Vet Cl path. MSc 2023	# Bone marrow	Mohammed A.Y. Al-Amery

Origin and development of red blood cells:

There are three types of cellular elements in the blood; erythrocytes (**red cells**), leukocytes **(white cells)**, and thrombocytes (**platelets)**. Each has its own functions and differs clearly from the others.

Most of the blood cells are formed in the bone marrow.

The examination of marrow specimens often provides information of great value in the diagnosis and treatment of hematologic disorders.

Blood forming system:

A. **In Embryo;** early from a mesenchyme, endothelium, liver, spleen, thymus and lymph nodes.

B. **After birth**; transfer gradually to the bone marrow.

Note; The active bone marrow is red in color and is called **red marrow**, whereas, non-productive resting marrow is called **yellow marrow**.

Hematopoiesis:

Is the formation of blood cellular components. All cellular blood components are derived from haematopoietic **stem cells**.

Stem cells: are reside in the medulla of the bone (bone marrow) and have the unique ability to give rise to all of the different mature blood cell types and tissues.

Stem cells are self-renewing cells: when they differentiate, at least some of their daughter cells remain as stem cells, so the pool of stem cells is not depleted.

In adult organisms, stem cells and progenitor cells act as a repair system for the body, replenishing adult tissues.

The character of stem cell in a Wright's stained preparation are large (20-30 u in diameter) with abundant, light blue, opaque cytoplasm. The cell borders are typically irregular, have one to two nucleoli with the nucleus being round to oval, with a finely to coarse reticulated chromatin pattern.

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للاطلاع **Note:** Adult stem cells are frequently used in medical therapies, for example in bone marrow transplantation. Stem cells can now be artificially grown and transformed (differentiated) into specialized cell types with characteristics consistent with cells of various tissues such as muscles or nerves. Embryonic cell lines and autologous embryonic stem cells generated through Somatic-cell nuclear transfer or dedifferentiation have also been proposed as promising candidates for future therapies.

There are three known accessible sources of autologous adult stem cells in humans: bone marrow, adipose tissue, and blood. Stem cells can also be taken from umbilical cord blood just after birth. Of all stem cell therapy types, autologous harvesting involves the least risk. Adult stem cells are frequently used in various medical therapies (e.g., bone marrow transplantation). Stem cells can now be artificially grown and transformed (differentiated) into specialized cell types with characteristics consistent with cells of various tissues such as muscles or nerves. Embryonic cell lines and autologous embryonic stem cells generated through somatic cell nuclear transfer or dedifferentiation have also been proposed as promising candidates for future therapies.

Myelopoiesis:

- The prefix **"myelo"** refers to all aspects of bone marrow activity and is not limited to granulocytic elements alone.
- Consists chiefly of erythropoiesis, granulopoiesis, and thrombopoiesis.
- Monocytes appear to be formed in the marrow as well as elsewhere.
- Lymphopoiesis occurs in extramedullary sites such as the spleen, thymus, and lymph nodes.
- The myeloblast, myelocyte & metamyelocyte refer to specific stages in granulocyte maturation.

Erythropoiesis:

Is the process which produces nonnucleated red blood cells in the **peripheral blood** and the number of bone marrow produced erythrocytes is equal to the number of cells that die. It is stimulated by decreased O_2 in circulation.

- **Erythropoietin** is hormone secreted by kidneys which stimulates proliferation and differentiation of red cell precursors.
- **Erythron**s; All of the immature and mature erythrocytes in the body.

Examination of the bone marrow:

A-Types of bone marrow;

Bone marrow which is active red in color (**red marrow**), whereas, non-productive resting marrow is **yellow marrow**. Yellow marrow consists of : endothelial, reticular and fat cells.

In the adult, yellow marrow fills most of the shaft of the long bones. Demands for increased production of blood cells are met by conversion of yellow marrow into red marrow. Red marrow expansion or regression is made possible through its ability to interchange with fat cells.

Red marrow is found throughout life in flat bones (**vertebrae, ribs, pelvis and bones of the head)** and the proximal ends of long bones **(femur and humerus)**.

B-Indications for Bone Marrow Examination:

1. Whenever anemia, neutropenia, or thrombocytopenia is identified and no cause is apparent.
2. Whenever excessive production of neutrophils or lymphocytes is present and no cause is apparent.
3. Can lend added support to the suspicion that hypoplasia, hyperplasia, or neoplasia of the marrow is associated with the disorder of the peripheral blood.

C-Sites for Bone Marrow Aspiration

1. Small Animals - Crest of ileum
2. Large Animals - Rib or sternum

D-Equipment Needed

- using a Steinman pin to make a hole in the bone cortex and an ordinary syringe and needle to aspirate the marrow. The one marrow needle for iliac crest, a needle approximately **5/8"** long, 18 G is desirable. For the femoral site, a longer needle **1 1/2", 16 G** is more useful.

E-Aspiration Technique

للاطلاع 1. Prepare the site selected as for aseptic surgery. If the iliac crest is to be used, its thick craniodorsal aspect can be located by placing the thumb and forefinger on either side of the wing of the ilium. If the femur is to be used, the aspiration site is the trochanteric fossa located medial to the palpable greater trochanter of the femur.

2. Enter the medullary cavity by carefully rotating the needle **(with its stylet securely in place)** back and forth until it is firmly seated in the bone. Caution: Do not penetrate the marrow cavity deeper than necessary since this will rupture blood vessels and the marrow aspirate will be contaminated with peripheral blood.

3. Remove the stylet from the bone marrow needle and attach a dry 10 ml syringe with air tight fitting plunger. Establish a vacuum in the syringe by withdrawing the plunger. Marrow should slowly enter the syringe. Collect only a few drops and release the vacuum. Caution: prolonged application of vacuum to the marrow will cause rupture of blood vessels and contamination of the specimen with peripheral blood.

F-Fixing and Staining the Marrow Films

1. If the marrow films are to be sent out for examination, they should be fixed by immersion for 5 minutes in absolute methyl alcohol. If they will be examined within 24 hours, fixation may be omitted.

2. If they are to be stained and examined in the office, the usual blood stain may be satisfactory. The staining time should be increased to 2-3 times the usual required for blood films.

G-Interpretation of Bone Marrow Examination

Not; Accurate classification of the immature cells requires much practice.

1. A differential count is made of 500 nucleated cells, and record the names of all cell types.

2. The G:E ratio (**also called the M:E ratio**) is determined by dividing the number of nucleated red cells into the sum of all cells belonging to the granulocyte series, **(proportion of granulocytes to nucleated red cells).**

a. An increased G:E ratio indicates that the granulocytes are relatively more numerous than usual. This indicates either granulocytic hyperplasia or erythrocytic hypoplasia.

Correlation of the bone marrow findings with the peripheral blood findings usually indicated.

b. A decreased G:E ratio has the opposite significance from an increased ratio, i.e., increased erythrogenesis or decreased granulopoiesis.

c. A G:E ratio of 1:1 would mean that nucleated cells of both series are present in equal numbers.

d. The G:E ratio as applied to anemia must be made in light of the leukocyte count. In the dog, when total leukocyte count within normal limits, a G:E ratio of less than 1:1 means intensification of erythrogenesis, while a G:E ratio greater than 2.5:1 reflects depression of erythrogenesis.

Maturation sequence (series):

Immature cells are generally larger and become smaller as they mature. The nuclei of the immature cells are relatively large in relation to the amount of cytoplasm and become smaller with maturity. The chromatin of the nucleus in immature cells is delicate, fine and stippled. As the cell matures, the chromatin becomes coarse, clumped and compact. Nucleoli are found in the nucleus.

Erythrocytes Maturation sequence (series):

1 Rubriblast

2 Prorubricyte

3 Rubricyte

4 Metarubricyte

5 Reticulocyte

6 Erythrocyte

Rubriblast:

- First recognizable cell in the erythrogenic series.
- A large round cell which contains a large round nucleus with a thin rim of royal blue cytoplasm.
- The nuclear chromatin is delicate and stippled.
- The nucleus contains one to several nucleoli.
- The cytoplasm is somewhat scanty and stains very basophilic.

Prorubricyte:

- Cell is similar to the Rubriblast but smaller in size.
- Nucleoli are usually absent, but remnants of the nucleoli may still be present.
- The chromatin material is somewhat coarser.
- A perinuclear clear zone may be observed.
- The cytoplasm stains basophilic.

Rubricyte:

- Cell is smaller than the Prorubricyte.
- Nuclear chromatin material is coarsely clumped separated by light streaks giving the so-called cartwheel appearance.
- Nucleus is round and stains very dark.
- Cytoplasm stains very **basophilic** in the early rubricyte stage, but the blue color is diluted out by the **pink** color of hemoglobin as it matures toward the next stages.
- This cell can be further subdivided according to the amount of hemoglobin in the cytoplasm into **basophilic, polychromatophilic** and **normochromic** rubricyte.

- Virtually last Rubricyte within erythrocytic series capable of cell division, but stop division when critical hemoglobin concentration is reached, while deficiency of hemoglobin in cell can result in extra divisions and smaller cells. **(eg: Iron deficiency results in microcytes.)**

Metarubricyte:

The nucleus is small, pyknotic, and appears as a dark blue homogeneous mass without any distinct chromatin structure. The cytoplasm stains similarly to the mature erythrocyte. The cell is not found in the peripheral blood of normal healthy animals. When observed in peripheral blood, it denotes a response to an anemic condition of at least 72-96 hours duration.

Except horse does not release nucleated red cells into the peripheral blood under any circumstances.

Reticulocyte (polychromatophilic cell):

- This cell is larger than the mature erythron and is non-nucleated.
- Cell may contain a small round nuclear remnant called a Howell-Jolly body.
- Cytoplasm stains slightly basophilic with Wright's stain. However
- Stained with a **supravital-stain** such as **new methylene blue** or **brilliant cresyl blue**, precipitated ribosomal RNA (reticulum) can be demonstrated within the cell.
- Cells are not found in health peripheral blood of the horse, cow, sheep, and goat. This means that the reticulocyte ripens in the bone marrow in these species.
- Dogs and cats may normally have **1-1.5%** reticulocytes in the peripheral blood.
- The cell does not participate in normal rouleaux formation or pathologic agglutination; is more resistant to crenation and lysis; is less susceptible to mechanical trauma; has a great excess of membrane in relation to its contents, but is able to synthesize hemoglobin.

Erythrocyte:

- These are the mature non-nucleated red blood cells.
- Cell stains buff or reddish color.

Control or eryhtropoiesis

Erythropoietin is stimulates the erythropoiesis. It is produced by the kidney and/or liver in most species. Its release determined by tissue oxygen levels.

Erythropoietin also causes an increased release of reticulocytes into the circulation (reticulocytosis).

Endocrine glands such as the pituitary, thyroid, adrenals, and gonads affect erythropoiesis. These glands are responsible for general metabolic activity and cellular oxygen requirements.

Essential materials for red blood cells production;

1. Proteins

- Needed for the production of globin in hemoglobin.
- Abnormal erythropoiesis may occur in a protein deficient diet.

2. Minerals

a. Iron is an integral part of the hemoglobin molecule and synthesis.

b.Copper is a factor for the enzyme ALA dehydrase which is required in the synthesis of heme.

c.Cobalt is essential for ruminants to synthesis B12 in the rumen. Excess Cobalt results in polycythemia (**produces tissue anoxia).**

3. Vitamins

Essential vitamins for erythropoiesis are in the B series. All may not be required by every species of animal, but deficiencies of the following may lead to development of anemia:

- Riboflavin (B2)
- Pyridoxine (B6)
- Niacin or nicotinic acid
- Folic acid
- Thiamine
- B12

Origen and development of granulocytes

- Granulocytes develop in the bone marrow from undifferentiated stem cells.
- Granulocytes are readily differentiated from the nucleated erythrocytes by their fine, reticulated chromatin structure and bluish cytoplasm.
- Maturation of the granulocytic series is characterized by the development of granules, initially formed in the **progranulocytes** and called **"primary or azurophil"** granules. These granules are peroxidase positive.
- Myelocyte stage is characterized by **"specific" or secondary** granules persist throughout the maturation process. (Basophils have granules with an affinity for blue or basic dye; eosinophils that are stained reddish-orange with the acid dye eosin are; and neutrophils with granules do not stain intensely with either dye).

Granulocytes Maturation sequence (series); The stages are:

1 Myeloblast

2 Progranulocyte

3 Myelocyte -- Basophil, eosinophil, neutrophil

4 Metamyelocyte -- Basophil, eosinophil, neutrophil

5 Band Cell -- Basophil, eosinophil, neutrophil

6 Segmented Cell -- Basophil eosinophil, neutrophil

1 Myeloblast

- The first cell that can be recognized in the granulocytic series.
- The most immature granulocytic precursor
- Possesses a relatively large, round to oval nucleus, with one to several nucleoli.
- The chromatin of the nucleus is finely stippled or has a light ground glass appearance.
- The cytoplasm is somewhat scanty, basophilic, and does not contain granules.

2 Progranulocyte

- The nuclear chromatin material is coarser and more clumped than that of a myeloblast.
- Remnants of the nucleoli may still be present.
- The cytoplasm is less basophilic than the myeloblast and contains dark stained non-specific granules called **"primary or azurophilic granules.**" These granules are peroxidase positive.

3 Myelocyte

- The nucleus remains round to oval and the chromatin material is more closely clumped.
- Contains **"secondary"** or specific granules, are identified by their staining properties as neutrophils, eosinophils, and basophils. These granules are peroxidase negative.
- Myelocyte and all subsequent cells of the granulocytic series been characterized as neutrophils, eosinophils and basophils.
- Myelocyte stage is the last stage of cell division and the first cell capable of phagocytosis.

4 Metyelocyte

- The nucleus is indented and often resembles a kidney bean.
- Nucleoli are not present and the nuclear chromatin material is coarser and clumped.
- Cytoplasmic granules are also present.

5 Band cell

- This cell has a horseshoe shaped nucleus.
- The opposite sides of the nucleus are more or less parallel.
- The nuclear chromatin material is markedly clumped.
- Band differ from metamyelocyte by horseshoe nucleus and tendency for nuclear sides to be parallel.

Segmented Cell

The nucleus may be mono-lobed with clumped chromatin material, or may consist of several lobes separated by constrictions or by filaments. The cytoplasm stains very faintly.

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Leukocyte lysosomes; The term lysosome is used to describe intracellular membranous sacs containing acid hydrolytic enzymes. When leukocytes phagocytize, there is a release of lysosomal contents. Primary granules (azurophil granules) contain acid phosphatase, acid hydrolytic enzymes, basic protein and one-third of the lysozyme. Secondary granules (specific granules) contain alkaline phosphatase, lactoferrin and two-thirds of the lysozyme.

Origen and development of agranulocytes

The agranulocytic series is comprised of leukocytes devoid of specific granulation. These cells generally originate in the lymphatic system, but they may be produced elsewhere in the body. This series includes the lymphocytic and monocytic groups.

1 Lymphocyte Maturation sequence (series)

The lymphocytic series refers to the development of the lymphocytes. The cells arise mainly from the reticular tissue of the lymph nodes and lymphoid tissue from which they derive their name. Lymphocytes are normally found in the peripheral blood. The nucleus does not become segmented and specific granules do not develop. The stages in lymphocytic development are:

- **Lymphoblast**
- **Prolymphocyte**
- **Lymphocyte**

Lymphoblast

- Cell is similar to other blast cells. It is round or oval, very large, with a large round to oval reddish-purple nucleus.
- The nuclear chromatin material is fine and well distributed, more coarse than in myeloblasts.
- The nucleus contains one or two nucleoli.
- The cytoplasm is bluish and nongranular and forms a thin rim around the nucleus.

Prolymphocyte

- The nucleus is round or oval in shape but smaller than the lymphoblast.
- The nuclear chromatin is coarse and slightly clumped.
- Nucleoli or remnants of nucleoli may be present.
- Abundant amount of light blue cytoplasm around the nucleus.
- There may be a few azurophilic granules in the cytoplasm.

Lymphocyte

- Mature cell of lymphocytic series, the only cell form found in the peripheral blood in health.
- Lymphocytes vary greatly in size and may be classified as small, medium or large.
- The cells are easily distorted and often appear in irregular shapes in stained preparations.
- The nuclear chromatin is condensed to form large, discrete almost solid clumps, with thickening of the nuclear membrane.
- Nucleoli are absent.
- Non specific granules may be observed in the cytoplasm of these cells.

2 Monocytic Maturation sequence (series)

The monocytic series refer to the stages of development of the monocyte. These cells may be formed from RE cells, the most important sites of origin is the spleen.

The stages in the monocytic development are:

- **Monoblast**
- **Promonocyte**
- **Monocyte**

Monoblast

The cell is large with a round or oval nucleus. A nucleolus is present. The nuclear chromatin material is fine and well-distributed. There is a thin rim of clear blue cytoplasm around the nucleus. There are no granules present in the cytoplasm.

Promonocyte

The cell is somewhat smaller than the monoblast with the nucleus being irregularly-shaped.The nuclear chromatin material is fine and spongy. There may be a nucleolus or a remnant of the nucleolus present. The cytoplasm is grayish blue and may contain non-specific granules.

Monocyte

The cell is larger than a neutrophil in the thin portions of a smear. The shape of monocytes is variable. The nuclei are usually round or kidney-shaped, but may be deeply indented or have two or more lobes connected by narrow bands. Blunt pseudopods and digestive vacuoles may be present. Monocytes are most difficult to identify and to differentiate from other cells.

The characteristic features of the monocytes and the most helpful in diagnosis are the dull grayish-blue color of the cytoplasm, blunt pseudopods and the brain-like convolution of the nucleus.

Origen and development of Thrombocytes/platelets

Cells of the megakaryocytic system are peculiar in that the nucleus undergoes multiple mitotic divisions without cytoplasmic separation, thus producing giant polyploid cells. The multiple nuclei usually remain attached to each other and are often superimposed giving a lobular appearance. The cytoplasm undergoes maturation changes characterized by the development of granules and membranes, culminating in platelet differentiation and liberation.

The stages in thrombocyte development are:

- Megakaryoblast
- **Promegakaryocyte**
- **Megakaryocyte**
- **Thrombocyte (Platelet)**

1 Megakaryoblast

The cell is large, irregularly shaped with a single or several round or oval nuclei and with a blue, nongranular cytoplasm. Nucleoli are usually present.

2 Promegakaryocyte

This cell differs from the megakaryoblast in that there are bluish granules in the cytoplasm adjacent to the nucleus. The nucleus in this second stage of maturation has usually divided one or more times and the cell has increased in size.

3 Megakaryocyte

The cell is very large with relatively large amounts of cytoplasm, and multiple nuclei. The cytoplasm contains numerous small, uniformly distributed granules that are reddish-blue in color.

4 Thrombocyte (Platelet)

Platelets are fragments of the cytoplasm of megakaryocytes. They vary in size and shape from a barely visible structure to masses larger than red cells or leukocytes.The cytoplasm stains a light blue and contains variable numbers of small blue granules (**azurophilic**).