Introduction to Leukemia

LEUKEMIA

DEFINATION:

Leukemia (and other cancers, in general) is defined as a disease where a clone of a certain cell (malignant) **gains an uncontrolled growth (proliferation)** advantage over the normal cells. Leading to **an overgrowth abundance of that malignant cell**. In the case of leukemia, this uncontrolled proliferation leads to the predominance of the malignant cell in the bone marrow followed by its spread to peripheral blood, and possibly other tissues.

Consequences of uncontrolled malignant cell growth

1. Replacement of the normal cells in the bone marrow by malignant clone.
2. Interference with the function of the normal marrow cells.
3. Possibly invasion (metastasis) of the other organs e.g., liver, lungs, bones, etc.
4. Death due to complication caused by the abundance of the malignant cells.

Complications:

Death usually occurs due to severe infections and organ failures such as lungs, liver, kidneys, etc. leukemia is a dangerous disease and therefore its proper diagnosis is crucial. Quick and proper diagnosis of leukemia could mean the difference between life and death of patient. Some types of leukemia, if diagnosed early could be treated and even cured. In the majority of cases, proper and quick diagnosis, could add a few years to the patient's survival.

There are a few non-malignant (benign) situations where the clinical and lab findings are similar to that of leukemia. For example, chronic

leukemia (where myeloid or lymphoid) is characterized by an extremely high. WBC counts, however, such counts could also be seen in benign situations such as **Leukemoid Reaction (LR)** with WBC counts ($>150\times10^9$/L) most of which are mature neutrophils and their precursors a picture very similar to CML.

However, the pathology and implication of LR are very different from CML. LR is usually seen in severe infections, pregnancy, inflammation, intoxication, and others. Similarly, CLL could be confused with **Reactive Lymphocytosis** (also known as **Infectious Lymphocytosis**) seen in Infectious Mononucleosis (IM), and other viral diseases, on the other hands, acute leukemias have similar morphology on Geimmsa stained slides where it is difficult to classify the type of blats on the basis of morphology alone. That is, whether the blasts are of myeloid or lymphoid origin. Therefore, distinguishing among the different types of leukemia is achieved using additional techniques such as: cytochemistry (special stains), immunphenotyping, PCR, etc.

Classification of Leukemia

A few methods or systems were designed to help in the classification of leukemias, however, all of the systems are based on two major criteria; 1. according to the pathological nature of the malignant process (i.e., acute versus chronic), or 2. According to the type of cell involved (i.e., lymphoid versus non-lymphoid). Further classification or sub-divided of these two groups was designed to accurately assess diagnosis, and treatment. One of such systems is the universally used French-American-British (FAB) classification (see below, in ALL classification).

1. Acute versus Chronic

This classification depends on the pathological presentation of the disorder, where it is classified as either Acute, or chronic.

Acute:

The process is sudden (abrupt) with death usually occurring within months from the time of diagnosis (if no proper medical intervention is taken). Symptoms include fever, hemorrhage, weakness, anemia, repeated infections, fatigue, easy bruising, and other bleeding disorders. Acute leukemia affect all ages, however, Acute Lymphoid Leukemia (ALL) is usually a disease of young children (see ALL below, for more details).

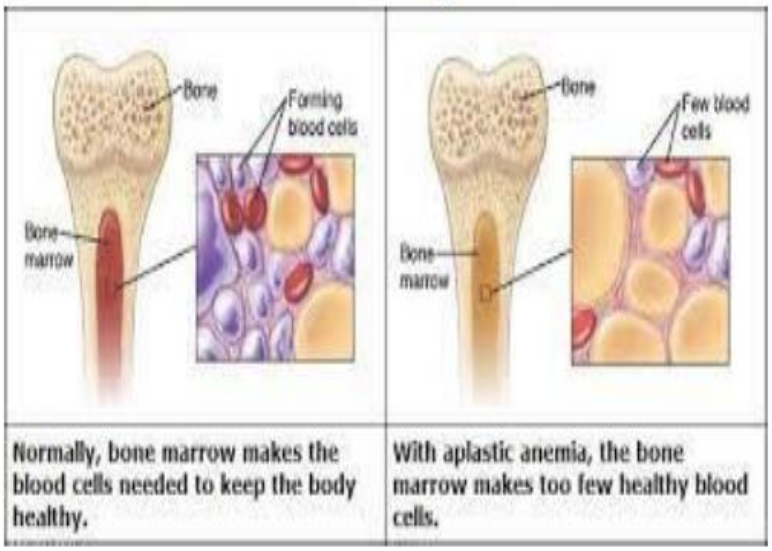
Chronic:

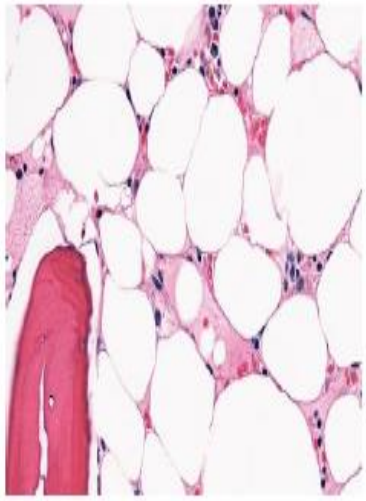
The process is more gradual (insidious) and subtle, taking years for death to occur. The symptoms are not so quick appearing until the late stages. Symptoms include malaise, fatigue, general weakness, anemia, pallor, splenomegaly and /or hepatomegaly. Chronic leukemia is a disease effecting adults, mostly. A typical feature of chronic leukemia (unlike acute ones) is an EXTREMELY ELEVATED WBC COUNTS.

2. Lymphoid versus Myeloid (also called non-lymphoid):

In this system, the leukemia process is classified according to the type of cell involved, which could be of: 1- Lymphoid origin, thus known as lymphoid (or lymphocytic) leukemia. 2- Myeloid origin, thus known as myeloid or myelocytic leukemia. This type is also known as Non-Lymphocytic Leukemia, i.e., AML (Acute Myeloid Leukemia) is also known as ANLL (Acute Non-Lymphoid Leukemia).

Aplastic anemia





Aplastic anemia, (hypoplastic) anaemia is defined as pancytopenia resulting from aplasia of the bone marrow. It is classified into primary (congenital or acquired) or secondary types, men and women are affected with equal frequency.

Pathophysiology

Bone marrow failure result from severe damage to the hematopoietic cell compartment. There is replacement of the bone marrow by fat.

Causes of aplastic anemia

Primary

1-Congenital (Fanconi, Shwachman (Diamond syndrome), Reticular dysgenesis, Amegakaryocytic thrombocytopenia, Familial aplastic anemias and Preleukemia).

2-Idiopathic acquired.

Secondary

1-Ionizing radiation: Accidental exposure (radiotherapy, radioactive isotopes, nuclear power stations).

2-Chemicals: Benzene, organophosphates, other organic solvents, and other pesticides.

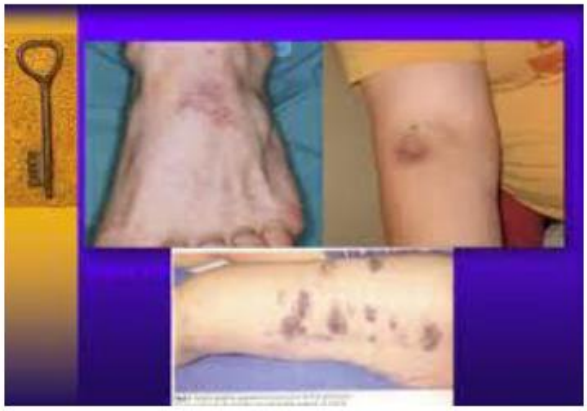
3-Drugs: Those that regularly cause marrow depression (e.g. busulfan, melphalan, cyclophosphamide, anthracyclines, nitrosoureas). Those that occasionally or rarely cause marrow depression (e.g. chloramphenicol, sulphonamides, anti-inflammatory, antithyroid, psychotropic, anticonvulsant/antidepressant drugs).
4-Viruses: Viral hepatitis.

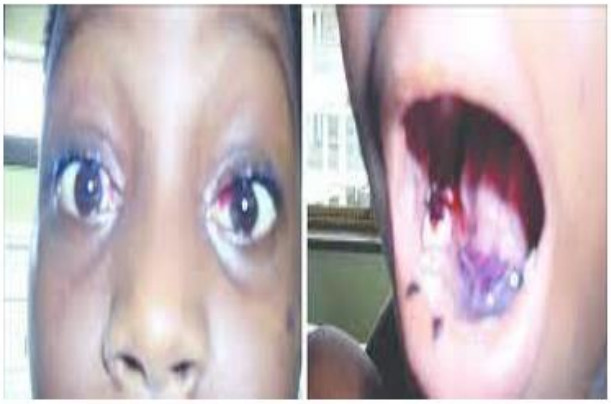
Laboratory findings

1-Blood:
Smear shows large erythrocytes and a paucity of platelets and granulocytes.
Reticulocytes are absent or few.
2-Bone marrow:
Fatty biopsy specimen may be grossly pale Dilute smear "Dry tap" instead suggests fibrosis or myelophthisis.

Clinical features

Bleeding is the most common early symptom. Easy bruising, oozing from the gums, epistaxis, heavy menstrual flow, and sometimes petechie (massive hemorrhage is unusual), symptoms of anemia are also frequent, including lassitude, weakness, and shortness of breath.





Lab:11 Hematology class: Three

By

Msc.Sarah Ali

Al-toosi University Collage

Department of Medical Laboratory Techniques

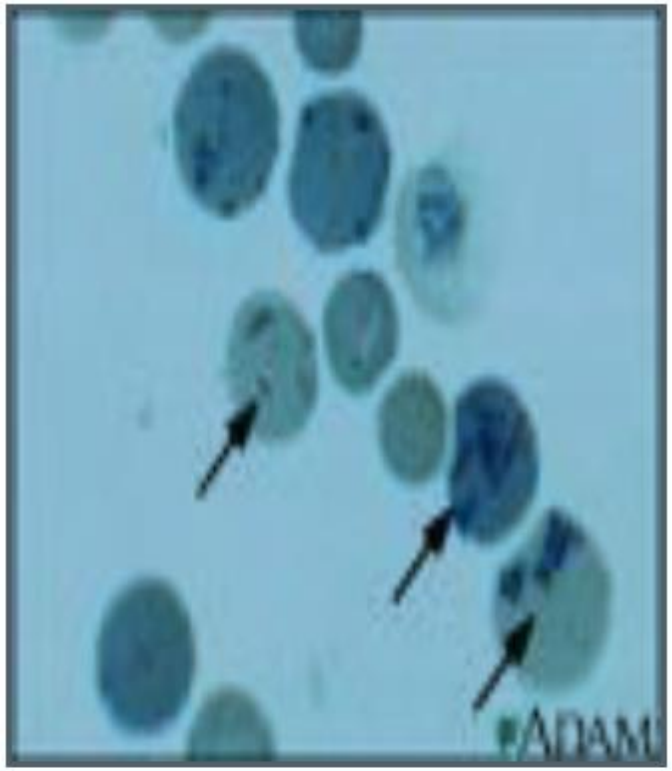
2022-2023

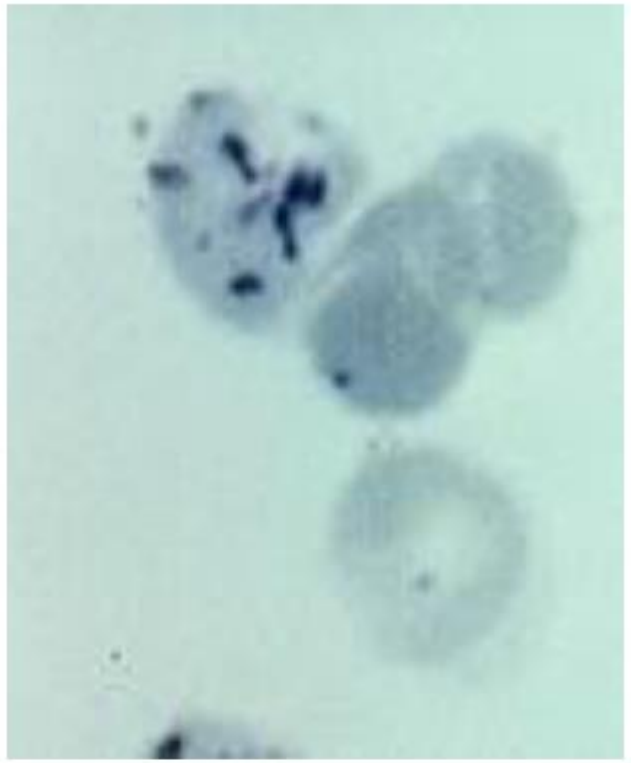
Reticulocyte

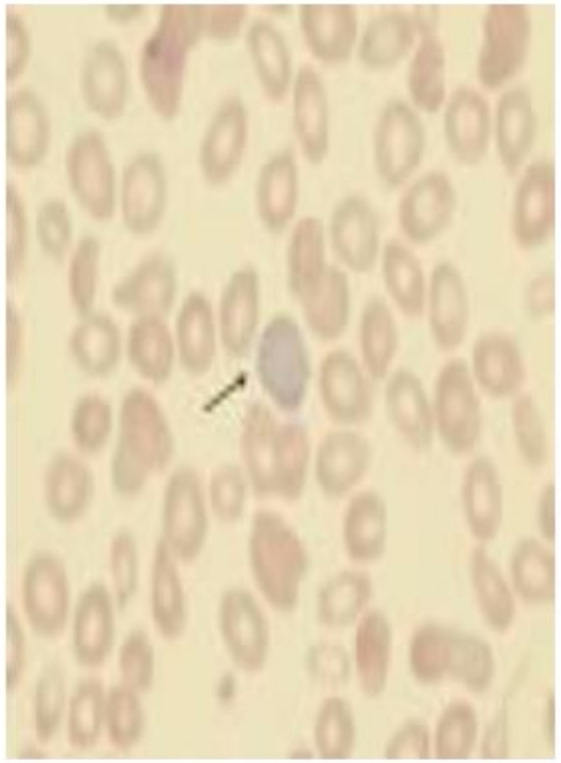
Definition:

- Immature RBC that remains 24hr in the circulation before they transform into mature RBC.

- They contains remnant of ribosome and RNA which were present in large amount in the cytoplasm in the precursors which they were derived.







Reticulocytes 40x	Reticulocytes 100x	Reticulocytes = polychromatohilic

Reticulocytes count

Purpose of testing:

- Reticulocyte count help in monitoring anemic patients under treatments
- Determine the state of increased erythropoietic activity
- Assess bone marrow activity

Principle of test

- The peripheral blood sample is stained with supravitalstain (brilliant cresylblue or New methyleneblue).
- These are **basic dyes** that have the ability to react with **ribosome** and **nucleic acids** of **reticulocytes** while it still alive.
- The neuclic acid-dye reaction form a **blue precipitate** of granules or **filaments**.

Staining method

- Deliver 2-3 drops of the dye solution into a 75x10 mm glass or plastic tube using Pasture pipet.
- Add twice the amount of the patients EDTA blood to the dye solution and mix.
- Keep the mixture for 37˚C for 15-20 min.
- Resuspend the Red cells by gentle mixing.
- Make film on the slide in the usual way.

Counting reticulocytes

1. Look into the slide area using 10x objective
2. Add a drop of oil
3. Move to 100x
4. Count all red cells (reticulocytes+ RBC) using a manual counter
5. Make sure not to count WBC (large nucleated cell)
6. The number of reticulocytesis counted also on outside paper to be able to calculate.

Calculations

$$\% \text{ retics} = \frac{\text{no. Of retics}}{\text{Total no. Of red cells}} \times 100$$

- Total no. of red cells= retics and RBC in 10 fields.
- If the count of RBC is 200-25-/ filed
- ⟶ meaning that in 5 filed we will have total of 1000 RBC and retics
- Thus calculation will be the following:

$$\% \text{ retics} = \frac{\text{no. Retics}}{1000} \times 100 \rightarrow \%\text{retics} = \frac{N}{10}$$

- *N.R in adults= 0.5% -1.5%* *N.R in neonates= 0.5 % -3%*

Source of errors:

- ❖ Improper mixing of the specimen with the stain
- ❖ Improper time of incubation of the stain with the dye.
- ❖ Improper count.
- ❖ Count Heinz body as reticulocytes.

Condition of high and low count of reticulocytes

Increased reticulocytes:

- Effective erythropioesis
- Anemic patient under treatment
- B.M assemant after bleeding

Decreased reticulocytes:

- Ineffective erythropiesis

  Ex: BM disease or chemotherapy associated .

  Hypoprolifrative conditions

Thanks for listening

Absolute leukocyte count

The absolute count for a particular type of blood cell is the total white blood cell count multiplied by the differential percentage for that cell type.

Normal value of absolute count of leukocytes

Cell	**Absolute count $\times 10^9$/L**
Neutrophil	1.8-7.7
Lymphocyte	1.0-4.8
Eosinophil	0.04-0.5
Monocyte	0.2-1.0
Basophil	0.01-0.1

Neutropenia

This implies a decrease in the number of neutrophils with an absolute Count <1500 cells/µl. Based on the count neutropenia can be mild (1000-1500 cells/ µl), moderate (500-1000 cells/ µl) and severe (<500 cells/µl).

Causes of neutropenia

- Bacterial infections such as typhoid, tuberculosis, gram- negative sepsis.
- Viral infections such as hepatitis B, cytomegalovirus, Epstein-Barr virus, human immunodeficiency virus, hepatitis C virus.
- Drugs
- Immune-mediated causes
- Transfusion reactions
- Neonatal alloimmune neutropenia
- Chronic autoimmune neutropenia
- Chronic idiopathic neutropenia
- Pure white cell aplasia

 Autoimmune disorders: systemic lupus erythematosus, rheumatoid arthritis, Wegener's granulomatosis.

- **Nutritional causes:** megaloblastic anemia, copper deficiency, zinc excess
- **Bone marrow disorders:** aplastic anemia, replacement by lymphoma, leukaemia,
- ✓ myelofibrosis, multiple myeloma
- ✓ Congenital or chronic neutropenias
- ✓ Cyclic neutropenia
- ✓ Inborn errors of metabolism

Differential Leukocyte Count (DLC)

Principle

 The differential blood count is based on the staining of nucleus and cytoplasm of the white blood cells.

 Staining of both nucleus as well as cytoplasm enables us to determine the morphology and other properties of cells.

 For differential count, generally the combination of polychrome methylene blue and eosin stains are used because of their selective staining properties; methylene blue stains nucleus while eosin stains cytoplasm.

 Staining is followed by quantitating the different types of cells

Equipment

 Micropipette
 Glass slides with cover slips
 Microscope
 Clean gauge or cotton

Sample

 Whole blood using EDTA or heparin as anticoagulant. However, capillary blood may also be used.

Reagents

 Stains – Commonly used satins are Leishman's stain, Wright stain, Giemsa stain, and Filed stain. However, only one stain is used while doing differential count. Generally, Wright's stain is used.

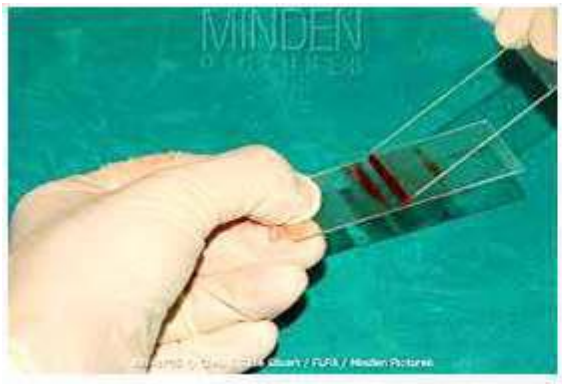
- 70% Ethanol
- Distilled water

Wright's Stain

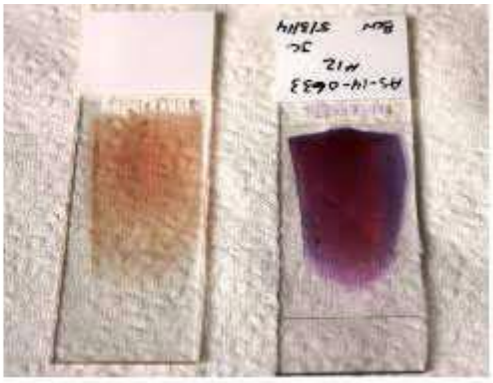
- It is a histologic stain that facilitates the differentiation of blood cell types.
- It is classically mixture of eosin (red) and methylene blue dyes.
- It is used primarily to stain peripheral blood smears, urine samples, and bone marrow aspirates and examined under light microscope.
- It is prepared by mixing 1.0gm of Wright's stain powder in 400ml absolute methanol. Thereafter 100ml phosphate buffered saline is added to the mixture.

Procedure

- Collect drops of blood on the end side of a glass slide.
- Spread the blood drop with another glass slide by placing it at an angle of 45 degree and move sidewise.
- Hold the spreader firmly and move it on the previous slide to the other end in a straight line with same force and pressure.
- Allow the glass slide to dry after formation of the smear.
- Air dry the slide or use any fixative to fix the smear.
- Stain the slides with a stain stated above.



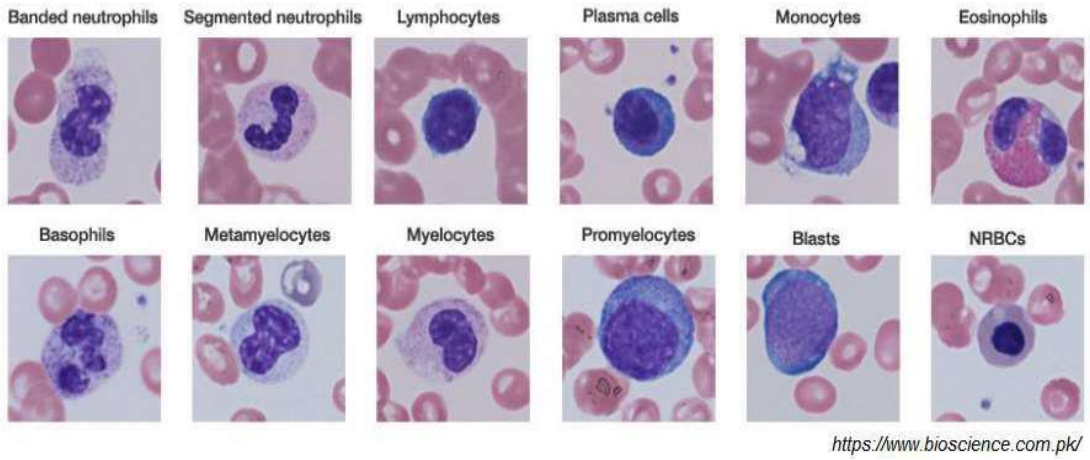
Smearing of glass slide



Smears on glass slides

Staining with Wright's stain

WBC types	Colour	WBC types	Colour
Erythrocytes	Yellowish – red	Eosinophils: Granules	Red or Orange – red
Neutrophils: Nucleus	Dark purple	Basophils: Nucleus	Purple to dark blule
Neutrophils: Cytoplasm	Pale – pink	Basophils: Granules	Very dark purple
Neutrophils: Granules	Reddish – lilac	Lymphocytes: Nuclei	Dark purple
Eosinophils: Nulclei	Blue	Lymphocytes: Cytoplasm	Sky blue
Eosinophils: cytoplasm	Blue	Platelets	Violet to purple granules



Staining of different subsets of WBCs (Wright-Giemsa stain)

Interpretation of results

Condition associated with increased or decreased number of WBC types		
Type of WBC	Increased number	Decreased number
Neutrophils	Neutrophilia	Neutropenia
Lymphocytes	Lymphocytosis	Lymphocytopenia
Monocytes	Monocytosis	Monocytopenia
Eosinophils	Eosinophilia	Eosinopenia
Basophils	Basophilia	Basopenia

Cell	%
Neutrophils	40-75
Lymphocytes	20-40
Eosinophils	2-6
Monocytes	2-10
Basophils	0-1

Precautions

- Always wear protective gloves/protective clothing/eye protection/face protection before handling the dilution fluid.
- Follow good microbiological lab practices while handling specimens and culture.
- Standard precautions as per established guidelines should be followed while handling clinical specimens.