THE IMPORTANCE OF A COMPLETE HEMOGRAM

Of all the diagnostic tests available, blood evaluation is one of the single most valuable tools in assessing the general health of the body. Blood, and the nutrients it carries, circulates through every living cell in the body. It stands to reason that it is an incredibly valuable indicator of disease, either local or systemic. The complete blood count (CBC) is the best and most convenient mechanism to detect abnormalities in our patient’s the blood. The CBC begins with the quantitative evaluation of erythrocytes, leukocytes and platelets, but it does not end there. It ends with the microscopic examination of the blood film to detect morphological abnormalities that provide valuable insight to various disease conditions.

QUANTITATIVE ANALYSIS

Erythrocytes

The red blood cell count (RBC), the packed cell volume (PCV) and the hematocrit all assess the same parameter, red blood cell mass in the body in relation to the volume of plasma. Elevations in these parameters indicate an absolute increase in the number of erythrocytes (erythrocytosis) or a relative decrease in the volume of plasma (hemoconcentration). Another term for erythrocytosis is polycythemia. Polycythemia may be secondary to other clinical conditions such as hypoxia, renal neoplasia, hyperthyroidism or splenic contraction. However, primary polycythemia (polycythemia vera) is a myeloproliferative disease and results from increased production of red cells from the marrow independent of erythropoietin production. Measurement of endogenous erythropoietin levels can give an indication of the etiology. Reduced red blood cell mass (anemia) can either result from a red cell production problem, hemolysis, blood loss, or a combination of the above. PCV alone can provide an accurate evaluation of red blood cell mass, but only with the addition of the RBC can all of the red cell indices be calculated.

The red cell indices, mean cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC), help us to classify an anemia as regenerative (blood loss or hemolysis) or nonregenerative (production problem), often giving us insight to the etiology. A nonregenerative anemia will typically have a normal MCV (normocytic) and MCHC (normochromic). This can be seen in a number of conditions resulting in decreased red cell production or in per acute blood loss of less than 3 to 5 days duration. The most common cause of a nonregenerative anemia in companion animal medicine is the anemia of chronic inflammatory disease. A regenerative anemia will have an increased MCV (macrocytic) and a decreased MCHC (hypochromic). However, because the indices used to evaluate regeneration are mean values, they will not increase until the population of immature erythrocytes (Reticulocytes) are abundant enough to push the mean values out of the reference range. Therefore, many regenerative anemias will have MCVs and MCHCs within the normal range. The most sensitive way to detect a regenerative response is by performing a reticulocyte count. Red Cell Distribution Width and evaluation of the blood film for polychromasia can markedly increase the sensitivity of detecting a regenerative response, particularly when MCV and MCHC are normal. A regenerative anemia may be seen in conditions resulting in blood loss or hemolysis. A particularly strong regenerative response is seen in animals with hemolytic anemia. Hemolysis results in the most dramatic changes in red cell indices. An anemia that is the result of chronic hemorrhage and iron deficiency will have a low MCV (microcytic) and low MCHC (hypochromic). In addition, cats with FeLV infection may experience a specific type of anemia arises from disturbances in red cell maturation, resulting in a macrocytic (elevated MCV) and normochromic (normal MCHC) abnormality.

Erythrocytes of Dogs Vs. Cats

The erythrocytes of the dog are approximately 7 µm in diameter. They have a discoid shape and on a smear this is demonstrated as a central pallor that is approximately 1/3 of the diameter of the red cell. A central pallor of ½ the diameter of the red cell or more is indicative of hypochromasia and iron deficiency anemia. This is typically the result of chronic blood loss. Cells with no central pallor may be seen. If these cells are smaller in diameter and darker staining with no central pallor a diagnosis of spherocytosis can be made. These are often seen in patients with immune-mediated hemolytic anemias, but may also be seen in patients with fragmentation hemolysis, coral snake bites or zinc toxicosis. Dogs may release reticulocytes from the marrow under conditions when the red cell mass is adequate. In the
dog, an absolute reticulocyte count of >80,000 cells / µl is considered an exaggerated response and indicative of a blood loss or hemolytic process.

The erythrocytes of the cat are smaller than that of the dog, measuring no more than 6 µm in diameter. Because of the smaller size, the central pallor of the cat erythrocyte is small, and often absent. Because of this, spherocytes cannot reliably be identified in the peripheral blood smear. If the central pallor of a feline erythrocyte is 1/3 the diameter of the red cell or more, that is an indication of hypochromasia and iron deficiency anemia associated with chronic blood loss.

The hemoglobin molecule of feline erythrocytes has 8 sulfhydryl groups, more than any other domestic species. Because of this, the feline hemoglobin molecule is highly susceptible to oxidative damage to such compounds as onions, acetaminophen, propylene glycol, and other causes of oxidant damage. In addition, certain disease processes such as lymphoma, hyperthyroidism and diabetes mellitus may result in oxidant damage to feline erythrocytes. These compounds and diseases result in Heinz body formation which reduces erythrocyte life-span. However, since the feline spleen is non-sinusoidal, erythrocytes with Heinz bodies are not lysed prematurely and are well tolerated by the circulatory system.

There are two types of reticulocytes in the cat, aggregate and punctate. Under normal condition, the cat has less than 1% aggregate reticulocytes and approximately 10% punctate reticulocytes. In anemias, where there is an increased demand for red cell mass, the aggregate reticulocytes will initially increase to an absolute count of 60,000 cells / µl or more. As these cells mature, the level of punctate reticulocytes rise. If the demand for early release of erythrocytes from the bone marrow is met, the aggregate reticulocytes with drop to normal levels, while the punctate reticulocyte level will remain elevated for a week or more. In the dog, there is no distinction between aggregate and punctate reticulocytes.

**Leukocytes**

The leukogram begins with the total white blood cell count (WBC). However, this number alone does not provide vital information needed to assess patient health. Many animals will have a normal WBC with significant abnormalities in the leukogram. Those hematology analyzers that can accurately perform a differential leukocyte evaluation provide superior information regarding individual leukocyte numbers to detect increased numbers of immature neutrophils, decreased numbers of mature neutrophils and increased or decreased numbers of lymphocytes, monocytes, eosinophils and basophils. These changes can occur without alterations in the total WBC, and in some cases, these changes are even more significant when the WBC is normal. Automated analyzers that utilize laser technology are better at providing accurate differential leukocyte counts than are older impedance counters because they separate cells based on their ability to cause light refraction. Impedance counters separate cells based on size alone. Some analyzers are capable of “flagging” atypical cells in circulation which could include reactive monocytes and lymphocytes or neoplastic hematopoietic blast cells.

Increased numbers of immature cells in circulation is termed a “left-shift”. The type of left shift present can be of prognostic value since it serves as an indication of how well the animal’s innate immune system is responding to the disease process. A left-shift may be degenerative, regenerative, or transitional, depending upon the total leukocyte count and the number of immature neutrophils in circulation.

*Regenerative left shift* – a left shift in which there is typically a neutrophilia and there are a higher numbers of mature cells than immature. This is a favorable response where the bone marrow has had sufficient time (3 –5 days) to respond to peripheral demands for neutrophils.

*Degenerative left shift* – a left shift in which there are more immature neutrophils (bands, metamyelocytes and myelocytes) than mature neutrophils (segmented). Total neutrophils counts are typically low or only slightly elevated. This indicates that the reserve of mature neutrophils in the bone marrow has been depleted, has had insufficient time to respond, or cannot meet the overwhelming demand for neutrophils. In most species this is an unfavorable prognostic indicator.

*Transitional left shift* – a leukogram that has a moderate to marked neutrophilia, but the immature forms still outnumber the mature forms or conversely, in situations where there is a neutropenia but mature forms still outnumber immature forms. These findings need to be interpreted in light of sequential hemograms or changes in status of the patient to determine if they indicate recovery or worsening of the disease.

**Platelets**
Platelet counts are difficult to obtain using any automated hematology analyzer, particularly in cats. This is because when platelets are activated they form clumps that cannot be counted. In addition, because of size similarity, impedance counters cannot distinguish large platelets from erythrocytes, a common occurrence in some dogs and most cats. Hematology analyzers that use laser technology are better at distinguishing platelets from erythrocytes because they do not identify them based on size. However, no analyzer can give an accurate assessment of platelet numbers when significant clumping has occurred. For this reason, all animals with abnormally low platelet counts should have a blood film evaluated to estimate platelet numbers from the smear and to identify the degree of platelet clumping. Two breeds of dogs (Greyhounds and King Charles Cavalier Spaniels) have platelet counts that are normally below the reference interval for other dogs.

The quantitative assessment of the hemogram is important, but the hemogram is not complete without a microscopic evaluation of the blood film.

CYTOLOGICAL ABNORMALITIES IN THE CANINE & FELINE BLOOD FILM

Introduction

The evaluation of a blood smear will allow the practitioner to gain rapid, valuable information regarding the health of the patient when the evaluation is performed in a systematic fashion. The important clinical information needed for the hematologic evaluation of an animal can all be obtained by estimating cell numbers and evaluating the morphologic changes in erythrocytes, leukocytes and platelets. The value of these findings, many of which are not recognized by automated cell counters, cannot be overemphasized.

Blood Collection and Slide Preparation

Vacutainer tubes containing EDTA should be filled to the designated amount. Partial filling of vacutainer tubes with blood may cause artifactual changes in cell morphology and numerical values. Blood smears should be prepared as quickly as possible in order to minimize artifactual changes in erythrocytes and leukocytes such as red cell crenation, leukocyte vacuolation and nuclear swelling and pyknosis. In addition, prolonged exposure to EDTA may make it more difficult, or even impossible to identify infectious agents, such as Mycoplasma haemofelis (formerly Haemobartonella felis), in the blood of infected cats. The coverslip technique for making smears is preferred over the glass slide technique. This technique minimizes traumatic injury to cells during slide preparation. This technique produces a more even distribution of cells, allowing more accurate estimation of leukocyte and platelet numbers. Smears should be rapidly dried with a blow drier to eliminate artifacts of air-drying red blood cells. This is particularly important when attempting to identify red cell parasites such as haemoplasmas or evaluation of erythrocyte shape changes.

Scanning the Smear

The first step in the evaluation of a blood smear is to scan the slide using a 10X or 20X objective. With regard to the red blood cells, we should observe red cell density and presence of rouleaux or agglutination. (Rouleaux may be differentiated from agglutination by saline test where 1 drop of blood is mixed with 0.5 to 1.0 ml of physiological saline solution and observed on wet mounts, unstained. Rouleaux will disperse with saline dilution.). With regard to nucleated cells we should confirm that the mature neutrophil is the predominant cell type. The presence of any left-shifted neutrophils or large or atypical leukocytes, as well as the presence of any nucleated erythrocytes should also be recorded. Platelet clumps should also be identified at this magnification because they will affect how we interpret platelet numbers later in the evaluation. This is particularly crucial in the evaluation of the feline blood film because platelet clumping is a common occurrence in this species. Leukocyte numbers may be estimated using the following
Erythrocyte Evaluation

Erythrocyte evaluation begins with the search for agglutination or rouleaux formation using a scanning objective. Erythrocyte morphology should be evaluated using the 100X, oil immersion lens in an area of the smear where red cells are evenly spaced, usually slightly behind the feathered edge. Red blood cells are evaluated for changes in size, shape, color and inclusions. Erythrocytes are normally very uniform in size. Typically, there is minimal variation in the size, shape or color of the erythrocytes in the blood smear. In most domestic species, red cells are normally a biconcave disc shape (discocyte). However, the central pallor (a paler staining area in the center of the erythrocyte) that is normally prominent in the blood cells of dogs of often not seen in the cat. This is associated with the smaller erythrocyte size in the cat (5-6 µm) than in the dog (7 µm). Therefore, in the feline species, spherocytosis (small, round, dense cells with no central pallor) cannot reliably be identified by examination of a blood film. Spherocytosis can only reliably be identified in the canine.

**Anisocytosis** is defined as a variation in cell size. This usually indicates the presence of abnormally large erythrocytes (macrocytes) which are commonly seen in regenerative anemias. Macrocytes and anisocytosis may be seen in nonregenerative anemias in cats with FeLV infection and some preneoplastic (dyplastic) and neoplastic (leukemias) diseases. Animals with regenerative anemias from any cause typically have marked anisocytosis due to the large polychromatophilic erythrocytes present. Dogs with IMHA usually have marked anisocytosis due to the presence of small spherocytes and large polychromatophilic cells.

**Poikilocytes** are abnormally shaped cells (See Attached Figure). There are several different types of poikilocytes each with a different specific cell morphology. Different types of poikilocytes suggest the occurrence of specific disease processes (See chart on last page). Most poikilocytes are due to pathologic changes in erythrocytes, however, **echinocytes** (crenated red cells) may be formed iatrogenically from insufficient blood in EDTA tubes or delay in slide preparation from collected blood. Low numbers of echinocytes are often seen in the peripheral blood of cats. Two of the most commonly observed shape abnormalities are schistocytes and acanthocytes. **Schistocytes** are small, irregularly shaped red cell fragments (Figure above). They result from mechanical trauma to circulating erythrocytes and, thus, are considered the hallmark of red cell fragmentation. In dogs, they are most frequently seen with DIC, microangiopathic hemolytic anemia, and neoplasms, particularly HSA; the latter can result in both DIC and microangiopathic hemolytic anemia. Schistocytes have been identified in 25% to 50% of dogs with HSA. However, other conditions, such as congestive heart failure, glomerulonephritis, myelofibrosis, chronic doxorubicin toxicosis, and increased red cell fragility associated with severe iron deficiency, may also result in the formation of schistocytes. **Acanthocytes**, or spur cells, are irregularly shaped erythrocytes containing membrane spicules that are unevenly
distributed around the red cell surface. Unlike schistocytes, however, acanthocytes have not been fragmented and are similar in size to normal erythrocytes. These cells result from alterations in the cholesterol and/or phospholipid concentration in the red cell membrane and are seen in dogs with severe iron deficiency anemia, diffuse liver disease, portocaval shunts, high-cholesterol diets, or HSA. The mechanism underlying acanthocyte formation in dogs with HSA is not completely understood. Coexistence of acanthocytes and schistocytes in a dog with anemia is highly suggestive of HSA.

The density of the red color in erythrocytes is dependent on the concentration of hemoglobin. Changes in hemoglobin concentration can be in the form of polychromasia or hypochromasia. Polychromasia is defined by the presence of basophilic appearing erythrocytes. Polychromatophilic erythrocytes are seen in regenerative anemias when immature erythrocytes with decreased hemoglobin and increased amounts of RNA are released from the bone marrow. Numbers of polychromatophilic cells correlate well with numbers of reticulocytes. There are two types of reticulocytes in feline blood, aggregate reticulocytes and punctate reticulocytes (these are discussed in more detail below). Polychromasia or the number of reticulocytes present is the only peripheral blood findings that can be used to determine if an anemia is regenerative or not. Erythrocytes are termed hypochromic if they stain less intensely red than normal. Generally, the hemoglobin in hypochromic patients is concentrated around the periphery of the cells causing a larger than normal central pallor. Normal canine erythrocytes have a central pallor with a diameter that is equal to approximately one-third of the diameter of the cell. In iron deficient dogs, the central pallor of most of the erythrocytes is half the diameter of the cell or greater, allowing the morphologic identification of hypochromasia. Hypochromatic erythrocytes result from decreased hemoglobin, most often associated with iron deficiency. Most iron deficiencies in domestic animals are due to chronic blood loss. Normal feline erythrocytes have a very small or no central pallor. In iron deficient cats, the central pallor is visualized in most, if not all of the erythrocytes and usually occupies 1/3 to ½ the cell diameter, allowing the morphologic identification of hypochromasia. Most (>95%) of all iron deficiencies are the result of prolonged or chronic blood loss. This is typically due to either external parasitism or GI neoplasms.

**Reticulocytes and Reticulocyte Stain**

In order to accurately quantitate the regenerative response of an anemia, a reticulocyte count must be performed. This is done by adding an equal volume of blood and New Methylene blue stain to a tube and incubating at room temperature for 15 minutes. A blood film is then prepared from the mixture and dried. Erythrocytes are counted as either mature red blood cells or as a reticulocyte if there is precipitated RNA seen. Normal dogs will have less than 1% reticulocytes and a dog with a good regenerative response should have >1%, preferably 2%, corrected reticulocyte count. The actual percent reticulocytes counted needs to be corrected for the degree of the anemia by using the following formula: (% retic) x (Patent PCV) / 45 or normal PCV in dog.

Since two types of reticulocytes are seen in the cat they must be distinguished between punctate reticulocytes and aggregate reticulocytes. Punctate reticulocytes have individualized reticulum in the cells (see small dots in erythrocyte in lower right of figure). In normal cats, 1% to 10% of the erythrocytes can be punctate reticulocytes. These are more mature forms of the reticulocyte are increased numbers are seen when regeneration has taken place within the past few days. Often only the punctates are elevated in cats with mild anemia or in cats where a regenerative response has taken place but is no longer active. Aggregate reticulocytes have clumped or aggregated...
reticulum in the cells (see clumped arrangement of blue material in erythrocytes in upper left of the figure). In normal cats, 0.0% to 0.5% of the erythrocytes are aggregate reticulocytes. These are the more immature forms of reticulocytes and correspond with polychromatophilic erythrocytes in the peripheral blood. Increased numbers >1% indicate active regeneration. The actual percent reticulocytes counted needs to be corrected for the degree of the anemia by using the following formula: (% retic) x (Patent PCV) / 35 or normal PCV in cat.

**Erythrocyte Inclusion**

Various erythrocytic inclusions can be seen in peripheral blood films. **Heinz Bodies** are identified on Romanowsky-type stains such as Diff Quik as pale, round inclusions or knob-like projections on red cells (Figure right). They are the result of oxidant damage to the globin portion of the hemoglobin molecule. They are more readily identified on wet mount preparations using New Methylene Blue Stain (Figure on right) where they appear as dark, blue refractile bodies. Heinz bodies are most prominent in the feline because the hemoglobin molecule in this species has 8 reactive sulfhydryl groups, more than any other species. These molecules are very susceptible to oxidative damage and low numbers (<1%) of Heinz bodies are often seen in normal cats. Large numbers of Heinz bodies in the cat may be seen in such conditions as diabetes mellitus, ingestion of onions, hyperthyroidism, lymphoma and acetaminophen administration.

**Howell-Jolly Bodies** are small, dark, round nuclear remnants seen on Wright-Giemsa or Diff-Quik stains (Figure right). Low numbers (<1%) may be normal in cats, due to the nonsinusoidal nature of the cat spleen. Increased numbers are seen in regenerative anemias, splenectomized animals, or animals receiving glucocorticoids or chemotherapeutic agents. Basophilic stippling is recognized as multiple, small dot-like inclusions in erythrocytes. They are most often seen in regenerative anemias, especially hemolytic anemias. However, they are also observed in the blood of animals with lead poisoning. Nucleated red blood cells are usually seen in regenerative anemias (See figure right), however they are not used to evaluate a regenerative response. Nucleated red blood cells in the absence of polychromasia is a dysplastic change and would indicate a nonregenerative response. This may be observed in such conditions as lead poisoning, bone marrow damage due to septicemia, or myeloproliferative diseases (particularly erythroleukemia due to FeLV). When the number of nucleated erythrocytes exceeds 5-7 / 100 WBC's, the leukocyte count must be corrected for these cells. The formula for this correction is Corrected WBC = (100) X (WBC) / (100) + # NRBC's.

Some erythrocyte inclusions are the result of infection with hemoparasites. Common hemoparasites of the cat include organisms in the genera *Mycoplasma* (the haemoplasmas) and *Cytauxzoon* (feline). Two distinctly different genotypes hemoplasmas (Old terminology *Haemobartonella felis*) have been identified in cats, based on 16S rRNA gene sequences. One genotype has been referred to as the *H. felis* large (Hflg) variant (Renamed to *Mycoplasma haemofelis*) (Figure right), because the organisms reportedly appear larger than the second variant, which was previously referred to as the *H. felis*, small (Hfsm) variant (Renamed *Mycoplasma haemominutum*). These
parasites are epiplectic and may be seen as dots, chains or ring forms (see figure to right). Blood smears must be prepared rapidly from cats suspected of having haemoplasma infection and it is preferential not to place blood in EDTA since EDTA will dislodge parasites from the erythrocyte membrane making identification impossible. Even with quality blood film preparation, parasites may not be visualized in blood even when animals are markedly anemic. Organisms tend to appear in blood during discrete parasitemic episodes of one or more days duration, and they can disappear from blood in a matter of two hours or less in infected cats. In many instances, a rapid decrease followed by a rapid increase in hematocrit occurs in association with the appearance and disappearance of organisms from the blood. Autoagglutination may be seen in blood samples after they cool below body temperature and the direct Coombs’ test may be positive at 37°C. Total and differential leukocyte counts are variable and of little diagnostic assistance. Bizarre monocytes, sometimes demonstrating erythrophagocytosis, may be seen in acute feline hemoplasmosis.

*Mycoplasma haemocanis* (Formerly *Haemobartonella canis*) is reported to be the causative agent of hemoplasmosis in dogs. Clinical signs are usually mild or unapparent in dogs unless they have been splenectomized. Although splenectomy is generally required before clinically significant hemoplasmosis occurs in dogs, cases have been described in nonsplenectomized dogs with concurrent Ehrlichia, Babesia, bacterial, or viral infections. Hemoplasmosis has also occurred in dogs given immunosuppressive drugs and in dogs with splenic pathology. Rare cases have occurred in spleen-intact dogs in which no evidence for immunosuppression was found.

*Cytauxzoon felis* is a tick-transmitted, protozoal hemoparasite that infects the red blood cells of cats. Cytauxzoonosis was first recognized as a fatal infection of domestic cats in southwestern Missouri (1973-1975), but has now been observed in many states in the Southeast. Infected cats become anemic, but reticulocyte counts are not increased in response to the anemia. Cats become thrombocytopenic during late stages of disease. White blood cell counts are variable, but leukopenia generally develops terminally. Parasitemia occurs late in the disease. When routinely-stained blood films are examined, organisms appear in erythrocytes either as rounded "signet ring" bodies 1 to 1.5 microns in diameter or as bipolar, oval or "safety pin" bodies 1 by 2 microns (See Figure above). The cytoplasm of the protozoan stains a light blue, while the nucleus appears red to purple. In the late stages of the disease, up to 25% of erythrocytes may contain protozoa.

Canine Babesiosis can be caused by either *Babesia canis* or *Babesia gibsoni*. *B. canis* is a large *Babesia* (See figure to right) and The severity of the disease varies with age of the animal and strain of *Babesia* involved. The course of disease may be acute and fulminating, subclinical or chronic. Puppies are most often clinically affected and may develop severe anemia. *B. gibsoni* is a small *Babesia* similar to *B. microti*, the etiological agent of human and rodent Babesiosis. This organism causes clinical signs in adults or puppies. The Organism is endemic in Africa, the Middle East and Asia. The first report in the US was in 1968, however, the infected Bull Terrier likely contracted the agent while in Malaysia. In 1979 the organism was isolated from a dog that lived in Connecticut and never traveled outside the US. Infected dogs have subsequently been identified in several states. Beginning in 1998, there has been a rapid increase in the number of cases reported, predominantly in Pit Bull Terriers and American Staffordshire Terriers. A small *Babesia* has been recognized in California that causes severe clinical disease. Although initially classified as *B. gibsoni* morphologically, this California organism is distinctively different from *B. gibsoni* based on molecular biology studies.
Platelet Evaluation

Platelet evaluation begins with the search for clumps using a scanning objective (Figure upper right). If platelet clumps are observed, quantitative assessment of platelets will be falsely reduced. An estimation of platelet numbers is done using the 100X oil emersion lens. The formulas for estimation of platelet numbers are (Dog): 
\[
\text{# Cells} / \mu l = (\text{# cells per 100X oil field}) \times 15,000,
\]
and for the (Cat):
\[
\text{# Cells} / \mu l = (\text{# cells per 100X oil field}) \times 20,000.
\]
The technique of platelet evaluation is particularly useful in evaluating platelets in the cat since automated cell counts are often unreliable in assessing platelet numbers for this species. The overlap in platelet and erythrocyte size, along with the propensity for feline platelets to clump or aggregate makes it very difficult to obtain accurate platelet counts using most automated hematology analyzers. Megaplatelets (Figure lower right), platelets as large as or larger than erythrocytes, may indicate platelet regeneration due to a peripheral destruction or consumption of platelets. However, in the cat, platelets are often larger than erythrocytes due to both the larger size of the platelet (than in dogs) and the smaller size or the erythrocyte. Thrombocytopenia may result from a production problem in the marrow, or a loss in the peripheral circulation due to destruction or consumption of platelets. A bone marrow evaluation may be necessary to make this distinction.

Leukocyte Evaluation

Leukocyte evaluation begins using a scanning objective by observing the mature neutrophil as the predominant cell type and identifying the presence of immature neutrophils (bands, metamyelocytes or myelocytes) or reactive changes in monocytes and lymphocytes. Large, immature blast cells should also be identified at this time. These pathological changes and other changes are then evaluated more closely using the 100X, oil immersion lens.

**Left shift** – the presence of excessive numbers of immature neutrophils (> 300 bands / \(\mu l\) of blood) in the peripheral blood. Most of these cells are band neutrophils with fewer metamyelocytes (Figures on right), myelocytes and progranulocytes (promyelocytes) in decreasing order of frequency. When the most immature forms (metamyelocytes and myelocytes) are relatively few compared to the band neutrophil population, the left shift is said to be pyramidal and complete. This is a favorable response. The extent of the left shift (how immature the cells are) will indicate the severity of the disease. The magnitude of the response (numbers of immature cells) will indicate the ability of the bone marrow to respond to the disease.

**Regenerative left shift** – a left shift in which there is typically a neutrophilia and there are more mature cells than immature. This is a favorable response where the bone marrow has had sufficient time to respond to peripheral demands for neutrophils.

**Degenerative left shift** – a left shift in which there are more immature neutrophils (bands, metamyelocytes) than mature neutrophils (segmented). Total neutrophils counts are typically low or only slightly elevated. This indicates that the reserve of mature neutrophils in the bone marrow has been depleted, has had insufficient time to respond, or cannot meet the overwhelming demand for neutrophils. In most species this is an unfavorable prognostic indicator.

**Transitional left shift** – a leukogram that has a moderate to marked neutrophilia, but the immature forms still outnumber the mature forms or conversely, in situations where there is a neutropenia but mature forms still outnumber immature forms. These findings need to be interpreted in light of sequential hemograms or changes in status of the patient to determine if they indicate recovery or worsening of the disease.
**Right Shift** – a leukogram with abnormally mature neutrophils in circulation. Neutrophils with 5 or more lobes in the nucleus are considered hypersegmented and usually represents aging of the cell in circulation. This is defined as a dysplastic change and is identified by the presence of neutrophils with 5 or more lobes in the nucleus. This is a maturation abnormality in cell aging, not toxicity. Hypersegmentation is most commonly associated with steroid administration, but may also be seen in myeloproliferative diseases or blood smears made from blood left in the vacutainer tube too long. It may occasionally be seen in dogs with reactive neutrophilias or poodles with hereditary macrocytosis.

**Toxic Change** - One important change in neutrophils is the presence of cytoplasmic toxicity. It is particularly important to evaluate toxicity of neutrophils in patients with a leukocytosis, leukopenia or a left-shift. Toxicity is a cytoplasmic change which is usually associated with the presence of bacterial infections or toxins. It results from a maturation arrest in cell development, and therefore occurs in bone marrow precursor cells. Toxicity is semi-quantitated in order of increasing severity from +1 to +4. A +1 Toxicity is defined by the presence of Döhle bodies; small, basophilic aggregates of RNA in cytoplasm of cells. This may be normal if seen in low numbers of neutrophils in cats. A +2 Toxicity is defined as Döhle bodies and diffuse cytoplasmic basophilia. A +3 Toxicity would contain all of the above plus foamy cytoplasmic vacuolation, and a +4 Toxicity would have all of the above plus giantism and/or nuclear lysis.

**Reactive Change**

Lymphocytes do not develop toxicity, but may become reactive as a result of some antigenic stimulation from an infectious agent, neoplasm, or immune-mediated disease. The cytoplasm of reactive lymphocytes becomes more intensely basophilic, almost a royal blue. Reactive monocytes may also be seen if the cytoplasm becomes more intensely basophilic and vacuolated. This usually indicates a chronic inflammatory process or may be seen with hemoplasmas in the cat.

**Leukemoid reaction** – a reactive leukocytosis that consists of an extremely high leukocyte count or an extreme left shift to the extent where it resembles a leukemia (See Figure to right). This change typically involves the neutrophils, but may on occasion occur in other cell lines such as lymphocytes or eosinophils.

**Neoplastic Cells in Circulation**

Neoplastic cells, primarily those of hematopoietic origin, can also be identified in the peripheral blood. These cells would alert the clinician to the possibility of leukemia, however, in many cases this diagnosis is confirmed by evaluating the bone marrow. Hematopoietic blast cells may be of erythroid, granulocyte, monocyte, or rarely megakaryocyte origin (myeloproliferative disease), or lymphoid origin (lymphoproliferative disease). In general, blast cells are identified as large cells with nuclei often 2 or more times the size of erythrocytes. They often have abnormal nuclear morphology including high N:C ratio, diffuse, altered chromatin pattern, and prominent nucleoli. The cytoplasm of these cells is often deeply basophilic. The presence of blast cells in the peripheral circulation would alert the clinician to the possibility of an acute leukemia, whereas the presence of unexplained elevations in mature leukocytes (neutrophils, lymphocytes, monocytes, eosinophils, or basophils) would suggest the possibility of a chronic leukemia. In addition to being classified as acute or chronic depending on the maturity of the neoplastic cell, leukemias are also classified as a
myeloproliferative (erythroid, myelogenous, or monocytic leukemia) or a lymphoproliferative (lymphoid leukemia) depending on the cell line from which the neoplastic population arises. Since leukemia is defined as a bone marrow neoplasm of the hematopoietic cells, a bone marrow evaluation is often required to make a definitive diagnosis. A list of the various types of acute and chronic leukemias is provided. In general, chronic leukemias have a better long-term prognosis than acute leukemias and lymphoid leukemias have a better prognosis than myeloproliferative disorders.

**Lymphoid Leukemias**
- Acute Lymphoblastic Leukemia (ALL)
- Chronic Lymphocytic Leukemia (CLL)

**Acute Myeloid Leukemias**
- Acute myelogenous leukemia (AML)
- Erythroid leukemia
- Acute myelomonocytic leukemia
- Acute monocytic leukemia
- Megakaryoblastic leukemia

**Chronic Lymphoid Leukemia**
- Chronic Lymphocytic Leukemia (CLL)

**Chronic Myeloid Leukemias**
- Chronic myelogenous leukemia (CML)
- Eosinophilic leukemia
- Basophilic leukemia
- Polycythemia vera

**Acute Myelogenous leukemia**

**Acute Lymphocytic Leukemia**

**Chronic Lymphocytic Leukemia**
## Red Cell Morphologic Changes

<table>
<thead>
<tr>
<th>Appearance</th>
<th>New Term</th>
<th>Old Term</th>
<th>Associated Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthocyte</td>
<td>Spur Cell</td>
<td>Target Cell</td>
<td>Liver disease, Splenic disease, Fragmentation disorders</td>
</tr>
<tr>
<td>Coocyte</td>
<td>Target Cell</td>
<td>Tear Drop Cell</td>
<td>Liver disease, Iron deficiency anemia, Splenic disease</td>
</tr>
<tr>
<td>Dacryocyte</td>
<td>Tear Drop Cell</td>
<td>Blommae Disc</td>
<td>Fragmentation disease, Myelofibrosis</td>
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<td>Discocyte</td>
<td>Blommae Disc</td>
<td>Sick Cell</td>
<td>Normal RBC</td>
</tr>
<tr>
<td>Drepanocyte</td>
<td>Sick Cell</td>
<td>Nonpathologic in goat and deer</td>
<td></td>
</tr>
<tr>
<td>Echinocyte</td>
<td>Burr Cell</td>
<td>Crenated Cell</td>
<td>Artifact, Uremia, Pyruvate kinase deficiency</td>
</tr>
<tr>
<td>Elliptocyte</td>
<td>Orbacocyte</td>
<td>Helmet Cell</td>
<td>Normal for some species (llama, avian, reptile, fish)</td>
</tr>
<tr>
<td>Keratocyte</td>
<td>Helmet Cell</td>
<td>Thin cell</td>
<td>Fragmentation hemolysis, Iron deficiency anemia</td>
</tr>
<tr>
<td>Leptocyte</td>
<td>Thin cell</td>
<td>Iron deficiency anemia, Liver disease, Regenerative anemia</td>
<td></td>
</tr>
<tr>
<td>Schistocyte</td>
<td>(Schizocyte)</td>
<td></td>
<td>DIC, Vasculitis, Fragmentation hemolysis, Iron deficiency anemia</td>
</tr>
<tr>
<td>Spherocyte</td>
<td></td>
<td></td>
<td>Immune-mediated hemolytic anemia, Burns, Fragmentation hemolysis, Snake venom toxicity, Acute zinc toxicity</td>
</tr>
<tr>
<td>Stomatocyte</td>
<td>Mouth Cell</td>
<td></td>
<td>Inherited stomatocytosis (Alaskan malamute, Miniature Schnauzer)</td>
</tr>
</tbody>
</table>