All Cells that end well- A Guide to Abnormal CBCs

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With the utilization of automated blood cell counting machines, other tests are often included as part of the CBC. Test such as the platelet count, red cell indices, reticulocyte count, etc... The three tests of the CBC which indicate if anemia is present are the RBC count, PCV, and Hemoglobin determinations. These values give the numeric factors related to the red blood cells. From these test results the volume (size), weight of hemoglobin, and the concentration of hemoglobin in just one red blood cell can be calculated. These tests are referred to as the red blood cell indices. The RBC count is decreased in all types of anemia's, certain leukemia's, and after or during hemorrhage. It may also be decreased after fluid therapy. It can be increased above normal in cases of dehydration, certain heart diseases, some types of acute poisoning and sometimes iron therapy. Some species and breeds of animals have high counts normally such as goats and greyhounds. Some animals are born with excessively high red cell counts; this is referred to as primary polycythemia. Others may develop this type of condition later in life as a result of a disease process; this is referred to as secondary polycythemia.

The Red Blood Cell

The primary function of red blood cells is exchange of gases between the tissue and the lungs. To help with this exchange, the red blood cells contain a gas-carrying protein called hemoglobin, surrounded by a protective cell membrane. Because hemoglobin is composed of protein attached to an iron molecule, the red blood cell can also be seen as an area of iron storage. Hemoglobin is the pigment that gives RBCs their red color. Red blood cells are technically called **erythrocytes**. They are manufactured in the bone marrow. The formation of red blood cells is called **erythropoiesis**. This process is subject to a feedback control which is dependent on the hormone **erythropoietin**, which is produced by the kidneys. This hormone stimulates the bone marrow to produce new erythrocytes; it does not stimulate white blood cell or platelet production. When the count is consistently low the animal is considered to be **anemic**. If the patient has a persistently high RBC count, this is referred to as **polycythemia**. Examination of the erythrocyte morphology is an important part of every blood smear differential exam. In carrying out these observations the following characteristics should be evaluated: size, shape, color, presence of inclusions and cellular arrangement. The red cell evaluation should be carried out in an area where the cells are evenly distributed and not crowded and overlapping (the monolayer of the smear). Any variation from normal should be noted, graded and reported. Abnormalities should be graded using the terms: occasional, slight, moderate or marked; or by using a numerical scale 1+, 2+, 3+, 4+. The terms used for each factor are as follows:

Erythrocyte Size

A. Anisocytosis - variation in size, both smaller and larger than normal.
B. Microcytosis - smaller than normal in size.
C. Macrocytosis - larger than normal in size.

Erythrocyte Inclusions

A. Howell-Jolly Bodies - nuclear remnants
B. Heinz Bodies - denatured hemoglobin caused by oxidative damage
C. Basophilic Stippling

D. Distemper inclusion bodies - found in distemper
E. Parasites
G. Nucleated RBCs - young erythrocyte containing nucleus

Erythrocyte Shape

A. Poikilocytosis- changes in cellular shape
B. Rouleaux- stacking of cells commonly seen in cats and horses
C. Agglutination- clumping together of cells

Hematopoiesis is the process of blood formation primarily in the bone marrow. This procedure involves the actual formation of the red and white blood cells in addition to platelets. The hematopoietic system is composed of a number of organs having functions in addition to blood formation. This system is widely distributed throughout the body and the organs involved are components of other systems. Extramedullary hematopoiesis is the formation and development of blood cells outside the bone marrow, as in the spleen, liver, or lymph nodes.

Bone marrow: produces erythrocytes and granulocytic leukocytes including neutrophils, eosinophils, and basophils; produces platelets, monocytes, lymphocytes (primarily B lymphocytes), stores iron
Lymph nodes: stores and recirculates lymphocytes.
Spleen: stores erythrocytes; destroys old erythrocytes; extramedullary hematopoiesis
Liver: stores iron; converts free bilirubin (hemoglobin) to direct bilirubin for excretion by the body; extramedullary hematopoiesis
Kidney: produces erythropoietin which stimulates RBC production by the bone marrow
Reticuloendothelium system (RES): A diffuse system constituting all phagocytic cells of the body except granulocytes including the cells lining the sinusoids of the spleen, lymph nodes, and bone marrow along with the fibroblastic reticular cells of hematopoietic tissues.
Thymus: produces lymphocytes (primarily T lymphocytes)

<u>The Granular Leukocytes</u>:

<u>The segmented neutrophil</u> - This cell is classified as a granular white cell because the cytoplasm contains granules. Its function is defense and phagocytosis in the body. The neutrophil is responsible for phagocytosis and destruction of invading organisms. It is active against bacteria, fungi, yeast, algae, parasites and viruses. It has a distinctly lobed nucleus contained in the cytoplasm. The nucleus will stain purple, the cytoplasm granules will be blue and pink (depending on the species). Some animals show more bluish granules while others will stain pinker; a combination of two can be seen depending on the acidic and basic content of their cells. The number of lobes found on the nucleus varies with the species. Some may have four or more lobes. Segmented neutrophils are sometimes referred to as polys, segs, and polymorphonuclear (PMNs) cells. The band neutrophil is an immature neutrophil which is found in very low numbers in the blood normally. It can be found in the range of zero to four in normal healthy patients. In immature neutrophils the cytoplasm is granular with a non-lobed nucleus. It is usually in the form of a horse-shoe shape but can have other shapes as the cell continues to mature. This cell is sometimes referred to as a “stab” or “band” or “non-seg” because the nucleus has not formed any distinct lobes. Some laboratories refer to any stage of immature neutrophils as non-segs.

<u>The eosinophil</u> - This cell is classified as a granular white cell because the cytoplasm contains large

granules. It is a major component of systemic hypersensitivity reactions. The granules found within the cell contain various enzymes, cytotoxic proteins and cytokine mediators. One of these enzymes is *histaminase* which inactivates histamine. Eosinophils have a major role in parasites. This cell has a lobed nucleus which can consist of two or more lobes. The cytoplasm contains large granules in which the number and intensity will vary between species. The nucleus stains a purple color and the granules will stain a distinct red color.

The basophil - This cell is classified as a granular white cell because the cytoplasm contains granules. The granules contain histamine, heparin and other substances released in response to the presence of allergens. Basophilia often occurs concurrently with eosinophilia. This cell helps with immediate hypersensitivity reactions, synthesis of several cytokines that initiate or modulate inflammatory response, and inhibits coagulation through the release of heparin. It has a lobed nucleus which can consist of two or more lobes depending on the species. The cytoplasm contains large granules that stain a blue-black color and the cytoplasm will stain dark purple.

The Agranular Leukocytes –

Lymphocyte - This white cell is classified as an agranular cell because the cytoplasm contains no obvious granules. Lymphocytes are composed of T cells and B cells. T cells are typically found throughout the blood and lymph system, B cells are typically found in secondary lymph tissue (lymph nodes). No morphologic differences can be seen between these two cells. B cells differentiate into plasma cells, T cells form and release cytokines. Peripheral lymphocytes serve as memory cells for the immune system. The cell is round with a smooth round nucleus. The cytoplasm is smooth. The nucleus stains a dark purple with the cytoplasm staining a light blue. The cell can be small or large depending on the age of the cell and the species of animal. This cell functions in antibody production. Reactive lymphocytes represent a response to antigenic stimulation. These cells are larger than neutrophils, vary in size and have a dark blue cytoplasm.

Monocyte- This white cell is classified as an agranular cell because the cytoplasm contains no obvious granules. In defense against microbial invasion, they are less efficient phagocytes than neutrophils. They are involved in the initiation of the immune response. The nucleus is large and usually of a kidney bean to segmented shape with a rough texture. The cytoplasm stains a gray to blue-gray color. The nucleus stains a lacey purple color. Monocytes may also contain vacuoles in the cytoplasm. It is a large, irregular shaped cell with small projections extending from the cytoplasm. Circulating monocytes can differentiate into macrophages if there is a demand for phagocytosis in the blood. The nuclei become round to oval in shape, the cytoplasm is more abundant, and vacuoles become more prominent and may contain phagocytized material. If blood is allowed to stand in EDTA, some monocytes differentiate into macrophages and phagocytize damaged RBCs and even other leukocytes

Erythrocytes - These cells stain a brownish red. In mammals they do not contain a nucleus when mature, immature cells may contain nuclear fragments or even a whole nucleus. Avian and reptiles have nucleated mature red cells in their circulation.

Thrombocytes - These are not individual cells but rather cellular fragments of a megakaryocyte which stain a light blue on a blood smear. Formation of platelet plugs help stop bleeding, contribute materials for secondary hemostasis and fibrin formation, promote vascular healing through platelet derived growth factor (PDGF), stimulates endothelial cell migration and smooth muscle production.

Some animals have small platelets while others have bigger platelets with some granulation. Avians

and reptiles have large nucleated platelets. Platelets can occur in clumps in instances of drawing trauma, delayed addition of anticoagulant, improper anticoagulant/blood ratio and various disease processes.

Terminology:
Shift to the left - occurs when there is an increase in the differential count of immature segmented neutrophils (bands, metamyelocytes, myelocytes, etc...).
When there is an increase above normal of the granulocytes the ending is "philia"

Neutrophilia - increase in neutrophils
Eosinophilia - increase in eosinophils
Basophilia - increase in basophils

When there is an increase in the agranulocytes the ending is "cytosis".

Lymphocytosis - increase in lymphocytes
Monocytosis - increase in monocytes
Erythrocytosis - increase in erythrocytes
Thrombocytosis - increase in thrombocytes

The ending "penia" is used to denote a decrease in a particular cell type.

Neutropenia - decrease in neutrophils
Lymphopenia - decrease in lymphocytes
Erythropenia - decrease in erythrocytes
Thrombocytopenia - decrease in thrombocytes

The Differential Blood Smear
One of the most important tests performed in hematology is the differential blood smear. This procedure involves making a smear of blood on a microscope slide, air drying and staining it. Observations of the erythrocytes, thrombocytes and leukocytes are made microscopically under oil immersion. Three factors are accomplished:

1. Classification of WBCs
2. Evaluation of RBCs
3. Estimation of platelet numbers

Nucleated RBC Correction
When more than 5 nucleated red blood cells (nRBC) are found on the differential smear, the WBC count must be corrected! nRBC's are not lysed with the diluting fluids normally used to perform the WBC count. They are counted as WBCs on the manual hemacytometer and automated counters artificially increasing the WBC count. A separate count of nRBC's must be conducted as the 100 WBCs are classified on the differential count. The number of nRBCs is counted during the WBC differential. The total is used to correct the leukocyte count of the patient using the formula given below. The formula is used when there are more than 5 nRBCs per 100 white cells counted. It is normal to find up to 5 nRBCs in blood.

$$\frac{\textbf{WBC count x 100}}{\textbf{100 + number of NRBC's}} = \textbf{Corrected WBC count}$$

References:
Sirois, Margi. Laboratory Procedures for Veterinary Technicians 6th ed. Mosby Elsevier 2015