**Clinical pathology**

* Clinical pathology: it means application of different lab. methods on a properly collected sample to evaluate the health or to study the disease in the living or dead subjects; and the subsequent use of the obtained results in making accurate diagnosis and prognosis for the solution of the clinical problem. It makes a link between patient, clinician and lab.
* It includes: Clinical haematology, Clinical biochemistry, Clinical parasitology and Clinical microbiology.

CLINICAL HAEMATOLOGY

* It is the study of blood and blood forming organs. In clinical practice, it Includes the evaluation of cellular components of the blood; which is about (45%) of total blood Volume, the rest is plasma, the fluid part of the blood in which cellular components are suspended.
* Plasma is composed of 90% water, 10% dissolved substances as plasma proteins, electrolytes, hormones, vitamins and other organic compounds.

HAEMATOPOIESIS (HAEMOPOIESIS):

It is the process of blood cell production and platelets which continues throughout life, replacing aged cells (which are removed from the circulation).

In prenatal life:

1. First quarter of gestation: It takes place in the yolk sac, outside the embryo.
2. In the second quarter of gestation: The liver mainly takes part and to a lesser extent the spleen, start of the bone marrow and lymphoid organs to take part in haematopoiesis in mammals.
3. At the time of birth nearly all blood cells are produced in the bone marrow (MEDULLARY HAEMATOPOIESIS) and haematopoiesis immediately or gradually stops in the liver and spleen.
4. In certain disease conditions, when there is great need for blood cells these two organs retain their ability for manufacturing blood cells (EXTRA MEDULLARY HAEMATOPOIESIS).

BONE MARROW

It is a large organ about two thirds of the liver size in dogs .In growing animals bone marrow of all bones is haematopoietically active, when growth stops haematopoiesis remains in the marrow of flat bones (skull, pelvic bones, sternum, vertebrae and ribs) and epiphysis of long bones it stops in the shaft (diaphysis) .The bone marrow has two compartments:

1. The vascular compartment: Composed of blood sinuses lined by special type of endothelial cells and crossed by special type of cells known as the adventitial cell or reticular cell together with macrophages both have important rules in regulating and maintaining haematopoiesis throughout life.
2. The extra –vascular compartment: It is the haemopoietic compartment containing precursors of all blood cells, macrophages, reticular cells which have many pseudopods that may completely encircle the developing blood cells ,in older animals they became loaded with fat producing fatty ,yellow inactive marrow that can turn to active red marrow in need. In addition that there is a group of accessary cells including macrophages, lymphocytes, and natural killer (NK) cells. Also extracellular matrix like collagen.

Haemopoietic growth factors:

They are cellular products produced to regulate and control haemopoiesis:

1. POIETINS:

1. Erythropoietin (EPO); It is a hormone-like circulatory glycoprotein produced mainly in the kidney; lesser amount is produced in kupffer cells in the liver. EPO stimulate erythropoiesis in response to hypoxia.
2. Thrombopoietin (TPO): It is synthesized in the kidney and in the liver; it stimulates platelets production on different levels in the bone marrow (BM).

2.Colony stimulating factors(CSFs):They are glycoproteins act directly on haemopoietic sub – populations in the BM ,produced from adventitial cells, T lymphocytes, macrophages and stromal cells, e.g. stem cell factor(SCF), granulocyte colony stimulating factor(G-CSF), macrophage colony stimulating factor(M-CSF), GM-CSF…..etc.

3. Interleukins: It is a family of proteins produced by different cells (cytokines) like fibroblasts, macrophages, activated T lymphocytes, endothelial cells... Etc. they control some aspects of haematopoiesis & immune response.

Blood cells are:

1. Red blood cells or erythrocytes (RBCs).
2. White blood cells (WBCs):
3. Mononuclear WBCs (monocytes & lymphocytes) also knew as agranulocytes.
4. Polymorphonuclear WBCs (neutrophils, eosinophil, and basophils) also known as granulocytes.

3. Platelets or thrombocytes, they are not true cells but cytoplasmic fragments of the giant BM megakaryocytes.

THE ERYTHRON

* It is a term applied to the circulating RBC mass, RBCs precursors in the bone marrow and erythropoiesis stimulating factors. RBCs, are biconcave discs, actually, they are bags that can deformed into almost any shape. They loss their nuclei before leaving the BM.
* Function: Transport of oxygen to the tissues &transport of carbon dioxide from tissues to the lungs, this is mediate by haemoglobin that fills these corpuscles. In addition rbcs have a buffering activity since they contain large quantities of the enzyme Carbonic anhydrase.
* Haemoglobin (HB) consists of: Haem & Globin, each complete haemoglobin unit is a tetramer or globular unit made up of four subunits; each subunit contains haem conjugated to a polypeptide chain of the globin.
* Haem: is an iron containing porphyrin derivatives (ferrous iron), there are four haem units in each (Hb) molecule.
* Globin : Are polypeptides, two pairs of polypeptides in each haemoglobin molecule, they are of special amino acid sequences .Each two chains are identical 2 α chains and 2 β chains in Adult Hb (Hb -A) .Fetal Hb (Hb- F) contains 2 α and 2 ϒ . Hb-F is soon replaced by Hb-A after birth; Hb type depends on the type of globin chain which is determined by amino acid sequences.
* Oxygen molecule reversibly attached to the ferrous iron in the haem molecule, it is oxygenation and not oxidation, oxidation of ferrous to ferric change haemoglobin to methaemoglobin which is unable to bind oxygen.
* RBCs may be considered as cell membranes containing haem globin and protective enzymes system that is responsible for ATP production, haemoglobin protection and anaerobic glycolysis. RBCs in mammals lack nuclei, ribosomes, mitochondria, rough endoplasmic reticulum, so they depend on anaerobic glycolysis to obtain energy needed.
* Erythropoiesis: pluripotent haemopoietic stem cell (PHSCs) Pluripotent myeloid stem cell (PMSCs) Burst forming unit erythroid (BFU-E) Colony forming unit erythroid (CFU-E) Rubriblast 2-Prorubricytes 4-Rubricytes (basophilic) 8-Rubricytes (Polychromatophilic) 16-Rubricyte (normochromic) 32- Metarubricyte 32-Reticulocyte 32-Erythrocyte.





**Morphology:**

Mammalian RBCs are discoid –shaped.

* Benefits: Flexible, Maximum, surface/volume ratio, Size – uniform.
* Diameter of rbcs varies according to animal sp. that of the dog is the largest and that of the sheep and goats are the smallest.



Evaluation of erythron :

1. RBC count/μl of blood ; decrease in rbcs means anaemia while increase in rbcs means polycythemia.

2. Packed cell volume(PCV%) also known as microhaematocrit value (Hct %) , it is the most efficient method for evaluating the erythron.

3. Meassurment of haemoglobin concentration ( Hb g/dl).

4. Erythrocyte indices: used for typing of anaemia, it is a calculation obtained from rbc count, PCV and Hb values. It includes:

* 1. Mean corpuscular (cell) volume(MCV) = (pcv % × 10)/( count), measured in Femtoliter (fl), increase in MCV indicates macrocytic type of anaemia due to increase in size of rbcs as in responsive anaemia which is characterized by increase in the number of large- sized immature rbcs in the peripheral circulation.
  2. Mean corpuscular (cell) haemoglobin(MCH) = (Hb g/dl x10)/(rbc count) measured in Pico gram (pg).
  3. Mean corpuscular haemoglobin concentration (MCHC) = (Hb g/dl x100)/ (PCV %) it is measured in gram/deciliter (g/dl), decrease in MCHC indicates hypochromic anaemia, increase in MCHC is not detected, if it is observed it is artifactual.

5. Stained peripheral blood film examination:

A. Abnormal rbcs morphology:

i) Abnormalities of shape (Poikilocytosis): It means major deviations from normal shape of erythrocytes for the particular animal sp. (minor deviations are normal).

1. Leptocyte :Thin, flat, hypochromic rbcs with increased surface area and normal cell volume.They are of two types, target and folded cells, mostly observed in chronic diseases, iron deficiency anaemia, haemolytic anaemia, liver diseases, thalassemia. Small number is normal in the blood of dogs.

2. Acanthocyte: Spiculated rbcs with irregularly spaced and variably-sized spicules, it is formed when cholesterol is present in excess to phospholipid in rbc cell membrane. It is mostly due to increase in blood cholesterol, presence of abnormal plasma lipoprotein and liver diseases.

3. Crenated RBC: Spiculated, their spicules are relatively evenly spaced and of similar sizes ,it is considered as an artifact resulting from excess EDTA ,prolonged sample storage or delay in the dryness of blood films.

4. Stomatocytes: Cup -shaped rbcs that have elongated or slit- like central pallor. It is mostly seen thick stained blood films as an artifact or in hereditary stomatosytosis, liver diseases or chronic anaemias.

5. Spherocyte: RBCS lack central pallor with smaller diameter than normal and biconvex surface with spherical shape , it results from cell swelling and/ or loss of part of cell membrane; as in immune mediated haemolytic anaemia e.g. blood parasite infection, snake bite, zinc toxicity, it causes anisocytosis, decrease in MCV.

6. Schistocyte: Fragment of an rbc with two or three pointed extremities, they are smaller than normal rbcs. Its observation in a stained blood may indicates ;severe iron deficiency anaemia , DIC in dogs fibrin strands may split its rbcs, not the smaller rbcs of cats and horses.

7. Sickle cell: Spindle – shaped rbcs ,it is considered normal in deer and young goat(in vitro phenomena due to high o tension + pH between 7.6-7.8. Sickle cell anaemia in man is due to abnormality in amino acid sequences of the β globin chain of haemoglobin (HB-S).

8. Dacrocyte: Tear drop-shaped rbcs with single elongated or pointed extremities Seen in blood of dogs and cats with myeloproliferative disorders and dogs with hypersplenism.

ii) Abnormalities and major deviations in size of rbcs (anisocytosis): Slight anisocytosis is normal in certain animal sp. as cow, less frequently in cat sheep and goat.

Mostly it is due to the presence of different populations of cells e.g. macrocytes (reticulocytes) in responsive anaemia; or the production of small- sized ( microcytes) rbcs as in iron deficiency anaemia. Spherocytes may also leads to anisocytosis.

B. Abnormality in arrangement of rbcs:

1. Rouleaux: It means adhesion of rbcs together like a stalk of coins .This is mostly due to changes in plasma protein con., as increase in fibrinogen and γ –globulin in inflammatory conditions .Extensive rouleaux is normal in equine , moderate to slight rouleaux is normal in cat & dog.

2. Agglutination: The aggregation or clumping of rbcs in together in clusters not in chains .It is caused by immunoglobulins bound to rbcs surfaces, as in immune – mediated anaemia.

C. Abnormalities in colour of rbcs:

1. Polychromasia: The presence of bluish –red rbcs in stained blood film , due to the presence of a combination of Hb (red) and ribosomes(blue).They are immature rbcs (reticulocytes) present in low number in normal dog(1%-1.5%) ,increased polychromasia indicate reticulocytosis and responsive anaemia.Equine blood does not show polychromasia in disease or in normal conditions.

2. Hpochromasia: RBCS with decrease in Hb content and increase in central pallor. It is associated with decrease in MCHC. Increased hypochromasia is associated with iron deficiency anaemia and chronic blood loss.

3. RBC inclusions: It means the presence of abnormal bodies inside rbcs.

a) Nucleated rbcs: Presence of immature rbcs in peripheral circulation e.g. rubricytes, metarubricytes is seldom in the blood of normal adult mammals, It is seen in regenerative anaemia, haematopoietic neoplasia and various inflammatory conditions.

b) Howell-Jolly body: They are small dark blue nuclear remnant (DNA in nature) formed in the bone marrow and it should be removed by pitting activity of the spleen. May be present in low number in rbcs of normal cats and horses, also it is associated with regenerative anaemia and blood films of splenectomized animals.

c) Heinz body: Large aggregates of oxidized precipitated Hb attached mostly to the internal surfaces of rbc cell membranes, they stain pale pink with Romanowsky,s stains, dark blue with supra-vital stains. Normal cat blood film may show few Heinz bodies (5-10%).In pathological conditions it is associated with dietary causes as consumption of large quantities of onion by small and large animals, kale and other Brassica sp. in ruminants, copper toxicity in sheep .It is known as Heinz body haemolytic anaemia.

d) Basophilic stippling: Blue staining punctuate inclusions stained with Romanowsky,s stains ,it represents RNA of polyribosomes, occur in regenerative anaemia in ruminants and lead toxicity in canine, punctuate may be fine or coarse.

e) Infectious agents: As protozoa e. g. Babesia, Theileria, Malaria. Bacteria as the rickettsial microorganism, anaplasma. Viral inclusions e.g. Canine distemper viral inclusions appear as red or orange mostly rounded bodies (it should be differentiated from Howell-Jolly bodies.

1. (in lead toxicity in all other species).

Expected causes of non – responsive anaemia:

* Acute blood loss of more than three days duration, and some cases of short term chronic blood loss before iron deficiency develop.
* Haemolytic diseases e.g. deficiency of certain important rbc enzymes like pyruvate kinase. Immune-mediated haemolytic anaemia. Infection associated haemolysis as babesiosis, bacillary haemoglobinuria, anaplasmosis etc.
* Chemical or toxin - induced haemolytic anaemia.