Leukocyte Disorders: What Your Analyzer Cannot Tell You

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Introduction
Leukocytes respond to infection, neoplasia, injury, and other insults. Identification of alterations in the blood concentration of the different types of leukocytes can provide useful diagnostic and prognostic information. While many of the available automated hematology analyzers provide accurate total WBC concentration and WBC differential in healthy animals, erroneous results often occur in ill animals that have abnormal cells in circulation. Some analyzers provide flags that will indicate problem samples, making review of a blood smear critical in these cases; however, microscopic examination of a blood smear should always be performed any time a CBC is performed. The first step when reviewing a smear is to confirm that the automated leukocyte count and differential are correct. If the smear review identifies a mismatch, then a manual differential should be performed. The number of cells included in the differential should increase from a minimum of 100 cells to 200 or 300 cells in animals with a significant leukocytosis. Some information is not readily available from an analyzer and can be best identified by smear review. Smear review is required to identify nucleated RBCs (included in WBC count by most automated analyzers), basophils (not detected by automated analyzers), leukoagglutination, mast cells, toxicity, immature neutrophils, neoplastic cells or organisms. For optimal results, smears should be made as soon as possible following blood collection to prevent the deterioration of cells that occurs with storage of blood. The feathered edge should be scanned for large structures (e.g., mast cells, neoplastic cells, platelet clumps, organisms); however, cells are frequently lysed or distorted in this area. The differential and assessment of morphology should be performed in the monolayer region (deep to the feathered edge) where the erythrocytes are close together but not overlapping. In this region, the leukocytes can adequately spread, allowing optimal assessment of nuclear and cytoplasmic features. Avoid the deeper areas of the smear where the cells do not spread well enough to evaluate morphology.

Types of Leukocytes
Segmented neutrophils are the predominant leukocyte in the blood of many mammals. They have a lobed nucleus with 1 or more focal areas of constriction. Nuclei contain condensed chromatin and stain dark purple. The cytoplasm typically is grainy pink and contains granules that range from indistinct to small and red. Band neutrophils are absent to infrequent in healthy animals; increased concentration is termed a left shift and supports inflammation. Band neutrophils have a horseshoe shaped nucleus that lacks focal areas of constriction and contains lighter staining, less dense nuclear chromatin. Neutrophilia occurs with inflammation, neoplasia, and elevated glucocorticoids or catecholamines. Neutropenia occurs with acute overwhelming tissue demand (e.g., infection or other cause of inflammation), immune-mediated neutrophil destruction, or impaired bone marrow production.
Eosinophils are in low concentration in the blood of healthy animals. They are often larger than neutrophils, have a lobulated nucleus, and contain distinct red cytoplasmic granules that vary in shape between species. Eosinophils with non-staining granules are found in some dogs (especially Greyhounds). They are of no reported pathologic significance but can be misidentified in both automated and manual differentials, causing lack of recognition of an eosinophilia. Band eosinophils are found in blood but are not usually quantitated separately from segmented eosinophils. Eosinophilia occurs with hypersensitivity/allergic disorders, parasitic infections, idiopathic eosinophilic disorders and some types of neoplasia (e.g., mast cell neoplasia, eosinophilic leukemia). Eosinopenia is of questionable diagnostic significance because of the low concentration expected in healthy animals.

Basophils are uncommon in the blood of most healthy mammals and cannot be reliably detected by automated analyzers. Basophils have a lobulated nucleus and grey-lavender to purple cytoplasm containing purple granules. In some species (e.g., horses, ruminants), purple granules are abundant and often obscure the nucleus. Cat basophils contain large numbers of pale, oval, lavender granules. The basophils of dogs contain few to no granules and may be difficult to differentiate from monocytes. Basophils from dogs have a long, ribbon-like, light staining nucleus and a lavender hue to the cytoplasm. Basophilia usually accompanies eosinophilia. Basophilia without eosinophilia occurs with basophilic leukemia, other myeloproliferative disorders, or disorders of lipid metabolism. Basopenia is of no diagnostic significance.

Mast cells are rare in the blood of healthy mammals. Mast cells contain dark purple cytoplasmic granules similar to basophils; however, mast cells have a round nucleus. They are often concentrated along the edges of the smear. Mastocytosis occurs in patients with mast cell neoplasia or with a wide assortment of non-neoplastic inflammatory disorders.

Lymphocytes are common in blood and have a round to oval, dark staining nucleus, a thin rim of homogenous blue cytoplasm, and a high nuclear:cytoplasmic (N:C) ratio. In dogs and cats, lymphocytes are generally larger than erythrocytes but smaller than neutrophils. In horses and ruminants, there is a mixture of small lymphocytes and larger lymphocytes. A minority of circulating lymphocytes contain small numbers of reddish purple granules in the cytoplasm. An increase in granular lymphocytes occurs with lymphoid leukemia or with some inflammatory disorders. Lymphocytosis occurs with increased catecholamines, chronic infections or inflammatory diseases, or lymphoid leukemia. Lymphopenia occurs with acute inflammation, glucocorticoids, loss of lymphatic fluid, and immunodeficiency syndromes.

Monocytes are found in low concentration in the blood of healthy animals. They have an oval, kidney bean, horseshoe or convoluted nucleus that contains less densely stained chromatin than neutrophils. They have abundant blue-grey, finely granular cytoplasm that may be vacuolated or contain a fine dusting of red granules. Monocytes can be difficult to differentiate from immature toxic neutrophils or large, reactive lymphocytes. Monocytosis occurs with inflammation (acute or chronic), neoplasia, and glucocorticoids. Monocytopenia is of no diagnostic significance.

Abnormal Leukocyte Morphology
The concentration of immature neutrophils in blood increases with inflammation and is termed a
left shift. Immature stages of neutrophils include (in order of decreasing maturity): (1) band cells; (2) metamyelocytes (kidney bean shaped nucleus); (3) myelocytes (oval to round nucleus); (4) promyelocytes (round, light staining nucleus; fine red cytoplasmic granules); and (5) myeloblasts (light staining nucleus with visible nucleolus). An inflammatory left shift should have an orderly maturation progression where the numbers of the more mature stages exceed the number of each sequentially immature stage. When maturation progression becomes inverted (e.g., myeloblasts or promyelocytes are present in the highest number), leukemia should be suspected. The neutrophils of animals with Pelger-Huet anomaly are hyposegmented and must be differentiated immature neutrophils to avoid incorrectly diagnosing a severe left shift. Pelger-Huet syndrome is usually inherited and of no pathologic significance. The nuclear chromatin of Pelger-Huet cells is similar to that found in segmented neutrophils.

Neutrophil toxicity indicates an increased rate of production in the bone marrow in response to inflammatory cytokines that results in maturation abnormalities. Toxic neutrophils are common with sepsis but occur with other inflammatory disorders. As a general rule, the presence of toxic change indicates more severe inflammatory disease in the patient. Morphologic criteria of toxicity include Döhle bodies (blue cytoplasmic inclusions), diffuse cytoplasmic basophilia, foamy cytoplasm, retained primary granules (toxic granulation), giant neutrophils, and asynchronous nuclear maturation. Severe toxicity makes it difficult to differentiate neutrophils from monocytes.

Neutrophils collected in EDTA may develop clear, distinct cytoplasmic vacuoles with storage; however, these cells lack the basophilia and Döhle bodies found with toxic change. Other artifacts associated with a delay in making smears include uneven distribution of cytoplasmic granules, swollen nuclei (making it difficult to recognize a left shift), pyknosis, karyorrhexis, and hypersegmented neutrophils (greater than 5 distinct nuclear lobes). Hypersegmented neutrophils also occur in vivo as an idiopathic finding, with myeloproliferative disorders or when there is prolonged transit time in the blood due to excess glucocorticoids or chronic inflammation.

The presence of aggregates of leukocytes in a smear is termed leukoagglutination. A major concern with leukoagglutination is that the automated WBC concentration and differential may not be reliable. Prompt processing of blood following collection may minimize leukoagglutination.

Leukemia is a neoplastic proliferation of blood cells in the bone marrow. In acute leukemia, immature cells (e.g. blast cells) are found in the blood without evidence of normal maturation progression. As a general rule, blasts cells have a light staining nucleus with one or more prominent nucleoli. It is difficult to differentiate the lineage of blast cells (e.g., lymphoid, erythroid, myeloid) on routinely stained smears. Immunophenotyping and/or cytochemical staining may be required to make a definitive diagnosis. In chronic leukemia, the neoplastic cells retain the ability to mature. Chronic leukemia is difficult to diagnosis based upon blood smear examination alone because the neoplastic cells may be morphologically normal. It is suspected when an underlying cause for leukocytosis cannot be identified.

Reactive lymphocytes are cells that have undergone immune stimulation. They are a non-specific finding and can be found in low concentrations in healthy animals. They tend to
increase with inflammation. Morphologic features of reactive lymphocytes include increased size, increased quantity of dark blue cytoplasm, and enlarged nuclei that may be cleaved, lobulated or convoluted. Nuclear chromatin varies from coarsely clumped to stippled. Reactive lymphocytes may have prominent nucleoli and be difficult to differentiate from neoplastic cells. Atypical lymphocytes are similar in size or larger than a neutrophil. They may have immature chromatin pattern and increased amounts of light blue cytoplasm. These may be neoplastic cells or may be cells responding to inflammation. Morphologic changes in lymphocytes may follow prolonged storage in EDTA and include nuclear lobulation, cytoplasmic vacuolization or smudging.

A variety of cytoplasmic inclusions may be found in circulating leukocytes. Phagocytized erythrocytes or hemosiderin may be found in neutrophils or monocytes in patients with hemolytic anemia (particularly immune-mediated). Organisms in leukocytes include bacteria, fungi (e.g., *Histoplasma capsulatum*), protozoa (e.g., *Hepatozoa* spp., *Leishmania* spp., *Toxoplasma* spp.), morulae from rickettsial species, or viral inclusions. Rarely, patients with hereditary disorders (e.g., Chediak-Higashi syndrome, lysosomal storage disorders) may have cytoplasmic granules or vacuoles.

**Suggested References**