

## **THE PERIPHERAL BLOOD SMEAR IS A VALUABLE COMPONENT OF THE CBC**

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Examination of the peripheral blood smear begins on low power. The feathered edge should be scanned for platelet clumps, large cells and microfilaria. The body of the smear should be scanned for proteinaceous background material, identification of the monolayer and again, large cells and microfilaria. A white blood cell estimate can also be performed at low power. Getting an accurate number at this level is challenging so the emphasis should be placed on whether the white blood cell count is increased, decreased or adequate. A rough estimate can be obtained by counting the cells per field and multiplying by 100-150. Within the monolayer, examination of the red blood cell density and red blood cell arrangement is important. Both agglutination and rouleaux can be observed at low power and may actually be missed at higher magnification. The red blood cell density is important because a decreased density is consistent with anemia. Red blood cells should be evenly spaced from each other and almost touching. Increased space between the red blood cells indicates anemia.

At higher power (40x, 50x or 100x) more specific information about red blood cells, white blood cells and platelets can be evaluated. Red blood cell morphology should be evaluated at higher power. Different morphologic changes can indicate very different disease states in the patient, or even artifact. Polychromasia indicates erythrocytes which are grayish-blue in color due to the presence of RNA, ribosomes and other organelles. Reticulocytes are often polychromatophilic. All polychromatophils are reticulocytes but not all reticulocytes are polychromatophilic. Anisocytosis indicates variation in cell size and may indicate the presence of small or large cells. Reticulocytes are often larger than mature erythrocytes so they are described as macrocytes. Other causes of macrocytosis include feline leukemia virus, folic acid deficiency (uncommon in animals) and macrocytosis of toy and miniature poodles. Microcytosis or small red blood cells can be observed with several different disease processes, the most common of which is iron deficiency. Due to the impaired heme synthesis, the red blood cells undergo an additional division. Microcytosis will often precede hypochromasia. Diseases associated with iron deficiency or iron misuse include chronic blood loss, dietary deficiency in neonates and portosystemic shunts. Other causes of microcytes include hereditary microcytosis (Akita and Shiba Inu) and familial dyserythropoiesis of English Springer Spaniels<sup>1</sup>. Hypochromasia can also be observed in patients with iron deficiency and indicates a decrease in hemoglobin content. If severe enough, this may result in a decrease in the mean corpuscular hemoglobin concentration or MCHC. Membrane abnormalities or poikilocytosis can result in various morphological

abnormalities. Spherocytes are red blood cells which have lost their biconcave shape. On a blood smear, they lack central pallor and they can appear a little smaller. They are commonly associated with immune-mediated hemolytic anemias but have also been reported in dogs with spectrin deficiency<sup>2</sup>. Acanthocytes are spiculated red cells with irregularly spaced spicules. These cells can be observed in patients with liver disease, portosystemic shunts or high-cholesterol diets. In contrast echinocytes are also spiculated red blood cells, but have regularly spaced spicules. These are generally considered an artifactual change; however, they have been observed in dogs with glomerulonephritis, lymphoma, hemangiosarcoma and other neoplasms, immune mediated hemolytic anemia, pyruvate kinase deficiency, rattlesnake envenomation and doxorubicin toxicosis among others<sup>3</sup>. Cats likely have echinocytes with many of these diseases as well but are specifically reported in the literature associated with chronic doxorubicin administration. Schistocytes are red blood cell fragments usually resulting from direct physical damage to the erythrocyte secondary to vascular abnormalities or turbulent blood flow. The shape of the fragments may vary from pointed or triangular to spiculated. Microangiopathic fragmentation has been described in dogs in a number of different disorders including disseminated intravascular coagulation (DIC), glomerulonephritis, hemangiosarcoma, myelofibrosis, dyserythropoiesis and chronic doxorubicin toxicosis. Other poikilocytes include target cells or codocytes, which can be observed with iron deficiency, liver disease or as an artifact, or dacryocytes (tear-drop shaped red blood cells) which have been reported in dogs with myelodysplastic disease and myelofibrosis. Keratocytes have one or two spicules and are thought to represent blister cells which have opened, whereas eccentrocytes have a peripheralization of their hemoglobin.

The erythrocytes should also be examined for inclusions. Nucleated erythrocytes are commonly observed in anemic patients as part of the regenerative response. However in the absence of anemia or polychromasia, bone marrow and splenic disorders should be considered. Howell-Jolly bodies are nuclear remnants and appear as a small basophilic inclusion. These structures can accompany a regenerative response. Basophilic stippling are part of the regenerative response to anemia in some species such as cattle, however in cats and dogs, this observation is less common. In these species, lead toxicity should be considered especially if the patient is not anemic or has increased numbers of nucleated red blood cells. Oxidative damage can result in a number of morphologic changes to the red blood cells including the formation of Heinz bodies. Heinz bodies are small aggregates of oxidized hemoglobin which can occur as hemoglobinized projections extending from the cell or as pale areas within the cell but close to the cell membrane. Staining the sample with new methylene blue or other vital stains colors the Heinz bodies pale blue, allowing them to become much more obvious. Cats are more susceptible to Heinz body formation because of their hemoglobin

structure; therefore a healthy cat can have low numbers of Heinz bodies in circulation. Many toxins have been reported to cause Heinz bodies. Hemic parasites also appear as red blood cell inclusions. In dogs two forms of babesial organisms can be identified; *Babesia gibsoni* is a small, signet ring shaped organism with an eccentrically placed nucleus and *Babesia canis* is a piroplasmic organism, much larger than *B. gibsoni*, generally having two organisms/cell. In cats, *Mycoplasma hemofelis* and *M. hemominutum* are common extracellular parasites. These organisms appear as small cocci or chains of cocci on the erythrocyte membrane. *Cytauxzoon felis* is an intraerythrocytic parasite that is regionally specific and appears as a small, signet ring shaped organism, similar to *B. gibsoni*.

A differential count of 100-200 cells should be performed at high power, in the monolayer of the blood smear. The white blood cells observed in the peripheral blood include neutrophils, eosinophils, basophils, monocytes, lymphocytes and less commonly, plasma cells, mast cells and blasts. In dogs and cats, the neutrophil is the predominant white blood cell. Neutrophils are granulocytes and originate from the same precursor cell as eosinophils and basophils. Neutrophils have a multilobulated nucleus with pale, basophilic cytoplasm. During an inflammatory response, toxic changes may occur which can change the appearance of the cytoplasm. Basophilic cytoplasm, foamy cytoplasm, döhle bodies and azurophilic granules are common changes that can occur with inflammation. Additionally, if there is increased tissue demand for white blood cells, particularly neutrophils, bands and metamyelocytes or hyposegmented neutrophils may be observed in circulation. Bands have a U shaped nucleus with no indentation and metamyelocytes have a reniform shaped nucleus. Neutrophils can also have a hypersegmented nucleus. Hypersegmented neutrophils are generally thought of as older cells and can be observed in patients with chronic inflammation, myeloproliferative disorders or receiving glucocorticoids. Eosinophils are also granulocytes and have a lobulated nucleus and contain numerous eosinophilic granules. In dogs these granules are round and in cats they are rod shaped. Eosinophilia can be observed with parasitic infestation, allergic or hypersensitivity responses and marked eosinophilia (>10,000/ l) can be seen in paraneoplastic responses, hypereosinophilic syndrome, eosinophilic pulmonary granulomas, hepatozoonosis and eosinophilic leukemia<sup>4</sup>. Basophils are not commonly observed in peripheral blood but can accompany eosinophils. In cats with eosinophilia and basophilia, heartworm infestation should be considered. Basophils have a lobulated nucleus with grayish-blue cytoplasm. In dogs, few basophilic granules are observed. In cats, basophils are filled with pale, lavender granules which are difficult to visualize with Dif-Quik type stains.

Lymphocytes are not granulocytes. These cells are small and round with a scant rim of basophilic cytoplasm and a round nucleus which fills the cytoplasm. Variable

lymphocyte morphology can be observed in peripheral blood. Reactive lymphocytes are seen with antigenic stimulation. These cells are larger and have increased amounts of deeply basophilic cytoplasm. Observation of "atypical" lymphocytes is a common notation on CBCs. These cells have an increased amount of basophilic cytoplasm and are slightly larger than classic lymphocytes. The clinical significance of these lymphocytes is uncertain but if they are present in low numbers, they likely represent a reactive population. However if the patient has a significant lymphocytosis ( $>20,000/\mu\text{l}$ ) and most or all of the lymphocytes appear atypical, a neoplastic proliferation should be considered. Monocytes are larger than lymphocytes and have a moderate rim of basophilic cytoplasm, often containing discrete cytoplasmic vacuoles and a variably shaped nucleus, from round to reniform to lobulated. These cells are often part of an inflammatory response, but in dogs increased monocytes can be observed as part of a stress response.

Other white blood cells that can be observed in peripheral blood include mast cells, plasma cells and blasts. Mast cells are round cells with a round nucleus containing numerous metachromatic granules. These cells can be seen in peripheral blood with a number of different diseases including mast cell neoplasia, parvoviral enteritis, fibrinous pericarditis, bacterial peritonitis, aspiration pneumonia, acute pancreatic necrosis, IMHA, allergic dermatitis and gastric torsion<sup>5</sup>. Presence of significant mastocytosis ( $>10,000/\mu\text{l}$ ) is more consis

mast cell tumor, systemic mastocytosis or mast cell leukemia. Plasma cells are infrequently observed in the peripheral blood but can be observed as part of an immune response, particularly post-vaccination. Blasts in the peripheral blood are a fairly common finding, especially in patients with lymphoma or other myeloproliferative or lymphoproliferative disorders. Blasts are larger cells with varying amounts of basophilic cytoplasm, round nuclei with a stippled chromatin pattern, and prominent nucleoli. Morphologically, we cannot consistently distinguish a large lymphocyte from myeloblast or myelomonocytic precursor<sup>6</sup>. Immunophenotyping of these cells is essential. Acute leukemias can have a marked leukocytosis, often  $>100,000$  cells/ $\mu\text{l}$  with a predominance of neoplastic cells. In patients with lymphoma, there may only low numbers of neoplastic cells in circulation, sometimes representing 1-2% of the nucleated cell population. Identification of these cells in the peripheral blood does not necessarily indicate neoplastic infiltration of the marrow; however, the recognition of the cells in circulation however is very important.

Platelets are cytoplasmic fragments of megakaryocytes. They can be estimated on high power (100x). In the monolayer, platelets should be counted in 10 fields and the average is calculated. This value is multiplied by 15,000-20,000. The blood smear should also be evaluated at low power for platelet clumps. The presence of platelet

clumps will decrease automated platelet counts as well as the estimate. Generally, platelets are smaller than red blood cells. They are a basophilic disc of cytoplasm with varying numbers of granules. However, in thrombocytopenic patients, giant platelets can be observed and indicate that the marrow is responding or attempting to respond to the thrombocytopenia.

In conclusion, examination of the peripheral blood smear is an essential component of a CBC. Abundant information can be gathered from the blood smear, including subtle changes such as toxic changes in the neutrophils, hemic parasites in the erythrocytes or low numbers of neoplastic cells or mast cells that may help to make a diagnosis.

### **References**

<sup>1</sup>Allen L, et al. **J Am Vet Med Assoc** 1999;214:218-221. <sup>2</sup>Slappendel RJ, et al. **J Vet Intern Med** 2005;19:187-192. <sup>3</sup>Weiss DJ, et al. **Vet Clin Pathol** 1990;19:114-118. <sup>4</sup>Marchetti v, et al. **Vet Clin Pathol** 2005 Sep;34(3):259-63. <sup>5</sup>McManus PM. **J Am Vet Med Assoc** 1999;215:355-357. <sup>6</sup>Gauthier MJ. **J Vet Intern Med** 2005;19:193-199.