Erythrocytes

A. Erythrocyte maturation

• Red blood cells are the predominant cell type in the peripheral blood.

• They originate in the bone marrow (and other sites of hematopoiesis) where they mature until they are released into the peripheral blood.

• The process of erythrocyte maturation is called erythropoiesis.

• Cell size decreases during RBC maturation.

• Mature RBCs have a lower mean cell volume (MCV) parameter than immature RBCs.

• Adequate erythropoiesis requires production of erythropoietin (EPO).

• Most EPO is produced by renal tubular epithelial cells. Kidney disease may prevent adequate production of EPO that can lead to abnormally low numbers of RBCs in the peripheral blood.

• Excess production of EPO is rare but may occur with some neoplastic diseases. Excess levels of EPO may cause a patient to have abnormally high numbers of RBCs in the peripheral blood.

• Androgen increase Epo release. In contrast, estrogens and corticosteroids decrease Epo release, but their effect is probably not clinically significant.

• As erythrocytes mature, the cells become smaller, the cytoplasm stains less blue (basophilic) and more red (eosinophilic) with Romanowski-type stains, and the nucleus condenses.

1. Rubriblasts are the first identifiable immature form of an RBC. These cells are relatively large and have scant, deeply basophilic cytoplasm, a round nucleus, and a prominent nucleolus. Abnormal release of rubriblasts into the peripheral blood is extremely rare but can occur with acute erythroid leukemia.
2. Rubricytes are more mature and slightly smaller than rubriblasts but are larger and less mature than metarubricytes. Their cytoplasm is slightly less basophilic than a rubriblast and they have a round nucleus, but they lack a nucleolus.
3. Metarubricytes also are called nucleated red blood cells (nRBCs). These cells are more mature and smaller than rubricytes. Metarubricytes are similar in size

to reticulocytes which are only slightly larger than mature erythrocytes. They have polychromatophilic cytoplasm. nRBCs contain a small, very condensed nucleus. nRBCs can be released into the peripheral blood when an animal is anemic or is hypoxic. Inappropriate release of nRBCs is associated with various splenic and bone marrow diseases as well as with iron deficiency, and copper deficiency.

4. Reticulocytes are just slightly larger and slightly more basophilic than mature RBCs. They have polychromatophilic cytoplasm. Reticulocytes are expected to be released into the peripheral blood when an animal is anemic or hypoxic. Healthy cats have less than 0.5% aggregate reticulocytes in the peripheral blood. Healthy dogs have less than 1% aggregate reticulocytes in the peripheral blood.
5. Mature RBCs of mammals are small, biconcave, eosinophilic cells that lack a nucleus. They contain a large amount of *hemoglobin* (Hb) that contributes to the red coloration of RBCs. Feline, equine , and bovine RBCs are relatively small, making it difficult to see central pallor in the RBCs. The diameter of a mature canine RBC is approximately 7.0 mm, which is large enough to appreciate central pallor in canine RBCs. Mature RBCs circulate in blood vessels (life spin) for approximately 73 days in cats, 100 days in dogs, 145 days in horses, 150 in sheep days , and 160 days in cattle before they are removed from circulation and replaced by newly formed, mature RBCs. In health the senescent RBC are removed from circulation by Phagocytosis and Intravascular lysis

B. Erythrocyte functions

RBCs are vitally important cells found in all vertebrate animals. RBCs transport O_2 to all cells of the body so that oxidative metabolism can occur. RBCs also transport carbon dioxide (CO_2), a toxic by-product of oxidative metabolism, away from cells. In addition, RBCs help to maintain the pH of the blood within a narrow window to optimize the chemical reactions that occur in the body.

C. Erythrocyte parameters and indices

• The number of RBCs present in a blood sample, the size of the RBCs, and the amount of Hb present within the RBCs are examples of erythrocyte parameters and indices that are reported in a CBC.

• Erythrocyte parameters include hematocrit (Hct), Hb concentration, MCV, MCH, MCHC, and RBC distribution width (RDW).

• PCV is measured after centrifugation of whole blood and indicates the percentage of the blood volume composed of cells (including erythrocytes, leukocytes, and platelets).

• Hb is measured by colorimetric techniques or by determining the optical density of oxyhemoglobin in the sample. Automated hematology analyzers report the Hb concentration in a whole blood sample.

• The RBC count can be determined using a hemocytometer; however, automated counters are more accurate for mammalian species.

• Factors that decrease PCV, Hct, Hb, and RBC count include anemia and overhydration.

• Factors that increase PCV, Hct, Hb, and RBC count include dehydration, splenic contraction, and absolute polycythemia.

• Mean corpuscular/cell volume (MCV) is measured directly by automated cell counters. MCV can be calculated using the formula: MCV (fL) = [Hct (%) $\times$ 10]$\div$ RBC count (millions/ μ L). The MCV of RBCs varies greatly with species. Patients with a decreased MCV have a microcytosis. The term for an increased MCV is macrocytosis.

• Mean corpuscular/cell hemoglobin concentration (MCHC) is a measurement of RBC hemoglobin content that corrects for RBC volume. It can be calculated using the formula: MCHC (g/dL) = [Hb (g/dL) $\times$ 100] $\div$ Hct (%). RBCs with a low MCHC are hypochromic.

a) Causes of hypochromasia include reticulocytosis, iron deficiency, copper deficiency, and lead toxicity.
b) Causes of hyperchromasia include hemolysis and oxyglobin administration. However true hyperchromasia (a red blood cell containing increased amounts of Hb) does not occur.

• Mean corpuscular hemoglobin (MCH) is the average amount of Hb in RBCs. The calculation for MCH is: MCH (pg) = [Hb (g/dL) $\times$ 10] $\div$ RBC count (millions/ μ L). MCH and MCHC are similarly affected by disease.

• Red cell distribution width (RDW) is determined by some automated cell counters. It indicates the degree of anisocytosis (size difference) in RBCs.

RDW = [(standard deviation of the MCV) ÷ MCV] × 100. Either microcytosis or macrocytosis may increase RDW.

D. Erythrocyte arrangement

• In healthy animals, RBCs are evenly distributed throughout the monolayer of a blood smear.

• Rouleaux occurs when RBCs are stacked in lines. Rouleaux is common in horses that have a decreased membrane charge in health. Moderate and mild rouleaux may be present in feline and canine blood in health, respectively. Marked rouleaux may be observed during inflammatory and neoplastic diseases. Increased fibrinogen and globulin concentrations may cause rouleaux.

• Agglutination is the aggregation of RBCs into grape-like clusters. Agglutination is an abnormal finding in any species. Agglutinated RBCs do not disperse when blood is diluted with saline and indicates ongoing immune-mediated disease.

E. Erythrocyte morphology

• Abnormalities in the size and shape of RBCs in a blood smear have been associated with specific disease processes.

• Anisocytosis is the term for differences in cell size (microcytes and macrocytes). In healthy patients, RBCs will be the same diameter throughout the blood smear.

• Basophilic stippling occurs when dense aggregates of residual RNA remain in immature RBCs.

• nRBCs are metarubricytes. Appropriate nRBC release is observed in strongly regenerative anemia and hypoxic conditions. Inappropriate nRBC release is associated with abnormal RBC maturation and diseases of the bone marrow and spleen.

• Howell–Jolly bodies are basophilic nuclear remnants in RBCs.

• Heinz bodies are areas of denatured and precipitated Hb that are caused by oxidative damage to the RBC. Heinz bodies stain pale red with Romanowski-type stains and stain blue with NMB stain .

• Poikilocytosis is the term for varied RBC shapes. RBCs of healthy cats, dogs, horses, and cattle appear round on a peripheral blood smear. Specific types of shape changes are associated with specific disease processes.

1. Dacryocytes Are teardrop-shaped erythrocytes that may result from the inability of the erythrocyte to return to its pre-existing shape after deforming in the blood vessels.
2. Acanthocytes are RBCs with irregular spicules. This type of poikilocyte is caused by an altered ratio of lipid to cholesterol molecules in the RBC membrane. Differential diagnoses include diseases such as liver disease, cancer, and disseminated intravascular coagulation (DIC).
3. Schistocytes are RBC fragments that indicate the RBCs have been sheared by intravascular fibrin or turbulent blood flow.
4. Spherocytes are rounded RBCs with a normal MCV but a smaller appearance on a blood smear. Spherocytes are not evaluated in feline, equine, and bovine blood because the RBC diameter of these species is already small. Spherocytes in dogs are smaller in diameter and lack the central pallor that can be seen in normal canine RBCs. The top differential diagnosis in a dog with large numbers of spherocytosis should be hemolytic anemia.
5. Ghost cells are pale remnants of RBCs that are lysed within blood vessels during intravascular hemolysis.
6. Target cells (codocytes) are erythrocytes that have deeply stained (pink) centers and borders, separated by a pale ring, giving them a target-like appearance. They are associated with liver disease, iron deficiency anemia.
7. Stomatocytes have a thick ring of Hb around the edges of the RBC and an oval area of central pallor. Diseases associated with Stomatocytes formation include iron deficiency and liver diseases.
8. Sickle cell, cell with a sickle or crescent shape, caused by the presence of a high concentration of an abnormal haemoglobin known as haemoglobin S

F. Erythron

The erythron is the portion of a CBC that reports RBC numbers, indices, and/or morphology. Here, the medical terminology used to describe abnormalities in the erythron.

I. Polycythemia (Erythrocytosis)

Is an increase in the Hct, RBC count, and Hb concentration.

1. Relative polycythemia : erythrocytosis occurs because of relative including

a. Dehydration

A decrease in plasma volume causes a relative increase in the Hct, RBC count, Hb concentration and plasma protein concentration. Mechanisms of relative polycythemia including water loss caused by vomiting, and diarrhea.

b. Redistribution of RBC

Excitement causes epinephrine release and splenic contraction, which lead to high Hct in general circulation. This effect is common in the horse and cat.

2. Absolute polycythemia: increase erythropoiesis expands the total RBC mass.

a. Primary absolute pol. : is a myeloproliferative disorder of stem cells.

b. Secondary absolute polycythemia: is caused by increased erythropoietin secretion.

1. Appropriate compensatory erythropoietin secretion occurs during chronic hypoxia (low PO_2) which occurs in the following instances :

a. high altitude
b. Chronic pulmonary disease
c. Cardiovascular anomalies

2. Inappropriate erythropoietin secretion (normal PO_2, no hypoxia) occur in some cases of hydronephrosis or renal cysts , Epo secretion neoplasms .

II. Anemia

defined as a decreased number of RBCs in the peripheral blood. This causes a decrease in Hct, RBC count, and Hb. Many disease processes cause anemia. Overhydration can also decrease Hct, RBC count, and Hb due to the increased amount of fluid in the plasma, which decreases the number of erythrocytes per microliter of blood. Typical clinical signs of anemia include pale mucous membranes, weakness, tachycardia, syncope, weak pulse, increased sensitivity to cold, and shock.

Classification

Can be classified according to

1. Morphological classification

a. Macrocytic anemia

- Macrocytic normochromic
- Macrocytic hypochromic

b. Normocytic anemia

- Normocytic normochromic
- Normocytic hypochromic

c. Microcytic anemia

- Microcytic normochromic
- Microcytic hypochromic

2. Etiological anemia

a. Hemorrhagic anemia (Blood loss anemia)
b. Hemolytic anemia: excessive destruction of RBC or shortened life span.
c. Aplastic anemia: depression of bone marrow.
d. Nutrition anemia: nutritional deficiency.

3. Classification according to bone marrow response.

a. Regenerative anemia (responding)

b. Non regenerative anemia (non responding)

Laboratory findings

1. Hemorrhagic anemia : anemia occurs following acute and sub-acute hemorrhages and in generally as normocytic

a. Signs of increased bone marrow activity are present (nucleated RBC appear in peripheral circulation 72- 96 hrs following blood loss).

b. The morphological changes include anisocytosis and poikilocytosis .

c. The degree of dropin total hemoglobin level is dependent up on the quantity of blood cell lost.

2. hemolytic anemia : the laboratory finding depending up on the amount of blood destroyed and the rate of destruction such as Babeseosis, laptospirosis, Bacellary Hb-uria, Equine infection anemia, copper and lead poisoning , postpurturent Hb- uria .

a. If destruction rapid the number of destruction RBC is great free Hb my be present in plasma and Hb- uria may or my not appear .

b. Icterus is not always present.

c. Signs of increased bone marrow activity is present ,this activity is manifested morphologically by anisocytosis, poikilocytosis, reticulocytosis, polychromatophilia, basophilic stippling and the appearance of nucleated RBC in the peripheral circulation .

d. Leukocytesis and thrombocytosis are may be present.

3. Bone marrow depression : non regenerative anemia associated with depression of the bone marrow are caused by several factors including physical and chemical agents such as exposure to X rays, bracken fern poisoning, excess administration of estrogen .

Laboratory finding

1. A decrease of erythropoiesis in bone marrow is usually lead to progressive fall in the total RBC count and Hb content.
2. Decrease in total leukocytosis observed.
3. Examination of HCT tube will often reveal clear, colorless and watery plasma.
4. Reticulocytes and nucleated RBC are not observed in the peripheral circulation.