RED BLOOD CELL MORPHOLOGICAL ABNORMALITIES IN PERIPHERAL BLOOD

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Systematic evaluation of red blood cell (RBC) morphology in a blood smear includes assessment of abnormalities in pattern, size, color and shape, identification of inclusions and quantification of nucleated red blood cells (NRBC). Although some information is available from automatic analysis, morphological smear examination can not be replaced by any other test.

Abnormalities in pattern:
Agglutination - three-dimensional aggregations of RBC resembling grapelike clusters that are formed due to the binding of antibodies to RBC surface (immune-mediated hemolytic anemia (IMHA), EDTA pseudo-agglutination in cats).
Rouleaux formation - RBC aggregations forming linear arrangements resembling stacks of coins indicating hyperglobulinemia and/or increased fibrinogen concentrations (under reactive conditions or in lymphoproliferative disorders).

Abnormalities in size (anisocytosis, macrocytosis, microcytosis):
Abnormalities in RBC size in a blood smear correspond to changes in diameter and not in volume as measured by hematologic analysers. Cells can appear smaller but be of normal volume whereas other cells appear bigger because they are thinner and more spread. Low numbers of larger or smaller RBC do not increase, resp. decrease the mean cell volume (MCV) out of reference interval. Beside sideropenic anemias, microcytosis is a common laboratory finding (85% of dogs) in patients with portosystemic shunt (Niles et al. 2001). Macrocytosis without reticulocytosis is a rare finding.

Abnormalities in color (polychromasia, hypochromasia, ghost cells):
Hypochromasia - RBC with enlarged central pallor due to decreased hemoglobin (Hb) content caused by defective Hb production or inhibition of Hb production. The iron deficiency anemia in dogs is accompanied by RBC shape abnormalities suggesting fragmentation as a result of diminished deformability. Hypochromic RBC must be distinguished from torocytes, an artifactual RBC change that mimics hypochromasia but has no diagnostic relevance.
Polychromasia - immature RBC (reticulocytes) with diffusely basophilic cytoplasm due to high amounts of ribosomes corresponding to active erythropoiesis.
 Ghosts cells - hemolysed RBC with the remaining membranes seen as “ghosts” in intravascular hemolysis due to oxidant injury, hypophosphatemia, erythroparasites, bacteria (Clostridia, Leptospira) or snake envenomation (Masserdotti 2009).
Abnormalities in shape (poikilocytes, echinocytes, acanthocytes, schistocytes, keratocytes, eccentrocytes, pyknocytes, spherocytes, target cells, stomatocytes, elliptocytes, drepanocytes):

Poikilocytes - general term for RBC with abnormal shape. Low numbers of misshapen RBC can be seen in blood from clinically healthy dog or cat.

Echinocytes - spiculated RBC with numerous short, sharp or blunt projections of uniform length and evenly spaced around the cell membrane. Echinocytes are often formed as artefacts (resulting from cell aging or exposure to excessive concentrations of EDTA). Echinocytosis is expected when increased pH or cell dehydration (low intracellular potassium), in inherited red blood cell disorders (decreased ATP production), in renal diseases, under drugs expanding the outer leaflet of the membrane (salicylates, phenylbutazone, furosemide, doxorubicin) or after snake envenomation (resulting from ATP depletion by ATPase enzymes and alteration of membrane by phospholipases) where echinocytes with 92% positive dogs were a consistent finding (Hackett et al. 2002).

Acanthocytes - RBC with irregularly sized spicules characterized by 2-20 membrane projections unevenly distributed over the cell surface. Acanthocytes occur through fragmentation injury (disruption due to abnormal vasculature) or alterations in lipid composition of the RBC membrane (abundant membrane due to high cholesterol to phospholipid ratio). Due to their rigidity, they are less deformable and may have a shorter life span resulting in hemolytic type of anemia. Acanthocytes were observed in dogs with hemangiosarcoma (HSA), lymphoma, osteosarcoma, gastrointestinal, musculoskeletal, renal, immune-mediated and different other diseases (Warry et al. 2013). Mean number of acanthocytes in HSA with 54% positive dogs was significantly higher than in other disorders (Tant et al. 2004).

Schistocytes - red blood cell fragments reflecting mechanical injury to erythrocytes associated with conditions in which the normally smooth endothelium is irregular, the vascular lumen is crossed by fibrin strands or the blood flow is turbulent. Schistocytes suggesting evidence of microangiopathic hemolysis are seen in disseminated intravascular coagulation (DIC), vasculitis, vascular neoplasm, glomerular diseases, portosystemic shunts, iron deficiency. In dogs with HSA they occured in 46% of patients, in which severe schistocytosis was presented by primary spleen or liver neoplasia (Hammer et al. 1991). Schistocytes are rarely seen in cats with DIC, more common they occur in liver diseases.

Keratocytes - RBC with one or two “horn-like” projections or with a “bite-shaped” defect in the cell outline. Keratocytes can indicate fragmentation injury associated with microangiopathic hemolysis (DIC, vasculitis, HSA) or mechanical fragility (iron deficiency anemia), oxidant injury or liver disease.

Eccentrocytes - RBC with dense Hb contracted to one side of the cell leaving a pale eccentric area formed as a result of direct oxidative damage to the RBC membrane and cytoskeleton. The prevalence of eccentrocytosis in diseased dogs is generally low (1.4%) and is associated with drug administration, onion/garlic ingestion, vitamin K antagonist intoxication, diabetes mellitus, T-cell lymphoma and severe infections with young dogs and whippets more likely to be affected (Caldin et al. 2005).

When the thin membrane of the eccentrocyte is removed or ruptures, a small cell lacking central pallor is formed. These “pyknocytes” can be mistaken for spherocytes.

Spherocytes - erythrocytes of a spheric form that appear smaller in diameter and more dense with reduced area of central pallor. They are produced when antibody coating RBC bind to
the Fc portion of macrophages resulting in partial phagocytosis of the RBC. The remnant has a reduced surface area to volume ratio and assumes a sphere shape. Moderate to marked spherocytosis is characteristic of immune-mediated hemolytic anemia. Although low numbers can be seen in some dogs and others may have no spherocytes at all. In dogs with regenerative IMHA spherocytosis is seen in 89% but there are not as frequently seen in non-regenerative forms of IMHA or the precursor-directed IMHA (Weinkle et al. 2005). Low numbers of spherocytes can be seen in many other acquired diseases as fragmentation anemias, oxidative injury, coral snake envenomation, bee stings, hemophagocytic syndrome.

*Target cells* (codocytes or leptocytes) - RBC having a condensed Hb within the area of normal central pallor, resembling a “bullseye” and reflecting an increased surface to volume ratio. Their deformability is normal but they are more resistant to osmotic lysis. Increased numbers of target cells can be an indicator of a balanced increase in cholesterol and phospholipids in the RBC membrane occurring in liver disorders or hypothyroidism (Goodfellow et al. 2008).

*Stomatocytes* - RBC with an elongated “mouth-like” or “slit-like” area of central pallor. An occasional stomatocyte might be seen in several pathological conditions (liver disease, lead poisoning) or with higher percentage (up to 19%) in hereditary stomatocytosis (Bonfanti et al. 2004). Stomatocytes are usually macrocytic and hypochromic, osmotically and mechanically fragile because of the increased volume to surface area ratio.

*Elliptocytes* - elongated RBC. Elliptocytosis can indicate liver disease (lipidosis in cats), myelofibrosis (non-regenerative forms of IMHA) or inherited/congenital red blood cell membrane abnormality.

*Drepanocytes* - sickle-shaped RBC. The sickling is thought to be an *in vitro* phenomenon due to variants of hemoglobin forming insoluble, elongated polymers when blood exposed to oxygen or due to alkalosis.

**Inclusions:**

*Howell-Jolly bodies* - small nuclear remnants persisting usually as a single basophilic perfectly round inclusion within the cytoplasm. In canine blood they are not routinely observed because they are effectively removed in the spleen. Occasionally they can be seen in healthy cats because of the non-sinusoidal spleen which is less efficient at removing such RBC. In both species Howell-Jolly bodies are commonly observed in regenerative anemias, compromised or absent splenic function (corticosteroids, splenectomy) or erythroid dysplasia (myelodysplastic syndrome).

*Basophilic stippling* - small aggregations of ribosomal RNA in the cytoplasm that may occur in regenerative anemias, lead poisoning or dyserythropoiesis (Williams and Williams 1990, Lukaszewska and Lewandowski 2008).

*Cabot rings* - rarely observed ellipsoid or figure 8 structures originating from mitotic spindles described in association with severe dyserythropoiesis (Lukaszewska and Lewandowski 2008).

*Heinz bodies* - precipitation of hemoglobin as a result of oxidative injury in form of a round inclusion(s) attached to the inner erythrocyte membrane usually extending above the surface. Small Heinz bodies can be observed in healthy cats (in amount ≤10%), but not in blood from healthy dogs. They stain prominently with new methylene blue or brilliant cresyl blue. RBC containing Heinz bodies distorting the membrane have reduced lifespan. Increased numbers of Heinz bodies may be seen in cats with diabetes mellitus, lymphoma or hyperthyroidism. In dogs Heinz body formation is associated with onion and garlic ingestion, zink, copper and
skunk mask toxicity as well as propofol, vitamin K₃, benzocaine, phenylhydrazine or acetaminophen administration (Yamato et al. 2005, Zaks et al. 2005, Bexfield et al. 2007).

**Nucleated RBC:**
Nucleated RBC are most commonly identified in the context of regenerative (mainly hemolytic) anemia. However they can be seen in many other conditions without anemia as altered splenic function, bone marrow disorders, heat stroke, septicemia/endotoxemia, lead poisoning and different organ diseases. Their finding reflects increased erythropoiesis, decreased removal from circulation or bone marrow injury. Erythroblastosis occurring in almost 42% of dogs with systemic inflammatory response syndrome is associated with significantly higher mortality (Müller et al. 2014). With 90% positive dogs evidence of NRBC was the most prevalent abnormality in patients with heat stroke with significantly higher NRBC and proportion of rubricytes found in non survivals (Aroch et al. 2009).