

## Erythrocytes

### A. Erythrocyte maturation

1. Red blood cells are the predominant cell type in the peripheral blood.
2. They originate in the bone marrow (and other sites of hematopoiesis) where they mature until they are released into the peripheral blood.
3. 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.
4. 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.
5. 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.
  - a. 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.
  - b. 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.
  - c. 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 lead toxicity, iron deficiency, and copper deficiency.
  - d. Reticulocytes are just slightly larger and slightly more basophilic than mature RBCs. Owing to the combination of blue and red staining in these cells, they commonly are

called polychromatophils. 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.

- e. 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. There is an area of central pallor in the RBCs of some species, which is caused by the biconcave shape of the erythrocytes. The diameter of a mature canine RBC is approximately 7.0  $\mu\text{m}$ , Feline 5.8  $\mu\text{m}$ , Bovine 5.5  $\mu\text{m}$ , Equine 5.7  $\mu\text{m}$ . Mature RBCs circulate in blood vessels for approximately 73 days in cats, 100 days in dogs, 145 days in horses, and 160 days in cattle before they are removed from circulation and replaced by newly formed, mature RBCs.

## **B. Erythrocyte functions**

RBCs are vitally important cells found in all vertebrate animals. RBCs transport  $\text{O}_2$  to all cells of the body. RBCs also transport carbon dioxide ( $\text{CO}_2$ ) 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 indices**

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

2. Mean corpuscular hemoglobin (MCH) is the average amount of Hb in RBCs. The calculation for MCH is:  $\text{MCH (pg)} = [\text{Hb (g/dL)} \times 10] \div \text{RBC count (millions}/\mu\text{L})$ .

3. 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:  $\text{MCHC (g/dL)} = [\text{Hb (g/dL)} \times 100] \div \text{Hct (\%)}$ . RBCs with a low MCHC are hypochromic. RBCs with increase MCHC are polychromic

- Causes of hypochromasia include, iron deficiency, copper deficiency, and lead toxicity.
- Causes of hyperchromasia include hemolysis and oxyglobin administration.

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

$RDW = [(standard\ deviation\ of\ the\ MCV) \div MCV] \times 100.$

#### **D. Erythrocyte arrangement**

1. In healthy animals, RBCs are evenly distributed throughout the monolayer of a blood smear.
2. Rouleaux occurs when RBCs are stacked in lines. Rouleaux is common in horses that have a decreased membrane charge in health. In certain diseases, normal membrane surface charge may be partially masked by excess protein (hyperfibrinogenemia, hyperglobulinemia) that decreases the repelling negative surface charges of the erythrocytes. Rouleaux and an increased ESR will be observed.
3. 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**

1. Abnormalities in the size and shape of RBCs in a blood smear have been associated with specific disease processes.
2. 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.
3. Basophilic stippling occurs when dense aggregates of residual RNA remain in immature RBCs.
4. nRBCs are metarubricytes. Appropriate nRBC release is observed in strongly regenerative anemia and hypoxic conditions. Inappropriate nRBC release is associated with diseases of the bone marrow and spleen.
5. Howell–Jolly bodies are basophilic nuclear remnants in RBCs.
6. Heinz bodies are areas of denatured and precipitated Hb that are caused by oxidative damage to the RBC. Heinz body formation can lead to intravascular hemolysis.
7. 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.
  - a) Echinocytes (crenate erythrocytes) are the most common type of poikilocyte in a peripheral blood smear. These cells are RBCs that have several evenly spaced

spicules. This shape is nearly always due to drying artifact that occurs during preparation of the blood smear.

- b) 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).
- c) Schistocytes are RBC fragments that indicate the RBCs have been sheared by intravascular fibrin or turbulent blood flow .
- d) 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.
- e) Eccentrocytes are RBCs with Hb condensed to one side of the cell. This is caused by oxidative damage to the Hb molecule.
- f) Keratocytes contain a torn vesicle to one side of the RBC and often form during oxidative damage to RBCs.
- g) Ghost cells are pale remnants of RBCs that are lysed within blood vessels during intravascular hemolysis .
- h) Leptocytes are thin RBCs with an increased area of central pallor . These cells often appear folded on themselves. Diseases associated with leptocyte formation include iron deficiency and liver diseases.
- i) Target cells (codocytes) are leptocytes that look like a target with Hb around the cell edges and at the center of the cell.
- j) Stomatocytes have a thick ring of Hb around the edges of the RBC and an oval area of central pallor.

## **F. Erythron**

The erythron is the portion of a CBC that reports RBC numbers, indices, and/or morphology.

### **1. Anemia**

Is 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.

### **Classification**

A. Classification according to size (MCV) and Hb concentration (MCHC) of the erythrocyte

1. The MCV categorizes the anemia as normocytic, macrocytic, or microcytic. The average erythrocyte volume is within the reference interval, increased, or decreased, respectively.

2. The MCHC categorizes the anemia as normochromic, hypochromic, or hyperchromic. The Hb concentration is within the reference interval if the erythrocytes are normochromic. Hb concentration is decreased if the erythrocytes are hypochromic. Hb concentration is increased if the erythrocytes are Hyperchromasia.

B. Classification according to bone marrow response

1. Regenerative anemia

The bone marrow actively responds to the anemia by increasing production of erythrocytes.

### **Laboratory findings**

- (1) Polychromasia
- (2) Reticulocytosis with anisocytosis. Macrocytosis increased (MCV)
- (3) hypochromasia decreased (MCH and MCHC)
- (4) Basophilic stippling of erythrocytes in ruminants

2. Nonregenerative anemia



Nonregenerative anemia suggests the lack of an erythroid response in the bone marrow. Lack of response could be the result of inadequate time for erythropoiesis to occur as well as conditions such as chronic inflammation, renal disease, and endocrine disorders.

#### 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. Polychromasia, reticulocytosis, and basophilic stippling are inadequate to absent.

#### C. Classification according to major pathophysiologic mechanisms

1. Blood loss( hemorrhagic anemia)
2. Accelerated erythrocyte destruction by intra-or extravascular hemolysis (Hemolytic anemia)
3. Reduced or defective erythropoiesis

#### **Anemia from blood loss (hemorrhagic anemia)**

Anemia occurs following acute and sub-acute hemorrhages and in generally as normocytic

#### Laboratory findings

1. The Hct initially is within the reference interval because all blood components (i.e., cells and plasma) are lost in similar proportions.
2. Hypoproteinemia (decreased plasma protein concentration) also may be observed.
3. Thrombocytosis, Platelet numbers usually increase during the first few hours after hemorrhage.
4. Neutrophilic leukocytosis
5. Signs of increased erythrocyte production (e.g., polychromasia, reticulocytosis) become evident by 48 to 72 hours

#### **Hemolytic anemia**

The laboratory finding depending up on the amount of blood destroyed and the rate of destruction such as Babesiosis, leptospirosis, Bacillary Hb-uria, Equine infection anemia, copper and lead poisoning , postpartum Hb- uria .

## Laboratory findings

1. Plasma protein concentration is within the reference interval or increased (hyperproteinemia).
2. Neutrophilic leukocytosis and monocytosis may occur.
3. Evidence of Hb degradation may be present.
4. Abnormal erythrocyte morphology ( poikilocytes e.g., Heinz bodies, spherocytes, polychromatophilia, reticulocytosis , basophilic stippling and the appearance of nucleated RBC in the peripheral circulation,).

## **2. Polycythemia**

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

### **I. Relative polycythemia.**

The total RBC mass is normal. Causes of relative polycythemia include the following:

#### **A. Dehydration**

1. A decrease in plasma volume causes a relative increase in the Hct, RBC count, Hb concentration and plasma protein concentration.

#### **2. Mechanisms of relative polycythemia**

a. Water loss caused by vomiting, diarrhea, excessive diuresis, water deprivation, perspiration.

b. Internal fluid loss in shock via increased vascular permeability

c. Loss of fluid by effusion into body cavities

#### **B. Redistribution of erythrocytes**

1. Excitement causes epinephrine release and splenic contraction.

2. This effect is common in the horse and cat.

### **II. Absolute polycythemia**

Increased erythropoiesis expands the total RBC mass. Plasma volume and plasma protein concentration are within the reference interval.

A. Primary absolute polycythemia ( polycythemia vera or primary erythrocytosis) is a myeloproliferative disorder of stem cells.

1. Clinical pathology findings include the following:

- a. Erythropoietin concentration is within the reference interval or decreased.
- b.  $PO^2$  is within the reference interval.
- c. Thrombocytosis and leukocytosis occasionally accompany the erythrocytosis.

B. Secondary absolute polycythemia is caused by increased Epo secretion.

1. Appropriate, compensatory Epo 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 Epo secretion ( normal  $PO^2$  , nohypoxia) occur in some cases of hydronephrosis or renal cysts , Epo secretion neoplasms .