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...
formula. **Formula:** \( \frac{\text{# cells/µl}}{= (\text{Ave. # of cells per field}) \times (\text{Objective power})^2} \). The objective used to estimate leukocyte numbers should be one where approximately 5 - 10 leukocytes are seen per field. **Example:** If an average of 5 cells were counted for each 50X field, the total leukocyte count would be \((5) \times (2,500) = 12,500 \text{ cells/µl}\).

**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 epicellular 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 parasitic 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): # Cells / μl = (# cells per 100X oil field) X 15,000, and for the (Cat): # Cells / μl = (# cells per 100X oil field) X 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 / μl 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 (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) 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 represent 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.

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Acute Myelogenous leukemia  Acute Lymphocytic Leukemia  Chronic Lymphocytic Leukemia
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<td>Drepanocyte</td>
<td>Sickle Cell</td>
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<td>Nonpathologic in goat and deer</td>
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<td>Burr Cell</td>
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<td>Pyruvate kinase deficiency</td>
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<tr>
<td>Elliptocyte</td>
<td>Ovalocyte</td>
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<td>Normal for some species (llama, avian, reptile, fish)</td>
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<tr>
<td>Keratocyte</td>
<td>Helmet Cell</td>
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<td>Fragmentation hemolysis</td>
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<td>Leptocyte</td>
<td>Thin cell</td>
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<td>Liver disease</td>
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<td>Burns</td>
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<td>Fragmentation hemolysis</td>
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<td>Snake venom toxicity</td>
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<td>Acute zinc toxicity</td>
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<tr>
<td>Stomatocyte</td>
<td>Mouth Cell</td>
<td></td>
<td>Inherited stomatocytes (Alaskan malamute, Miniature Schnauzer)</td>
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</table>
**Hemoparasites of the Dog and Cat**

**And Hematology Case Challenges**

**Cell Tropism**

- Red blood cells
- Leukocytes – granulocytes and mononuclear
- Platelets
- Most are vector-transmitted infections
  - Direct inoculation: Blood transfusion

**The Red Blood Cells**

**The Dog**
- Bacterial: *Mycoplasma* & *Bartonella*
- Protozoal: *Babesia*

**The Cat**
- Bacterial: *Mycoplasma*
- Protozoal: *Cytauxzoon*

**Hemotrophic Mycoplasmas**

Formerly *Haemobartonella*

- Extracellular (surface) RBC parasites
  - Causes immune-mediated (primarily extravascular) destruction of RBCs by host
  - Coombs'-positive anemia
- 2 species in cats; 2 in dogs
  - *M. haemofelis* (formerly large *H. felis*)
    - Most pathogenic (anemia)
  - *M. haemominutum* (small form)
    - Mild clinical signs if any (many infections unapparent)
  - *M. haemocanis* (canine isolate)
    - Clinical disease only in immunocompromised or splenectomized dogs
    - 99% sequence homology to *M. haemofelis* (16srRNA gene)
  - *M. haemoparvum* – small form recently isolated from dogs
    - Similar to *M. haemominutum*

**M. haemofelis**

- Believe to be vector borne, primarily transmitted by fleas
- Mechanical transmission is also possible
- Risk factors – outdoor cats, cat-bite abscesses, FeLV positive status
- Clinical signs vary from unapparent disease to severe anemia and death
  - Fever, pale mucus membranes, icterus, splenomegaly

**M. haemofelis in Peripheral Blood**

- Attaches to erythrocyte surface and indents membrane
- Causes membrane damage and pock-like lesions even upon detachment
- May contribute to immune-mediated component of red cell destruction
- Cyclic parasitemia
Laboratory Findings

- Regenerative anemia → ↑ MCV, ↓ MCHC
  - unless complicated by FeLV infection
  - Combination may promote myeloproliferative disorders (erythrodysplasia / erythroleukemia)
- Autoagglutination and Coombs’ – anemia
- Hyperbilirubinemia (extravascular hemolysis)

Diagnosis

- Serology not available
- Identification of organism or organism DNA
  - Peripheral blood film: Fresh smear not in EDTA
    - Collect blood in heparinized capillary tube
  - PCR analysis of peripheral blood

M. haemocanis

- Clinical disease only seen in immunosuppressed or splenectomized dogs
  - Fever, pale mucus membranes, icterus

PCR Analysis

- Highly sensitive PCR for M. haemofelis and M. haemominutum
  - 14% healthy control cats positive for haemoplasmas (subclinical infections)
- PCR also available for M. haemocanis and M. haemoparvum, presumably just as sensitive although not evaluated
Treatment of Haemoplasmas
- Doxycycline @ 5 mg / kg; q12h for 3-4 weeks
- Enrofloxacin (10 mg / kg q24h) may be effective in cats that don’t tolerate Doxy.
- Treated or untreated animals may remain carriers but seldom relapse with disease
- Azithromycin not effective

The Neutrophils
- The Dog
  - Bacterial: Anaplasma and Ehrlichia
  - Protozoal: Hepatozoon (rare)
- The Cat
  - Bacterial: Anaplasma

Anaplasma phagocytophilum
- Tick-transmitted, obligate intracellular bacterium
- Causative agent of granulocytic anaplasmosis
- Can infect a wide variety of hosts
  - Humans, dogs, cats, horses, ruminants

Clinical Signs
- Golden and Labrador retrievers over-represented
- Three publications
- Breed popularity vs. susceptibility
- Lameness and joint pain; inflammatory polyarthritis
- Indistinguishable from Lyme disease and E. ewingii
- Meningitis
- Gastrointestinal signs
- Respiratory signs (pneumonitis)
- Humans: initially, flu-like symptoms

Diagnosis
- Presumptive diagnosis
  - Identification of morulae in peripheral blood or synovial fluid (1–10 weeks post-infection)
  - Ehrlichia ewingii or Anaplasma phagocytophilum indistinguishable
- Confirmation
  - Serology
  - PCR analysis

Synovial fluid analysis
- Important in dogs with clinical evidence of joint disease
- Neutrophilic predominance (nondegenerate)
- Intracytoplasmic morulae in neutrophils (1%–10%)
SNAP® 4Dx® Plus Test
- Can be used in dogs and cats but only approved for use in dogs
- Acutely infected animals may be negative
- Cross-reactivity with serum from animals infected with *A. platys*
  - Approximately 1/2

PCR analysis
- Very sensitive for detecting acute infection
- Can detect infection prior to seroconversion
- False negative, particularly in subclinically infected dogs
- Not effective way of confirming positive serology in a healthy dog

Therapy
- Drug of choice: doxycycline / minocycline
- Optimal dose and length of therapy not firmly established
- Current recommendation
  - 5 - 10 mg/kg twice a day for 30 days
- Rifampin and levofloxacin *in vitro*

Therapy
- Drug of choice: doxycycline / minocycline
- Optimal dose and length of therapy not firmly established
- Current recommendation
  - 5 - 10 mg/kg twice a day for 30 days
- Does not cross blood / brain barrier as well as doxy

The Platelets
- *Anaplasma platys*
- Canine and human pathogen
- Feline?

Infectious Cyclic Thrombocytopenia
- *Anaplasma platys*
- Tick vector – Brown dog tick
- Only intracellular organism known to specifically infect platelets
Clinical Signs

- Mild clinical disease or subclinical infection in most dogs (persistence)
- Fever, anorexia, sometimes petechiation or epistaxis
- 1 to 2 weeks post-infection

Diagnosis and Treatment

- Visualization of organisms in peripheral blood
- Confirmation
- PCR analysis
- Cross reactivity *Anaplasma phagocytophilum* on SNAP 4Dx Plus assay
- TM: Doxycycline

And that’s all I have to say about Hemoparasites

*Forest Gump 1994*

Hematology Case Challenges or Maybe Not!

Sadie

- 7 year old, female, spayed golden retriever
- Presentation (Emergency)
  - Episodic weakness (twice) over 2 week period
  - Acute collapse day of presentation, but recovered

Physical Exam Findings

- Pale mucus membranes, tachycardia (120 BPM)
- CRT prolonged @ 4 sec.
- Abdominal distension
  - Effusion and/or mass was difficult to determine on abdominal palpation
- PCV 19%
- TPP 5.4
- Plan: CBC
CBC Results

<table>
<thead>
<tr>
<th>WBC</th>
<th>19.1 (6.0 – 17.0) x 10^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuts</td>
<td>15.2 (3.0 – 11.5) x 10^3</td>
</tr>
<tr>
<td>Bands</td>
<td>0.900 (0.0 - 0.3) x 10^3</td>
</tr>
<tr>
<td>Lym.</td>
<td>0.700 (1.0 – 4.8) x 10^3</td>
</tr>
<tr>
<td>Mon.</td>
<td>2.3 (0.15 – 1.35) x 10^3</td>
</tr>
<tr>
<td>Eos.</td>
<td>0.0 (0.1 – 1.25) x 10^3</td>
</tr>
<tr>
<td>RBC</td>
<td>2.51 (5.4 – 7.8) x 10^6</td>
</tr>
<tr>
<td>HGB</td>
<td>6.8 (13.0 – 19.0) g/dL</td>
</tr>
<tr>
<td>HCT</td>
<td>18.2 (37.0 – 54.0) %</td>
</tr>
<tr>
<td>MCV</td>
<td>76.2 (66 – 75) fL</td>
</tr>
<tr>
<td>MCHC</td>
<td>36.3 (34.0 – 36.0) g/dL</td>
</tr>
<tr>
<td>Pts</td>
<td>25.0 (150 – 430) x 10^3</td>
</tr>
</tbody>
</table>

Reticulocyte count (6%) = 150,600 / µl (>80,000 = regenerative)

Blood film evaluation

- Regenerative anemia (polychromasia)
- Poikilocytosis
  - Acanthocytes
  - Schistocytes
  - Thrombocytopenia

Findings from Blood Film Evaluation

- Hallmark of fragmentation hemolysis
- Fragmentation of cells passing through tortuous or abnormal vessels
  - DIC
  - Neoplasia (HSA, Thyroid ACA)
    - Up to 50% of dogs with HSA
  - Vasculitis
  - Thromboembolism (Cushing's, HWD)
  - Cavalt Syndrome
  - Glomerulonephritis
- Increased fragility of erythrocytes
  - severe iron deficiency anemia
  - Doxorubicin

Abdominal Ultrasound

- Free abdominal fluid
- Large mass in cranial abdomen (14 cm)
  - Cavitated with mixed echogenicity
  - Appeared to be associated with the spleen
Abdominal Fluid

- Color – opaque and red
- PCV – 15%
- WBC – 12,500 cells / μl
- Interpretation – hemorrhagic effusion
  - Erythrocytes, macrophages, mesothelial cells, neutrophils
  - No neoplastic cells seen

No firm diagnosis?

- Hemangioma, hemangiosarcoma, hematoma, lymphoma, others?
  - Imaging and fluid analysis not helpful
- Hematological abnormalities indicate HSA
  - Anemia seen in 80% of dogs with splenic HSA
  - Dogs with splenic masses and evidence of anemia, fragmentation hemolysis and thrombocytopenia
  - Significantly greater risk of having HSA (90%)

Hemoabdomen and HSA

- Spontaneous and may be sporadic
  - DIC – causes bleeding tendencies
  - Thrombocytopenia
  - Rupture of neoplastic vessels

CBC findings that support Dx of HSA

- Anemia – (80%) of dogs
  - Hemolysis and/or hemorrhage
- Thrombocytopenia – (75%) of dogs
  - DIC or microangiopathic disease in fibrin filled neoplastic vessels
- Schistocyte formation – (up to 50%) of dogs
  - Hallmark of red cell fragmentation
  - DIC or microangiopathic disease in fibrin filled neoplastic vessels

Hemostasis Profile

- PT and APTT – normal
- FDPs - negative
- D-dimers – (ref. range < 250 ng/ml)
  - Not useful in this case due to hemoabdomen
  - Can result in d-dimer levels > 1,000 ng/ml in dogs without evidence of TE disease
Next Step?

- Need to confirm diagnosis
  - Take the dog to surgery
  - Ultrasound guided FNA of mass

Fine-needle Aspiration of Splenic Mass

- Potential for definitive, presurgical diagnosis
- Potential for complications
  - Seeding the abdomen with tumor cells
  - Hemorrhage
  - Dog is already bleeding likely due to rupture of neoplastic vessels, not DIC

Cytologic Features of HSA

- Low to moderate cellularity
- Hemorrhage
- Oval to spindle-shaped cells, arranged individually or in small clusters
- Pale, lightly basophilic, veil-like cytoplasm
- Small, punctate, clear cytoplasmic vacuoles
- Cytomegaly and bizarre nuclear features of malignancy

Plan for Sadie

- Owners elected surgery and chemo if possible
- Sadie was transfused (PCV 26%)
- Surgery was performed and a 14 cm x 16 cm mass was identified in the spleen
- Multiple, red-purple, raised nodules were present in all lobes of the liver (not seen on ultrasound)
- The spleen and biopsies taken from the hepatic masses were submitted for histopathology
- Final Dx: hemangiosarcoma

Treatment

- Chemotherapy was initiated approximately 1 week post-op (once histopath confirmed a diagnosis) (PCV 35%)
- 21 day cycle of VAC
  - Vincristine 0.75 mg/m² BSA (IV) (Day 8 & 15)
  - Doxorubicin 30 mg/m² BSA (IV) (Day 1)
  - Cyclophosphamide 200 - 300 mg/m² BSA (PO) (Day 10)
- Sadie received 4 cycles of therapy
**Prognosis**

- Long-term prognosis extremely poor
- Death from exsanguination from rupture of metastatic site
- Surgery alone rarely curative with MST of 1 to 3 months
- Multi-drug chemotherapy MST 6 to 9 months

**Sadie**

- Sadie was found dead in her bed 9 months after splenic surgery
- Likely the result of ruptured metastatic lesion

---

**Penny: 8 year old, F/S**

**Long-haired Dachshund**

![Rhinocerocyte](image)

**Presentation to Referring Veterinarian**

- 3 week history of lethargy
- Eating and vomiting grass and grass roots
- Sleeping a lot
- Presently on monthly heartworm medication
- Vaccinations current

---

**Penny’s Exam**

- Temp. 101.5°F
- Respiration 35 PM
- HR 90 BPM
- Pale mucus membranes
  - No history of trauma or evidence of hemorrhage
- Fecal parasites check negative
- Heartworm test negative
- CBC & Biochemical profile

**CBC Results**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Normal Range</th>
</tr>
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<tbody>
<tr>
<td>HCT</td>
<td>12.2%</td>
<td>(37-55)</td>
</tr>
<tr>
<td>RBC</td>
<td>2.0 M/µl</td>
<td>(5.5-8.5)</td>
</tr>
<tr>
<td>Hgb</td>
<td>4.9 g/dl</td>
<td>(12-18)</td>
</tr>
<tr>
<td>MCV</td>
<td>62.0 fl</td>
<td>(60-77)</td>
</tr>
<tr>
<td>MCH</td>
<td>24.5 pg</td>
<td>(18-30)</td>
</tr>
<tr>
<td>MCHC</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>RDW</td>
<td>15.9%</td>
<td>(14.7-17.9)</td>
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<tr>
<td>Platelet</td>
<td>819 K/µl</td>
<td>(175-500)</td>
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<tr>
<td>WBC</td>
<td>6.58 K/µl</td>
<td>(5.5-16.9)</td>
</tr>
<tr>
<td>Neut</td>
<td>4.23 K/µl</td>
<td>(2-12)</td>
</tr>
<tr>
<td>Lym</td>
<td>1.52 K/µl</td>
<td>(1.0-4.9)</td>
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<tr>
<td>Mono</td>
<td>0.67 K/µl</td>
<td>(0.3-2.0)</td>
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<tr>
<td>Eos</td>
<td>0.13 K/µl</td>
<td>(0.1-1.5)</td>
</tr>
<tr>
<td>Baso</td>
<td>0.04 K/µl</td>
<td>(0-0.1)</td>
</tr>
</tbody>
</table>
CBC Results

- HCT 12.2% (37-55)
- RBC 2.0 M/µl (5.5-8.5)
- Hgb 4.9 g/dl (12-18)
- MCV 62.0 fl (60-77)
- MCH 24.5 pg (18-30)
- RDW 15.9% (14.7-17.9)
- Platelet 819 K/µl (175-500)

- WBC 6.58 K/µl (5.5-16.9)
- Neut 4.23 K/µl (2-12)
- Lym 1.52 K/µl (1.0-4.9)
- Mono 0.67 K/µl (0.3-2.0)
- Eos 0.13 K/µl (0.1-1.5)
- Baso 0.04 K/µl (0-0.1)

- Reticulocyte 0.6%
- Absolute retic 11.6 K/µl

Biochemical Profile

- WNL

Problem List

- Severe nonregenerative anemia
- Thrombocytosis
- Pica
- Vomiting
- Plan

Penny’s Presentation

- 1 month history of lethargy and decreased appetite
- Eating grass and grass roots and vomiting them
- Physical exam
  - BW 4.6 kg
  - Quite but alert
  - Temp 102.1
  - HR 80, Resp. 32
  - MM white with no CRT available
- Normal fecal color
  - Fecal occult blood test (Hemocult) negative

Problem List

- Severe nonregenerative anemia
- Thrombocytosis
- Pica
- Vomiting
- Plan

- Refer to UF-VMC for further evaluation
Diagnostic Plan

- MDB
  - CBC, UA, Biochemical profile
  - Reticulocyte count
  - Coombs’ test
  - Cross match

CBC Results

- HCT 15.1% (37-55)
- WBC 6.92 K/µl (6.0-17)
- RBC 2.31 M/µl (5.4-7.8)
- Neut 4.5 K/µl (2-12)
- Hgb 5.4 g/dl (13-19)
- Bands 0.21 K/µl (0-3)
- MCV 66.4 fl (66-75)
- Lym 1.80 K/µl (1.0-4.9)
- MCH 23.4 pg (18-30)
- Mono 0.45 K/µl (0.3-2.0)
- MCHC 35.8 g/dl (34-36)
- Eos 0.1 K/µl (0.1-1.5)
- RDW 12.1% (11-13)
- Baso 0.0 K/µl (0-0.1)
- Platelet 735 K/µl (150-430)
- Plasma Protein 6.9 g/dl

- Reticulocyte 0.1%
- Absolute retic 2.3 K/µl

Iron Levels

- Serum iron 374 ug/dl (70-264)
- TIBC 559 ug/dl (246-504)
- % saturation 67 (19-79)

Biochemical Profile

- If Penny was bleeding (e.g. chronic hemorrhage) what abnormality would you see in the profile?
  - Low total protein (albumin, pos. globulin)
  - High BUN, normal creatinine
- Penny was normal, no abnormalities seen

Diagnostic Plan

- Cross-matched transfusion
- Adapted to chronic anemia
- Concern for anesthesia
- General anesthesia
- Imaging
  - Thoracic and abdominal radiographs (neoplasia?)
  - Ultrasound of abdomen
- Bone marrow collection and evaluation

Results

- Pre transfusion PCV 15.1%
- Post transfusion PCV 20.0%
- Imaging: no abnormalities seen
- Bone marrow aspirate
  - Dry tap (Why?)
- Core biopsy of marrow
  - Role core on slide for cytological evaluation
  - Submit core for histopathology
- Sent patient home awaiting biopsy results
Bone Marrow Evaluation

- Erythroid hyperplasia with left-shifting
- Maturation arrest at rubricyte/metarubricyte stage
- Erythrophagocytosis of precursor cells
- Tentative diagnosis: Nonregenerative (Central) immune-mediated anemia

Core Biopsy

- Myelofibrosis, multifocal, moderate
- Erythroid hyperplasia
- Hemosiderosis
- Megakaryocytic hyperplasia with left-shift

Tentative Diagnosis and Plan

- Nonregenerative IMA
- Direct Coombs’
- Tick titers
- Await results and begin therapy
Recheck

- Return visit 1 week after diagnostic evaluation
- Penny more active after transfusion, but getting lethargic again
- PCV 15% with low number of spherocytes on blood smear
- Direct Coombs’ negative at 1:2
- Serology for *E. canis* and *A. phagocytophilum* - negative

Treatment Plan

- Azathioprine  2mg/kg for two weeks
- Prednisone  1 mg/kg BID
- Famotidine 5 mg per day (pepcid AC)

2 week Recheck

- Penny more alert than before
- PCV 22%
- No spherocytes noted on smear
- Continue on current dose until PCV normal

Nonregenerative IMHA

- Estimated 33% to 58% of IMHA are nonregenerative
- Anemia typically severe, (median PCV 11%) with majority being <20%
- Age 10 mo. to 12 yr. (median 6.5 yr.)
- Female over-represented in most studies
- Dachshunds over-represented at UF (> 10 cases)
- Two forms
  - PRCA: more severe less common form
  - Evidence of erythropoiesis in bone marrow (>90%)

Stokol et al., JAVMA 216:14291436, 2000. (43 cases)

Chronicity of Anemia

- Important in establishing diagnosis
- Most dogs have clinical signs of 7 or more days
  - Lethargy, anorexia, pallor, weakness, pica, vomiting
- Animals tolerant of very low red cell mass

Laboratory Analysis

- Normocytic, normochromic anemia
  - Spherocytes seen in small percent (16%), numbers vary
- 0 to 7 NRBC’s / 100 WBC (no / rare polychromasia)
- Normal leukocyte count
  - 50% had mild left-shift
- Platelets most often increased
  - Decreased in 22% of cases
- Direct Coombs’ test positive in 30 to 50% (low titer 1:4)
- ANA positive in 23%
Bone Marrow Evaluation

(Stokol et al.)

- Bone marrows difficult to aspirate in 21 of 43 dogs
  - No spicules
  - Dry tap
- 16 dogs had core biopsies, all had myelofibrosis (reversible)
- Erythroid precursors may be absent (5% PRCA), normal numbers, or increased (most common)
- Erythroid hyperplasia was common among dogs with myelofibrosis
- Maturation arrest at rubiricytes and metarubricytes with few to no polychromatophilic cells
- Most dogs had large amounts of iron

Treatment for NRIMHA

(Stokol, et al., JAVMA 216:1429-1436;2000)

- Combination chemotherapy
  - Pred. & Cytoxan (73% remission)
  - Pred. & Azathioprine (52% remission)
  - Pred. alone (25% remission)
- Response rate
  - Complete remission (55%)
  - Partial remission (18%)
  - Poor response (27%)

Response Time and Mortality

- Response to treatment seen in 1 to 10 weeks
  - Median 2 weeks
- 18 cases with extensive follow-ups
  - 5 off all medication within 2 years
  - 9 on alternate day pred / azathioprine for 3 years
  - 6 relapsed when drug dosage / frequency was reduced
- If drugs are reduced, maintain at reduced level for prolonged period of time (60 days?)
- Mortality rate 28%

Summary

- NR-IMHA should be considered in dogs with severe, chronic, nonregenerative anemia
- Normal WBC and normal or increased platelets
- Bone marrow evaluation may aid in confirming diagnosis
  - Fibrosis, maturation arrest, erythrophagia
- Myelofibrosis may complicate marrow aspiration
- Treatment should include combination chemotherapy
- Response to therapy may take weeks to months
A Practical Guide to:

The Collection and Cytological Evaluation of Tissue Aspirates

Materials Needed

ASPIRATION TECHNIQUE

- 2 possible methods
  - Needle with attached 6 cc syringe
    - Larger masses
    - Masses that are difficult to exfoliate
    - Do not pump the plunger
  - Needle without attached syringe
    - Allows better control for smaller masses
    - Less traumatic

Aspiration procedure
Cytologic Stains

- Three-step staining set
- Diff Quik®
- Wright-Giemsa
- “Wright-Giemsa Stain Kit”
- Volu-Sol, Inc. Item #VWG-300
- www.volusol.com

Interpretation (Pondering the Material)

5 Categories of Tissue Lesions

- **Inflammatory lesion** - neutrophils
- Cystic lesion – amorphous material
- Hemorrhagic lesion – phagocytized RBCs
- **Neoplastic lesion** – monomorphic cell population
- Mixed cell population – both inflammatory and noninflammatory cells
Inflammatory Lesions

- Neutrophils above those expected from blood contamination
- Three types of inflammation
  - Purulent inflammation
  - Pyogranulomatous inflammation
  - Eosinophilic inflammation

Purulent Inflammation

- Greater than 85% neutrophils
- Look for degenerative changes
- Bacterial infections

Karyolysis

Pyogranulomatous Inflammation

- Greater than 15% to 40% macrophages
- Fungal infections
- Foreign bodies
- Intracellular bacteria
  - Mycobacteria spp.
  - Bartonella spp.

Fungal Hyphae
GMS Stain
**Eosinophilic Inflammation**
- Greater than 10% eosinophils
  - Allergic / hypersensitivity
  - Parasitic
  - Eosinophilic granuloma complex
- Breed and Species
  - Cats, Huskies, Rottweiler

**Cyst Formation**
- Follicular cyst (EIC)
- Apocrine cyst
- Sebaceous cyst

**Hemorrhagic Lesion**
- Hematoma
- Seroma
- Neoplasia
  - Hemangioma
  - Hemangiosarcoma
Neoplasia

- Monomorphic population of cells
- Benign vs malignant

Benign Neoplasia / Hyperplasia

- Uniformity in nuclear and cytoplasmic size
- Uniformity in N:C ratio
- Consistent size, shape, and number of nucleoli

Characteristics of Malignancy

- Anisokaryosis
- High or variable N:C ratio
- Variable nucleoli
- Coarse, clumped chromatin
- Increased Mitotic activity
- Pleomorphism
- Nuclear molding
- Multinucleation
Special Considerations

- The cell population should contain 3 or more of the nuclear criteria for malignancy
- Presence or absence of inflammation
- Exceptions to the rules
  - Location of lesion
  - Specific tumor types
  - To be discussed later
Categories of Neoplasia

- Epithelial
- Mesenchymal
- Round cell
- Neuroendocrine

Epithelial Tumors

- Usually exfoliate easily
- Cells tend to occur in clumps or clusters
- Distinct cytoplasmic borders
- Cytoplasmic membranes adherent to each other displaying tight cell junctions

Mesenchymal Tumors

- Usually exfoliate poorly
- Cells individually arranged
- Indistinct cytoplasmic borders
- Wispy, spindle-shaped cytoplasm
**Round Cell Tumors**

- Usually exfoliate easily
- Individually arranged, round cells
- Distinct cytoplasmic borders
- Definitive cytologic diagnosis is often attainable

**Round Cell Tumors**

- Histiocytic tumors
- Lymphoma
- Mast cell tumor
- TVT
- Plasmacytoma
- Melanoma

**Histiocytoma**

- Benign, cutaneous tumor primarily of young dogs less than 3 years of age
- Moderate amounts of pale cytoplasm
- Pleomorphic nuclei with variable N:C ratio
- Nuclei have fine chromatin pattern with indistinct nucleoli
- Infiltration with lymphocytes and spontaneous regression

**Lymphoma**

- Malignant neoplasm of peripheral lymphoid tissues
- Scant amounts of deeply basophilic cytoplasm
- Lymphoglandular bodies often seen in the background
- Nuclear morphology may vary depending on maturity or immunophenotype of the neoplastic cell
Plasmacytoma

- Lymphoid neoplasm affecting primarily dogs and occasionally cats
- Site predilection: oral cavity, GI tract, ears, and digits
- Cells contain variable amounts of deeply basophilic cytoplasm with small nucleus which is often eccentrically located
- Perinuclear, clear, Golgi zone
- May have marked anisokaryosis with occasional binucleation and multinucleation
- Usually considered benign

Mast Cell Tumor

- Potentially malignant neoplasm
- Most common cutaneous tumor of dogs
- Characterized by round cells with variable numbers of small, purple (metachromatic) granules
- Inflammation with eosinophils is often present

Cytologic Classification

- **Well-differentiated**
  - nuclei uniform in size
  - abundant cytoplasmic granulation
- **Moderately differentiated**
  - moderate anisokaryosis with variable N:C ratio
  - low to moderate cytoplasmic granulation
- **Poorly differentiated**
  - cytoplasmic granulation is sparse
  - Moderate to marked anisokaryosis and variable N:C ratio
Melanoma

- Potentially malignant neoplasms of neuroectodermal origin
- May appear as epithelioid, spindle cell, balloon cell or signet-ring cell types
- Characterized by the presence of variable numbers of dark green to black cytoplasmic granules
- Malignant potential of tumors evaluated using 2 criteria

Cytologic Differentiation of Melanomas

- Most well differentiated (heavily pigmented) melanomas are considered benign
  - Cutaneous location
  - Individually arranged or in small clusters
  - Nucleus often occluded by cytoplasmic vacuolation

Cytologic Differentiation of Melanomas

- Poorly differentiated
  - Amelanotic
  - All malignant
  - Epithelial or spindle-cell appearance
Neuroendocrine Tumors

- Tumors of the endocrine and chemoreceptor glands
  - Thyroid, parathyroid, endocrine pancreas, adrenal, carotid body and aortic body
- Appears cytologically as free nuclei in background of cytoplasm
- Nuclear criteria for malignancy not helpful in predicting biological behavior

Canine Thyroid Tumors

- Most often do not fulfill the nuclear criteria for malignancy
- Most are malignant

Feline Thyroid Tumors

- Similar to canine
- Uniform population of nuclei in background of cytoplasm
- Few distinct cytoplasmic borders
- Pink material (colloid)
- Most are benign

Mixed Cell Population

- Lesion that contains both inflammatory and noninflammatory cells
- Inflammation causes reactive changes in epithelial and mesenchymal cells that mimic malignancy
- May require histopathology
Summary

- A systematic approach to cytology provides useful diagnostic information
- What information can be gained about the lesion
- The information obtained will depend on the sample collected, the slide preparation, and skills of interpretation
Cytology in the Evaluation of Lymphoid Tissue in the Dog and the Cat
A. Rick Alleman, DVM, PhD
DACVP, DABVP

Lymp Nodes

Lymph node sampling and cytology is quick, easy, and usually rewarding. Cytologic samples of peripheral and/or internal lymph nodes may be collected by fine-needle aspiration biopsy or nonaspiration fine-needle biopsy techniques. Sampling can also be performed by imprints or scrapings from lymph nodes that have been surgically removed or at necropsy.

Lymph node cytology is an excellent way to evaluate a lymphadenopathy whether it is a single, multiple, or a generalized lymph node enlargement. If multiple lymph nodes are enlarged, more than one should be sampled. A lymph node away from the mouth or any site of inflammation should be aspirated as well as any lymph node close to a site of inflammation. Generally, if no lymph nodes are enlarged, lymph node cytology is generally not helpful. In addition to be unrewarding, aspiration of a nonenlarged node is difficult and usually results in aspiration of perinodal fat with little or no lymphoid tissue present. Nonetheless, normal sized lymph nodes may be aspirated on occasion to investigate the potential for metastatic disease.

Selection of a Node.

The lymph nodes generally palpated in dogs and cats include the submandibular, prescapular, and popliteal lymph nodes. Popliteal and prescapular lymph nodes are preferred biopsy sites for animals with generalized lymphadenopathy. When possible, avoid submandibular lymph nodes since they are frequently reactive due to constant exposure to antigens from the oral cavity. Also, it is best to avoid extremely enlarged lymph nodes since they may yield misleading information due to the presence of necrosis or hemorrhagic tissue. A moderately enlarged lymph node is preferred.

Sample Collection and Preparation.

Aspiration Procedure.

For cutaneous lymph nodes, the skin over the node to be aspirated needs no special preparation. It is prepared as one would prepare the skin for giving an injection. The aspiration technique requires the use of a 22 gauge needle and a 6 or 12 cc syringe. A 22 gauge butterfly catheter may be substituted for small or hard to reach nodes. When possible, insert the needle toward the periphery of the node, avoiding necrotic centers. A slight negative pressure is applied and the needle is advanced into the lesion and then redirected, if the lymph node is large enough, in a fan-like pattern until material appears in the hub of the needle. Do not pump the plunger of the syringe as this will damage the fragile lymphoid cells. During redirection of the needle, care should be taken not to withdraw the needle from the lymph node. When material appears in the hub of the needle, the plunger is released and the needle is withdrawn from the node and skin. The needle should be removed from the syringe. Air is then drawn into the syringe, and the needle is replaced onto the syringe. The aspirated material is then gently expelled onto a clean glass slide. A second clean slide is gently laid on top of the material, parallel to the first slide. The material is allowed to diffuse out, and the slides are gently slid apart. Slides are air-dried and are then stained using Diff Quik or some comparable Romanowsky-type stain.

Cytological Interpretation of Lymph Node Aspirates.

If the previously described guidelines are adhered to, there is generally good correlation between cytologic and histologic diagnosis. Any enlarged lymph node may be aspirated for the purpose of classifying the lesion into the following classifications:

1. Normal lymph node.
2. Reactive (lymphoid hyperplasia).
3. Inflammation (lymphadenitis).
4. Lymphoid neoplasia (lymphoma).
5. Metastatic disease.
6. Edema (lymphedema).

**Normal Lymph Node**

Normal lymph nodes contain 75-90% Small, well-differentiated lymphocytes. These cells measure 7 to 10 μm or 1 to 1.5 times the size of erythrocytes. They contain a thin rim of cytoplasm and the nucleus is roundish to oval sometimes indented. It has dense clumps of dark chromatin and has no visible nucleolus. Normal nodes usually contain 5 – 10% Intermediate (medium) lymphocytes (approximately 9 to 15 μm in diameter, about the same size as a neutrophil) and <5% Lymphoblasts. Lymphoblasts are generally greater than 15 μm in diameter, or 2 to 5 times the size of an erythrocyte and are larger than a neutrophil. Lymphoblasts have a moderate amount of basophilic cytoplasm that may appear granular because of the dark-staining protein-rich areas and lighter staining areas of some organelles. A clear perinuclear area in the cytoplasm representing the Golgi apparatus may occasionally be visible. Nuclear shape is variable, ranging from round to irregular, and generally has a stippled chromatin pattern. Single to multiple nucleoli are often visible. Plasma cells, macrophages, neutrophils and mast cells are occasionally seen in very low numbers in normal nodes.

![Normal Lymph Node](image)

Figure 1. Normal lymph node. Note the predominance of small well differentiated lymphocytes, the low numbers of intermediate lymphocytes and the rare lymphoblasts.

**Reactive Lymphoid Hyperplasia (RLH), or Reactive Lymph Node.**

In a reactive node, small, well-differentiated lymphocytes are still the predominant population, but increased numbers of intermediate lymphocytes and increased numbers of lymphoblasts is usually present, particularly in the cat. However, the lymphoblast population typically will not exceed 10 to 20% of the total lymphoid population of a reactive node. The most striking feature in reactive nodes from dogs is the presence of Plasma cells. Plasma cells are medium-sized round to oval cells with a single eccentrically placed round nucleus. The nucleus of a mature plasma cell is the same size and color as a small lymphocyte but the cytoplasm is much more abundant. The cytoplasm is deeply basophilic and generally have a visible Golgi apparatus appearing as a clear area located between the nucleus and greatest volume of cytoplasm. Infrequently, the cytoplasm is filled with clear or pale blue-staining vacuoles that are actually packets of immunoglobulin. These are Mott cells, and the vacuoles are Russell bodies. Neutrophils, eosinophils and/or macrophages may be mildly increased due to antigenic stimulation producing an inflammatory reaction. Low numbers of mast cells are often seen in reactive nodes, especially in the cat or in animals with primary or concurrent dermatological conditions. If reactive lymphoid hyperplasia is observed in: A regional lymph node, areas drained by the node(s) should be examined for causes of local antigenic stimulation (infection, inflammation, neoplasia). If the patient has a generalized RLHs systemic infection or other causes of antigenic stimulation should be investigated.
Figure 2. Reactive lymph node. Although a limited sample is observed, note the predominance of small well differentiated lymphocytes, the increased number of plasma cells, intermediate lymphocytes and lymphoblasts.

**Note:** A specific condition is reported in the feline patient known as Distinctive Peripheral Lymph Node Hyperplasia (DPLH) in which affected cats present with a generalized lymphadenopathy (See below under Feline Lymphoma) (Figure on right). Cytologically, large numbers of lymphoblasts are observed, many of which appear very anaplastic. However, the nodes are believed to be reactive since the condition spontaneously regresses without therapy in 1 to 17 weeks (JAVMA, Vol 190, No. 7, April 1, 1987, pg. 897-899). Since lymphoma in cats rarely presents as a generalized lymphadenopathy, the diagnosis of multicentric lymphoma in the cat should be made with caution, and confirmed histologically (J. Amer. Vet. Med. Assoc. 190 (2):897-899, 1987 and Vet. Pathol, 23:286-392, 1986).

**Inflammation (lymphadenitis).**

In lymphadenitis, the predominant non-lymphoid inflammatory cell population categorizes the type of inflammation present. **Suppurative inflammation** is characterized by the presence of increased numbers of neutrophils beyond what may be expected from any blood contamination present. Here, greater than 5% of nucleated cells are neutrophils. This is usually the result of a bacterial infection either in the node (abscessed lymph node) or in an area being drained. **Eosinophilic inflammation** is characterized by an inflammatory reaction that contains an eosinophilic infiltration, usually accompanied by a mild increased numbers of neutrophils +/- low numbers of macrophages. An eosinophilic lymphadenitis is most commonly caused by an allergic dermatitis, and is typically seen in the inguinal or popliteal lymph nodes. Other common causes of eosinophilic lymphadenitis include other non-dermatologic allergic/hypersensitivity reactions, eosinophilic granuloma complex, parasitic diseases, eosinophilic gastroenteritis, hypereosinophilic syndrome, and mast cell tumors. In rare cases, lymphoma cells may secrete chemotactic factors that result in an eosinophilic infiltration. **Pyogranulomatous inflammation** contains a significant macrophage component, with or without the presence of neutrophils. This type of inflammation typically results from fungal infections (blastomycosis, coccidioidomycosis, cryptococcosis, or sporotrichosis) protozoal infections (cytauxzoonosis, toxoplasmosis, or leishmaniasis) mycobacterial infections, *Nocardia/Actinomyces, Bartonella* in dogs. A mild pyogranulomatous inflammation may also be observed in lymph nodes that drain areas of chronic inflammation or neoplasia. (Figure on right is pyogranulomatous lymphadenitis with Blastomycosis organisms)
**Lymphoid neoplasia (lymphoma).**

Lymphoma is suspected whenever 30% of the cells population from a lymph node aspirate is lymphoblasts, though, typically, the lymphoid population will likely be between 50% to 90%. When >50% lymphoblast cells are present, a cytological diagnosis of lymphoma can be reliably made. Lymphomas may be classified by their tissue of origin (e.g. renal, thymic, intestinal etc.), with multicentric lymphoma being the most common type observed in dogs. However, knowing the "cytologic type" of lymphoma present may give some indication of the grade of malignancy, the potential for response to chemotherapy, and the potential, or explanation for paraneoplastic syndromes such as hypercalcemia. The most accurate means of typing lymphoma is by using lymphocyte markers that will determine the subset of lymphocytes involved in the neoplastic process (e.g. B-cells, T-cells such as CD4 or CD8, or Natural Killer cells).

**Canine Lymphoma**

In canine lymphoma, the predominant cell type is the immature lymphoblast. Only rarely will the small, well-differentiated lymphocytes become neoplastic. Lymphoblasts are large cells with nuclei that vary in size from 2 to 5 times the size of erythrocytes with a deeply basophilic cytoplasm that is more abundant than that of small or intermediate lymphocytes. The chromatin pattern is more diffuse and paler staining than in the well-differentiated lymphocyte. A variable number of distinct or indistinct nucleoli are frequently visible. (Figure right, canine lymphoma).

**Note:** The previous administration of glucocorticoids can drastically alter the lymphocytes population within a lymph node. Lymphoblasts are very sensitive to the cytotoxic effects of glucocorticoids much more so than mature lymphocytes. This may iatrogenically decrease the differential lymphoblast count below 30% to 50% of the population therefore making a lymphoma diagnosis difficult.

**Cytological Typing of Canine Lymphomas**

Some diagnostic and prognostic information may be obtained by evaluating the cytologic features of the neoplastic lymphoblast population and identifying them as either immunoblastic, lymphoblastic, or small, noncleaved lymphomas. **Immunoblastic lymphomas** have a lymphoblast population that has a plasmacytoid appearance with eccentrically located nuclei and a perinuclear clear zone "Golgi Zone". Nuclei often contain a single, prominent nucleolus. This is a B-cell lymphoma which is typically responsive to initial protocols of chemotherapy, however, relapse is common. The **Small Noncleaved** contains lymphoblasts that have round nuclei which are 1.5 to 2.5 times the size of erythrocytes and are centrally located within the cytoplasm. Small, multiple, prominent nucleoli are usually seen. These may be B-cell or T-cell in origin, and generally not as responsive to chemotherapy as the immunoblastic type. In **Lymphoblastic** lymphomas, the neoplastic lymphoblasts have characteristic nuclei that are often cleaved or convoluted. Nucleoli are often absent, however, when present are small and obscure. The cytoplasm is usually scant. This is a T-cell lymphoma and may be associated with paraneoplastic hypercalcemia. These are usually the least responsive to chemotherapy and the presence of hypercalcemia and associated renal failure worsens the prognosis. Many thymic lymphomas are of this type and are of T-cell origin. Most of the lymphomas in dogs are a high grade malignancy. The characteristic cytological features of the 3 most common types are presented.

**Feline Lymphoma**

The same criteria for diagnosing lymphoma are used in dogs and cats. When a lymph node aspirate or mass is aspirated and found to contain a population of lymphocytes of which 50% or more are blast cells, lymphoma can reliably be diagnosed. However, two complicating factors make the diagnosis
of lymphoma in the cat more difficult than in the dog: 1. Lymphomas in the cat are more frequently composed of a population of well-differentiated lymphocytes, which is rarely observed in dogs, and 2. as mentioned previously, a condition known as “Distinctive Peripheral Lymph Node Hyperplasia” (DPLH) that clinically, cytologically and histologically may resemble multicentric lymphoma has been reported to occur in young cats(J. Amer. Vet. Med. Assoc. 190 (2):897-899, 1987 and Vet. Pathol, 23:286-392, 1986). In addition, Multicentric lymphoma, involving only the peripheral lymph nodes, is common in the dog, but rare in the cat. Therefore, a diagnosis of lymphoma cannot be made when evaluating aspirates taken from cats with only generalized peripheral lymphadenopathy.

**Anatomic forms of feline lymphoma**

Lymphoma involving the internal organs occurs with relative frequency in the cat. Various forms may include mediastinal, hepatic, alimentary, renal, ocular, and primary CNS lymphoma. There may be a relationship between alimentary and renal lymphoma and with renal lymphoma and CNS metastasis. When aspirates from masses in any of organs yield a dense population of lymphoid cells, lymphoma should be suspected. When the lymphocyte population consists of primarily lymphoblasts, as is seen in many cases, the cytologic diagnosis of lymphoma can reliably be made. However, many lymphomas of liver or intestinal origin are composed of small, well-differentiated, normal-looking, neoplastic lymphocytes (Figure to right; small-cell hepatic lymphoma top, and small cell intestinal lymphoma second). Many lymphomas in the cat are composed of T-cells transformed by the FeLV virus, but most arising from the gastrointestinal tract are FeLV-negative B-cell lymphomas.

An unusual form of alimentary lymphoma classified as **large granular lymphoma (LGL)** is also reported in the cat (third Figure on right). It is characterized by a population of individually arranged round cells with fairly abundant cytoplasm. The cytoplasm contains a focal accumulation of azurophilic granules (resembling mast cell granules). These tumors usually involve the small intestine and are believed to be of cytotoxic T-cell or natural killer cell origin. The focal accumulation of the granules may help to distinguish this neoplasm from the intestinal form of MCT also seen in the cat. LGLs generally have less cytoplasm, fewer, larger granules, and no or few eosinophils as compared to mast cell neoplasms. LGL stain positively for lymphoid tissue markers and with PTAH (phosphotungstic acid-hematoxylin), and negative with toluidine blue, mast cell tumors stain just the opposite.

**Feline Hodgkin’s-like Lymphoma.**

This condition resembles the condition in humans and has generally been recognized in older cats (> 6 yrs). Most affected animals presented with a mass in the ventral cervical region, submandibular and/or prescapular node enlargement. As in humans, only a single node or group of nodes is generally involved with eventual contiguous nodal advancement. The cytologic diagnosis is very difficult since the neoplastic cells (Reed-Sternberg cells and their variants) only comprise 1% to 5% of the cells in the affected lymph node, the rest of the cells are non-neoplastic lymphocytes, macrophages, and granulocytes. The diagnosis needs to be confirmed histologically and various histological types of the disease exist (Vet. Pathol. 38:504-511, 2001. Vet Clin Path 37(3): 323-327, 2008).
Immunophenotyping and PARR Analysis for diagnosis of lymphoma

**Immunophenotyping** is the most accurate way to determine cell type involved (B-cell vs. T-cell). This analysis can provide both prognostic and therapeutic information. One of three techniques (Immunohistochemistry, immunocytochemistry or flow cytometry) can be used to identify the surface protein markers that are present on a population of neoplastic lymphocytes.

Surface protein markers are identified as **Cluster Differentiation** markers or (CD) CD3, CD4, CD8 for T-cell markers and CD21 & CD79a for B-cell markers. These techniques are not designed to identify the lymphocyte population as neoplastic or non-neoplastic. The diagnosis of lymphoma must first be made cytologically or histologically prior to immunophenotyping.

- **Immunohistochemistry:**
  - **Tissue sections** go through a series of steps so that antigens located on the cell surface, cytoplasm, or nucleus are bound to antibodies that are then exposed to enzymatic reactions that lead to a color change on the bound surface. If the expected color is detected, the cells are considered positive for that antigen. The antibodies available do vary between preparations (i.e. frozen tissue vs. formalin-fixed tissue) so it is best to discuss the desired tests with the diagnostic laboratory to ensure satisfactory results.

- **Immunocytochemistry:**
  - **Tissue aspirates** from an enlarged node are placed in a saline solution of specific volume and sent to lab for processing.

- **Flow cytometry:**
  - **Tissue aspirates** of lymph nodes or bone marrow that are be collected in anticoagulant (EDTA tubes) and submitted to the appropriate labs. Surface markers on cells are evaluated and lymphocyte populations are classified as B-cell or T-cell in origin.

**Important Note:** While immunohistochemistry, immunocytochemistry and flow cytometry aids in revealing the cell types in the sample (B-cell or T-cell), they do not classify the population as neoplastic. As such, a diagnosis of neoplasia (in this case lymphoma) should be made by other means (cytology, histology). Below is a table of common markers used to further characterize lymphomas as B cell or T cell.
PCR for Antigen Receptor Rearrangements (PARR) (DNA analysis)

This technique is used to help identify a population of lymphocytes as neoplastic or non-neoplastic. The assay tests for clonality in the antibody receptor or T-cell antigen receptor. Not for typing as B or T cells because some Bs have T cell antigen rearrangements etc. The technique is good for diagnosing canine lymphoma, however the sensitivity in diagnosing feline lymphoma is about 60%, meaning that about false negative results will be obtained in about 40% of feline lymphomas. This test can be performed at the Clinical immunology laboratory at CSU 970-491-1170, among other institutions.

Table 1: Molecular markers used in the characterization of lymphoid neoplasms

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Likely Marker Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>B cells</td>
<td>Pos: <strong>CD79a</strong>, <strong>CD21</strong>, CD20, CD19 Neg: CD3</td>
</tr>
<tr>
<td>T cell</td>
<td>Pos: <strong>CD3</strong>, CD4, CD8 Neg: CD79a</td>
</tr>
<tr>
<td>Immature Cell</td>
<td>Pos: <strong>CD34</strong>, CD4+/CD8+ (T cells) Neg: CD 45 (dim uptake)</td>
</tr>
<tr>
<td>Mature Cell</td>
<td>Pos: CD45 (bright), CD20 (B cells), CD4 or CD8 (T cells) Neg: CD34</td>
</tr>
</tbody>
</table>

*These markers can be evaluated with various techniques, including immunohistochemistry, immunocytochemistry, and flow cytometry.

Table 2. Veterinary diagnostic labs with molecular diagnostic capabilities *(FYI Only)*

<table>
<thead>
<tr>
<th>Diagnostic Lab</th>
<th>Services Available</th>
<th>Contact Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCSU</td>
<td>Flow Cytometry PARR</td>
<td>Clinical Immunology Lab, Attn.: Linda English, Rm. B-324, 4700 Hillsborough St. Raleigh, NC 27606 (970) 491-1170 (919) 513-6363</td>
</tr>
<tr>
<td>Kansas State</td>
<td>Flow Cytometry</td>
<td>Clinical Immunology/Flow Cytometry Lab, Attn.: Dr. Wilkerson, 1800 Denison Ave., Manhattan, KS 66506 (785) 532-4617</td>
</tr>
<tr>
<td>Colorado State</td>
<td>Flow Cytometry PARR</td>
<td>Clinical Immunopathology, Veterinary Diagnostics Laboratory, 300 West Drake, Fort Collins, CO 80523 (970) 491-6138</td>
</tr>
</tbody>
</table>
**Metastatic Disease.**

Knowledge of the areas drained by specific lymph nodes is critical in determining the presence of metastatic disease. It is also important to remember that the absence of obvious metastatic disease in a cytology specimen does not rule out the possibility of early metastasis. Since many tumors will enter the nodes through afferent, subcapsular vessels (Figure on right), or begin as focal accumulations, early metastatic disease might be missed on cytologic preparations. **Metastatic disease is characterized by** the presence of a homogenous cell population not normally found in a lymph node. These cells usually appear anaplastic and display obvious characteristics of malignancy. The remaining lymphoid population may appear reactive, however, the neoplasia may replace (efface) the lymph node parenchyma totally, making cytological identification of the swelling as a lymph node difficult. The absence of lymphadenopathy does not rule out the presence of metastatic disease. Mast cell tumors, amongst other neoplastic processes, are renown to metastasize without creating lymphadenopathy. The presence of lymphadenopathy in a lymph node draining an area with a tumor does not automatically indicate metastasis has occurred. Lymph nodes draining an area where a tumor is located often become reactive in response to the regional inflammatory process induced by the neoplasm. In addition, many lymph nodes may be normal in size and have significant metastatic disease. This is particularly true of metastatic mast cell tumors.
**Rick Alleman, DVM, PhD, DABVP**

**Biographical Sketch:** Dr. Alleman received his DVM from Louisiana State University and practiced companion animal medicine and surgery for 9 years in private small animal practice in New Orleans. During that time he was board certified as a Diplomate of the American Board of Veterinary Practitioners with a specialty in companion animal practice. Dr. Alleman then completed a clinical pathology residency at the University of Florida and became a Diplomate of the American College of Veterinary Pathologists with a specialty in clinical pathology and completed his PhD degree in molecular biology of vector-borne diseases. He is a former Professor, Service Chief, Resident Coordinator and Director of Laboratories at the University of Florida, College of Veterinary Medicine. He is currently Manager of Lighthouse Veterinary Consultants.