Anemia: Beyond the Numbers

Jennifer S. Thomas, DVM, PhD, Diplomate ACVP
Associate Professor
Department of Pathobiology and Diagnostic Investigation
Diagnostic Center for Population and Animal Health
College of Veterinary Medicine
Michigan State University
E. Lansing, MI 48824-1314

Introduction
Anemia results from hemorrhage, destruction of circulating erythrocytes, and/or decreased production in the bone marrow. Results from automated analyzers provide objective measures that indicate the severity of anemia, the size of erythrocytes, and the hemoglobin concentration in erythrocytes. Microscopic review of a blood smear provides additional valuable diagnostic clues about the underlying cause of anemia. In general, erythrocytes are evaluated for size, shape, distribution, color, inclusions, and the presence of nucleated erythrocytes (nRBCs). Erythrocyte distribution is assessed on low magnification. Erythrocyte morphology is best assessed on high magnification (100x oil objective). A well-made smear with an adequate monolayer region is required for optimal assessment of erythrocyte morphology. Cells are often distorted near the feathered edge. In the thick areas of the smear, the cells overlap and are difficult to evaluate.

Changes in Erythrocyte Color
The cytoplasm of erythrocytes from healthy animals should be uniformly red in color. In some species, such as dogs, a central pale region (central pallor) is common. Polychromatophilic erythrocytes are immature erythrocytes that are larger and bluer in color using Romanowsky type stains (e.g., Wright’s stain, Diff-Quik®). They correspond to aggregate reticulocytes identified using a supravital stain such as New Methylene blue (NMB); however, it is likely that not all aggregate reticulocytes are identified as polychromatophils on a routinely stained smear. Quantitation of reticulocytes on a NMB smear can provide a more objective measure of reticulocyte concentration. Some automated analyzers provide reticulocyte concentration. Healthy dogs may have up to 1% polychromatophils in circulation while fewer are expected in healthy cats. Polychromatophils are not expected in the blood of healthy horses or ruminants. Increased polychromatophils (termed polychromasia) indicate that the anemia is regenerative (i.e., the bone marrow is responding appropriately by increasing erythroid production and releasing immature cells into circulation). Regenerative anemias result from blood loss or hemolysis. An absence of polychromasia indicates either impaired bone marrow production or that the bone marrow has not had enough time (typically 3 to 5 days) to respond. Horses do not consistently release reticulocytes into circulation even with regenerative responses.

Hypochromasia refers to erythrocytes that have pronounced central pallor surrounded by a pale-staining, thin rim of hemoglobin. Hypochromasia indicates decreased hemoglobin concentration in erythrocytes, most commonly due to impaired hemoglobin production secondary to iron deficiency (e.g. chronic external blood loss). Torocytes are erythrocytes with normal hemoglobin concentration that can be confused with hypochromic erythrocytes. Torocytes are an artifact of smear preparation. They have a punched out central pale region that abruptly transitions to a thick, dense outer rim of hemoglobin.
Variation in Size
Anisocytosis is the term used for variation in erythrocyte size. The degree of anisocytosis in healthy animals varies between species but is usually mild. Increased anisocytosis occurs when macrocytes (large erythrocytes) and/or microcytes (small erythrocytes) are present. Macrocytosis usually occurs with regenerative anemia due to increased reticulocytes and is associated with polychromasia. Macrocytosis without polychromasia is supportive of regenerative anemia in horses. Macrocytosis without polychromasia is occasionally found in disorders associated with abnormal erythrocyte production (e.g., FeLV infection in cats). Microcytosis is usually associated with iron deficient states but may also occur with liver disease (especially portosystemic shunt in dogs). Spherocytes are erythrocytes that appear to be densely staining microcytes on a blood smear; however, they have a normal cell volume. They appear as microcytes because a portion of their cell membrane was removed without concurrent loss of cytoplasmic contents, limiting their ability to spread on a glass slide. Spherocytes are easiest to recognize in dog blood smears. Be cautious in diagnosing spherocytes in other species.

Evaluation of Distribution
Rouleaux are the coin like stacking of erythrocytes. It is common in blood smears from healthy horses and cats where it is not considered abnormal. In other species, rouleaux tend to occur in patients with elevated globulins (e.g., fibrinogen, immunoglobulins and other acute phase proteins) secondary to inflammation or lymphoproliferative neoplasia. Dilution of blood with saline will cause rouleaux to disperse.

Agglutination is the grape-like clustering of erythrocytes that occurs with increased antibody deposition on erythrocyte membranes. Agglutination may be found in animals without evidence of anemia; however, it supports immune-mediated destruction of erythrocytes in animals with anemia. Agglutination does not disperse when blood is diluted with saline.

Abnormal Erythrocyte Shape
Mammals erythrocytes are usually round (exceptions include camelids) and have a smooth membrane. Poikilocytosis refers to the presence of abnormally shaped erythrocytes. It is generally classified according to the type of poikilocytosis present. Poikilocytes may be artifacts secondary to sample handling and smear preparation, occur in young healthy animals of some species (e.g., calves), or be indicative of specific disorders.

Acanthocytes have membrane projections that are irregular in size and distribution. They are often associated with liver disease, lipid disorders, or with splenic or renal disorders that result in fragmentation (e.g., hemangiosarcoma in dogs) Acanthocytes may be confused with echinocytes. Echinocytes have membrane projections that are more regular in size and distribution. Projections may be sharp or blunted. Echinocytes are usually artifacts (crenated cells); however, they tend to be more numerous in some disorders (e.g., renal failure,
envenomation, neoplasia). It is often difficult from blood smear review to determine if echinocytosis are artifacts, though pathologic echinocytosis may also be associated with spherocytosis.

Codocytes have excess membrane and resemble a target because they have a central zone of hemoglobin, a middle pale zone, and an outer rim of hemoglobin. They are a non-specific finding most commonly associated with regeneration and accelerated erythroid production. They are also found without polychromasia in conditions associated with iron deficiency, liver disease, renal disease, or lipid disorders.

Eccentrocytes occur with oxidative damage that causes abnormal fusion of the erythrocyte membrane. They have hemoglobin dispersed to one side and a crescent shaped clearing on the opposite side of the cell. Eccentrocytes may develop into pyknocytes if the fused portion of the membrane is removed. Pyknocytes appear as small, densely stained erythrocytes (similar to a spherocyte) with small projections.

Schistocytes (schizocytes) are small erythrocyte fragments that vary in size and shape. They suggest fragmentation associated with mechanical trauma due to turbulent blood flow or thrombosis in the vascular system (e.g., disseminated intravascular coagulation, vasculitis, hemangiosarcoma, endocarditis). They are also found in patients with iron deficiency anemia, liver failure, glomerulonephritis or dyserythropoiesis. Blister cells (prekeratocytes) and keratocytes (helmet cells) also occur with erythrocyte fragmentation as well as liver disease, chemical injury, iron deficiency anemia, or myelodysplastic syndrome. Blister cells have a clear round zone near the periphery of the cells. Keratocytes have 1 or 2 projections on the periphery that are remnants from a ruptured vesicle.

Spherocytes are small, densely staining erythrocytes. They are usually associated with immune-mediated hemolysis but may also be found with other disorders (e.g., erythrocyte parasites, blood transfusion, envenomation, dyserythropoiesis) where a portion of the cell membrane is removed.

**Erythrocyte Inclusions**

Heinz bodies are aggregates of oxidized hemoglobin that attach to the membrane. They are pale red, round structures that often protrude from the cell surface. They are easier to identify in NMB stained blood. Heinz bodies suggest exposure to some type of oxidant except in cats where Heinz bodies may be found in normal cats. Increased numbers of Heinz bodies are found in cats with diabetes mellitus, hyperthyroidism, and lymphoma. Howell-Jolly bodies are small nuclear remnants that may be found in healthy animals. Increased Howell-Jolly bodies occur with regenerative anemia or in animals that have poorly functioning spleens or are receiving glucocorticoid therapy.

Basophilic stippling are erythrocytes with diffuse small, blue inclusions containing aggregates of residual RNA. It is a non-specific finding that occurs most frequently in regenerative anemia, especially in cats and cattle. Basophilic stippling without evidence of regeneration occurs with lead toxicity. Siderocytes are erythrocytes with iron inclusions. Unlike basophilic stippling, siderotic inclusions are focal accumulations of fine blue material often near the membrane.
Erythrocyte parasites found in blood smears include *Anaplasma* spp., *Babesia* spp., *Mycoplasma (Haemabartonella, Eperythrozoon)* spp., *Cytauxzoon* spp., and *Theileria* spp. Stain precipitate, Howell Jolly bodies or siderotic inclusions can be confused with parasites. Organisms on the erythrocyte surface may fall off following collection so blood smears should be made immediately after collection. Dogs with distemper virus have pale pink to blue cytoplasmic inclusions that are often more prominent with Diff-Quik® staining.

**Nucleated Erythrocytes**

Nucleated erythrocytes are occasionally found in blood smears of healthy mammals. Increased nRBCs (termed rubricytosis) occurs most commonly with regenerative anemia. In the absence of polychromasia, rubricytosis suggests bone marrow disorders (e.g., trauma, necrosis, inflammation, neoplasia), splenectomy, splenic disorders, lead toxicity or hemangiosarcoma.

**Classification of Anemia**

Blood loss anemia is often diagnosed based upon historical, clinical or laboratory evidence of hemorrhage. Decreased protein concentration occurs with acute hemorrhage, particularly external blood loss. Expected findings on a blood smear include polychromasia (if there has been enough time for a bone marrow response), anisocytosis, codocytosis, Howell Jolly bodies, basophilic stippling and nRBCs. In the absence of complicating factors, other morphologic abnormalities are not expected. If blood loss is chronic and leads to iron deficiency then hypochromasia, microcytosis, and poikilocytosis (e.g., keratocytes, schizocytes) are seen.

Hemolytic anemia results from increased erythrocyte destruction by macrophages or erythrocyte lysis in the vascular system. Polychromasia is often more marked than with blood loss. Careful examination of a blood smear for inclusions (e.g., organisms, Heinz bodies) or morphologic changes (e.g., agglutination, spherocytes, acanthocytes, schizocytes, keratocytes, eccentrocytes, pyknocytes) may reveal a cause for hemolysis.

Nonregenerative anemia results from decreased or ineffective erythroid production in the bone marrow. Polychromasia should be absent or minimal. Some disorders with ineffective erythropoiesis may be associated with macrocytosis (e.g., FeLV infection in cats) or microcytosis (e.g., iron deficiency, portosystemic shunts in dogs). Morphologic abnormalities may or may not be identified on a blood smear, depending on the underlying cause for bone marrow suppression.

**Suggested References**