"Help, my patient is anemic! A primer on CBC and blood smear investigation of anemia"
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Introduction:

Complete blood counts (CBCs) are frequently performed on clinically normal patients to evaluate patients for subclinical disease, especially in geriatric or pre-anesthetic/surgical patients, or on ill animals where the exact cause of illness is not readily apparent. Anemia is a decrease in circulating erythrocytes typically identified in veterinary medicine by a hematocrit or packed cell volume (PCV) below the reference interval.

Anemia is a clinical sign, not a specific disease, and as such it is always secondary to an underlying problem, ranging from chronic inflammation to infectious organisms, toxins, metabolic or endocrine diseases, nutritional deficiencies, neoplasia, and more. Correctly interpreting CBC and blood smear findings is essential for diagnosing and managing the right underlying problem(s). A rational diagnostic approach to anemia combines interpretation of laboratory data with a thorough knowledge of the associated pathophysiological mechanisms to derive a sensible list of differentials.

The following notes provide a step-wise conceptual framework for working through anemia, with an emphasis on hematology analyzer data and blood smear morphologic assessment. Although you cannot become an expert in hematology based on just one lecture, with practice and an orderly approach to blood smear evaluation, you will become more proficient in hematology interpretation!

Manual PCV & Hematology Analyzers:

The first step to laboratory assessment of anemia requires measuring red blood cell mass. A fast, simple, and inexpensive way to do this is a manual spun packed cell volume (PCV). A small amount of blood (often far less than 0.5 ml) is inserted into a glass capillary tube, one end is plugged with a non-porous material like clay, and the tube is centrifuged for 3-5 minutes. Then, the centrifuged tube is compared to a reference chart to estimate the PCV. A spun PCV is often said to be the most accurate measure of RBC mass, but it depends on technique, calibration of the centrifuge, proper timing, and correctly interpreting the chart. Other information you can obtain from this includes a refractometric total solids/protein (which can be important for assessing hydration status and some causes of anemia, such as blood loss) and qualitative information about the plasma. If the plasma has a pink tinge, this suggests hemoglobinemia from either pathologic intravascular hemolysis, or possibly iatrogenic artifactual hemolysis due to suboptimal collection. A yellow tinge suggests icterus due to hyperbilirubinemia, which may be due to hemolysis, hepatic disease, or obstructive cholestasis.

Hematology analyzers can provide more information about the circulating RBC mass and other blood cell lines than a simple PCV/TS. Automated analysis often provides multiple measures of RBC mass, including a red blood cell count/concentration ([RBC]), a hemoglobin concentration (HGB), and a hematocrit (HCT). In addition to direct measurements of RBC mass, automated counters provide other information. Mean cell volume (MCV) represents the average RBC size, and mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) describes the average
hemoglobin per RBC. The red blood cell distribution width or RDW is the coefficient of variation in cell size, and represents a quantitative measure of anisocytosis (variation in cell size. MCV, MCHC, and RDW are used in some classification schemes for anemia.

The two basic types of automated hematology analyzers are impedance counters and flow-cytometry based analyzers, although some machines utilize both methods. Impedance counters pass the blood cells through a microfluidic channel separating an electrical current and measure the frequency and magnitude of charge disruptions to provide number and type of cells. Impedance counters are affordable, rapid, and reliable, although the leukocyte differential they produce is not very accurate and these machines are more susceptible to certain artifacts. Flow cytometry based machines utilize lasers and certain dyes/reactions to measure number of cells, size, and parse them into categories based on their staining patterns. These machines have more advanced capabilities and provide more accurate cell differentials than impedance counters, but are quite a bit more expensive and may require more upkeep.

HCT is often interpreted and discussed as synonymous with PCV, but it is important to note that HCT is a mathematically calculated value derived from the formula $HCT = \frac{RBC \times MCV}{10}$. Therefore, anything that interferes with the measurement of RBC (such as agglutination excluding clumped erythrocytes from counting) or dramatically alters MCV (such as macrocytosis or microcytosis) can lead to a discrepancy between HCT and a spun PCV.

**Assessing the Chronicity & Severity of Anemia:**

It is important to gauge how threatening the anemia is to patient survival and/or quality of life, to differentiate underlying diseases, and to assess the necessity of therapeutic interventions (i.e. blood transfusion). The following are guidelines on severity of anemia for companion animal patients:

- **Mild anemia:** HCT 26-38% (dogs), 21-27% (cats)
- **Moderate anemia:** HCT 15-25% (dogs), 12-20% (cats)
- **Severe anemia:** HCT <14% (dogs), <11% (cats). [See Figure 1]

![Figure 1: Severe anemia with decreased RBC density (note the abundant white space).](image)

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In general, animals are more likely to be symptomatic from acutely developing anemia than those that have been more insidious, allowing time to acclimate to decreased oxygen carrying capacity. Clinical signs of acute, severe anemia include tachypnea, tachycardia, and exercise intolerance.

**Basic Approach to Reviewing a Peripheral Blood Smear:**

It is critical to review peripheral blood smears for a number of reasons. First, as a basic quality assessment/quality control measure to verify the numbers provided by the machine look reasonable. Many pathologies or in vitro artifacts can cause problems for the machine. For example, RBC agglutination may falsely decrease RBC and falsely increase MCV/MCHC/RDW, altering the hematocrit in the process. Platelet clumping in the sample tube commonly causes spurious thrombocytopenia.

Second, hematology analyzers are imperfect and may misclassify or completely ignore certain types of cells. The presence of nucleated red blood cells may be miscounted as leukocytes on many machines. When there are nRBCs present, the WBC count must be corrected according to the following formula: \( WBC_{CORR} = WBC \times \left( \frac{100}{100 + nRBC} \right) \). Some neoplastic cells may be so large and abnormal that they are completely excluded by the machine, and thus invisible to the clinician without reviewing the smear.

Finally, there are a number of subjective morphologic changes that cannot be determined without blood smear review. Toxic changes that support inflammation, infectious organisms, polychromasia, and pathologic findings like spherocytes are all examples of important pieces of information that cannot be provided by an automated cell counter.

You should review a blood smear with a consistent system to avoid missing findings. In general, we recommend initially scanning the slide at low power (10-20x objective) to verify it is well-stained and has appropriate areas for viewing cell density and morphology. Then, go to the “feathered edge” to look for platelet clumps, microfilariae, large neoplastic cells, schizonts, and other infectious organisms (Figure 2). Many of the cells at the feathered edge are overspread or ruptured, so this is a suboptimal area to evaluate leukocyte and erythrocyte morphology. After looking at the feathered edge, go to the “monolayer” where ~50% of cells are touching and are nicely spread out. While still on low magnification, ask yourself: Is the RBC density normal or decreased? Do the number of WBCs look increased, decreased, or normal? Can I see platelets scattered? Correlate your findings with the automated numbers. Then, go to higher magnification (40x-100x) and perform a 100-200 cell WBC differential count. Evaluate the morphology of all three cell lines. Look for infectious organisms and atypical cells. This process may be challenging at first, but with repetition you will become proficient!
Regenerative versus Non-Regenerative Anemias:

We classify anemias in veterinary medicine as regenerative or non-regenerative because along with assessing the severity and RBC indices/morphology, it often aids in determining the etiologic cause. An anemia is considered regenerative in most species (see exceptions below) if there is an appropriate bone marrow response, i.e. a reticulocytosis. If there is no reticulocytosis, it is considered either a non-regenerative anemia, or possibly too early to see regenerative effects because the bone marrow takes 3-7 days to reach maximal regenerative capacity.

Some newer automated hematology analyzers can directly measure the reticulocyte concentration using a fluorescent RNA dye. Alternatively, one can perform a manual reticulocyte count by incubating a drop of peripheral blood with 1-2 drops of New Methylene Blue stain for 15 minutes, then making a blood smear and counting reticulocytes. You count 1000 RBCs, tracking which are reticulocytes or not, and calculate a reticulocyte percentage. Then, this percentage is multiplied by the RBC concentration to yield an absolute reticulocyte concentration.

As a general rule, the absolute reticulocyte count is evaluated, rather than the reticulocyte percentage. The following are species-specific guidelines based on reticulocyte count for determining if an anemia is regenerative and if so, the vigor of the response:

- **Dogs:** Absolute reticulocyte count > 80,000/µL
  - Mild: 100,000-150,000
  - Moderate: 200,000-300,000
  - Marked: 400,000-500,000
- **Cats:** Absolute reticulocyte count > 60,000/µL
  - 100,000-200,000 is a moderate to marked regenerative response

In addition to these quantitative measurements of reticulocytosis, there are a number of RBC index changes and subjective morphologic observations that support a regenerative response. As

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reticulocytes are younger RBCs that are larger in size and contain less hemoglobin, the MCV tends to be high while the MCHC tends to be low in regenerative anemias. These anemias are often referred to as “macrocytic, hypochromic.” These reticulocytes also have more cellular RNA, so they stain blue-purple with Romanowsky-type stains (i.e. Diff-Quik), and they are called polychromatophils; when these are numerous, it is said there is moderate/marked polychromasia (Figure 3). As there tends to be a large amount of variation in RBC size (anisocytosis), the RDW tends to be increased as well. Other findings on a blood smear that can be associated with regeneration include nucleated red blood cells (nRBCs), Howell-Jolly bodies (small nuclear remnants), target cells (aka codocytes), and stomatocytes.

Figure 3: The red cell density is decreased, compatible with a moderate to severe anemia. Note the numerous large purple-gray staining polychromatophils (black arrows) and the wide variation in cell size (anisocytosis) suggesting a regenerative anemia.

Figure 4: Metarubricyte, a type of nucleated red blood cell (nRBC). This RBC precursor can be seen in circulation with regenerative anemias. nRBCs are counted as leukocytes by most machines, so the WBC count must be corrected.
**Regenerative Anemia Etiologies:**

In regenerative anemias, the bone marrow is able to mount an effective response to increased demand for decreased peripheral RBCs to attempt to restore homeostasis. The two broad categories of anemia that are regenerative include blood loss or hemorrhage, and red blood cell destruction or hemolysis. Multiple types of blood loss can result in anemia, from massive exsanguination to chronic gastrointestinal hemorrhage (i.e. melena) or hematuria, cavitory bleeding (i.e. hemangiosarcoma), etc. Initially, peracute blood loss may be difficult to recognize as the proportional loss of cells and plasma will result in minimal change to PCV/TP. However, over time, as fluid shifts from within tissue cells to the vasculature, the remaining red blood cells and proteins will be diluted, manifesting as decreased PCV/TP. Long-standing blood loss may lead to iron deficiency anemia, which often manifests as a microcytic, hypochromic anemia with marked anisocytosis, variable regeneration, thrombocytosis, and significant poikilocytosis (variation in red blood cell shape); see Figure 5 for an example.

![Blood smear from a case of classic iron deficiency anemia in a dog. Cirled RBCs show significant hypochromasia. Arrowheads indicate target cells (codocytes). The arrow is a schistocyte.](image)

Cases with regenerative anemia due to increased erythrocyte destruction (hemolysis) often have key morphologic findings that suggest an etiology. Immune-mediated hemolytic anemia (IMHA) is a common disease causing marked regenerative anemia. IMHA is often associated with macroscopic or microscopic agglutination as well as spherocytes (Figure 6). Intravascular hemolysis due to IMHA (or any other condition) will manifest with ghost cells (Figure 7), where faint outlines of lysed RBCs drained of hemoglobin are noted.
Many toxic causes of hemolytic anemia result from **oxidative damage** to RBC phospholipid membranes. These include common drugs/toxins like acetaminophen, NSAIDs, zinc, garlic/onions, Vitamin K, naphthalene, skunk musk, and many more. The two main morphologies with this type of anemia are **Heinz bodies**, which are denatured aggregates of hemoglobin, and **eccentrocytes** aka “blister cells” (Figure 8); ghost cells may also be seen as described in Figure 7. Also notably, endogenous oxidants produced by a variety of diseases (i.e. diabetic ketoacidosis, lymphoma, etc) can result in numerous Heinz bodies in cats.
Certain conditions can result in RBC shearing or fragmentation, including hemangiosarcoma, disseminated intravascular coagulation (DIC), thromboembolism, glomerulonephritis, severe heartworm disease, iron deficiency, vasculitis, and more. These conditions often result in a characteristic triad of fragmented cells, including schistocytes (Figure 5), keratocytes, and acanthocytes.

Numerous hemoparasities can also result in anemia through multiple mechanisms. Some blood parasites can result in secondary IMHA, such as *Ehrlichia canis*, *Anaplasma phagocytophilum*, etc. However, these organisms do not directly infect erythrocytes. Other infectious agents directly infect RBCs and cause hemolysis through direct damage and/or triggering splenic macrophage clearance. Prominent examples of these include *Mycoplasma hemofelis* in cats (Figure 9), *Babesia canis/gibsoni* in dogs.

Figure 8: Panel A = numerous homogeneous-staining protrusions on RBC membranes (circles) are Heinz bodies. These can be confirmed with New Methylene Blue staining (inset). Panel B = numerous eccentrocytes with a blister-like clearing can also be seen with oxidative damage.

Figure 9: Blood smear from a cat. Note the tiny cocci and ring shaped *Mycoplasma hemofelis* organisms in circled RBCs. These sometimes also form chains.
Non-Regenerative Anemia Etiologies:

It is always important to consider that an anemia resulting from hemorrhage or hemolysis may not be regenerative yet due to insufficient time for the body to respond. Initially, splenic contraction can slightly increase PCV and reticulocytes, but erythroid hyperplasia in the bone marrow takes 3-5 days (up to a week) to mount a maximal regenerative response. Blood smear morphology, chemistry parameters, and clinical/historical information can assist in discriminating these so-called “pre-regenerative” anemias.

True non-regenerative anemias (not just too early to see regeneration) result from direct or indirect interference with bone marrow hematopoiesis for one or multiple reasons. These anemias are often normocytic and normochromic (due to a lack of larger, hypochromic reticulocytes). One of the most common causes of non-regenerative anemia from causes outside the marrow is anemia of chronic disease aka anemia of inflammatory disease (AID). AID develops because inflammatory cytokines (such as IL-1, IL-6) interfere with iron regulation, decrease RBC lifespan, and blunt bone marrow response to pro-erythropoietic cytokines. AID is typically mild to moderate in severity, and usually lacks characteristic morphologic changes or alterations to other blood cell lines. Another common cause of non-regenerative anemia due to disease outside the bone marrow is anemia of renal disease. Chronic and/or severe renal disease results in decreased production of erythropoietin (EPO), which results in decreased RBC production in the bone marrow. A variety of other endocrine diseases (hypothyroidism, Addison’s disease) and nutritional deficiencies (iron, copper, cobalamin, folate) can result in mild non-regenerative anemia.

Non-regenerative anemias resulting from direct bone marrow pathology are often severe, and frequently accompanied by additional cytopenias (neutropenia, thrombocytopenia). Bone marrow tissue can be replaced by abnormal tissue (myelophthisis) in lymphoma, leukemia, metastatic neoplasia, myelodysplastic syndromes, and myelofibrosis. Some infectious diseases like chronic Ehrlichiosis, Leishmaniasis, Mycobacteriosis, parvovirus, feline panleukopenia virus, FIV, and FeLV can result in bone marrow inflammation (myelitis) and necrosis, and/or directly damage hematopoietic precursors. Finally, certain drugs (especially chemotherapeutic agents), toxins, and radiation may directly damage bone marrow and result in non-regenerative anemias (with or without other cytopenias).

References & Further Reading:

Ettinger SJ, Feldman EC. Textbook of Veterinary Internal Medicine, 7th ed. 2010. Saunders Elsevier; St. Louis, MO.


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