

Compendium

Anemia: Determining the Cause

Wendy Fleischman, DVM, DACVIM

VCA Veterinary Specialists of Northern Colorado

Abstract: Anemia is a common finding in small animal practice; however, the multitude of potential causes can make determining the underlying diagnosis a challenging and frustrating endeavor. With a basic understanding of red blood cell production and a systematic diagnostic approach, clinicians should be able to clearly define the cause of anemia in most cases.

Anemia refers to a reduced hemoglobin concentration due to decreased red blood cell mass. This can be expressed as a packed cell volume (PCV), hematocrit, red blood cell (RBC) count, or hemoglobin level below the reference interval. For simplicity, hematocrit or PCV will be used in this article.

Erythropoiesis and Erythrokinetics

A basic grasp of RBC production can contribute much to understanding the causes of anemia. In adult mammals, RBCs are produced primarily in the bone marrow.¹ The RBC mass can be divided into four phases or compartments: stem cells, progenitor cells, precursor cells, and mature erythrocytes.² A diagram illustrating the development of RBCs is provided in **FIGURE 1**.

Stem Cells

The hemopoietic stem cells are a small population of uncommitted, self-renewing germ cells. These cells can give rise to all types of blood cells (erythrocytes, leukocytes, and platelets). Injury to the stem cell compartment is an uncommon cause of anemia. In such a situation, the anemia is accompanied by deficiencies in the other two cell lines, a condition referred to as *aplastic anemia*, or, more correctly, *aplastic pancytopenia*. Reported causes of aplastic pancytopenia are listed in **BOX 1**.³⁻⁵

Progenitor Cells

Hemopoietic growth factors drive pluripotent stem cells to become progenitor cells committed to a specific cell line. The cytokines and hormones in the cells' environment then drive further differentiation and proliferation of the progenitor cells. In the case of erythrocyte progenitor cells, the hormone that causes differentiation and proliferation is erythropoietin.⁶ Erythropoietin is produced by renal interstitial cells in response to renal hypoxia.⁷ Erythropoietin inhibits apoptosis (programmed cell death) of the erythroid progenitor cells, allowing them to differentiate into erythroid precursor cells and, eventually, mature erythrocytes. Lack of erythropoietin or an inability of the progenitor cells to respond to erythropoietin are common mechanisms leading to nonregenerative anemia.

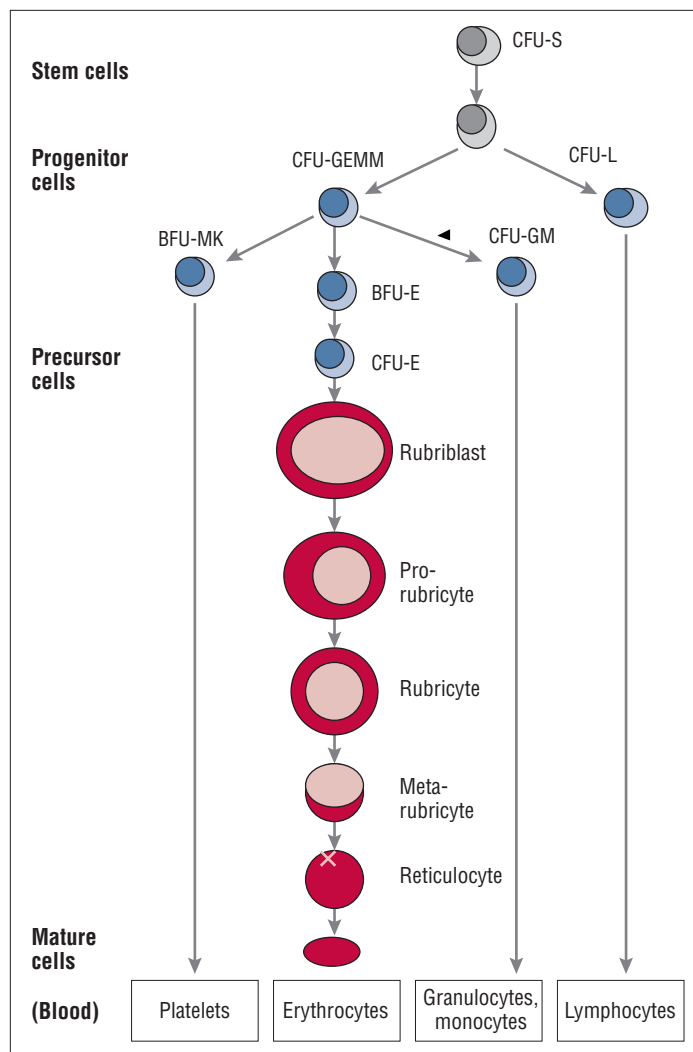


Figure 1. The development of RBCs. *BFU-E* = burst-forming unit erythrocytes; *BFU-MK* = burst-forming unit megakaryocytes; *CFU-E* = colony-forming unit erythrocytes; *CFU-GEMM* = colony-forming unit granulocytes, erythrocytes, monocytes, megakaryocytes; *CFU-GM* = colony-forming unit granulocytes, monocytes; *CFU-L* = colony-forming unit lymphocytes; *CFU-S* = colony-forming unit stem cells.

Precursor Cells

Precursor cells are fully committed to one cell lineage and make up most of the cells within the bone marrow. They develop along a preprogrammed pathway of proliferation and maturation.² Maturation and release of erythrocyte precursor cells can be enormously accelerated in response to erythropoietin. Uncommonly, vitamin deficiency or congenital hemoglobin or cytoskeletal defects can lead to dysfunctional maturation of the precursor cells, which can result in nonregenerative anemia.^{8,9} Destruction of precursor cells, such as occurs in pure red cell aplasia (PRCA) and immune-mediated ineffective erythropoiesis, causes a nonregenerative anemia.^{10,11}

Mature Erythrocytes

The final compartment of the RBC mass is composed of mature erythrocytes. Mature erythrocytes extrude their nuclei and cell organelles before being released into the bloodstream. Disease processes that alter this compartment to cause anemia are RBC loss (internal or external bleeding) and destruction (hemolysis). In most cases, anemia due to blood loss or destruction is regenerative.

Diagnostic Tests

The initial evaluation of anemia requires four diagnostic tests: measurement of the PCV or hematocrit, quantification of total plasma solids (TS; plasma protein and fibrinogen) concentration by refractometry, examination of a blood smear, and a complete blood count (CBC). Three of these tests can be performed immediately by almost all practitioners and yield a great deal of information. The PCV and TS values are determined after centrifugation of a small quantity of blood in microhematocrit tubes. The PCV identifies the presence of anemia and allows determination of severity. The TS concentration may help distinguish between blood loss and hemolysis. Examination of the serum in the microhematocrit tube will reveal hyperbilirubinemia or hemoglobinemia, if present. An algorithm for the diagnosis of anemia is presented in **FIGURE 2**.

The Blood Smear

Blood smear examination provides instantaneous information about the nature of the anemia through observation of RBC morphology. Additionally, many erythrocyte abnormalities that cannot be detected by other tests may be revealed on a blood smear. A few examples are the presence of spherocytes, autoagglutination, Heinz bodies, poikilocytes (i.e., abnormally shaped erythrocytes), erythroparasites, and nucleated RBCs. Spherocytes appear smaller than normal RBCs and lack central pallor. In dogs, the presence of spherocytes and autoagglutination suggests immune-mediated hemolytic anemia (IMHA).¹² Identification of spherocytes in cats is not possible because normal feline RBCs are smaller and demonstrate less central pallor than canine RBCs.¹³ Heinz bodies are small, round pieces of denatured hemoglobin that often protrude from the cell margin. Eccentrocytes are RBCs in which the hemoglobin has shifted to one side, leaving a clear zone along the other side. Ruptured eccentrocytes are called *pyknocytes*. Like spherocytes, pyknocytes are smaller than normal RBCs,

Box 1. Reported Causes of Aplastic Anemia (Aplastic Pancytopenia) in Dogs and Cats³⁻⁵

Infectious agents

- Parvovirus
- *Ehrlichia* spp
- *Anaplasma* spp
- FeLV
- FIV

Drug effects/hormones

- Hyperestrogenism (exogenous or endogenous)
- Chemotherapeutics, azathioprine, hydroxyurea
- Griseofulvin, fenbendazole, albendazole
- Trimethoprim/sulfadiazine, chloramphenicol
- Methimazole
- Phenylbutazone
- Thiacetarsamide
- Quinidine

Radiation

Idiopathic/immune-mediated

Myelophthisis

- Neoplasia
- Fibrosis

lack central pallor, and may be difficult to appreciate in cats. The presence of Heinz bodies, eccentrocytes, and pyknocytes indicates oxidative damage to RBCs, such as occurs with onion, acetaminophen, zinc, and other toxicoses as well as several metabolic diseases in cats.¹⁴⁻¹⁶

Echinocytes, which have short, evenly spaced spicules, are often the result of blood drying slowly on the slide but are also seen with certain conditions such as glomerulonephritis, lymphosarcoma, and rattlesnake envenomation.^{17,18} Acanthocytes have large, irregularly spaced projections and are most commonly associated with hemangiosarcoma in dogs and hepatic lipidosis in cats.^{19,20} Schistocytes and keratocytes are pieces of RBCs, the latter with spicules. These cells indicate RBC fragmentation, as is seen with disseminated intravascular coagulation and hemangiosarcoma.²¹ Nucleated RBCs may be noted with accelerated erythropoiesis or may suggest splenic disease, bone marrow disease, or lead poisoning.²²

The Reticulocyte Count

Reticulocytes are RBCs that have been released from the bone marrow before complete extrusion of all RNA and cellular organelles. They can be demonstrated in a blood smear using supravital

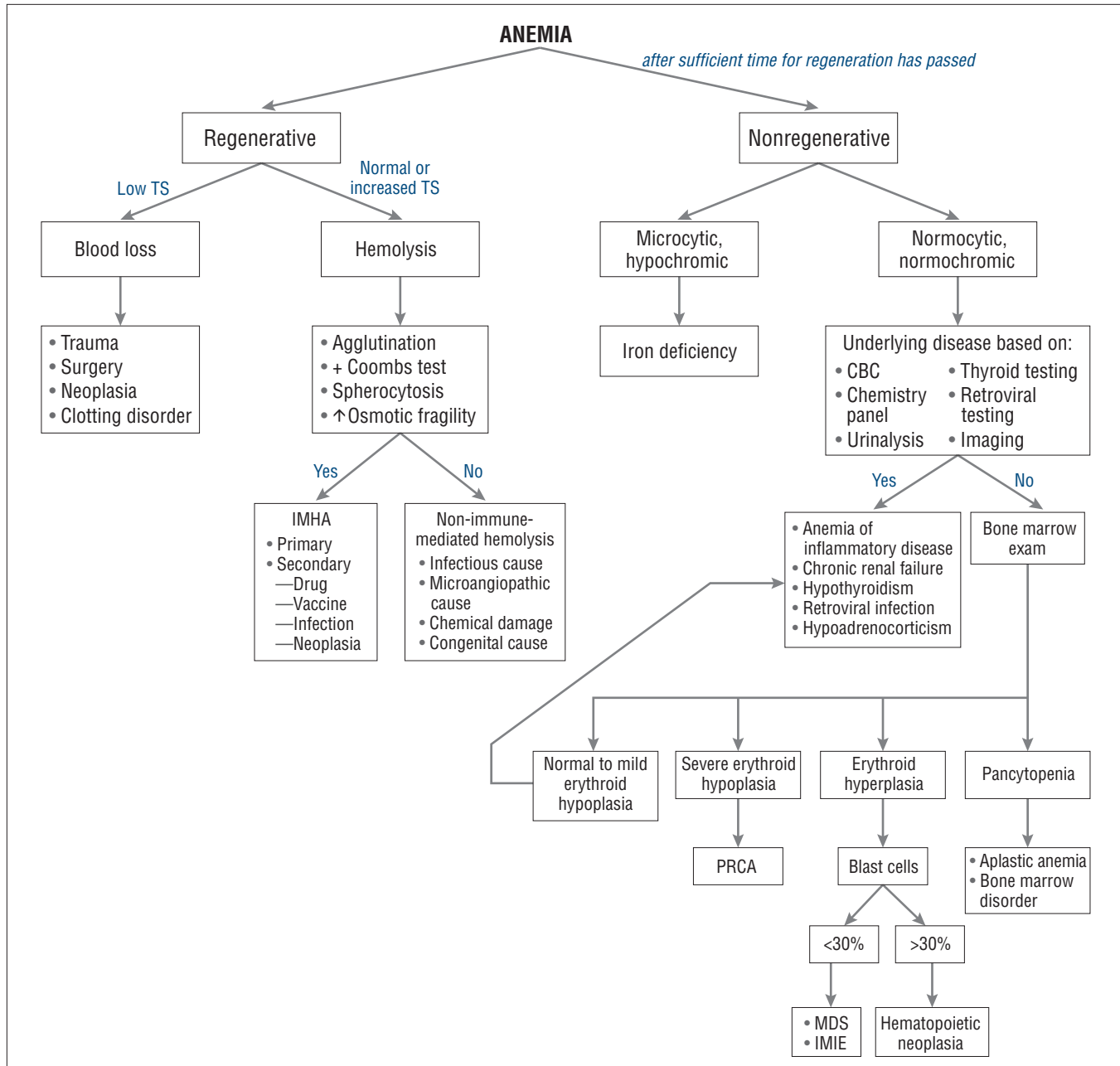


Figure 2. Diagnostic algorithm for anemia. *IMHA* = immune-mediated hemolytic anemia, *IMIE* = immune-mediated ineffective erythropoiesis, *MDS* = myelodysplastic syndrome, *PRCA* = pure red cell aplasia, *TS* = total plasma solids.

stains such as new methylene blue or brilliant cresyl blue. These stains precipitate the RNA in the reticulocyte, staining it blue. Reticulocytes stained with new methylene blue appear as erythrocytes with blue granules. Reticulocytes are generally not observed for 2 to 4 days after an acute episode of blood loss or hemolysis. The reticulocyte response generally peaks between 4 and 7 days after the insult and starts to decline at 2 to 3 weeks in dogs and 9 to 13 days in cats, assuming the underlying cause of the anemia has been treated.^{23,24}

There are two types of reticulocytes: aggregate and punctate. Aggregate reticulocytes are larger and less mature than punctate

reticulocytes. Cats are unique in that their punctate reticulocytes have a long half-life in blood and therefore can reflect an insult that occurred days to weeks prior to testing.^{23,24} Punctate reticulocytes do not accumulate in large numbers in dogs. Aggregate reticulocytes are considered an indicator of current regeneration in both species.

A reticulocyte count can be performed by incubating a small amount of EDTA-anticoagulated blood with an equal amount of a supravital stain such as new methylene blue for 10 minutes before making a blood smear slide. The number of reticulocytes seen

Box 2. Sample Calculation for Reticulocyte Response

1. Count the number of aggregate reticulocytes in 1000 RBCs (five 40× fields).
2. Divide the number of reticulocytes by 10 to obtain the uncorrected reticulocyte percentage.
3. To obtain the corrected reticulocyte percentage, multiply the uncorrected reticulocyte percentage by the ratio of the patient's PCV to the average normal PCV for the species (45 for dogs; 35 for cats).
4. To obtain the absolute reticulocyte count, multiply the uncorrected reticulocyte percentage as a decimal by the patient's RBC count.

Example: Three days after hemorrhage from severe thrombocytopenia, a dog has a PCV of 13% and an RBC count of $1.6 \times 10^6/\mu\text{L}$. The number of aggregate reticulocytes in 1000 RBCs is counted (109). Based on **TABLE A**, determine the regenerative status of the anemia based on the reticulocyte response.

1. Number of aggregate reticulocytes: 109
2. Uncorrected reticulocyte percentage = $109/10 = 10.9\%$
3. Corrected reticulocyte percentage = $10.9 \times (13 \div 45) = 3.1\%$
4. Absolute reticulocyte count = $0.109 \times 1.6 \times 10^6/\mu\text{L} = 174,400/\mu\text{L}$

Slight to moderate regeneration, likely because it is still early after the onset of hemorrhage.

Table A. Reference Guidelines for Assessing Reticulocyte Response

Degree of Regeneration	% Reticulocytes		Reticulocytes/ μL	
	Dogs	Cats	Dogs	Cats (aggr)
None			<60,000	<15,000
Slight	1–4	0.5–2	60,000–150,000	15,000–50,000
Moderate	5–20	3–4	150,000–300,000	50,000–100,000
Marked	>20	>4	>500,000	>200,000

CBC = complete blood cell count, PCV = packed cell volume, RBC = red blood cell.

per 1000 RBCs is then counted. A high-power field (40× objective) in which the RBCs are touching or just overlapping contains approximately 200 cells. The number of reticulocytes counted in five such fields is divided by 10 to yield the reticulocyte percentage (number of reticulocytes per 100 RBCs). In cats, aggregate reticulocytes are differentiated from punctate reticulocytes by the presence of clumped reticulum as opposed to individual pieces of reticulum. A corrected reticulocyte count for the degree of anemia is calculated by multiplying the reticulocyte percentage expressed as a percentage by the ratio of the patient's PCV to the average normal PCV for

the species. If the RBC is available from a CBC, the absolute reticulocyte count is obtained by multiplying the reticulocyte percentage expressed as a decimal by the RBC. Examples of reticulocyte calculations and reference guidelines for reticulocytes are provided in **BOX 2**.

Classification of Anemia by Regenerative Status

The initial step in elucidating the cause of anemia is to determine whether the anemia is regenerative or nonregenerative. Regenerative anemia indicates that the bone marrow is able to respond to the anemia appropriately and that the likely cause of anemia is blood loss or hemolysis. Nonregenerative anemia indicates bone marrow dysfunction and suggests a lack of erythropoietin, an inability of the progenitor cells to respond to erythropoietin, or a maturation defect of the precursor cells. An acute case of anemia due to blood loss or hemolysis may also appear nonregenerative for the first few days.

The blood smear examination and RBC parameters from the CBC provide a good foundation to begin evaluation for the presence of regeneration.^{23,25} Nonregenerative anemia is most commonly normocytic (normal mean cell volume [MCV]), normochromic (normal mean cell hemoglobin concentration [MCHC]), and homogeneous in cell volume (normal RBC distribution width [RDW]). RBC morphology is typically normal on a blood smear. Less commonly, nonregenerative anemia is microcytic (low MCV), hypochromic (low MCHC), and heterogeneous in cell volume (increased RDW). This situation is most commonly seen with anemia due to severe iron deficiency, a sequela of chronic blood loss. RDW is an index of the variation in RBC volume. An increase in RDW indicates heterogeneity in RBC size (volume). This can be due to an increase in small cells, an increase in large cells, or both.

In cases of regenerative anemia with an appropriate marrow response to increased erythropoietin levels, RBC production is accelerated and results in the release of reticulocytes into the bloodstream.²³ Changes in the RBC indices with regenerative anemia may include macrocytosis (high MCV), heterogeneous cell volume (high RDW), and hypochromasia (low MCHC). The decreased MCHC reflects a normal hemoglobin content in a larger-than-normal cell. On blood smear evaluation, RBC morphology shows varying amounts of anisocytosis (variation in volume) and macrocytosis (large cells) depending on the degree of regeneration and underlying process. Nucleated RBC (metarubricytes or late rubricytes) may also be noted with accelerated erythropoiesis.

Polychromatophils are immature RBCs that stain blue-purple on Wright-stained blood smears. Polychromasia on blood smear examination and reticulocytosis are the hallmarks of regenerative anemia.

The reticulocyte count must be interpreted in light of the degree of anemia. Minimal to mild reticulocytosis in the presence of severe anemia is considered nonregenerative if sufficient time for an adequate response has passed. Sequential hemograms and reticulocyte counts are instrumental in making this determination.

Box 3. Causes of Nonregenerative Anemia

Early blood loss or hemolysis

Erythrocyte hypoplasia

- Relative erythropoietin insufficiency—chronic renal failure
- Blunted marrow response to erythropoietin
 - Hypothyroidism
 - Hypoadrenocorticism
 - Hypopituitarism/decreased growth hormone
- Insufficient iron availability
 - Sequestration in mononuclear phagocytes—anemia of inflammatory disease (inflammation, infection, neoplasia)
 - Whole body depletion—chronic external blood loss (GI, respiratory, urinary)
 - Abnormal hepatic metabolism—portosystemic shunt
- Bone marrow disease
 - Myelophthisis (neoplasia, fibrosis)
 - Myelofibrosis (sequela of IMHA, pyruvate kinase deficiency, drug effects, neoplasia, necrosis)
 - Myelonecrosis (sepsis, hypoxia, neoplasia, drug effects, immune-mediated disease, infection, radiation)
 - Myelodysplasia (primary or secondary)^a
 - Aplastic anemia (infection, drug adverse effects, toxins, estrogen, radiation, idiopathic)
 - Pure red cell aplasia/immune-mediated ineffective erythropoiesis
 - Hemophagocytic syndrome (primary or secondary)
 - Erythroleukemia
 - Drug adverse effects/toxins
 - Infection (parvovirus, *Ehrlichia* spp, FeLV, FIV, sepsis/endotoxin)

Maturation defect

- Nutritional deficiency—cobalamin, folate, pyridoxine, copper
- FeLV infection
- Hereditary dyserythropoiesis

GI = gastrointestinal.

^aWeiss D, Smith S. Primary myelodysplastic syndromes of dogs: a report of 12 cases. *J Vet Intern Med* 2000;14(5):491-495.

Diagnostic Differentials

Once the regenerative status of the anemia has been determined, more specific tests are indicated to systematically eliminate the various causes of each category of anemia.

Nonregenerative Anemia

In most cases of true nonregenerative anemia (i.e., not hemorrhage or hemolysis that has not yet begun to regenerate), the anemia is mild to moderate.²⁶ Causes of nonregenerative anemia are listed in **BOX 3**. The most common cause of nonregenerative anemia in dogs and cats is inflammatory disease. Anemia of inflammatory disease (formerly *anemia of chronic disease*) can be a sequela of a multitude of inflammatory, infectious, traumatic, and neoplastic

Box 4. Causes of Blood Loss

Internal or external

- Coagulation or platelet disorders (anticoagulant rodenticide toxicosis, disseminated intravascular coagulation, congenital factor deficiency, thrombocytopenia, von Willebrand disease)
- Trauma
- Surgery

Internal

- Splenic rupture (hematoma, neoplasia, trauma)
- Hemorrhage from nonsplenic neoplasms

External

- Chronic bleeding—GI (neoplasia, polyp, ulcers, hookworms, inflammatory bowel disease), respiratory, urogenital
- Ectoparasites (usually juvenile patients)—fleas, ticks

diseases.^{27–30} Other causes of nonregenerative anemia include chronic renal failure, hypothyroidism in dogs, and primary bone marrow disease.³¹ Certain causes of nonregenerative anemia, such as FeLV infection, PRCA, and later-stage chronic renal failure (erythropoietin deficiency), may present with a more severe anemia. CBC and chemistry profile, urinalysis, and thoracic and abdominal imaging can help identify the presence of underlying disease, neoplasia, or renal failure. Retroviral testing is indicated for cats. Thyroid testing may be indicated for dogs. If no underlying disease can be identified, bone marrow evaluation is indicated to help identify conditions such as PRCA, myelodysplasia, leukemia, and aplastic pancytopenia.

Regenerative Anemia

If the anemia is determined to be regenerative, the next step is to differentiate blood loss from hemolysis. As previously stated, evaluation of the TS is often helpful. Decreased TS is more consistent with blood loss, whereas normal to increased TS is suggestive of hemolysis. Elevated TS in the face of anemia may suggest dehydration or hyperglobulinemia.

Causes of blood loss are listed in **BOX 4**. Most causes of blood loss can be easily identified. Blood loss related to trauma is suspected based on history. Physical examination and imaging of the thorax and abdomen can identify the presence of intracavitary hemorrhage in cases of trauma, tumor rupture, or a bleeding diathesis. Recovery of nonclotting blood on abdominocentesis indicates the presence of hemoabdomen. Results of a platelet count and clotting profile are used to eliminate a bleeding diathesis. Gastrointestinal (GI) bleeding may be indicated by the presence of hematemesis or melena or may be occult and difficult to confirm. Tests that may be used in cases of suspected GI bleeding include fecal analysis, abdominal imaging, and endoscopy. As discussed earlier, chronic bleeding may eventually lead to iron deficiency and, subsequently, microcytic, hypochromic, heterogeneous anemia.

In cases of regenerative anemia, hemolysis becomes the most likely disease process if blood loss is ruled out. Causes of hemolysis are listed in **BOX 5**. Hemolysis can be immune mediated or non-immune mediated. The most common cause of hemolysis in small animals is IMHA.³² IMHA can be primary or secondary to other diseases (e.g., infectious disease, neoplasia), drug adverse effects, or vaccine adverse reactions.^{12,33–37} The primary form may also be termed *idiopathic* or *autoimmune hemolytic anemia (AIHA)*. The diagnosis of IMHA is supported by a low hematocrit (typically <25%) with one or more of the following: autoagglutination, positive Coombs test result, spherocytosis (in dogs), and increased osmotic fragility.^{38,39} The presence of non-immune-mediated hemolysis may be suggested by the history (e.g., ingestion of onions, envenomation), by breed (e.g., phosphofructokinase or pyruvate kinase deficiency), by the presence of underlying disease (e.g., microangiopathic trauma due to hemangiosarcoma), or by findings on imaging (e.g., zinc toxicosis).

Hemolysis can also be classified as intravascular, extravascular, or a combination of both. Examination of serum and urine can sometimes aid in this determination. Intravascular hemolysis results in the release of hemoglobin into the blood that is then filtered into the urine, causing the serum and urine to develop a dark red color. The differential diagnosis for dark red urine includes hematuria, hemoglobinuria, and myoglobinuria. Hematuria can be differentiated from hemoglobinuria and myoglobinuria by centrifugation of the urine. With hematuria, the RBCs will form a pellet and the supernatant will clear after centrifugation, whereas with hemoglobinuria and myoglobinuria, the supernatant will remain pigmented. Hemoglobinuria can be differentiated from myoglobinuria by analysis of serum for creatine kinase activity, which is typically increased in cases of myoglobinuria. The presence of hemoglobinemia and hemoglobinuria in the setting of regenerative anemia is the hallmark of intravascular hemolysis.¹³ Causes of intravascular hemolysis include IgM-mediated IMHA, zinc toxicosis, phosphofructokinase deficiency, and some cases of oxidative damage such as occurs with onion ingestion.^{13,40–42}

Extravascular hemolysis, and to some degree intravascular hemolysis, results in the release of increased amounts of bilirubin into the blood. As affected RBCs are phagocytosed by the mononuclear-phagocyte system in the spleen and liver, hemoglobin in the RBCs is metabolized and water-insoluble (unconjugated) bilirubin is released into plasma, where it binds to albumin. Unconjugated bilirubin is taken up by hepatocytes for conjugation into a water-soluble (conjugated) form for excretion in bile. Massive hemoglobin breakdown, especially in the setting of concurrent hepatic injury from hypoxia, inflammation, or thrombosis, can lead to backflow of conjugated and unconjugated bilirubin into the plasma. Conjugated bilirubin is excreted into urine by the kidneys, often leading to a dark yellow urine. A small amount of bilirubinuria can be normal in dogs due to the ability of the canine kidney to conjugate bilirubin. Bilirubinuria in cats is always abnormal. The differential diagnosis of bilirubinuria and hyperbilirubinemia includes causes of hepatic dysfunction and biliary obstruction. Not all cases of extravascular hemolysis demonstrate bilirubinuria and

Box 5. Causes of Hemolysis

Immune-mediated

- Autoimmune
 - Primary/idiopathic IMHA
 - Neonatal isoerythrolysis
 - Incompatible blood transfusions
- Secondary
 - Infections (see next section)
 - Neoplasia
 - Drug effects (penicillins, cephalosporins, sulfonamides, methimazole)
 - Vaccination adverse reaction

Infectious

- Hemotropic mycoplasmosis
- Ehrlichiosis
- Babesiosis
- Cytauxzoonosis
- FeLV
- Septicemia

Fragmentation/physical damage to RBC membrane (microangiopathy)

- Mechanical (disseminated intravascular coagulation, caval syndrome, glomerulonephritis, hemolytic uremic syndrome)
- Abnormal endothelium (hemangiosarcoma, vasculitis, splenic torsion, hepatic disease)
- Thermal injury (heatstroke, severe burns)
- Osmotic injury (freshwater near-drowning)

Drugs, toxins, chemical damage to RBC membrane

- Zinc toxicosis (ingestion of pennies minted after 1983 or of zinc-containing ointments)
- Severe snake envenomation (crotalids)
- Severe hypophosphatemia
- Oxidants (ingestion of onions or onion products, garlic, acetaminophen, propylene glycol, vitamin K, benzocaine, methylene blue, naphthalene [mothballs])

Hemophagocytic syndrome

Congenital RBC abnormalities

- Defects of heme synthesis (rare)
- Membrane defects (stomatocytosis, increased osmotic fragility, nonspherocytic anemia)
- Enzyme defects (pyruvate kinase deficiency, phosphofructokinase deficiency)

hyperbilirubinemia due to the liver's enormous capacity for bilirubin metabolism.

An easy but crude in-house test to identify agglutination caused by IMHA is the saline dispersion test. For this test, clumps of

RBCs must first be seen on a glass slide containing one drop of whole or EDTA-anticoagulated blood and 2 to 5 drops of saline solution. To differentiate rouleaux (RBCs adhered in chains or stacks) from agglutination, a coverslip is placed over a drop of EDTA-anticoagulated blood on a new slide, and several drops of saline solution are placed along the edge of the coverslip. The RBCs are observed through a microscope to see whether clumps disperse or persist when the saline solution rushes in. Persistent agglutination indicates the presence of anti-RBC immunoglobulin on the surface of RBCs and supports a diagnosis of IMHA. It does not indicate whether the IMHA is primary/autoimmune or secondary. As this is a crude test, results should be considered as supportive only.

In dogs, the most common cause of spherocytosis is immune-mediated hemolysis as the immunoglobulin-covered RBC membranes are attacked by macrophages in the liver and spleen. Less common reasons for the presence of spherocytes include non-immune-mediated causes of hemolysis such as microangiopathic trauma, zinc toxicosis, and transfusion of stored blood.^{13,40} Spherocytes can also be seen with severe snakebite and bee-sting envenomation.^{43,44}

The direct antiglobulin or Coombs test identifies the presence of immunoglobulin or complement on the surface of RBCs.^{45,46} A positive result supports IMHA but does not distinguish whether it is primary or secondary. False-negative and, less commonly, false-positive results can occur. The utility of a Coombs test in cats has historically been considered dubious, but results of a 2006 study demonstrated reasonable sensitivity and specificity for the diagnosis of IMHA in cats.³⁵

In dogs, IMHA is usually primary or idiopathic.^{32,39,47} The diagnosis of primary/autoimmune IMHA is one of exclusion; thus, once IMHA has been diagnosed and an association with a drug adverse effect or vaccine adverse reaction has been eliminated, tests to rule out an underlying neoplastic or infectious disease are indicated, especially in older patients. Cats have long been considered more likely to have secondary IMHA,^{13,36,37,48} but primary IMHA is being recognized more frequently in this species.³⁵ It is important to rule out FeLV infection, FIV infection, and hemotropic mycoplasmosis (formerly *Hemobartonella* infection) in cats with IMHA and to consider feline infectious peritonitis, ehrlichiosis, anaplasmosis, drug effects, and neoplasia when appropriate.^{13,36,37,47,49,50}

Conclusion

The many causes of anemia in cats and dogs can make the road to a diagnosis seem daunting. However, a systematic approach most often yields the correct diagnosis. Information rapidly derived at initial evaluation (PCV/TS and blood film examination) can be used along with data from an automated CBC (erythrocyte count and indices) for this purpose. Additional tests, including reticulocyte count, sequential blood smear examinations, Coombs test, and saline dispersion, often help to further characterize the anemia.

References

1. Zon LI. Developmental biology of hematopoiesis. *Blood* 1995;86(8):2876-2891.
2. Sieff CA, Williams DA. Hematopoiesis. In: Handin RI, Lux SE, Stossel TP, eds. *Principles and Practice of Hematology*. Philadelphia, PA: Lippincott; 1995:171-224.
3. Weiss DJ. Aplastic anemia. In: Feldman BF, Zinkl JG, Jain NC, Schalm OW, eds. *Schalm's Veterinary Hematology*. 5th ed. Ames, IA: Blackwell Publishing; 2006:212-215.
4. Shelly SM. Causes of canine pancytopenia. *Compend Contin Educ Pract Vet* 1988;10(1):9-17.
5. Kearns SA, Ewing P. Causes of canine and feline pancytopenia. *Compend Contin Educ Pract Vet* 2006;28(2):122-134.
6. Jelkmann W. Erythropoietin: structure, control of production, and function. *Physiol Rev* 1992;72(2):449-489.
7. Semenza GL. Regulation of erythropoietin production. New insights into molecular mechanism of oxygen homeostasis. *Hematol Oncol Clin North Am* 1994;8(5):863-884.
8. Car BD. Erythropoiesis and erythrokinetics. In: Feldman BF, Zinkl JG, Jain NC, Schalm OW, eds. *Schalm's Veterinary Hematology*. 5th ed. Ames, IA: Blackwell Publishing; 2006:105-109.
9. Watson AJ, Canfield PJ. Nutritional deficiency anemias. In: Feldman BF, Zinkl JG, Jain NC, Schalm OW, eds. *Schalm's Veterinary Hematology*. 5th ed. Ames, IA: Blackwell Publishing; 2006:190-195.
10. Weiss DJ. Antibody-mediated suppression of erythropoiesis in dogs with red blood cell aplasia. *Am J Vet Res* 1986;47(12):2646-2648.
11. Stokol T, Blue J. Pure red cell aplasia in 9 cats (1989-1997). 1999;214(1):75-79.
12. Klag AR, Giger U, Shofer FS. Idiopathic immune-mediated hemolytic anemia in dogs: 42 cases (1986-1990). *J Am Vet Med Assoc* 1993;202(5):783-788.
13. Giger U. Regenerative anemias caused by blood loss or hemolysis. In: Ettinger SJ, Feldman EC, eds. *Textbook of Veterinary Internal Medicine*. 6th ed. St. Louis, MO: Elsevier Saunders; 2005:1886-1907.
14. Houston DM, Myers SL. A review of Heinz-body anemia in the dog induced by toxins. *Vet Hum Toxicol* 1993;35(2):158-161.
15. Lee KW, Yamato O, Tajima M, et al. Hematologic changes associated with the appearance of eccentrocytes after intragastric administration of garlic extract to dogs. *Am J Vet Res* 2000;61(11):1446-1450.
16. Christopher MM. Relation of endogenous Heinz bodies to disease and anemia in cats: 120 cases (1978-1987). *J Am Vet Med Assoc* 1989;194(8):1089-1095.
17. Weiss DJ, Kristensen A, Papenfuss N, McClay CB. Quantitative evaluation of echinocytes in the dog. *Vet Clin Pathol* 1990;19(4):114-118.
18. Brown DE, Meyer DJ, Wingfield WE, Walton RM. Echinocytosis associated with rattlesnake envenomation in dogs. *Vet Pathol* 1994;31(6):654-657.
19. Hirsch VM, Jacobsen J, Mills JH. A retrospective study of canine hemangiosarcoma and its association with acanthocytosis. *Can Vet J* 1981;22(5):152-155.
20. Christopher MM, Lee SE. Red cell morphologic alterations in cats with hepatic disease. *Vet Clin Pathol* 1994;23(1):7-12.
21. Hammer AS, Couto CG, Swardson C, Getzy D. Hemostatic abnormalities in dogs with hemangiosarcoma. *J Vet Intern Med* 1991;5(1):11-14.
22. Knecht CD, Crabtree J, Katherman A. Clinical, clinicopathologic, and electroencephalographic features of lead poisoning in dogs. *J Am Vet Med Assoc* 1979;175(2):196-201.
23. Fernandez F, Grindem C. Reticulocyte response. In: Feldman BF, Zinkl JG, Jain NC, Schalm OW, eds. *Schalm's Veterinary Hematology*. 5th ed. Ames, IA: Blackwell Publishing; 2006:110-116.
24. Perkins P, Grindem C, Cullins L. Flow cytometric analysis of feline reticulocytes. *Am J Vet Res* 1995;56(12):1564-1569.
25. Neiger R, Hadley J, Pfeiffer DU. Differentiation of dogs with regenerative and non-regenerative anemia on the basis of their red cell distribution width and mean corpuscular volume. *Vet Rec* 2002;150(14):431-434.
26. Waner T, Harrus S. Anemia of inflammatory disease. In: Feldman BF, Zinkl JG, Jain NC, Schalm OW, eds. *Schalm's Veterinary Hematology*. 5th ed. Ames, IA: Blackwell Publishing; 2006:205-209.
27. Feldman BF, Kaneko JJ, Farver TB. Anemia of inflammatory disease in the dog: clinical characterization. *Am J Vet Res* 1981;42(7):1109-1113.
28. Feldman BF, Kaneko JJ. The anemia of inflammatory disease in the dog I: the nature of the problem. *Vet Res Commun* 1981;4(4):237-252.
29. Ottenjann M, Weingart C, Arndt G, Kohn B. Characterization of the anemia of inflammatory disease in cats with abscesses, pyothorax, or fat necrosis. *J Vet Intern Med* 2006;20(5):1143-1150.

30. Weiss DJ, Krehbiel JD. Studies of the pathogenesis of anemia of inflammation: erythrocyte survival. *Am J Vet Res* 1983;44(10):1830-1831.
31. Feldman BF. Non-regenerative anemia. In: Ettinger SJ, Feldman EC, eds. *Textbook of Veterinary Internal Medicine*. 6th ed. St. Louis, MO: Elsevier Saunders; 2005:1908-1917.
32. Barker RN. Anemia associated with immune responses. In: Feldman BF, Zinkl JG, Jain NC, Schalm OW, eds. *Schalm's Veterinary Hematology*. 5th ed. Ames, IA: Blackwell Publishing; 2006:169-177.
33. Reimer ME, Troy GC, Warnick LD. Immune-mediated hemolytic anemia: 70 cases (1988-1996). *J Am Anim Hosp Assoc* 1999;35(5):384-391.
34. Duval D, Giger U. Vaccine-associated immune-mediated hemolytic anemia in the dog. *J Vet Intern Med* 1996;10(5):290-295.
35. Kohn B, Weingart C, Eckmann V, et al. Primary immune-mediated hemolytic anemia in 19 cats: diagnosis, therapy, and outcome (1998-2004). *J Vet Intern Med* 2006;20(1):159-166.
36. Peterson ME, Hurvitz AI, Leib MS, et al. Propylthiouracil-associated hemolytic anemia, thrombocytopenia, and antinuclear antibodies in cats with hyperthyroidism. *J Am Vet Med Assoc* 1984;184(7):806-808.
37. Norris JM, Bosward KL, White JD, et al. Clinicopathological findings in cats with infectious feline peritonitis in Sydney, Australia: 42 cases (1990-2002). *Aust Vet J* 2005;83(11):666-673.
38. Weiss D, Tvedten H. Erythrocyte disorders. In: Willard MD, Tvedten H, eds. *Small Animal Clinical Diagnosis by Laboratory Methods*. 4th ed. St. Louis, MO: Elsevier Saunders; 2004:38-62.
39. Balch A, Mackin A. Canine immune-mediated hemolytic anemia: pathophysiology, clinical signs, and diagnosis. *Compend Contin Educ Vet* 2007;29(4):217-225.
40. Gurnee CM, Drobatz KJ. Zinc intoxication in dogs: 19 cases (1991-2003). *J Am Vet Med Assoc* 2007;230(8):1174-1179.
41. Harvey JW. Pathogenesis, laboratory diagnosis, and clinical implications of erythrocyte enzyme deficiencies in dogs, cats, and horses. *Vet Clin Pathol* 2006;35(2):144-156.
42. Desnoyers MD. Anemias associated with Heinz bodies. In: Feldman BF, Zinkl JG, Jain NC, Schalm OW, eds. *Schalm's Veterinary Hematology*. 5th ed. Ames, IA: Blackwell Publishing; 2006:178-184.
43. Walton RM, Brown DE, Hamar DW, et al. Mechanisms of echinocytosis induced by *Crotalus atrox* venom. *Vet Pathol* 1997;34(5):442-449.
44. Wysoke JM, Bland van-den Berg P, Marshall C. Bee sting-induced haemolysis, spherocytosis, and neural dysfunction in three dogs. *J S Afr Vet Assoc* 1990;61(1):29-32.
45. Jones DRE, Gruffydd-Jones TJ, Stokes CR, Bourne FJ. Use of a direct enzyme-linked antiglobulin test for laboratory diagnosis of immune-mediated hemolytic anemia in dogs. *Am J Vet Res* 1992;53(4):457-465.
46. Slappendel RJ. The diagnostic significance of the direct antiglobulin test (DAT) in anemia dogs. *J Vet Immunol Immunopathol* 1979;1(1):49-59.
47. Piek CJ, Junius G, Dekker A, et al. Idiopathic immune-mediated hemolytic anemia: treatment outcome and prognostic factors in 149 dogs. *J Vet Intern Med* 2008;22(2):366-373.
48. Messick JB. Hemotropic mycoplasmas (hemoplasmas): a review and new insights into pathogenic potential. *Vet Clin Pathol* 2004;33(1):2-13.
49. Breitschwerdt EB, Abrams-Ogg ACG, Lappin MR, et al. Molecular evidence supporting *Ehrlichia canis*-like infection in cats. *J Vet Intern Med* 2002;16(6):642-649.
50. Lappin MR, Breitschwerdt EB, Jensen WA, et al. Molecular and serologic evidence of *phagocytophilum* infection in cats in North America. *J Am Vet Med Assoc* 2004; 225(6):893-896.



3 CE Credits

This article qualifies for 3 contact hours of continuing education credit from the Auburn University College of Veterinary Medicine. CE tests must be taken online at Vetlearn.com; test results and CE certificates are available immediately. Those who wish to apply this credit to fulfill state relicensure requirements should consult their respective state authorities regarding the applicability of this program.

1. Erythropoietin, the hormone responsible for stimulating production of RBCs in the bone marrow, is produced by the
 - a. adrenal glands.
 - b. pituitary gland.
 - c. lungs.
 - d. kidneys.
2. A _____ suggests the presence of regeneration.
 - a. normal MCV
 - b. high MCV
 - c. low MCV
 - d. normal RDW
3. Which statement regarding reticulocytes is correct?
 - a. Reticulocytes are generally observed the day after an episode of acute blood loss.
 - b. They are usually counted on Gram-stained smears.
 - c. They indicate an appropriate response by the bone marrow to a decreased RBC mass or hypoxia.
 - d. They indicate a regenerative anemia regardless of the severity of the anemia and the level of reticulocyte response.
4. The most common etiology of IMHA in dogs is
 - a. secondary to ehrlichiosis.
 - b. an autoimmune response.
 - c. secondary to penicillin administration.
 - d. secondary to neoplasia.
5. Bone marrow examination would be indicated for a patient with
 - a. reticulocytosis secondary to IMHA.
 - b. nonregenerative anemia secondary to chronic renal failure.
 - c. nonregenerative anemia for which an underlying disease cannot be identified after initial blood work, urinalysis, and imaging studies.
 - d. anemia secondary to hemoabdomen.
6. Which statement regarding erythropoietin insufficiency is correct?
 - a. It can result in severe regenerative anemia.
 - b. It does not result in anemia.
 - c. It results in moderate regenerative anemia.
 - d. It can be the result of chronic kidney disease.
7. Which of the following would be expected to result in regenerative anemia?
 - a. anticoagulant rodenticide intoxication
 - b. chronic infection
 - c. hypothyroidism
 - d. myelofibrosis
8. What is the most likely diagnosis for a dog with weakness, pale mucous membranes, a PCV of 20% (reference range: 36% to 50%), a TS value of 4.0 g/dL (reference range: 5.2 to 7.8 g/dL), anisocytosis, and polychromasia?
 - a. IMHA
 - b. acute blood loss
 - c. chronic renal failure
 - d. PRCA
9. Which blood smear finding does not support a diagnosis of IMHA?
 - a. autoagglutination
 - b. spherocytosis
 - c. metarubricytosis
 - d. rouleaux formation
10. Which is the least likely diagnosis for a cat with a normocytic, normochromic, nonregenerative anemia that is still nonregenerative 1 week after initial presentation?
 - a. coagulopathy
 - b. chronic renal failure
 - c. FeLV infection
 - d. PRCA