CLINICAL PATHOLOGY (PATH – 404) - PART II

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ANEMIA DUE TO DEFECTIVE ERYTHROPOIESIS

Factors Involved in Defective Erythropoiesis

1) Abnormal maturation of erythrocyte:
It occurs in
- ✓ Erytheremic myelosis
- ✓ Erythroleukemia
- ✓ Microcytosis of poodles

2) Disorder of haeme (Hb) synthesis:
When bone marrow exposed to
- ✓ Chloromphenicol
- ✓ Copper deficiency
- ✓ Lead toxicity
- ✓ Molybdenum toxicity
- ✓ Reduced absorption of iron from the GIT

3) Disorder of nucleic acid synthesis
- ✓ Deficiency of vitamin B_{12}
- ✓ Folic acid deficiency

Broadly speaking, there are two types of anemia: i) Primary Anemia 2) Secondary Anemia
i) *Primary anemia*: due to damage to the bone marrow
ii) *Secondary anemia*: due to damage to the extra bone marrow tissue.

Factors Required for Normal Erythropoiesis

i) Presence of precursors (Multi & unipotential)
ii) Availability of nutrients (i.e. B-complex, B_{12}, Folic acid and iron)
iii) Stimulatory factors (i.e. erythropoietin, GSF, IL-III)
iv) Micro-environment of bone marrow

General Mechanism of Defective Erythropoiesis

- Primary and secondary bone marrow failure
  - ✓ Primary bone marrow failure from intra-marrow disease (bone marrow damage) results in inadequate production of stem and progenitor cells
  - ✓ Secondary bone marrow failure occurs from extra-marrow causes (outside the bone marrow) such as lack of nutrients or growth factors (Epo, colony-stimulating factors, or cytokines)
- Bone marrow failure leads to PRCA or aplastic anemia. In PRCA only erythroid stem cells are damaged. In aplastic anemia all type of cells are damaged and there is granulocytopenia and thrombocytopenia.

Differentiation of Anemia or Diagnostic Approach towards Anemia Caused by Reduced / Defective Erythropoiesis

This practical approach to the diagnosis of anemia is based on;

a) Morphology of erythrocytes
b) No. of neutrophils
c) Platelet numbers
d) M : E ratio or cellularity of bone.

(1): Normocytic, Normochromic Anemia with

a) Normal to increased neutrophils
b) Decreased Platelet count
c) Increased M.E ratio caused by hypocellularity of erythroid stem cells.

Causes:

(A) Anemia due to lack of Erythropoietin in certain disorders:

a) *Chronic renal disease*
   I. The degree of anemia is roughly proportional to the severity of uremia
   II. Erythropoietin deficiency caused by destruction of Epo-secreting peritubular interstitial cells
   III. Hemolysis caused by factors in uremic plasma
   IV. Gastrointestinal hemorrhage from abnormal platelet function and vascular lesions
   V. Inhibitors of erythropoiesis in uremic plasma
b) *Endocrinopathies* e.g. hypoadrenocorticism, hypoandrogenism and hypopituitrism
   I. Some of these hormones (e.g. androgens) may enhance the action of erythropoietin.
   II. In other cases the exact mechanism of anemia is unknown.

(B): Anemia of inflammatory disorders:

a) The onset of anemia may be as short as 3-10 days.
b) Aid is mediated by a variety of cytokines involved in inflammatory or neoplastic processes, including tumor necrosis factor, IL-alpha, IL-1beta, and interferon-γ.

c) Diminished marrow responsiveness to erythropoietin.

(C): Feline leukemia virus (FeLV) associated with non regenerative anemia:

I. Erythroid stem and progenitor cells are selectively killed by FeLV.
II. The anemia may be macrocytic due to asynchronous maturation

(D): Pure red cell aplasia (PRCA)

I. There is loss if erythroid precursors in bone marrow.
II. PRCA appears to be immune mediated
III. It is non regenerative autoimmune hemolytic anemia.

(2): Monocytic, Normochromic Anemia with neutropenia and Variable M.E ratio

- Aplastic anemia or pancytopenia:

✓ This is a disease of the multipotential stem cell or bone marrow microenvironment that leads to pancytopenia and an acellular fatty bone marrow.

✓ Concomitant deficiencies in erythropoiesis, granulopoiesis and thrombocytopenia usually precede the development of anemia because of the shorter life span of leukocytes and platelets.

- Causes of aplastic anemia:

✓ Phenylbutazone toxicity, albendazole toxicity, trimethoprim-sulfadiazine toxicity and chloramphenicol toxicity in cats.

(3): Microcytic, Hypochromic Anemia with variable neutrophils and platelet counts and usually a hyper cellular marrow with a variable M.E ratio.

Causes:

✓ Iron deficiency
✓ Pyridoxine deficiency: This vitamin is a cofactor in heme synthesis. Its deficiency leads to a failure to utilize iron
✓ Cu deficiency: no absorption of iron from intestine
✓ Dyserythropoiesis in dog

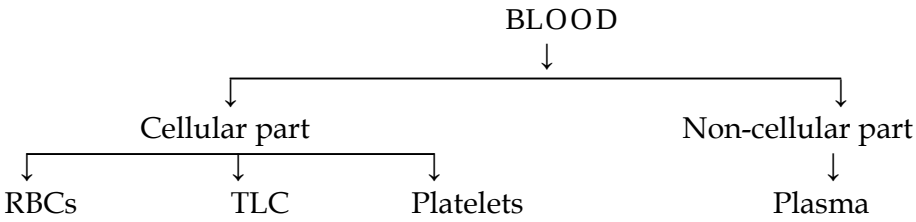
(4): Macrocytic, Normochromic Anemia with variable neutrophils and platelet counts.

M.E ratio is usually low because of hypercellualr erythroid marrow.

Causes:

✓ Ruminants grazing on soil rich in Mo or deficient in Co.
✓ Zn or folic acid deficiency megaloblastoid erythroid precursors are observed in the bone marrow
✓ Feline leukemia virus infection
✓ Macrocytosis of poodles. This hereditary condition is uncommon. Neither anemia nor reticulocytosis occurs. Erythrocyte counts typically are within the low end of the reference interval.

HEMATOLOGY



Blood Collection Sites

1. Jugular vein:-
   Commonly used site in large animals including small and large ruminants, but occasionally used in dog, cat, rabbit, rat, guinea pig and birds
2. Cephalic vein:-
   It is located in the forelimb region. This site of blood collection is mostly used in dogs.
3. Ear vein
   A prominent ear vein is present in the marginal area of dorsal surface of the ear of the animals. This site is mostly selected in rabbits, guinea pigs, monkey, cats and small dogs. *Note:* An insulin syringe is used for this purpose.
4. Toe or toe nail :-
   Toe or toe nail is clipped for this purpose in small dogs, pups, guinea pigs and other small wild animals.
5. Tail :-
   Site is selected 3-4 inches away from the parineal area. Two methods are used for blood collection as follows:
   i) Venipuncture
   ii) Amputation – is mostly adapted in rats, mouse, and fish.
   Note: Tail is dipped in warm water (at 45 C) for I min before collection of blood. This results into enhancing blood flow towards the collection site.
6. Heart :-
   Blood can be collected direct from the heart – mostly in birds and reptiles.
7. Femoral, saphenous and tibial vein :-
   Femoral vein is present in the medial thigh while saphenous and tibial veins are located at lower region of the hind limb.
8. Mammary vein :-
   In dairy animals like cattle and buffalo, it is located anterior to udder and lateral to the linea alba. In cattle as it is much prominent, it is good site for blood collection.
9. Anterior vena cava: Mostly used in pigs.
10. Retro-orbital venous plexus:
    Mostly in rat, mouse and guinea pig.
    This site is less traumatic and blood can collected repeatedly. For blood collection, 20 gauge needle is used, it is passed 3-4 mm behind the eye via medial or lateral canthus after anaesthetizing the animal. The direction of the needle should be towards the larynx.
11. Wing vein or comb: in birds
12. Web vein: Web is between toes and foot pad and used in guinea pigs.

Containers for Blood

It depends on the quantity of the blood and use of the collected blood.

a) Test Tubes:
   Commonly, test tubes are used for clotted blood.
b) Vessels for unclotted blood:
   Glass vials with stoppers with or without anticoagulant called " Vaccutainer " are used for unclotted blood. Vaccutainer are commercially available in the market of different capacities (2-50 ml).

ANTICOAGULANTS

An anticoagulant is a substance that prevents coagulation; that is, it stops blood from clotting.
Different anticoagulants are used as follows:

1. EDTA [Ethylene Diamine Tetra Acetic acid]
   It is available in tow forms/salts i.e. Na+ and K+
   Mode of Action: - EDTA chelates calcium ions to form a soluble complex.
   Quantity Used :- 10-20 mg / 10 ml of blood
   Advantages:
   a) Excellent preservative power for up to 6 hours.
   b) Recommended for natural hematological procedure.
   c) Preserve cellular elements better than heparin and oxalate.
   Disadvantage:
   a) More than 2 mg / ml cause shrinkage of the cells.
   b) Mostly K+ salt of EDTA is used. But it caused drop in the blood pH.

2. Heparin
   This anticoagulant is an acid mucopolysaccharide which is considered to be the anticoagulant of reference for red cell morphology.
   Mode of Action:- Stops the formation of thrombin from prothrombin by anti-thrombin plasmin therefore stopping formation of fibrin from fibrinogen.
   Quantity Used: 1-2 mg / 10 ml of blood
   Advantages:
   a) Least effect on size and morphology of RBCs due to which there is no hemolysis of RBCs.
   b) Recommended for blood gas analysis i.e. PH, Blood bicarbonates determination and partial pressure of Carbon dioxide and oxygen.
   Disadvantages:
   a) It may cause clumping of white blood cells.
   b) It is unsuitable for blood smears because it interferes with the staining reaction.
   c) It is quite expensive one anticoagulant.
   d) It does not prevent clotting more than 8 hours.
   e) It is not suitable for prothrombin determination test.

3. Sodium citrate (Na –citrate)

Mode of Action:- Same as that of EDTA
Quantity Used: 10-20 mg /10ml of blood
Advantage:
a) It is used for blood transfusion
Disadvantages:
a) It interferes with the blood chemical tests.
b) It causes shrinkage of the cells.
c) It prevents clotting only for few hours.

4. Potassium oxalate (K – oxalate)
Mode of Action: Same as that of EDTA
Quantity Used: 20 mg / 10 ml of blood
Advantage:
a) Very soluble anticoagulant
Disadvantages:
a) It causes 6-8% shrinkage in cell volume, due to which blood parameters such as PCV (packed cell volume) and MCV (Mean corpuscular volume) also hampered.
b) It is a poisoning substance which interfere with the electrolyte distribution form precipitation of blood proteins.
c) It increases the blood pH.

5. Sodium oxalate:
Mode of Action: - Same as that of EDTA
Quantity Used: 20 mg /10 ml of blood
Advantage:
a) It is recommended for prothrombin time determination test.
Disadvantages:
Same as that of K-oxalate

6. Ammonium and potassium oxalate:
Mode of Action: Same as that of EDTA
Quantity Used: 1ml or 20 mg for 10 ml of blood
Preparation:
Ammonium oxalate: 1.2 gram
Potassium oxalate: 0.8 gram
Dissolve in 100 ml of distilled water.
Advantages:
a) It is used for most hematological procedures.
b) It causes less distortion of cells than other oxalates
Disadvantages:
a) It is not used for BUN (blood urea nitrogen) determination.

7. Lithium oxalate:
Mode of Action: Same as that of EDTA
Quantity Used: 20 mg / 10 ml of blood
Advantage:
a) More soluble than Na- and K- oxalates.

Disadvantages:
Same as that of K-oxalate

8. Lithium citrate:
Mode of Action: Same as that of EDTA
Quantity Used: 30 mg /10 ml of blood
Advantage:
a) It is used for determination of blood mineral constituents.

Errors during Blood Collection

1) Hemolysis:
It may occur due to;
a) Wet syringe or container
b) Failure to remove needle before filling collection vials
Hemolysis can interfere with the results of the following tests;
i) Serum lipase
ii) Serum bilirubin
iii) Icterus index
iv) Urea nitrogen
v) Inorganic phosphate
vi) Alkaline phosphatase
vii) Acid phosphatase
viii) Transaminases
ix) Lactic dehydrogenases
x) Arginase
xi) Blood pH
xii) Chloride concentration
xiii) Prothrombin concentration
2) Lipemia
If patient is not fasted for an adequate time before the collection of blood. Then, it may produce faulty elevated values for;
i) Total proteins
ii) Transaminases
iii) Icterus index
iv) Homoglobin
v) Amylases
3) A Fibrin Clot can occur when blood is centrifuged too soon after blood collection or when speed of centrifuge is too fast.
4) Blood should be collected when animal is at rest and without undue excitation to avoid alteration in hemogram and blood chemistry values.
5) Blood smears should be made within 15 minutes after collection. Best smears are made immediately from fresh blood.
6) Don't store blood smears in refregitors.
7) Clotting of blood may result due to;
a) Taking too much time for blood collection
b) Failure to agitate the blood sample immediately after collection.

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