non-regenerative anemia, but the anemia usually is not the primary reason for presentation and the diagnosis of these endocrine diseases usually is by history, clinical signs, and other laboratory testing.

REFERENCES
