CLINICAL PATHOLOGY (PATH-404)

CLASS LECTURES

FOR

MID TERM Examination

(PART I)

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Pathology:

It is the study of changes in the anatomy, histology, physiology and biochemistry of a cell in living individual. Or Study of phenomenon of development of disease is called pathology.

When any pathological organism enter in the body it causes change in anatomy, histology, physiology, and biochemistry e.g. in case of cloudy swelling of hepatocytes, SGOT and SGPT increase in blood; it is a biochemical change. Alkaline phosphatase level increases when there is damage to the cardiac cells.

Clinical Pathology:

Branch of pathology in which we study the changes in the chemical composition the body fluids which help in confirmation or diagnosis of a disease.

Objectives of Clinical Pathology

1. Disease diagnosis:

   It helps in diagnosis of a disease especially when disease is in its sub-clinical stage e.g. animal suffering from TB is normal but its milk yield decreases day by day or there is decrease in body weight. Tuberculin is prepared antigen of TB.

   In case of Brucellosis take serum of suspected animal and perform Rose Bengal test. If agglutination occurs, it is a case of Brucellosis.

2. Differential Diagnosis:

   It helps in differential diagnosis. If urine is red in color, it may due to hematuria or hemoglobinuria. In hematuria, intact red blood cells are seen. In case of hemoglobinuria there are lysed RBCs. It occurs in babesiasis, theleriasis, and parturient hemoglobinuria.

   Take urine in test tube and centrifuge it, RBCs will be settled down and urine will be at the upper layer if RBCs are intact, this shows hematuria. If there are destructed RBCs, then there will be suspended RBCs in urine.

3. Determination of the fate of drug:

   In coccidal infection, give anticoccidial drug. After a week check the number of eggs in the feacal sample. If no. is low then drug has worked in the body.

4. Prognosis of the disease:

   For example in a disease, WBCs increase in the blood. To know whether animal is suffering from infection or not, take blood sample and do TLC (total leukocytes count); if WBCs are increased it means animal is able to fight against infection and prognosis is favourable; if they are decreased then body is unable to fight and prognosis is unfavourable or poor

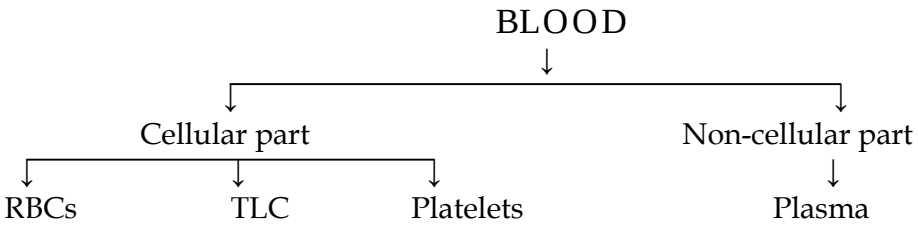
5. Side effects of a drug/medicine:

   Chloramfanicol: If it is given for long time, it depresses the activity of bone marrow leading to aplastic anemia. It can be confirmed by RBCs count or bone marrow M.E ratio. No. of myeloid and no. of erythroid cells changes and ME decreases; it shows aplastic anemia.

6. Health monitoring:

   It helps in the monitoring of health of a flock. Hematology, fecal examination, urine examination, body weight, feed consumption, and serological tests are used to

diagnose the sub-clinical form of diseases. If feed intake decreases, it means something is wrong with the flock.



Erythrokinetics

A study of the kinetics of red blood cells from their generation to destruction.

1) Stem cells:

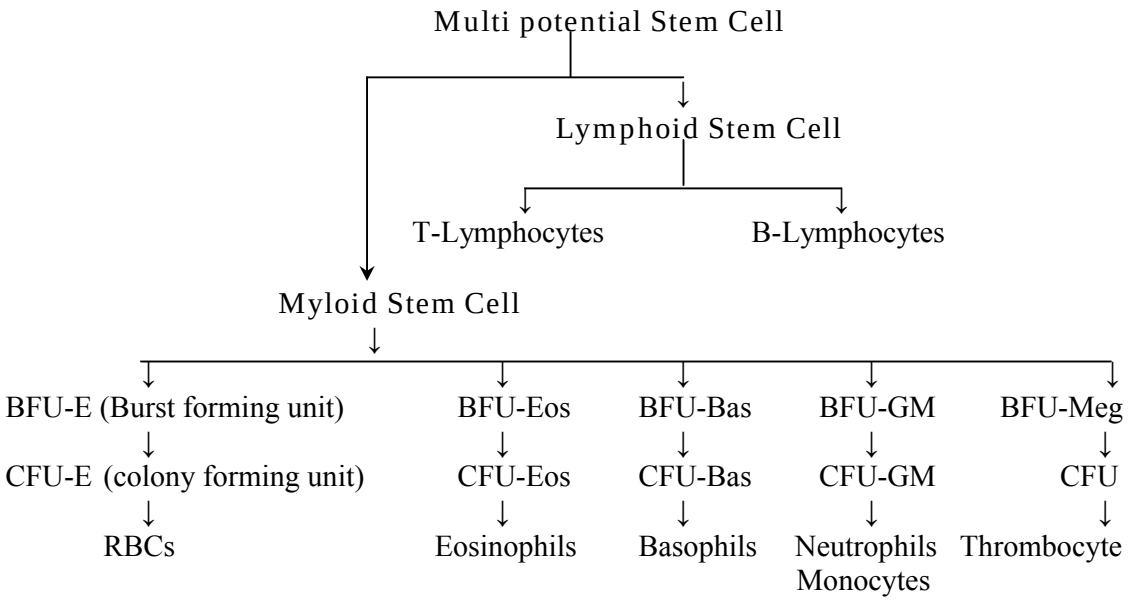
They may be multipotential, unipotential or pluripotential. Growth stimulating factor (GSF) – release from bone marrow play key role in the activation of these cells to multiply. These cells can not be differentiated with H& E staining.

2) Progenitor cells:

These cells have limited activity of self renewal / differentiation. These cells divide themselves into different types of precursors (see next). Some of the progenitor cells are unipotential while the others are multipotential and these can not be differentiated with normal and special staining.

3) Precursors:

They have no ability for self differentiation and these can be identified. They are responsible for production of different types of other blood cells.



Stem cells are pluripotential stem cells; they differentiate and divide into more than two types of cells.

- Lymphoid Stem Cell
- Myeloid Stem cell

These two cells are *progenitor* cells.

Erythropoiesis

Production of erythrocytes is known as erythropoiesis.

Production of erythrocytes occurs extravascularly in bone marrow parenchyma in mammals. In birds it occurs within vascular sinuses of the bone marrow (intravascular).

When last stage is attained (i.e. reticulocytes), they pass through endothelial cells and enter into the blood circulation.

Rubriblast → Prorubricytes → rubricytes → metarubricytes → reticulocytes → Erythrocytes

Regulation of Erythropoiesis

Erythropoiesis is controlled by certain factors.

1) Erythropoietin (EPO)
   Erythropoietin is released from peritubular interstitial cells of the kidney but 10-15 % of erythropoietin is also released from specific hepatocytes.
   Erythropoietin inhibits the apoptosis of progenitor cells i.e. BFU and CFU which causes the rapid differentiation and division into precursors and erythrocytes.
   It also accelerates hemoglobin synthesis.
2) Interleukin-3 (IL-3), GM-CSF, and G-CSF enhance the multiplication of progenitor cells leading to increased erythropoietin production.

I Interleukin-3 (IL-3) is released from activated T-lymphocytes.
GM-CSF released from activated T-lymphocytes, macrophages, endothelial cells and fibroblasts.
G-CSF is released from macrophages, monocytes, neutrophils, endothelial cells and fibroblasts. (Fibroblast and activated T-lymphocytes enhances multiplication of progenitor cells.)

3) Androgen stimulates the release of erythropoietin. Estrogen & progesterone inhibit release of erythropoietin but not clinically significant.
4) Hormones of thyroid and pituitary cause the high tissue demand for oxygen and more release of erythropoietin.

Morphological Abnormalities of Erythrocytes (RBCs)

There are three different types of RBCs, on the basis of:

- Change in size
- Change in shape
- Presence of inclusions

1) Size abnormalities (Anisocytosis): RBCs with a variation in size and diameter are known as *Anisocytes*.
2) Shape abnormalities
   Poikilocytes- There are two further types of RBCs;
   i) Leptocytes : RBCs with decreased volume in relation to their diameter
   ii) Spherocytes: RBCs of spherical shape; increased volume in relation to their diameter.
   Acanthocytes – RBCs with rounded projections on its surfaces – seen in liver diseases of dog and cat. These should be differentiated from Crenation.
   Crenation: Such RBCs are usually seen when blood is mixed in hypotonic solution or in delayed drying of blood smears. In this abnormality, RBCs are presented with pointed projections on its surfaces.

3) Presence of Inclusions:
   a) *Reticulocytes*: Immature RBCs in bone marrow having DNA remnant in their cytoplasm are known as reticulocytes. These are found in all types of recovery phases of anemia.
   b) *Punctate basophilia* (Basophilic stippling bodies); when there is aggrega-tion of basophilic staining material in the cytoplasm of the RBCs.
   c) *Polychromatophilic cells* (Metarubricytes): There is diffused basophilia in the cytoplasm of the RBCs. These are seen in all types of degenerative anemia as well as in the hemolytic anemia of dog and cat.
   d) *Heniz bodies*: RBCs having single or multiple, round or irregular, rough or fine refractile inclusions in the cytoplasm. These can be seen in phenothiazine and wild onion poisoning.

Anemia

It can be defined as "Absolute fall in hematocrit, erythrocyte count and hemoglobin concentration is called anemia".

History:

The following are the findings which will be important:

History of medication, vaccination, blood transfusion, exposure to toxic chemicals and plants, prior pregnancy and reproduction status.

Signs:

Paler mucous membrane, increase heart rate, depression, weakness, low oxygen carrying capacity of blood, loss of stamina, decrease tolerance, shock if 1/3rd blood loss, heart murmur caused by reduced viscosity and increase increased turbulence of blood flow, icterus, hemoglobinuria, hemoglobinemia, petechial hemorrhage, fever.

Lab findings:

Decrease in Hb, RBC, and PCV.

Classification of Anemia

Classification of anemia depends upon:

- ✓ Etiology
- ✓ Morphological characteristics of cells
- ✓ Bone marrow response

Morphological Classification of Anemia

Whenever there is decrease in hematocrit, there may be decrease in MCV or MCHV.

MCV (Mean Corpuscular Volume)

It ensures that it is a type of anemia based on bone marrow depression. From erythrocyte indices you can go for diagnose the type of anemia. MCH is specified for each specie. It can be increased or decreased or normal than reference value.

It can be calculated as;

MCV = PCV x 10 / avg. no. of erythrocytes = fl (unit)

Here,

PCV = Packed cell volume, fl = femto liter

If MCV increases, cell is called macrocytic. In this case immature cells come in circulation because erythropoiesis, occurring in bone marrow is trying to compensate. It is also called regenerative anemia because there is regeneration of erythrocytes.

If MCV decreases: cell is called microcytic, when hyper mature cells
If MCV is normal: cell is called normocytic, regenerative or not regenerative.

MCH (Mean Corpuscular volume of Hemoglobin)
It can be calculated as;

MCH = Hb concentration x 10 / avg. no. of erythrocytes = pg (pico gm)

MCHC (Mean Corpuscular Hemoglobin Concentration)
It can be calculated as:

MCHC = Hb concentration x 100 / PCV

If MCHC increases, it will be hyperchromic anemia. It occurs only when excessive damage of hemoglobin, excessive intravascular hemolysis and give reddish color plasma – in case of hemoglobinemia

If MCHC decreases, it will be hypochromic anemia. It occurs when excessive loss of blood and iron not available in diet.

Normal MCHC: If hemorrhage has occurred and there is availability of iron which will be utilized again for formation of Hb in bone marrow. At that there is normochromic anemia

Classification of Anemia (Summarized Table)

On morphological basis, there are 6 different types of anemia as follows;

Anemia	MCV (cytic)	MCHC (chromic)
Normocytic, Normochromic	Normal	Normal
Normocytic, Hypochromic	Normal	↓
Macrocytic, Normochromic	↑	Normal
Macrocytic, Hypochromic	↑	↓
Microcytic, Normochromic	↓	Normal
Microcytic, Hypochromic	↓	↓

Etiological Classification of Anemia

Hemorrhagic anemia:
Escape of blood from blood vessels. It may be internal hemorrhage (due to blood sucking parasites) or external hemorrhage.
Hemolytic anemia:
It is due to hemolysis of erythrocytes.
Bone marrow depression anemia:
It is a type of anemia produced due to reduced or defective bone marrow.

Hemorrhagic anemia

Loss of blood may be due to any accident, fracture, or a surgery. It may be acute or chronic.

Acute Hemorrhagic Anemia

It develops as a result of sudden loss of blood in injuries, fracture etc.

There may be loss of handsome amount of blood, but initially PCV and heart activity is normal but after 3-4 hours an hypoxic condition develops, and hematocrit (Hct) will increase upto 80 % because old erythrocytes come in circulation caused by spleenic

contraction due to release of epinephrine and blood becomes hyper viscous. And now there will be hypovolemia and all hematocrit parameters will increase.
12-48 hours post hemorrhage; hemetocrit will decrease and hypovolemia is recovered. Blood proteins are reduced. Thrombocytosis occurs to stop hemorrhage. Neutrophilic leukocytosis will also occur.
2-3 days post haemorrhage; reticulocytes will start appearing in blood. It indicates that Erythropoeisis has started. Thrombocytes and blood protein come to normal. Blood proteins are utilized for hemoglobin synthesis and later on erythrocytes come on normal level. 2-3 weeks are required for dog to recover from acute haemorrhagic anemia.

Chronic Hemorrhagic Anemia

In chronic hemorrhagic anemia all hematocrit parameters are reduced before the appearance of signs of anemia. There is persistent thrombocytosis and regenerative response is less intense than in acute haemorrhage. Blood proteins may be decreased but if slow then may remain in range. Hypovolemia may develop. There will be deficiency of iron. If iron is not available there will be no erythrocytic response and no immature erythrocytes in circulation.
In case of external hemorrhage there is loss of blood from body. In case of internal hemorrhage there is loss of blood within body.
In internal hemorrhage reticulocyte response is more progressive as compared to in external haemorrhage.
When hemorrhage occurs within the body cavity there is more regeneration because the escaped blood (haeme + globin) will be reabsorbed in lymphatics and reutilized for synthesis of erythrocytes and Hb.

Hemolytic Anemia

When hemolysis of erythrocytes. It may be intravascular and extravascular. Number of RBCs decreased but hematocrit is increased because hemoglobin remains in blood so it causes hemoglobinemia.
These only appear in case of acute hemolytic anemia. Signs of hemorrhage are absent. Icterus may develop. There may be hemoglobinemia and hemoglobinuria if within blood vessels.

Clinical Findings:

Clinical signs of hemorrhage are absent. Reticular response is present (immature cells in blood). It is more intense than hemorrhagic anemia. Plasma protein is normal or slightly increased. It is due to increased hemoglobin degradation i.e. Mean carpuscular hemoglobin (MCH) and Mean carpusular hemoglobin concentration (MCHC) increased. In acute cases there is neutrophilocytosis and in chronic cases there is monocytosis.
Increase bilirubin in blood expresses excessive degradation. There are morphological abnormalities in erythrocytes (Heinz bodies, spherocyte, piokilocyte and erythrocyte containing parasites).

Etiology and Mechanism of Extravascular Hemolysis

Normally erythrocytes complete their life span then they are taken up by macrophages of spleen and liver. But abnormal process is characterized by abnormal excessive phagocytosis.

Ab and C_3b mediated hemolysis:

The hidden Ag on the cell membrane of erythrocytes is exposed. Lymphoid system recognizes it and there is formation of Ag-Ab complex which activates the complement and C_3b attaches on Fc receptor. Macrophages recognize it and cause phagocytosis of erythrocytes.

Sometimes this phagocytosis remains incomplete due to which there is partial damage to the RBCs and we can observe spherocytes in the circulation.

Immune mediated hemolytic anemia (IMHA):
Mechanism is not correctly known.
Somehow RBCs are recognized as antigen by immune system and antibodies are produced against RBCs. As a result, hemolysis occurs. It is caused by viral or bacterial infection or medication.

Anemia by some Infectious Agents:
i) *Mycobacterium hemolytica* infection: Cell membrane is changed or hidden Ag is exposed or C3b is activated during chronic infection. It leads to cross interaction with erythrocytes.
ii) Drugs such as Penicillin: absorption occurs on the cell membrane of RBCs and act as antigen known as Hepton, antibodies produced are known anti-drug antibodies.

Decreased Deformity of RBC:
Changes occur in cell membrane; internal viscosity is high ultimately RBCs become spherocytes. These enter spleen and phagocytosed by macrophages.

Reduced glycolysis and low production of ATP:
RBCs affected by this condition are predisposed to removal from vasculature by spleenic macrophages. It occurs with normal aging. This reduction is accelerated in hereditary pyruvate kinase and phosphofructokinase deficiency anemia.

Spleenomegaly/increased macrophage activity:
When there is enlargement of spleen, there is increased activity of macrophages and RBCs are more prone to phagocytic activity.

Lab findings/blood picture:

- Reticulocytic response.
- Hemoglonemia and hemoglobinuria are absent.
- Bilirubin may be high showing excessive extravascular hemolysis.
- Bone marrow response is there (when hemolysis is slow), showing compensating mechanism for RBC destruction.
- Neutrophilic leukocytosis (acute), monocytic leukocytosis (chronic).

Etiology and Mechanism of Intravascular Hemolysis

Antibody and complement (C_3b) mediated/immune mediated hemolysis:
Hidden antigen is exposed and increase in antibodies, Ag-Ab complex will activate C_3b and C9 will be activated which produces large defects on the surface of RBCs and escape of Hb becomes easy.

Physical injury to RBCs:

- ✓ Increase fibrin strands in blood causes traumatic injury to erythrocytes e.g. micro angiopathic anemia, disseminated intravascular coagulation vesiculitis, and hemongioma.

Oxidative injury:

- ✓ Heinz bodies increased and denature the hemoglobin and functional ability of RBCs is inhibited.
- ✓ Due to formation of cross linkages between cell membrane across the cytoplasm which disfigure the cell.
- ✓ Due to Oxidation of Hb methemoglobin is formed and oxygen carrying capacity is reduced.

When Heinz bodies or accenterocytes, it shows antioxidants in body.

Osmotic Lysis:

- ✓ Hypophosphotemia: osmosis will be increased leading to lysis.
- ✓ Diuretic phase of diabetes mellitus may lead to phosphate depletion.
- ✓ Administration of hypotonic fluid

Alteration in the cell membrane of RBCs:

It increases hemolytic activity due to;

- ✓ Snake venom
- ✓ Castor beans containing ricin
- ✓ Phospholipase (toxin produced by bacteria).
- ✓ Blood parasites (babeisiasis)

Lab Findings:

- Regenerative response 2-3 days post hemolysis.
- Hemoglobinemia and hemoglobinuria: 12-24 hours post hemolysis.
- Bilirubin in blood: 8-10 hours post hemolysis
- Hemosidrinuria
- Abnormal erythrocytes like keratoocytes, accenterocytes, spherocytes, heinz bodies and erythrocytes having parasites.

Anemia due to Reduced or Defective Erythropoeisis

When bone marrow is exposed to infection; it results into reduced or defective erythropoiesis.

Factors Involved in Reduced Erythropoeisis

1) Any chronic infection.
   - ✓ Chronic inflammation
   - ✓ Neoplasia

2) Cytotoxic damage to bone marrow.
   - ✓ Cytotoxic cancer drugs
   - ✓ Furazolidone
   - ✓ Phenylbutazone
   - ✓ When bone marrow is exposed to radiation

3) Lack of availability of erythropoietin; which occurs in

- ✓ Chronic renal infection.
- ✓ Hypopituitrism
- ✓ Hypoadrenocorticism
- ✓ Hypoandrogenism

4) Immune mediated diseases.
Pure red cell aplasia (PRCA). In this condition progenitor cells of erythrocytes are damaged congenitally.

5) Other Infection agents; Like
   - ✓ Feline leukemia
   - ✓ Feline pan leukopenia
   - ✓ Parvovirus
   - ✓ Trichostrongylyoids

6) Myelopathies (diseases of myeloid tissues)
   - ✓ Lymphocytic leukemia
     There is increased count of WBCs in circulation and increased progenitors proliferation for B and T-lymphocytes in bone marrow – ultimately depress the activity of progenitors of erythrocytes.
   - ✓ Metastatic neoplasia
   - ✓ Myeloproliferative disorder (abnormal erythrocytes are formed)
   - ✓ Osteoporosis (activity of bone marrow is depressed).

[Upcoming Notes (PATH 404) >>> 18 Oct 2010, Monday]