HEMATOLOGY LECTURE NOTES

White Blood Cells Disorders

For

BACHELOR OF MEDICAL LABORATORY SCIENTISTS (BMLSC)

First edition

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Preface

Blood is the window to the body; it is the predictor of vitality and long life
Hematology is about relationships; the relationships of the bone marrow to the systemic circulation, the relationship of the plasma environment to the red cell life span and the relationship of hemoglobin to the red cell
Hematology is a difficult subject to master, because it forces students to think in an unnatural way.
In its most fundamental form, hematology is the study of blood in health and in disease.
This text book is written especially lab technologists to understand the most diagnostic information about the blood tests result, how correlated with diseases, etc
I try to make it more simple, more meaningful, because the most students are coming from Arabic schools and they meet many problems to understand the English language, with all that they try several times to make themselves better and better, I have queried many students over my 20 years of teaching and asked them what they desire to see in a textbook? What is the matter with them? The most of them reply that we get it more difficult science, but we love it and when we understand it becomes more interesting, we needs more efforts, and continuously reading.
I hope that this text travels with you as you continue your career in the laboratory professions and I hope that the information motivates you to read and learn more and more, god help you to serve yourself and your co citizen...

Dr. Abdulrazzaq Othman Alagbare
Acknowledgments

Deeply grateful for everyone who had written a book of hematology, or published on the internet, and I take from him, without prior permission, put this is a science for all, maybe it is difficult to me to get permission from them, but I still sorry, I still thanks everybody, maybe my thanks it is not enough but I hope them to forgive me for everything.

My target to give knowledge for students

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<tr>
<td></td>
<td>● Lymphoma</td>
<td></td>
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Section 1

Benign Leukocytes

Introduction:

White blood cells are a group of cells circulating in the peripheral blood may be divided into two broad groups:

1-the phagocytes → Neutrophil, Eosinophil, Basophil and Monocyte
2- the immunocytes → lymphocytes, their precursor cells and plasma cells

- Only mature phagocytic cells and lymphocytes are found in normal peripheral blood
- Their main function to protect the body and fight infections such as Viruses, Bacteria, Fungus, and others, the WBC activity site mainly in the inflamed tissues
- They are closely connected with two soluble protein systems of the body: immunoglobulins and complements

Leukopoiesis means the states of

- the production
- development
- differentiation of all white blood cells

Stages of Leukocyte Maturation

Granular cells development steps

Myeloblast->Promyelocyte (Progranulocyte) ->Myelocyte->Metamyelocyte->Band-> ->Segmented Neutrophil (Eosinophils and Basophils)
White Blood Cells types

They are 2 types

**Myelocytic Lineage**

Production of Granulocyte and Monocytes
Begins with myeloblast
Formed in bone marrow

**Lymphocytic lineage**

- Production of T and B lymphocytes
- Begins with lymphoblast
- Produced mainly in various lymphogenous tissues

**White Blood Cells storage**

Most neutrophils live and stored in the bone marrow. Normally, about three times WBC are stored in the marrow

Only about 5% live in the blood

In the circulation are

- 50% circulated in the circulation
- 50% in the marginal pool stored (in the wall of the vessels), the come out when needed
Cells distribution

1-In bone marrow: RBC:WBC=1:5
2-In Circulation: RBC:WBC=500:1

Diapedesis

Definition: Process by which Neutrophils and Monocytes come out of blood vessel wall

Pores of vessel wall are smaller than cells

Small portion of the cell squeezes through the pores

WBC - Life Span

Granulocytes - Life Span

After being released from bone marrow
- In blood 4 to 8 hours
- In tissues 4 to 5 days where needed

During serious tissue infection, total life span shortened

Monocytes - Life Span

- In blood 10 to 72 hours
- In tissues, they become tissue Macrophages, can live for 15-20 DAY
- Tissue macrophages provide continuing defense
Lymphocytes- Life Span

Continual circulation of lymphocytes
Lymphocytes enter circulation with lymph from the lymph nodes and other lymphoid tissue
After a few hours, they go out of blood and back into the tissues by diapedesis
Again re-enter lymph and return to blood
Life spans (weeks or months) depends on the body's need for these cells

Leucocytes in the Peripheral blood

Divided into

Phagocytes
- Granulocytes
- Neutrophils
- Eosinophils
- Basophils

Mononuclear phagocytic cells
- Monocytes
- Macrophage and dendritic cells

Lymphocytes
- B-cells
- T-cells

Relative absolute count or Differential leukocyte count (DLC)

Definition:
- It is the % of each type of WBC in the peripheral blood,
- Disadvantage: don’t give the true and actual count of each cell’s type
- For that must use the absolute count

<table>
<thead>
<tr>
<th>Cell</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils</td>
<td>40-65%</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>25-40%</td>
</tr>
<tr>
<td>Monocyte</td>
<td>3-8%</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>2-5%</td>
</tr>
<tr>
<td>Basophil</td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>
**Absolute leukocyte count:**

Definition: is the total of all the granular and non-granular white cells in the peripheral blood. 4000-10000/cum

**Names**

- Total leukocytes count (TLC)
- White cells count (WCC)
- White Blood Cells (WBC)

Normal range: 4000-10000/µL

**Cell** | **Absolute count/u/L**
---|---
Neutrophils | 2000-8000
Lymphocyte | 1000-4000
Monocyte | 150-800
Eosinophil | 100-600
Basophil | 40-100

All they are mature and functional cells. Each one has absolute normal count.

**How calculated the absolute count?**

Use the following formula

Absolute count = Diff % × Total WBC count

**Differential leukocyte count (DLC) or the relative count importance:**

1. Diagnosis and follow up cases
2. Indicator of chemotherapy Radiotherapy
3. Viral or Bacterial Infection?
4. Normal or abnormal blood cells presence
Types of DLC:
- **Normal DLC**
  When the person is healthy has the 5 normal white blood cells
- **Abnormal DLC**
  The person is not healthy, And the cells may include
  1. Normal + abnormal cells
  2. All are abnormal

**Cells included in DLC**
All nucleated cells in the peripheral blood normal or abnormal
Details
- Mature white blood cells in the peripheral blood.
- Immature white blood cells in peripheral blood
- NRBC (the nucleated RBC)
- Abnormal cells (of metastatic cancer)

**DLC over 100%**
The DLC must count 100 WBC on the PBS, but there are cases needs to count 200 WBC as follow, if there:
- WBC >35.0 × 10⁹/L
- Lymphocytes > 40% or <17 %
- Monocytes >12%
- Blasts (first-time for the patient)

**Critical values of WBC**
Critical values the values which are out of the lower or higher limits of normal reference.
- WBC Low 3.0 X10⁹/L
- High 25.0 X10⁹/L
- Also a case of presence of blast cells as first time for the patient

**Leukocyte indices on the CBC report are**
- LYM% (LY%) (lymphocyte) - relative (%) content (the norm is 25-40%) lymphocytes.
- LYM # (LY #) (lymphocyte) - the absolute content (norma1, 2 - 3,0 h10⁹ / l (or 1,2-3,0 x).
- NEUT% (NE%) (neutrophils) - relative (%) content of neutrophils.
- NEUT # (NE #) (neutrophils) - the absolute content of neutrophils.
- MON% (MO%) (monocyte) - relative (%) content of monocytes (normal 0,04-0,11).
- MON # (MO #) (monocyte) - the absolute content of monocytes (normal 0,1-0,6 10⁹ cells / l).
- EO% - relative (%) content of eosinophils.
- EO # - absolute content of eosinophils.
- BA% - relative (%) content of basophils.
- BA # - absolute content of basophils.
- IG % 0.36% and IG#
The peripheral blood cells

Platelets
Eosinophil
Neutrophil
Lymphocyte
RBC
Monocyte
Basophil
Band form

Plasma cells in the bone marrow only

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Section 2

Quantitative abnormalities of White Blood Cells

Introduction

- The white blood cells are **short lived** (phagocytic)
- Their abnormalities are **dramatic**
- The most important in the quantity abnormalities is to study the absolute count not the relative count only.

**More important to be careful to the**

- neutropenia in which the absolute neutrophils count is less than 2.0 x10⁹/L.

**Abnormalities in leukocyte number can be divided into**

- Increases count in each cell type.
- Decreases count in each cell type.

It is more accurate to think in terms of **absolute number** of a specific cell type rather than a percentage

- Absolute count it is a **true case** of increase of the number of any type of cells
- **The relative** case means that the DLC is over the normal but there is no increase of the absolute count of that cells

**Leucocytes in the human body**

Divided into two systems

1- Phagocytic system are
   - Neutrophils, Eosinophils, Basophils
   - Mononuclear phagocytic cells

2- The non-phagocytic system
   - Lymphocytes
   - B-cells .T-cells

**Leukocyte counts**

- The normal **adult** human leukocyte count in peripheral blood is 4.0-10.0 x 10⁹/L.
- A white blood count of 11.0 x 10⁹/L or more suggests **leukocytosis**
Granulocytes:
The Granular cells

**Definition:** Granulocytes are a group of white blood cells characterized by the presence of **granules in their cytoplasm**

**General proprieties**
- Contain **granules** in their cytoplasm
- They are 3 types:
  - Eosinophils "which red staining granules" 5%.
  - Basophils "which blue staining granules" 0.5-1%
  - Neutrophils "polymorphonuclear" cells 90-95%
- They constitute 65% of all white cells.
- They circulate in the blood and migrate into tissues particularly during inflammatory response.
- Contain **enzymes** in the granules
- **Lobulated** nucleus
- They are **mature and functional cells**
- White cells spend **only hours** in the circulating blood (4-5 hours)
- Most of the function of the white cells is performed in **the tissues**.
**Neutrophils properties**

<table>
<thead>
<tr>
<th>Property</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nucleus</strong></td>
<td>Polysgmented nucleus (lobes)</td>
</tr>
<tr>
<td><strong>Normal neutrophil nucleus</strong></td>
<td>3-4 lobes</td>
</tr>
<tr>
<td><strong>Band form</strong></td>
<td>2 lobes</td>
</tr>
<tr>
<td><strong>PMNs</strong></td>
<td>&gt;5 lobes</td>
</tr>
<tr>
<td><strong>Whole life</strong></td>
<td>5-7 days</td>
</tr>
<tr>
<td><strong>Life in the circulation</strong></td>
<td>4-5 hrs</td>
</tr>
<tr>
<td><strong>Enzymes</strong></td>
<td>Peroxidase reacting enzyme, and Alkaline phosphates</td>
</tr>
<tr>
<td><strong>Cytoplasm</strong></td>
<td>Contain granules</td>
</tr>
<tr>
<td><strong>Percentage</strong></td>
<td>40-65 %</td>
</tr>
<tr>
<td><strong>Function</strong></td>
<td>Kill bacteria</td>
</tr>
<tr>
<td><strong>Motion</strong></td>
<td>Active motile with amoeboid motion</td>
</tr>
<tr>
<td><strong>Neutrophils after death</strong></td>
<td>Called pus cells</td>
</tr>
<tr>
<td><strong>Storage place</strong></td>
<td>1- Mainly in Bone marrow</td>
</tr>
<tr>
<td></td>
<td>2- inside wall of vessels (veins)</td>
</tr>
<tr>
<td></td>
<td>3- spleen</td>
</tr>
<tr>
<td><strong>Absolute count in the peripheral blood</strong></td>
<td>2000-8000 µ/l</td>
</tr>
<tr>
<td><strong>50%</strong></td>
<td>in the circulation</td>
</tr>
<tr>
<td><strong>50 %</strong></td>
<td>In the storage</td>
</tr>
</tbody>
</table>
# Neutrophils distribution

- 50% in the circulation, 50% in the storage → marginal pool
- Circulating Pool

There is a continuous interchange, however, between the marginating and the circulating pools.

Marginating occurs to allow neutrophils to move from the circulating blood to the tissues through a process known as *diapedesis*.

## The neutrophils distributed in

- bone marrow
- marginal pool
- Circulating Pool
- Tissues
**Stages of granular white cell phagocytosis**

In the presence of bacterial or fungal infections, the neutrophil is activated to kill the offending organism steps

**A-CHEMOTAXIS:**
- chemical signals sent by foreign body,
- More neutrophils mobilize and rush to site of infection.

**B-OPSONIZATION:**
- needs the help of IgG + C3 to make it easily recognized by neutrophil and ingested.

**C-INGESTION:**
- The foreign body is engulfed by the neutrophilic pseudopod membranes

**D-KILLING:** the foreign body killed by the neutrophilic enzymes
Neutrophils abnormalities of females

**Drumstick**

Morphology:
Drumstick shaped nuclear appendage. ± 1.5 µm in diameter and attached to the nucleus by a filament. Inactive X chromosome of the female.

Found in:
Neutrophils of females
Males with Klinefelter syndrome

**Sessile Nodule**

Morphology:
Inactive X chromosome found as nodule on neutrophils of females.

Found in:
Neutrophils of females
### 2-Eosinophilic granulocytes

<table>
<thead>
<tr>
<th>Feature</th>
<th>Details</th>
</tr>
</thead>
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<tr>
<td><strong>Nucleus</strong></td>
<td>2 lobes</td>
</tr>
<tr>
<td><strong>Cytoplasm</strong></td>
<td>granular</td>
</tr>
<tr>
<td><strong>Granules content</strong></td>
<td>histamine, serotonin, and heparin etc.</td>
</tr>
<tr>
<td><strong>Granules</strong></td>
<td>big and yellow color</td>
</tr>
<tr>
<td><strong>Relative absolute count</strong></td>
<td>less than 2-6%</td>
</tr>
<tr>
<td><strong>Absolute count</strong></td>
<td>120-600 µ/l</td>
</tr>
<tr>
<td><strong>Function</strong></td>
<td>is defense <em>Against parasites</em>, and <em>allergic cases.</em></td>
</tr>
</tbody>
</table>

### 3-Basophilic granulocytes

- Nucleus: 2 lobes
- Cytoplasm: granular
- Granules: big and deep brown color obscure the nucleus
- content: histamine, serotonin, and heparin
- Relative absolute count: less than 1%
- Absolute count: 40-100
- Mast cells: the basophils in the tissue called mast cell
- Function: *increase vascular permeability*
Mast cells:

- The basophils in the tissue called mast cell, more specific cells and long lived 9-18 month
- They are widely distributed throughout the body and are larger than basophils, with a small round nucleus and more granules
- Contain acid phosphatase, alkaline phosphatase, and protease.

Function: plays a role in hypersensitivity reactions by binding IgE

**Mast cells** secrete vasoactive substances (prostaglandins and leukotrienes) during the inflammatory response.
4-Monocytes

One cell in the peripheral blood,

- It is **Immature** cell in the peripheral blood and **cannot fight infectious** agents
- It is the biggest cell in the peripheral blood,
- It’s nuclei is irregularly lobated,
- cytoplasm : abundant with fine granulation **NON VISIBLE contains** peroxidase, acid phosphatase, and arylsulfatase; this indicates that these granules are similar to the lysosomes of neutrophils
- Consist 3-8% of all WBC
- After they stay 2-3 days in the peripheral blood they migrate into the tissues to form **Macrophage**.
- Live 11-12 days

**Monocyte out of the circulation converted to**

1-Histiocytes → Tissue Macrophages in the Skin and Subcutaneous Tissues
2-and macrophages

Where?

in the endothelium of the body cavities; and take new name in that tissue viz

- Macrophages in the Lymph Nodes
- Alveolar Macrophages in the Lungs
- Kupffer Cells → Macrophages in the Liver Sinusoids
- Macrophages of the Spleen and Bone Marrow
- Microglia in brain
Function of Macrophage (Monocyte):

1. Defense against bacteria, fungi, viruses, and foreign bodies
2. Antigen presenting cell to the lymphocytes and other cells
3. Remove the dead cells from tissue and circulation
4- Phagocytosis by Macrophages

- Much more powerful phagocytes than neutrophils
- Can phagocyte more bacteria
- Engulf much larger particles (ex. leishmania and toxoplasma) even whole RBCs, malarial parasites
- Macrophages use nitric oxide (and reactive oxygen intermediates) to kill organisms they have ingested.

**Site:** tissues outside the vessels

**Mode of activity:** by phagocytosis

*macrophage (pink) attacking—Escherichia coli (green).*
**Lymphocytes**

**Types:** two major groups T and B lymphocytes  
- They are the second numerous cells in the peripheral blood  
- They called non granular  
- They has one rounded nucleus  
- Few rim of cytoplasm  

**General function:** responsible for the immunity in the body  

**Relative absolute count:** 25-40% of all WBC  
**Absolute count:** 1000-4000

**First group: Thymus-dependent T-lymphocytes**

1. Name: Thymus-dependent T-lymphocytes  
2. Percentage of all lymphocyte: 60-80%  
3. Site of production: Bone marrow  
4. Site of developing: thymus  
5. Function: The main immune cell responsible for  
   cellular immunity  
6. Life span: many years  
7. Circulation: the recirculates in each part of the body  

Diagrammatic cross section of a portion of the thymus, showing several lobules separated by connective tissue strands (trabeculae).
Function of T-Lymphocyte

During their maturation in the thymus they acquire the ability to recognize and distinguish self from non self "foreign tissues and infections agents". They are not capable of antibody production. Identified by their surface "CD = Cluster of Differentiation

Type of immunity: delayed-type hypersensitivity, or “immune cellularity” example as the tuberculin reaction

Types of T-Lymphocytes

Divided into

- Helper cells CD4+
- Suppressor cells. CD8+
- Natural killer -NK cells, CD3-

Second group - Bone marrow-dependent B-lymphocytes

- Name: Bone marrow-dependent B-lymphocytes
- Most B lymphocytes mature in the bone marrow Percentage of all lymphocyte: 20%
- Site of production: Bone marrow
- Site of developing: the secondary lymphoid organs (spleen, lymph nodes, etc)
- Function: humoral immunity (against viruses, bacteria, and allergens)
- Transformation: secreting plasma cells, to produce antibodies
- Life span: many years
- Circulation: the recirculates in each part of the body

Lymphatic vessels. Small lymphatic capillaries opening into the tissue spaces pick up interstitial tissue fluid and carry it into progressively larger lymphatic vessels, which carry the fluid, now called lymph, into regional lymph nodes. As lymph leaves the nodes, it is carried through larger efferent lymphatic vessels, which eventually drain into the circulatory system at the thoracic duct or right lymph duct.
Section 3

Abnormalities in leukocyte number

Quantitative changes of the WBC
Divided into two big categories

1-Leucocytosis - Quantitative changes
- Definition.. Is a raised white blood cell count (the leukocyte count) above the normal range due to elevation of any of a single lineage.
- Normal reference range (adult 21 years) 4.0 – 10.0 x 10³/L

2-Leucopenia - Quantitative changes
- Definition.. Total WBC lower than the reference range for the age is defined as leucopenia.
- Leucopenia may affect one or more lineages and it is possible to be severely neutropenic or lymphopenic without a reduction in total white cell count.

leukocytosis
The mechanism of leukocytosis can be of several forms
- Decreased marginal pool.
- Increase bone marrow production.
- Splenectomy → (Increase cells).

Classification of leukocytosis
Leukocytosis can be subcategorized by the type of white blood cell that is increased in number
- Leukocytosis-Neutrophilia.
- Leukocytosis-Lymphocytosis.
- Leukocytosis-Monocytosis.
- Leukocytosis-Eosinophilia.
Evaluation of leukocytosis

1- It may be a sign of illness
2- It is not a disorder, nor is it a disease
3- It is simply a laboratory finding.
4- A leukocyte count above 25 to 30 x 10⁹/L is termed a leukemoid reaction

Leukocytosis may indicate

1- Bacteria, viral or parasitic infection
2- Blood malignancy (Leukemia, PV)
3- Metastatic cancer
4- Normal reactive case
5- Drugs effects

Laboratory Signs of infection (cytotoxic)  

1- Presence of toxic granulation in neutrophils
2- Presence of toxic vacuolization in neutrophils
3- Increase of band form over 5% "left shift"
4- Increase Immature granulocytes (IG)
5- Presence of Dohle bodies

Other tests

1- Increase of ESR
2- Positive CRP test
3- LAP test positive
Granulocytosis
Increase in the count of all or one of the granulocytic components
- Neutrophils
- Basophils
- Eosinophils

Agranulocytosis
Decrease in the count of all or one granulocytic component

Neutrophilia
- Definition.. Increase in the number of neutrophils and / or its precursors
- In adults count >8.0 x 10^9/L but the counts are age dependent
- Increase may results from alteration in the normal steady state of
  1-Increase Production
  2-Demargination (marginal pool to circulating pool).

Toxic vacuolation: neutrophils showing phagocytosed bacteria in a case of severe septicaemia

Causes of Neutrophilia
- Infection, Bacterial - viral, fungal, spirochaetal, rickettsial).
- Inflammatory conditions, Autoimmune disorders
- Gout, Neoplasia, Metabolic conditions, Uraemia –Acidosis - Haemorrhage
- Corticosteroids, Marrow infiltration/fibrosis Cigarette smoking, Post-splenectomy
- Myeloproliferative disorders -leukemia. PV
Acute Neutrophilia

Acute neutrophilia – this occurs 4-5 hours after a pathologic stimulus

Results from an increased flow of cells from the bone marrow to the peripheral blood

*Bands and metamyelocytes may be seen*

Chronic neutrophilia –

This follows acute neutrophilia

The bone marrow starts to throw out younger and younger cells (a shift to the left)

Stress neutrophilia

- Definition: represents a shift of neutrophils from the marginating pool into the circulating pool
- results from physical action, other types of stress, and epinephrine injection.
- This case has the following
- It is transient
- No granulocyte production increase in the B.M

Terms describe the Neutrophils count and production:

- **Neutrophilia**: increase of the absolute count of the neutrophils over 8000 /µL (In all bacterial infection, toxins, drugs etc)
- **Neutropenia**: decrease of the absolute count of neutrophils lower than 2000 /µL (in aplastic anemia, toxins, drugs, cancers)
- **Shift to the left** Increase of the band form (indication of bacterial infection)
- **Agranulocytosis** must be written if absolute count is under 200/µL

Terms describe the Neutrophils content

1. **Toxic granulation**: Increases of the granules due to infection
2. **Hypersegmented Neutr.**: Increase nuclear segmentation over 5 lobes
3. **Toxic vacuolization**: presence vacuoles in the cytoplasm of neutrophils
4. **Dohle bodies**: are light blue-gray, oval, basophilic, leukocyte inclusions located in the peripheral cytoplasm of neutrophils.

Dohle bodies
Immature granulocytes (IG) - Reactive Left Shift –

Immature granulocytes (IG)

1. Means less mature forms Immature granulocytes indicate bone marrow is activated to fight off infection.
2. When immature granulocytes absolute numbers increase significantly,
3. this is a sign of infection, lead to increase the neutrophils absolute count

IG Cells are promyelocytes, myelocytes, and metamyelocytes

Divided into 2 types

1-sever → include metamyelocyte –myelocyte and band form
2-mild → include myelocyte and band form

indicates an increase in new cell production in this cell series

Normal IG: 0.36%

IG=2% indication of infection (Cases of septicemia in infants)

So higher so BAD

![Sever "Left Shift"](image1)

![Mild-moderate "Left Shift"](image2)
Leukemoid reaction

- is a marked elevation of the white cell count resembling leukemia
- but due to a benign condition
- The WBC count is often in a range of over 25.0 x10^9/L to 50.0 x10^9/L – and sometimes can be reach to 100.0 x10^9/L

Types:
1-Neutrophilic leukemoid reaction - adult
2-Lymphocytic leukemoid reaction - mostly children
3-Eosinophilic leukemoid reaction
4-Monocytic leukemoid reaction

The patient has no
- 1-Anemia
- 2-Bleeding
- 3-The case is acute
Section 4

**Leukopenia Neutropenia**

**Leukopenia**

Definition: Is a decrease in the number of circulating white blood cells (leukocytes) in the peripheral blood lower than 4000/cells/µL (for adult)

**Result of leukopenia? Increased risk for infection**

Causes of leukopenia

a) Chemotherapy,

b) radiation therapy

c) leukemia (as malignant cells overwhelm the bone marrow),

d) myelofibrosis

e) aplastic anemia

f) Medications

**Signs and symptoms of neutropenia**

*Due to low ability to fight bacterial infections*

- severe infections or sepsis
- Fever
  - Mouth ulcers ,
  - Diarrhea
  - Burning sensation when urinating
  - Sore throat

**Neutropenia**

a) Neutropenia defined as an absolute neutrophil count (ANC) (segmented + band neutrophils) less than 2000/ µl

b) Patients with neutropenia are more susceptible to bacterial infections

c) Patients with fevers and frequent infections.
Causes of Neutropenia
1. Aplastic anemia
2. Viral infections
3. Radiation
4. Autoimmune
5. Medication

Increased destruction
- autoimmune neutropenia.
- chemotherapy treatments,
- autoimmune diseases
- Sequestration in spleen

Neutropenia types
1. Severe acute neutropenia
2. Chronic Neutropenia
3. Chronic idiopathic neutropenia
4. Cyclic neutropenia
5. Congenital neutropenia
6. Drug-induced neutropenia
7. Autoimmune neutropenia

1-Acute Neutropenia
Severe acute neutropenia: An autoimmune reaction where the body produces antibodies to its own neutrophils which results in neutropenia.

2-Chronic Neutropenia
A patient has chronic neutropenia if the condition lasts for longer than 3 months.

3-Chronic idiopathic neutropenia
is a disorder in which the neutrophil count is less than $1.0 \times 10^9/L$, but results in few infections.

4-Cyclic neutropenia is characterized by periodic severe neutropenia, (Often <200/L) occurring in regular 21- to 30-day cycles.

5-Congenital neutropenia
1. Name: Kostmann's syndrome, Cause: autosomal recessive
2. Age: the first year of life, Severity: the patient has life threatening infections

6-Drug-induced neutropenia

7-Autoimmune neutropenia
1. Chronic, Autoimmune (antibodies directed against neutrophil antigens)
Neutropenia Classification - based on the absolute neutrophil count (ANC)

<table>
<thead>
<tr>
<th>Risk Category</th>
<th>ANC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Within normal limits</td>
<td>Normal</td>
</tr>
<tr>
<td>1</td>
<td>$\geq1500 - &lt;2000/mm^3$</td>
<td>Mild neutropenia</td>
</tr>
<tr>
<td>2</td>
<td>$\geq1000 - &lt;1500/mm^3$</td>
<td>Moderate neutropenia</td>
</tr>
<tr>
<td>3</td>
<td>$\geq500 - &lt;1000/mm^3$</td>
<td>Severe neutropenia</td>
</tr>
<tr>
<td>4</td>
<td>$&lt; 500/mm^3$</td>
<td>Agranulocytosis</td>
</tr>
</tbody>
</table>

Diagnosis of neutrophilia
1. CBC $\rightarrow$ count of Neutrophils absolute count $\rightarrow$ Blood film study $\rightarrow$ evaluation of band form presence over 5% $\rightarrow$ Toxic granulation in the cytoplasm and Toxic vacuolization
2. Bone marrow study
3. Uses of some stain such as -- A leukocyte alkaline phosphatase (LAP)

A leukocyte alkaline phosphatase (LAP) score
(sometimes called a neutrophil alkaline phosphatase or NAP score)
this is characteristically give a high % positive in mature neutrophils over 97%

Terms for neutrophils
- Neutrophilia: increase of the absolute count of the neutrophils over 8000 /µL, and the neutrophils DLC
- Neutropenia: decrease of the absolute count of neutrophils lower than 2000 /µL
- Agranulocytosis must be written if absolute count is under 200/µL
- Shift to the left: Increase of the immature granulocytes
- Toxic granulation: Increases of the granules due to infection
- Hypersegmented Neutr. Increase nuclear segmentation over 5 lobes
- Toxic vacuolization: presence vacuoles in the cytoplasm of neutrophils

Diagnosis of neutropenia
- A full blood count (low ANC)
- Bone marrow biopsy is often necessary
- Antineutrophil antibodies
Monocytosis
(monocytes > 1.0 x10^9/L in adults occurs in
1. in the recovery phase of infection,
2. myeloproliferative disorders.
3. **Monocytosis may result from**
   1. *viral, fungal, rickettsial, and protozoal infections.*

**Monocytopenia** (< 0.2 x10^9/μL) may be seen in the first few hours after giving prednisone, but by 12 hours returns to normal.

Eosinophilia
Eosinophilia is defined the absolute count is >700/μL eosinophils

**Causes of Eosinophilia**
1. **Allergy:** Atopic, Drug sensitivity
2. pulmonary eosinophilia
3. Infection: *Parasites*, recovery from infections
5. Skin disorders
6. Hypereosinophilic syndrome
**Idiopathic Hypereosinophilic Syndrome**

- An eosinophil count **greater than 1,500/µL** for at least **6 months**
- The leukocyte count usually ranges from **10,000 to 30,000/µL**, *with 30 to 70% eosinophils.*
- The eosinophils usually *look normal and mature on the blood smear*

**Criteria of diagnosis**

- Peripheral blood eosinophil >1.5 x 10⁹/µL
- Persistence of counts more than 6 months
- Absence of any obvious cause for eosinophilia

**Basophilia** ( > 0.2 X 10⁹/L/µL) is seen in

1. allergic reactions
2. chronic myelocytic leukemia
3. polycythemia vera
4. extramedullary hematopoiesis.
5. Basophilia is also reported in hypothyroidism.
Lymphocytes benign disorders

Lymphocyte function
The primary function of lymphocytes is immunologic:
- Forming antibodies; and securing immunity.
- Recognizing what is foreign, (non-self)
- Non-self or foreign substances may appear as bacteria, cell substances, proteins, or viruses

Points to remember
- All leukocytes make a one-way trip between blood and tissues.
- Lymphocytes only can recirculate between blood, tissue, and lymph fluid.
- Lymphocytes are the second most common type of leukocytes in adults (20–40% of WBC).
- The lymphocyte number is higher in children between the ages of 1 and 4 to have a relative lymphocytosis

Morphological appearance of lymphocytes
- Resting lymphocytes
- Reactive “transformed lymphocytes,” “atypicallymphocytes,” or “viral lymphocytes”
- Large granular lymphocytes, N.Killer cells
**Lymphocytosis**

1. Is an increase in the number of lymphocytes in the blood according to the patient age
2. **Relative lymphocytosis** is normal in children under age 2.
   - **Infants**: greater than 9000 per microliter
   - **older children**: greater than 7000 per microliter
   - **adults**: absolute lymphocytosis over 4000 per microliter

Causes **All** viral infection

---

**Lymphocytopenia:**

Decrease of the absolute count of lymphocytes lower than according to the patient age

- **Infant**: lower than 4000/cum
- **Young children**: lower than 3000/cum
- **Adult**: lower than 1000/cum

**May be seen with**

- corticosteroid Rx, chemo Rx, irradiation,
- Hodgkin's disease, HIV infection
- In HIV infection the lymphopenia is largely due to a loss of CD4 positive T-helper lymphocytes

**Reactive lymphocytes:**

- Used to describe transformed or benign lymphocytes.
- You should use the term “reactive”,
- The word “atypical” is used to describe malignant-appearing cells.
- A few reactive lymphocytes is not abnormal
Causes of Reactive lymphocytosis

1- Pertussis or whooping cough
2- Toxoplasmosis
3- Brucellosis
4- Infectious mononucleosis
5- CMV
6- Infectious lymphocytosis (coxsackie virus A or B6, echovirus, and adenovirus 12)
7- HTLV-1 (Human T Lymphocyte virus)
Resting Lymphocyte

Larg granular Lymphocyte (LGL)

Infectious mononucleosis

Lymphatic reactive state
Section 5

**Qualitative defects of granular white cells**

May be on
- Morphologic changes
- Functional changes

These changes are classified as either
  - Inherited
  - Acquired. (greater seen)

Qualitative changes of the white cell take place either in
  - the cytoplasm
  - or the nucleus

1- **Pelger-Huet anomaly**

This is benign congenital disorder

**What changes in the nucleus?**
- Bilobed and occasional unsegmented neutrophils
- don’t affect the cells function

2- **Hypersegmentation**

Defined as a segmented neutrophilic nucleus having more than five lobes,
  1. indication of Megaloblastic anemia
  2. antimetabolite cytotoxic therapy.

3- **May-Hegglin anomaly**

This is a congenital disorder
  1. Neutrophils contain basophilic inclusions of RNA
  2. leucopenia
  3. Thrombocytopenia and giant platelet are frequent
4-Alder’s granulation

This anomaly is *Inherited*

occurs in

- Granulocytes (Neutrophils),
- Monocytes
- and Lymphocytes

They contain *granules* which stain purple with Romanowsky stain

5-Chediak-Higashi syndrome

This is a congenital anomaly occurs in

- Granulocytes (Neutrophils),
- Monocytes,
- Lymphocytes
- and Platelets

They contain

**Giant granules**

1. **Neutrophils in Chediak-Higashi syndrome**, *Loss the Chemotaxis and phagocytosis ability*

2. **Platelets lack dense granules and platelet function is abnormal**

**Chediak-Higashi anomaly: characteristic giant granules within the cytoplasm of bone marrow myeloid precursors**
6- Myeloperoxidase deficiency (MPO)

*MPO* exist normally in
1- Neutrophils
2- Monocytes

**What is MPO deficiency?**
- Inherited
- Autosomal recessive

**How present the deficiency?**
Absence of the MPO

*Result of this absence* in the phagocyte cells *cannot kill the foreign* substances which engulfed

---

**Negative MPO**  
**Positive MPO**
Section 6

Hematological- malignant Disorders

leukemia

The leukemias are cancers of the WBCs involving
- Bone marrow,
- Circulating WBCs, and
- Organs such as the spleen and lymph nodes.

Etiology

Risk of developing leukemia is increased in patients with exposure to
- Ionizing radiation
- Chemicals (eg, benzene)
- Chemotherapy
- Infection with a virus
- Chromosomal translocations

Pathophysiology of leukemia

Malignant transformation usually occurs at
- The pluripotent stem cell level,
- Sometimes involves a committed stem cell with more limited capacity for differentiation.

More characteristics are:
- Abnormal proliferation
- Clonal expansion
- Diminished apoptosis (programmed cell death) lead to replacement of normal blood elements with malignant cells.

Manifestations of leukemia are due to

1. Suppression of normal blood cell formation
2. Organ infiltration by leukemic cells. → enlargement of the liver, spleen, and lymph nodes

Inhibitory factors produced by leukemic cells and replacement of marrow space may lead to

a) Suppress normal hematopoiesis, with ensuing anemia, thrombocytopenia, and granulocytopenia.
The leukemic cells are
- Trapped early from the B.M
- Proliferate without control (No effect of growth factors)
- Not able to carry out their function
- fetal disease if not treated

**Leukemia cells**

1-Leukemia for the following cells
1. Neutrophil
2. Eosinophil
3. Basophil
4. Monocyte
5. T-Lymphocyte
6. B-Lymphocyt

**2-RBC:**
Polycythemia Vera (PV)

**3-Thrombocytes:**
Essential Thrombocythemia (ET)

**4-Plasm cells**
Multiple myeloma (MM)

---

### 1- Myeloid group malignancies

<table>
<thead>
<tr>
<th>Granular cells</th>
<th>(N. E. and B) Leukemias</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocytes</td>
<td>Monocytic leukemia</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>Polycythemia vera</td>
</tr>
<tr>
<td>Platelets</td>
<td>Essential thrombocythemia</td>
</tr>
</tbody>
</table>

### 2- Lymphoid group malignancies

<table>
<thead>
<tr>
<th>B-lymphocytes</th>
<th>Leukemias</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-lymphocytes</td>
<td>Leukemias</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>Multiple myeloma</td>
</tr>
</tbody>
</table>

### French-American-British (FAB) Classification of Acute Leukemias

The FAB classification is based largely on.

1-Morphology of cells
2-Simple cytochemical stains reaction

But must be

1-At least **30%** of cells in the bone marrow or blood must be blasts.
2. and does not include cytogenetic abnormalities
Leukemia Classification

Leukemia were originally termed acute or chronic based on life expectancy but now are classified according to cellular maturity.

Leukemia could be Classified into

- Acute leukemia
- Chronic leukemia
- Myelodysplastic syndromes
- Leukemoid reaction

1-Acute leukemia

Acute leukemia consist of predominantly immature, poorly differentiated cells (usually blast forms). Acute leukemia are divided into

- Acute Lymphocytic Leukemia (ALL)
- Acute Myelogenous Leukemia (AML) types, which may be further subdivided by the French-American-British (FAB) classification

2-Chronic leukemia

- Chronic leukemia has more mature cells than do acute leukemias.
- They usually manifest as abnormal leukocytosis and mostly asymptomatic person.

Chronic leukemia are divided into

- Chronic lymphocytic leukemia – CLL
- Chronic myelogenous leukemia - CML

3-Myelodysplastic syndromes

Myelodysplastic syndromes involve progressive bone marrow failure but with an insufficient proportion of blast cells (< 30%) for making a definite diagnosis of AML; 40 to 60% of cases evolve into AML.

4-Leukemoid reaction

1. A leukemoid reaction is marked granulocytic or lymphocytic leukocytosis (ie, WBC > 25,000/μL) produced by normal bone marrow in response to systemic infection or cancer.

2. Although not a neoplastic disorder, it is benign case

3. A leukemoid reaction with a very high WBC count may require testing to distinguish it from CML
# Differences between acute and chronic leukemia

<table>
<thead>
<tr>
<th></th>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>All ages</td>
<td>Adults</td>
</tr>
<tr>
<td><strong>Clinical onset</strong></td>
<td>Sudden</td>
<td>Gradual</td>
</tr>
<tr>
<td><strong>Course of disease</strong></td>
<td>Weeks to months</td>
<td>Months to years</td>
</tr>
<tr>
<td><strong>Predominant cell</strong></td>
<td>Blasts</td>
<td>Mature forms</td>
</tr>
<tr>
<td></td>
<td>Some mature forms</td>
<td></td>
</tr>
<tr>
<td><strong>Anemia</strong></td>
<td>Mild-severe</td>
<td>Mild</td>
</tr>
<tr>
<td><strong>Thrombocytopenia</strong></td>
<td>Mild-severe</td>
<td>Mild</td>
</tr>
<tr>
<td><strong>WBC</strong></td>
<td>Variable</td>
<td>Increased</td>
</tr>
<tr>
<td><strong>Organomegaly</strong></td>
<td>Mild</td>
<td>Prominent</td>
</tr>
</tbody>
</table>

### Clinical Picture of acute leukemias

- Anemia
- Bleeding
- Infection

### Clinical Picture of chronic leukemias

- No anemia
- No bleeding
- No Infection

### Acute leukemia cause morbidity and mortality through:

- Deficiency in blood cell *number and function*
- Invasion of vital organs
- Systemic disturbances by *metabolic* imbalance

### Investigation needs to diagnose leukemia

- Complete blood count (CBC or FBC)
- Peripheral blood film inspection
- A bone marrow examination
- Flow cytometry or immunophenotyping studies
- Chromosomal analysis
- Cytochemical stains
1-Complete blood count show

1. Analyzing CBC
   - Thrombocytopenia?
   - RBC level/ anemia?

2. Observe cell lineage
   - Lymphoid or Myeloid?

3. Assess maturity of predominating cells

PBS: show

- Normochromic,normocytic anemia
- Thrombocytopenia
- Changes of white blood cells,
  - % of blast cells
  - Type of blast cells (myeloblast or lymphoblast etc)
  - morphologic feature (auer rods, vacuoles, the size of the nucleus etc)

2-Bone marrow aspiration in acute leukemia

- Bone marrow aspiration → a liquid sample of marrow for cells study (morphology, developing state of each cells type,)
- Mostly is hypercellular B.M and few cases hypocellular
- >10% to 90% leukemic blasts at diagnosis or during relapse.
- The blast must present in the peripheral blood, unless the WBC count is markedly decreased.

Normal marrow smear viewed at high magnification
Bone Marrow Tryphine Biopsy

Trephine biopsy → is a histological bone marrow tissue examination for structure study (architecture, cellularity and presence of fibrosis or abnormal infiltrates can be reliably determined).

Bone Marrow Tryphine Biopsy, It is a histological test for bone marrow tissue to obtain the blood cells **Indication:** → If the peripheral blood indicate

1-Hypocellular
2-Aplastic anemia
3-Metastatic cancer

Or the previous result indicate → Dry tap bone marrow aspiration

3-Flow cytometry or immunophenotypic studies

What is flow cytometry?

Definition: Flow cytometry is a method of measuring multiple physical and chemical characteristics of particles by optical means.

Measurement of physical, antigenic, functional properties of cells suspended in fluid

**Sample requirements**

A suspension of single cells or other particles in a suitable buffer

**Spacemen**

- Peripheral blood,
- Bone marrow cells
- Others

Monoclonal antibodies toward cell-type restricted antigens are used in this highly specific method

**CD markers Show and determine**

- blast cells
- mature cells
- myeloid Cells types and count
- lymphoid cells types and count
- Normal or abnormal

4- Chromosomal analysis

A chromosomal abnormalities study is very important *(diagnostic and prognostic for*

- AML
- ALL

It is critical in the diagnosis and treatment of AML. As in ALL
5-Leukemia: Laboratory Evaluation - Cytochemistry

Cytochemical stains are an important assistant to identifying and confirming a myelocytic acute leukemia.

Blood cells contain various

- enzymes,
- fats, and Carbohydrates
- other substances that can be identified by cytochemical means.

The widely used by FAB classification of acute leukemia is based on morphology and cytochemistry.

- Usually performed on bone marrow slides
- Helpful in differentiating lymphoid or myeloid lineage of blasts in Acute Leukemia

The most important cytochemical studies in the study of acute leukemia are

- Myeloperoxidase (MPO)
- Sudan Black B (SBB)
- Specific esterase (SE)
- Nonspecific esterase (NSE)
- Terminal deoxynucleotidyl transferase (TdT)
- Periodic acid Schiff stain PAS

**Myeloperoxidase (MPO)**

- Myeloperoxidase (MPO) is a lysosomal enzyme
- Activity is present in the primary granules and Auer rods of myeloid cells
- Separates myeloid and lymphoid blasts
- Stains late myeloblasts, granulocytes, monocytes less intensely
- Differentiates AML from ALL
- Granules stain reddish-brown
- Smears must be fresh
Sudan black B (SBB)
- SBB stains the lipid membrane surrounding the azurophilic granules
- Cells: of the myeloid and monocyte lineages
- negative reaction → Lymphocyte give negative reaction
- Positivity reaction in → in the myeloid lineage increases with maturation.
- Uses for → differentiate between acute myeloblastic and acute lymphoblastic leukaemia.

Esterases

Specific Esterase (Naphthol AS-D Chloroacetate)
1. It is an enzyme enzyme found in large amounts in moncytic
2. Activity is in cytoplasm
3. Positive in AML
4. Granules of myeloblasts stain blue-black

Nonspecific Esterase (Alpha-Naphthyl Acetate)
5. It is an enzyme found in large amounts in moncytic
6. Activity is in cytoplasm
7. Positive in AMML Stains moncytes
8. Differentiates myeloblasts from monoblasts
9. Granules stain orange red
Periodic acid-Schiff (PAS)

1. The presence of glycogen within the cytoplasm of many haemopoietic cells may be demonstrated by the PAS reaction.

2. Red to purple staining material indicates a positive reaction.

*Uses:* The PAS stain is used primarily to differentiate between *acute lymphoblastic and acute myelomonoblastic leukaemia.*

1. A negative reaction are seen in lymphoblasts while myeloblasts and monoblasts give a negative reaction.

![PAS stain showing block positivity on the bone marrow from a patient with B lymphoblastic leukaemia/lymphoblastic lymphoma](image)

Terminal Deoxynucleotidy transferase (TdT)

1. Primitive cell marker found in cell nuclei

2. Distinguishes ALL from malignant lymphoma

*Toluidine Blue:* Positive marker for basophils and mast cells

**Lab Techniques for Diagnosis and classification of neoplasms**

**Molecular Genetics**

1. This newer method of diagnosing leukemia consists of DNA probes and polymerase chain reaction (PCR)-based studies.

2. They are rapid and precise and are used to confirm chromosomal abnormalities that are not detected by conventional studies. Ex.BCR/ABL for CML
Cytogenetics (Chromosome studies)

The chromosomes in the malignant population identifies chromosome translocations which are specific for certain leukemia examples: Philadelphia chromosome \((t[9:22])\) is associated with CML.

<table>
<thead>
<tr>
<th>Cytochemical Reaction</th>
<th>Cellular Element Stained</th>
<th>Blasts Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloperoxidase (MPO)</td>
<td>Neutrophil primary granules</td>
<td>Myeloblasts strong positive; (AML)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Developing and mature granulocytes give positive MPO reactions.</td>
</tr>
<tr>
<td>Sudan Black B (SBB)</td>
<td>Phospholipids</td>
<td>Myeloblasts strong positive (AML)</td>
</tr>
<tr>
<td>Specific esterase (SE)</td>
<td>Cellular enzyme</td>
<td>Myeloblasts strong positive (AML)</td>
</tr>
<tr>
<td>Nonspecific esterase (NSE)</td>
<td>Cellular enzyme</td>
<td>Monoblasts strong positive AMML</td>
</tr>
<tr>
<td>Terminal deoxynucleotidyl transferase (TdT)</td>
<td>Intranuclear enzyme</td>
<td>Lymphoblasts positive ALL</td>
</tr>
<tr>
<td>Periodic acid Schiff stain PAS</td>
<td>glycogen and mucopolysaccharides.</td>
<td>Lymphoblasts positive ALL</td>
</tr>
</tbody>
</table>

**Toluidine Blue**: Positive marker for basophils and mast cells
Leukocyte Alkaline Phosphatase (LAP) OR Neutrophil Alkaline Phosphatase, NAP

Detects activity of the enzyme alkaline phosphatase in the cytoplasm of neutrophils...

- **Specimen used:** Fingerstick or heparinized blood smears; **NOT** bone marrow smears.
- **Interpretation:** *Neutrophils (bands and segs) are the only cells counted* and they are rated 0 - 4+ on the basis of the quantity and intensity of the precipitate within the cytoplasm.
- The sum of the ratings of 100 cells = the LAP score.
- Normal LAP score is over 70 cells

**Significance:**
Mainly performed to differentiate between
- Chronic myelocytic leukemia (malignant) and neutrophilic leukemoid reactions, most often due to a severe bacterial infection (benign).
- The LAP stain is **NOT** useful for acute leukemia with predominantly blast cells.

A high NAP score is seen in neutrophilia of
- Bacterial infection
- Leukaemoid Bacterial reactions,
- PRV

### Example:

<table>
<thead>
<tr>
<th>Rating</th>
<th>Count</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>1+</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>2+</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>3+</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>4+</td>
<td>5</td>
<td>20</td>
</tr>
</tbody>
</table>

**LAP Score** = 120
Cluster of differentiation

- Definition: it is a system or protocol used for the identification and investigation of cell surface molecules
- **What are a surface molecules?**
  They are small parts of the cells surface membrane mostly are proteins, based on this particles not existing in any other cell in the body
  - This part of the membrane cells has many names when described sometimes called protein, when you study physiology, receptor when used as place to connect with specific antibody for it, and finally may called antigen when we separate it and inject it in other living organism, such as mice to produce antibodies against it, and we use these antibodies to recognize the mother of that antigen which coming from

**This protocol given a number for each type of cells to know it.**

Abbreviations

- Cluster of differentiation (CD)
- Cluster of designation (CD)
- Classification determinant (CD)
  Numbered up to 371
- Each group of cells defined as (+) Positive or (-) Negative, symbol to indicate this marker exist or no For example, a "CD34+, CD31-"
- Each CD has a number, this number not given to another protein example CD4 only for the helper lymphocyte,
- What is the good things we get from this protocol?
  Simply we can recognize what type of cell is this. I will give you an example to understand The CD molecules are very useful in defining leukocytes, types, count
The methods for identified cells are many but one is important in our work in the lab. and always recall immunophenotyping.

We write and speak about Immunophenotyping.. what is this thing?

- Immunophenotyping is a technique used to study the protein expressed by cells used in laboratory diagnostic purpose allowing cells to be defined based on what molecules are present on their surface methods flow cytometry.

**What is flow cytometry?**

- Definition: Flow cytometry is a method of measuring multiple characteristics of particles by optical means.
  - physical characteristics, antigenic, functional properties of cells
  - and chemical characteristics

**The sample must be fluid such as**

- Peripheral blood,
- CSF,
- Bone marrow cells,

The method don’t test a solid mass or tissue you must first make a suspension of single cells or other particles in a suitable buffer.
<table>
<thead>
<tr>
<th>Type of cell</th>
<th>CD markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>stem cells</td>
<td>CD34+, CD31-, CD117</td>
</tr>
<tr>
<td></td>
<td>all leukocyte groups</td>
</tr>
<tr>
<td></td>
<td>Granulocyte</td>
</tr>
<tr>
<td></td>
<td>Monocyte</td>
</tr>
<tr>
<td></td>
<td>Thrombocyte</td>
</tr>
<tr>
<td></td>
<td><strong>T lymphocyte</strong></td>
</tr>
<tr>
<td></td>
<td><strong>T helper cell</strong></td>
</tr>
<tr>
<td></td>
<td>T regulatory cell</td>
</tr>
<tr>
<td></td>
<td>Cytotoxic T cell</td>
</tr>
<tr>
<td></td>
<td>B lymphocyte</td>
</tr>
<tr>
<td></td>
<td>Natural killer cell NK cells</td>
</tr>
</tbody>
</table>
Section 7

**Acute Myeloblastic Leukemia**

Stem cell disorder characterized by malignant neoplastic proliferation and accumulation of immature and nonfunctional hematopoietic cells in the BM

**Risk factors**
- Chemo/radiation
- Exposure to benzene
- History of MDS

**Also known as**
- Acute myelocytic leukemia
- Acute myelogenous leukemia
- Acute nonlymphocytic leukemia

**AML** is a malignant, clonal disease that involves proliferation of blasts

**Involved in**
- Bone marrow
- Peripheral Blood

**AML is:**
- It is a blood cancer
- Failure to produce normal cells (Neutrophil etc)
- Highly heterogeneous
- Mostly in adult with a median age of onset of 50 yr
- Belong to myeloid group cancer mainly Neutrophils
- There is no enough Neutrophils for bacteria killing

**FAB classification**
Morphologic classification of AML is based on
- Cellular differentiation (What type of cell?) (granulocyte, monocyte, erythroid, or megakaryocytic)
- Extent of maturation (Myeloblast, promyelocyte, granulocyte)

**Classified as**
- M0 Minimally differentiated
- M1 Myeloblastic
- M2 Myeloblastic with differentiation.
- M3 Promyelocytic
- M4 Myelomonocytic
- M5 Monoblastic
- M6 Erythroleukemia
- M7 Megakaryocytic
Pathophysiology

Uncontrolled growth of blasts in marrow leads to
- Stop normal cells production.
- Appearance of blasts in peripheral blood.
- Accumulation of blasts in other sites (CNS, Kidney, Liver, spleen).

Function disorders of many organs.

Metabolic effects of AML
- Increase in uric acid → uric acid nephropathy
- Release of phosphates → decrease in $\text{Ca}^{2+}$ and $\text{Mg}^{2+}$

Patient’s Symptoms (Clinical Features of AML)

- Anemia (weakness, fatigue, dyspnea on exertion)
- Bleeding (mucosal bleeding, purpura)
- Infection (neutropenia → infections)

Acute leukemia cause morbidity and mortality through: -

- Deficiency in blood cell number and function
- Invasion of vital organs
- Systemic disturbances by metabolic imbalance

Lab Features: Peripheral blood

1-WBC count:
variable at diagnosis → (1-200 x $10^9$/L)
1. >20% blasts present
2. Auer rods: fused primary granules in myeloblasts

2-RBCs
- Decreased
- Hgb < 10g/dL
- Inclusions reflect rbc maturation defects
- nRBCs present

3-Platelets
- Decreased
- Hypogranular, giant forms
- Megakaryocyte fragments

Auer rods are a linear projection of primary azurophilic granules, and are present in the cytoplasm of myeloblasts and monoblasts in patients with acute leukemia.
2-Bone marrow aspirate-and biopsy
- >20% blasts in BM
- myeloblast is the predominant cell

Decrease in normal
- erythropoiesis,
- myelopoiesis,
- megakaryocytes

3-cytochemistry staining
1- Sudan black stain (SBB): Positive
2- Myeloperoxidase stain: (MPO): Positive
3- Specific Esterase (ES): Positive

Positive: MPO
Positive: ES
White blood cells disorders for MLs

Flowcytometry or immunophenotypinag AML

diagnosis
identify antigens present on the blast cells

Positive for
1. CD 13
2. CD 33

- HLA-DR
- CD34

AML in brief

AML is:

- Name: Acute myeloblastic leukemia
- Abbreviation: AML
- Definition: It is a blood cancer of myeloid line of blood cells,
- Age: adult
- Risk factors: radiation, Genetic, chemical exposures
- Highly heterogeneous
- Failure to produce normal cells (Neutrophil etc)
- There is no enough Neutrophils for bacteria killing
- Mostly in adult with a median age of onset of 30-50 yr
- Belong to myeloid group cancer mainly Neutrophils
- The main cell affected: the granular precursors
  - Cells: myeloid granular cells (N, E, and B)
- Organ starting: Bone marrow
  - Bone production: abnormal blast cell, reduced production of Normal granular cells, RBC and Platelets
- Rate of production: rapidly and fetal without treatment
- Organs Distribution: peripheral blood
- Invasion of vital organs
- Cell in the peripheral blood > 70 blast
- Normal cells: less than 20-30%
- Infiltration: many organs
- Symptoms: insidious, anemia, bleeding and infection
Acute monoblastic and monocytic leukaemia (AMML)

Acute monoblastic and monocytic leukaemia can occur at any age but most commonly occurs in young adults.

**Type:** myeloid leukemia

**Age:** at any age commonly young adult

**PBS:**

1. Acute monoblastic leukemia → 80% monoblast
2. Acute monocytic leukemia → presence of 80% promonocytes and monocytes.

**NSE:** positive

**Immunophenotype:** CD14+, CD36+

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**Acute Monoblastic Monocytic Leukemia AMML**

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**B.M.** .. Acute Monoblastic Monocytic Leukemia AMML
Acute lymphoblastic leukemia (ALL)

- Acute lymphoblastic leukemia (ALL) is a malignant (clonal) disease of the bone marrow in which early lymphoid precursors proliferate and replace the normal hematopoietic cells of the marrow.
- ALL is the most common type of cancer and leukemia in children

Classification

- B-cell precursors (80 to 85% of cases)
- T-cell precursors (15 to 20% of cases).

Pathophysiology

1. Genetic mutations during development in utero,
2. Abnormal response of the immune system to infection in childhood
3. Stop normal cells production.
4. Appearance of blasts in peripheral blood.
5. Accumulation of blasts in other sites (CNS, Kidney, Liver, spleen).
6. Function disorders of many organs.

Metabolic effects of ALL

1. Increase in uric acid —> uric acid nephropathy
2. Release of phosphates —> decrease in Ca^{2+} and Mg^{2+}
Patient`s Symptoms (Clinical Features of ALL)

- Anemia (weakness, fatigue, dyspnea on exertion)
- Bleeding (mucosal bleeding, purpura)
- Infection (neutropenia → infections, fever)

FAB Morphological Classification of ALL--L1. L2. L3

- ALL is divided into three main types:
  1. T-cell Precursor T  ALL → L1, L2
  2. B-cell precursor ALL → L1, L2
  3. B-cell ALL (same as Burkitt lymphoma) → L3.

Acute lymphoblastic leukemia (ALL) Incidence *

- ALL-L1: small uniform cells childhood ............ consist 85%
- ALL-L2: large varied cells........... consist 14% mostly in adult
- ALL-L3: large varied cells with vacuoles (bubble-like features). .......... called Burkitt's) and consist 1%

Burkitt cell lymphoma/leukaemia: peripheral blood film showing Burkitt cells with a high N/C ratio,

Laboratory findings

<table>
<thead>
<tr>
<th>CBC Result</th>
<th>WBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Anemia (low Hb, PCV, RBC)</td>
<td>1. Normal count</td>
</tr>
<tr>
<td>2-Thrombocytopenia</td>
<td>2. Lower count</td>
</tr>
<tr>
<td></td>
<td>3. Higher count</td>
</tr>
<tr>
<td></td>
<td>Plus presence of abnormal white blood cells</td>
</tr>
</tbody>
</table>
**Blood smear study**

- RBC: Normocytic anemia
- PLTs: Low count
- WBC: Lymphoblast 90%
- Normal other cells 10%

**Bone marrow biopsy and aspirate:**

Must presence of >20% or more of all nucleated cells are blast

*bone marrow aspirate smear of patient with precursor B-cell acute lymphoblastic leukemia*

**Immunophenotyping of lymphocytes**

- Immunophenotyping is performed by flow cytometric techniques
- using monoclonal antibodies (mAbs)
- Represents a crucial moment in the diagnostic work-up of ALL

<table>
<thead>
<tr>
<th>Pan T markers</th>
<th>cCD 2-3-5, and CD7 (c= cytoplasm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-helper</td>
<td>CD4</td>
</tr>
<tr>
<td>T-suppressor</td>
<td>CD8</td>
</tr>
<tr>
<td>Pan B marker</td>
<td>cCD19 and CD22</td>
</tr>
</tbody>
</table>

**Immunophenotypic categories of acute lymphoblastic leukemia (ALL):**

<table>
<thead>
<tr>
<th>Types</th>
<th>FAB Class</th>
<th>Tdt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precursor B</td>
<td>L1,L2</td>
<td>+</td>
</tr>
<tr>
<td>Precursor T</td>
<td>L1,L2</td>
<td>+</td>
</tr>
<tr>
<td>B-cell</td>
<td>L3</td>
<td>-</td>
</tr>
</tbody>
</table>

**Cytochemistry staining for ALL**

<table>
<thead>
<tr>
<th>Periodic acid shift (PAS)</th>
<th>Glycogen</th>
<th>Lymphoblasts positive</th>
</tr>
</thead>
</table>

**ALL Cytogenetics and molecular biology**

- It is important step in the characterization of ALL.
- It is possible to identify alterations of the karyotype
- It has prognostic value

**ALL Genetic profile**

Method- Microarrays

1. It can define the genetic profile of each neoplastic form have prognostic value
2. It has prognostic value
To differentiate between AML & ALL

<table>
<thead>
<tr>
<th>AML has</th>
<th>ALL has</th>
</tr>
</thead>
<tbody>
<tr>
<td>❖ Auer rods</td>
<td>❖ No Auer rods</td>
</tr>
<tr>
<td>❖ SBB: Positive</td>
<td>❖ SBB: Negative</td>
</tr>
<tr>
<td>❖ MPO: Positive</td>
<td>❖ MPO: Negative</td>
</tr>
<tr>
<td>❖ SE: Positive</td>
<td>❖ SE: Negative</td>
</tr>
<tr>
<td>❖ CD 33, CD 13</td>
<td>❖ Tdt Positive</td>
</tr>
<tr>
<td></td>
<td>❖ PAS: positive</td>
</tr>
</tbody>
</table>
### ALL in general lines:

<table>
<thead>
<tr>
<th>Abbreviated as: ALL</th>
<th>There is no enough Lymphocytes to kill viruses, fungus and produce antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etiology - unknown</td>
<td>Low serum globulin, with Abnormal response of the immune system to infection in childhood</td>
</tr>
<tr>
<td>It is a blood cancer of white blood cells (Lymphocytes)</td>
<td>Appearance of blasts in peripheral blood over 80%</td>
</tr>
<tr>
<td>Failure to produce normal cells (Lymphocytes)</td>
<td>Accumulation of blasts in other sites (CNS, Kidney, Liver, spleen) causing functional disorders of many organs</td>
</tr>
<tr>
<td>Highly heterogeneous</td>
<td>Increase in uric acid $\rightarrow$ causing uric acid nephropathy</td>
</tr>
<tr>
<td>Belong to Lymphoid group cancer</td>
<td>Release of phosphates $\rightarrow$ with the result of decrease in $\text{Ca}^{2+}$ and $\text{Mg}^{2+}$ in the serum</td>
</tr>
<tr>
<td>85% of childhood leukaemias</td>
<td>Mostly in children (up to 15 years)</td>
</tr>
<tr>
<td>Commonest in the age 2-10 years - Peak at 3-4 years.</td>
<td></td>
</tr>
</tbody>
</table>
Section 8

The Chronic Myeloproliferative Disorders (CMPDs)

Introduction
- CMPDs are malignant Clonal stem cell disorder
- Each disorder has specific genetic abnormalities.
- Bone marrow fibrosis in all CMPDs
- Fibrosis is secondary phenomenon
- Many finally terminate into an acute myelogenous leukemia (AML).
- Loss of regulatory signals that control the production of the mature cells
- Most of these disorders are seen in older adults (50-70yrs aged)
- Could be also in children

All shared in
- splenomegaly, hepatomegaly, leukocytosis, thrombocytosis, erythrocytosis.

The CMPD classification
- Clonal haematopoietic disorders
- Proliferation of one of myeloid lineages
  - Granulocytic → Granulocyte Chronic myeloid Leukemia  CML
  - Erythroid → RBC  Polycythemia Vera (PV)
  - Megakaryocytic → Platelets Essential thrombocytopenia ET
  - Myeloid Metaplasia or → Myelofibrosis Myeloid Metaplasia (MMM)

Relatively normal maturation
Chronic Myeloid Leukemia

- Chronic myelogenous leukemia (CML), also known as chronic myeloid leukemia,
- Definition: is a myeloproliferative disorder characterized by increased proliferation of the granulocytic cell line without the loss of their capacity to differentiate.
- associated with a characteristic chromosomal translocation called the Philadelphia chromosome.
- It accounts for 20% of all leukemia affecting adults..

Philadelphia chromosome

is an acquired cytogenetic anomaly that is characterizes in all leukaemic cells in CML
90-95% of CML pt`s have Ph chromosome
Shared translocation of chromosome 22 and chromosome 9

Philadelphia chromosome BCR / ABL

1. BCR (breakpoint cluster region) gene on chromosome 22 fused to the ABL (Ableson leukemia virus) gene on chromosome 9
Ph chromosome is found on
2. myeloid, monocytic, erythroid, megakaryocytic,
3. B-cells and sometimes T-cell proof that CML derived from pluripotent stem cell

The Philadelphia Chromosome: t(9;22) Translocation
CML- phases

Disease Course of Chronic myelogenous leukemia classically occurs in three phases:

1- **A chronic phase** (last 4-5 years up to 15 years) → asymptomatic or have only mild symptoms of fatigue and the % Of blast is less than 5%

2- **An accelerated phase** (last 6-9 months) → a gradual increase in blasts in the blood or bone marrow, it is short, may transformed to AML or myelofibrosis, and the % Of blast is between 5-20%
3-Blast crisis (last for 3-6 months) → rapid progression and short survival, and the % of blast is more than 20% in the peripheral blood and bone marrow.
CML Diagnosis

1- CBC
shows increased granulocytes of all types, typically including mature myeloid cells.

- Basophils and
- Eosinophils are almost commonly increased; this feature may help differentiate CML from a leukemoid reaction

RBC:

a. Hb: is low
b. NRBC: may present

Platelet count:

- increase can exceed 1,000,000/uL
- Giant platelets may be present

PBS: the presence of precursor cells of the myeloid lineage.
In addition, basophilia, eosinophilia, and thrombocytosis can be seen
2-Bone Marrow study

1-Cellularity: Hypercellular, Increase reticulin fibrosis in 30-40%
2-Granulocytic hyperplasia blasts less 10%
3-Increase the M:E ratio Myeloid:erythroid ratio – 10:1 to 30:1 (N : 2:1)
4-Ph.chromosome test: positive [t(9;22)]

3-cytogenetics that detects the translocation t(9;22)

which involves the ABL1 gene in chromosome 9 and the BCR gene in chromosome 22
As a result of this translocation known as the Philadelphia chromosome chromosomal abnormality
Thus, this abnormality can be detected by
  o Routine cytogenetics,
  o BCR-ABL1 can be detected by fluorescent in situ hybridization, as well as by PCR

Fluorescence in situ hybridization using unique-sequence, double-fusion DNA probes for bcr (22q11.2) in red and c-abl (9q34) gene regions in green.
The abnormal bcr/abl fusion present in Philadelphia chromosome-positive cells is in yellow (right panel) compared with a control (left panel).
Other lab features:

Increased of:

- **serum vitamin B-12-binding protein (TC-I)**. The latter is synthesized by the granulocytes and reflects the degree of leukocytosis.
- **Serum uric acid** reflection of high bone marrow cellular turnover
- Lactate dehydrogenase -LDH
- Decrease → LAP score

Key Facts of CML

- CML is a chronic cancer of neutrophils
- Marked leukocytosis with all stages of granulocyte maturation
- Hepatosplenomegaly
- Thrombocytosis is common in chronic phase
- Three phases: chronic, accelerated, blast
- Philadelphia chromosome positive
- BCR-ABL fusion gene is present
- LAP score <10
Section 9

2-Polycythemia rubra vera (PRV)

Definition: Polycythemia vera (PV) is a chronic myeloproliferative disorder characterized by an increase in red cell mass, it is a cancer of the erythrocytes

Characterized by
Excessive production of all blood cells lines
1-RBC, PCV, Hb Male > 0.51 (50%)-Female > 0.48 (48%) | 2-WBC | 3-PLTs

independent of erythropoietin

Classification of polycythemia

1-Absolute polycythemia
   1. primary polycythemia (PRV)
   2. Secondary polycythemia

2- Relative polycythemia

1-Polycythemia rubra vera (PRV)

Names:
1-Polycythemia vera (P. Vera)
2-Polycythemia rubra vera (PRV)

Classified as negative Philadelphia chromosome

Pathophysiology of Polycythemia vera

1. Polycythemia vera (PCV), being a primary polycythemia,
2. Caused by neoplastic proliferation and maturation to produce what is referred to as panmyelosis of
   - Erythroid
   - Megakaryocytic
   - Granulocytic elements.

1. PRV is associated with a low serum level of the hormone erythropoietin (EPO).
2. PRV cells often carry a mutation in the tyrosine kinase (JAK2), which acts in signaling pathways of the EPO-receptor, Making those cells hypersensitive to EPO.

Genetic Causes of PRV

- The disease results from somatic mutation of a single stem cell (MSC) this mutation called “The JAK2 mutation” is present in haemopoietic cells in almost 100% of patients.
- the increase of “JAK2” in red cells is the diagnostic finding, in many patients
- JAK2 is a member of the Janus kinase family and makes the erythroid precursors hypersensitive to erythropoietin (EPO)

Janus-associated kinase 2 JAK2
The erythrocytes increase production effect on the health in PV

- In PRV the RBC not normal cells
- The RBC in PRV carry oxygen as the normal cells
- The oxygen saturation is normal in polycythemia vera
- Circulation time: increase up to twice the normal value
- Erythrocytes count: reach up to 11 million/cum $\rightarrow$ increase blood viscosity 5 times normal $\rightarrow$ caused rouleux formation of the RBC
- PCV: reach 70% up to 80%
- Blood volume: increase twice the normal

For many people, polycythemia vera may not cause any signs or symptoms. However, some people may experience:
- Itchiness, especially following a warm bath or shower
- Headache
- Dizziness
- Weakness
- Excessive sweating
- Painful swelling of one joint, often the big toe
- Shortness of breath
- Breathing difficulty when you lie down
- Numbness, tingling, burning or weakness in your hands, feet, arms or legs
- A feeling of fullness or bloating in your left upper abdomen due to an enlarged spleen

A feeling of fullness or bloating in your left upper abdomen due to an enlarged spleen

Ruddy facies of polycythemia

Burning pain in the feet or hands accompanied by erythema, pallor, or cyanosis - Microvascular thrombosis
Blood tests

An increase in the:
- Number of red blood cells
- Number of in platelets
- Number of white blood cells.
- Elevated hematocrit measurement,
- Elevated levels of hemoglobin,
- Very low levels of erythropoietin.

Laboratory finding

- Hb > 21g/dl
- Hct male > 60%, female > 55%
- RBC: > 6 million/cum
- WBC: > 12000/cum
- Platelets: > million/cum
- EPO: low

Peripheral blood

- RBC: rouleux formation
- WBC: increase with neutrophilia
- PLTs: increase count

Diagnostic Criteria for Polycythemia Vera

- Raised red cell mass: RBC count _ Hb _ PCV (Ht) 8 to 9 million and occasionally 11 million erythrocytes per cubic millimeter of blood (a normal range for adults is 4-6)
- Thrombocytosis (>400,000/µL)
- Leukocytosis >12 x10^9/µL without fever or infection
- JAK2 mutation positive
- Arterial oxygen saturation <92%

---------------------
- Raised of S.Uric Acid
- ESR (ZERO)
- Reduced of EPO
Treatment PV

- The most common treatment modality utilized in PV is **phlebotomy**.
- Reduction of blood volume (usually 1 unit of whole blood—450 cc), can be performed weekly or even twice weekly in younger patients to control symptoms.
- The **Hct target range is less than 45% for men, less than 42% for women.**
- A predictable **complication** is Iron deficiency anemia $\rightarrow$ 250 mg of iron is removed with each unit of blood.
- Survival is long (average 9 - 14 years).

2- **Secondary polycythemia**

Secondary polycythemia is an increase in red cell mass due to some other condition, such as
- High altitudes
- Cardiovascular diseases
- Chronic lung disease with hypoxemia
- Kidney cancer
- Drugs uses (EPO)

In secondary polycythemia, the oxygen saturation is *usually low* (that’s why the patient is making so many red cells – he or she needs to create more oxygen carrying capacity!)

**Lab finding**

1. Increase of Hb up to 20 g/dl
2. Normal PLT and WBC count
3. Increase EPO
4. Normal ESR
5. S.Uric acid normal

3- **Relative erythrocytosis**

It is a case of an increase of
- RBC mass. Hb .PCV
- It is not malignant case; the term is synonymous with ‘polycythaemia’

**Causes**
- Heavy smoking
- Dehydration and hemoconcentration.

**Lab finding:**

1. Increase Hb not over 19g/dl
2. Normal PLT and WBC count,
3. Normal EPO,
4. Normal ESR
5. S.Uric acid normal
### Differential diagnosis of polycythaemia

<table>
<thead>
<tr>
<th>Features</th>
<th>Polycythemia vera (PV)</th>
<th>Secondary polycythemia</th>
<th>Relative erythrocytosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>▪ Hb</td>
<td>Over 21gr/dl</td>
<td>Up to 20 gr/dl</td>
<td>Lower than 19 g/dl</td>
</tr>
<tr>
<td>▪ RBC mass</td>
<td>Increase +++</td>
<td>Increase ++</td>
<td>Increase ++</td>
</tr>
<tr>
<td>▪ WBC</td>
<td>Over 12000/cum</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>▪ Platelets</td>
<td>Over&gt; 450000/cum</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>▪ EPO</td>
<td>Decrease</td>
<td>Increase</td>
<td>Normal</td>
</tr>
<tr>
<td>1. Organomegaly</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>❖ Transformation</td>
<td>AML</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>1. Presence of JAK2.</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>• ESR</td>
<td>Zero</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>1. Serum u.acid</td>
<td>Increase</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>
Essential Thrombocythemia – ET

Essential thrombocythemia (ET) is a chronic myeloproliferative disorder characterized by panmyelosis, with a megakaryocytic predominance

- Cancer of megakaryocytes (Platelets)
- Increase of megakaryocytes without TPO increase,
- With the result of increase of PLT count in the peripheral blood and abnormal platelet function

Half of patients show the JAK2 mutation

Names:
1. Primary thrombocythemia
2. Essential thrombocythemia-ET

Essential thrombocythemia is characterized by the following

- A persistently elevated platelet count greater than 600,000/µL
- Megakaryocytic hyperplasia
- A clinical course complicated by thrombotic or hemorrhagic episodes or both

Points on ET

- Disease of middle aged persons
- Sometimes occurs in children
- Disease is inactive for 10-20 years or more.
- Transform to myelofibrosis
- May be transform to AML 10-20% of patients
- Discovered only by routine PB examination
- Neither splenomegaly nor hepatomegaly
- Recurrent abortions
- Cause of death: thromboembolic events

Symptoms of ET

- Bleeding: (at GTI, urinary tract, joint, and brain)
- Neurologic events: Headaches, paresthesias, visual disturbances etc
- Microvascular occlusions: (pain, acrocyanosis gangrene of the fingertips or toes)
- Large vessel thromboses

Complication:
1. Thrombosis
2. Bleeding.
3. Transformation to AML (rare)
4. Myelofibrosis
Diagnostic criteria OF ET
Platelet count must be >600 x 109/L (normal = 150 – 450).
- Hgb must be <13 g/dL or RBC mass must be normal (excludes PV).
- Philadelphia chromosome must be absent (excludes CML).
- Marrow must lack fibrosis (excludes chronic myelofibrosis).
- All other causes of thrombocytosis (e.g., iron deficiency anemia, cancer) must be excluded.

Peripheral blood findings
- PLTs count: over 600,000/cum could be reach > 1,000,000/cum
  thrombocytosis with giant platelets; bizarre forms
  increased MPV
  circulating megakaryocytes and megakaryocytic fragments

WBC
- Leukocytes: <20,000/ cum
- mild eosinophilia/basophilia

RBC
- RBC normal unless concurrent iron deficiency
Bone marrow:
1. Marked clustering of megakaryocytes
2. Half of patients show the JAK2 (Va1617Phe) mutation

Reactive Thrombocytosis
- Benign case of thrombocytosis
- PLT’s count over 600,000/Cum

Cases has thrombocytosis over million/cum
a) infection 22%
 b) Tissue damage 20%
c) Chronic inflammation 13%
d) Malignancy 6%
Myelofibrosis Myeloid Metaplasia (MMM)

Introduction

Chronic myelofibrosis
In this disorder, the myeloid cells proliferate rapidly with a high white count with a left shift, and a hypercellular marrow, features common to all myeloproliferative disorders.
But as the disease progresses, the marrow becomes replaced by fibrous tissue.
The hematopoietic precursors start to produce from other organs outside the marrow, in places like the liver and spleen. That called Extramedullary hematopoiesis.

Points to remember
- Hematopoietic stem cells grow out of control
- Age: middle age 50-70yrs
- Incidence: male and female are equal
- Risk factor: benzene and also to ionizing radiation breast cancer, prostate cancer, Hodgkin's disease
- Chronic: many years the patient is healthy
- In late stage, anemia, fever weight loss etc

“teardrop”
In squeezing through the tight fibrosis in the marrow and splenomegaly the red cells take on an unusual, “teardrop” shape.
Any case with tear drops needs bone marrow biopsy
A leukoerythroblastic reaction

Definition: it is a leukoerythroblastic reaction in the blood or it is a clonal stem cell defect characterized by

1-Leukoerythroblastic reaction:
which means presence in the peripheral blood of
   • Nucleated RBC
   • Immature granulocytes

2-Extramedullary hematopoiesis

3-Fibrosis of the bone marrow/reticulin silver stain

4-Teardrop RBCs

5-Absence of the Philadelphia chromosome

6-Hepatosplenomegaly

Laboratory picture of MMM

Called a leukoerythroblastic reaction in the blood characterized by
The presence in the peripheral blood of
   • Nucleated RBC
   • Immature granulocytes
   • Teardrop RBCs

Any case with tear drops needs bone marrow biopsy

CBC OF MMM

1-Anemia

2-WBC increase count

3-Platelets: increase in 50 % of cases

PBS

1-shows numerous teardrop

2-NRBC

3-immature granulocyte

4-precursors and giant platelets (Abnormal giant-sized megakaryocytes)
Bone Marrow

- 1-The bone marrow usually cannot be aspirated ("dry tap").
- 2-The biopsy can give the diagnosis
- 3-Philadelphia chromosome negative

Summary and points to remember about MMM

MMM is characterized by
1. marrow fibrosis,
2. extramedullary hematopoiesis
3. leukoerythroblastic blood smear.
4. MMM, the accelerating fibrosis –with leukopenia and thrombocytopenia
5. Bone marrow biopsy- dry tap.
6. MMM has the worst prognosis of all of the myeloproliferative disorders.
7. Can transform to acute leukaemia in 10-20% of cases
Section 10

**Lymphoproliferative Disorders**

**Introduction**

1. The chronic lymphoproliferative disorders are malignant clonal proliferations of relatively mature lymphoid cells.

2. These disorders are **not stem cell malignancies** (like chronic myeloproliferative disorders),

3. but malignancies that began in a lymphoid cell **at some particular stage of development**.

4. It’s like the cell just **gets fixed** at this particular stage and can’t mature any further.

**The main chronic lymphoproliferative disorders are:**

- Chronic lymphocytic leukemia (CLL)
- Hairy cell leukemia (HCL)
- Prolymphocytic leukemia (PLL)
- Large granulated lymphocyte leukemia.

**They are very different**

- clinically,
- morphologically
- and immunophenotypically.
Lymphoproliferative Disorders

Definition: is a malignant disease of lymphoid cells

Types:
  - T-Lymphocytes 2%
  - B-lymphocytes 96%

Characteristics
  - The lymphocytes are **not able to fight infection** very well.
  - Lymphocytes count increases in the blood and bone marrow
  - Enlargement of spleen Liver (Hepatomegaly), and lymph nodes
  - Invasion of the bone marrow

Chronic lymphocytic leukemia (CLL)

It is
  - Chronic lymphoproliferative disorder
  - Cell: B cells, Non adequate functional,
  - Has low Gamm golbulin production
  - Accumulated in the P. blood, bone marrow, spleen, liver and lymphnodes
  - Age: 55-70 yrs
  - Sex: frequent in males than females
  - Life: 2 yrs. up to 20 yrs.
  - Some dierapidly, within 2-3 years of diagnosis;
  - Discovered **incidentally** after a blood cell count is performed for another reason

25-50% of patients will be **asymptomatic** at time of presentation.

Symptoms include the following:

1. Enlarged lymph nodes, liver, or spleen
2. Recurring infections
3. Loss of appetite or early satiety
4. Abnormal bruising (late-stage symptom)
5. Fatigue
6. Night sweats
Peripheral blood show

CBC
1-RBC: mild anemia
2-WBC: over 50,000 / cum with lymphocytosis
3-PLT: slight decrease

P.B.S
shows the presence of blood smear show small lymphocytes &smudge cells
(Ruptured lymphocytes ("basket" or "smudge" cells)

The presence of a lymphocytosis in an elderly individual should raise strong suspicion for CLL.

Immunophenotype

• Negative for TdT,
• Positive for the B-cell antigens CD19, CD20, CD23
• Positive for monoclonal surface Ig (indicate B cells make immunoglobulin)
2-Hairy cell leukemia (HCL)

Hairy cell leukemia (HCL) is
1. A rare chronic lymphoproliferative disorder
2. Cells: small B-lymphocytes
3. Age: elderly (40-60)
5. Sites involved are blood, bone marrow, and spleen.

Diagnosis of Hairy cell leukemia (HCL)
The peripheral blood cell counts show Pancytopenia with decreased cell counts in all 3 cell lines.

- **Anemia** is usually severe and normochromic-normocytic in character.

- **Neutropenia and monocytopenia** are usually present in hairy cell leukemia, but an elevated white blood cell count (hairy cells) is found in 20% of cases.

- **Thrombocytopenia** is found in more than 80% of patients.

- Bone marrow aspirate is usually unsuccessful due to a "dry tap," with fried-egg appearance cells.
Flow cytometry on blood or bone marrow

Hairy cell leukemia CD markers

Indicate the HCL.

1. CD19
2. CD20
3. **CD22**
4. CD 11c—membrane adhesion
5. **CD103**

Characteristic cytochemical feature of hairy cell leukemia

a hairy cell that shows tartrate resistant acid phosphatase (TRAP) positivity
Section 11

**Adult T-cell leukemia/lymphoma (ATLL)**

**HTLV- Introduction**

- The human T cell lymphotrophic virus (HTLV) is a **retrovirus**.
- HTLV can infect various types of cells, including B cells, monocytes, and fibroblasts.
- The main cell target is **T cells** (both CD4+ and CD8+).
- One very interesting thing about HTLV is that it **spreads** within the host via cell-to-cell transmission using virus-induced cell-to-cell synapses.
- The most important viral protein in the oncogenesis of ATL is the functionally pleiotropic **viral protein Tax**, which both **promotes** the proliferation of and **inhibits the apoptosis** of infected T cells.

![Diagram of HTLV infection and ATL progression]

Natural course from HTLV-I infection to onset of ATL. HTLV-I is transmitted in a cell-to-cell fashion. After infection, HTLV-I promotes clonal proliferation of infected cells by pleiotropic actions of Tax and other viral proteins. Proliferation of HTLV-I-infected cells is controlled by cytotoxic T cells **in vivo**.
**Adult T-cell leukemia-lymphoma (ATLL) OR ATL**

A. **Definition:** Is a lymphoproliferative disease of malignant T-cells

B. **Caused by:** with the **human T-cell lymphocytotropic virus I (HTLV-I)**

C. Adult T-cell leukemia–lymphoma (ATL) is a peripheral T-cell malignancy,

D. Name:
   - Adult T cell leukemia –lymphoma (ATLL)
   - Adult T cell Leukemia (ATL)

*very aggressive disease with a mean survival of 8 months.*

**Transmission of HTLV-1**

Transmission of HTLV-1 is believed to occur

A. From mother to child.

B. Sexual contact.

C. Contaminated blood.

D. Blood transfusion.

E. Sharing of contaminated needles.

**The patient symptoms**

HTLV infections are usually **hidden and asymptomatic**, and usually do not cause disease until decades later.

**The patient has and complain from**

1. Lymphadenopathy, hepatomegaly, splenomegaly

2. more serious diseases like HTLV-associated myelopathy (HAM)

3. skin abnormalities. dermatitis

4. **hypercalcemia** and bone disease are common

**CBC and Peripheral blood smears from ATLL**

1. Anemia

2. Thrombocytopenia

3. WBC:over 500,000/cum

Abnormal T-lymphocytes.

deeply-lobulated nuclei, (Flower cells), a high nucleocytoplasmic ratio,

clumped chromatin and in apparent nucleoli
Other diagnostic criteria - **Immunophenotyping**

Immunophenotypically, ATL cells have a characteristic profile.

The cells are typically:
- **Strongly positive** for CD4 or CD8, CD25
- **Negative** for myeloid (CD13, CD33) and B cell lineage (CD10, CD19, CD20, CD21, CD22) markers

### Large granular lymphocytic leukemia

**DEFINITION**

Large granular lymphocytic (LGL) leukemia is a **chronic lymphoproliferative** disorder that exhibits an unexplained, chronic (> 6 months) elevation in large granular lymphocytes (LGLs) in the peripheral blood.

**Types** - It is divided into two main categories:

1. T-cell LGL (T-LGL) leukemia transformed CD8+ T-cell (10 years survival)
2. And natural-killer (NK)-cell LGL (NK-LGL) leukemia The NK type, is a very aggressive disease. With a short survival

**Site** : peripheral blood, bone marrow, spleen, and liver

**Laboratory finding**

**Blood**

1. **The lymphocytosis** is typically 2-20x10⁹/L
2. The neutrophil count is decreased
3. **The blood smear** shows a lot of large lymphocytes with abundant, clear cytoplasm and cytoplasmic granules.

**Bone marrow**

shows the same population of malignant cells as the blood, as well as the same decrease in neutrophils

**Laboratory finding**

a) hypergammaglobulinemia,
b) autoantibodies, and circulating immune complexes are commonly seen

**Immunophenotyping**

**The T-cell type of LGLL shows**

- positive for CD2, CD3, CD8 and CD57
- negative for CD4

**The NK type of LGL shows**:

- positive for CD2 and CD56 and negative for CD3, CD4 and CD8
Section 12

**Monoclonal Gammopathy (Hypergammaglobulinemia)**

1-Multiple Myeloma

- **Definition:** is a monoclonal malignancy of plasma cells
  1. **Age:** 40-70 years
  2. **Site:** Bone marrow
  3. **Cell:** plasma cell
- **Result of cancer:** produce abnormal protein called **para protein**

**Multiple myeloma pathology**

1. Normal bone marrow is **gradually replaced** by the malignant plasma cells leading to **pancytopenia**.
2. Most malignant plasma cells **actively** produce immunoglobulins.
3. In multiple myeloma, the normally controlled and purposeful production of antibodies is **replaced** by the **inappropriate production** of even **larger amounts** of **useless** immunoglobulin molecules. **para protein**
4. The normally **equal production** of light chains and heavy chains may be **imbalanced**.
5. The result is the release of **excess free** light chains or free heavy chains
Para proteinemia

Multiple myeloma characterized with

- Replacement of bone marrow with plasma cells accumulation
- Bones destruction
- Produce abnormal protein (“M” protein)

include the following immunoglobulin

- IgG: 50%
- IgA: 25%
- light chains: only 15% (light chain disease)
- IgD (1%) and IgE are rare

IgM myeloma is virtually nonexistent

1-Multiple Myeloma Patient’s symptoms

- common symptoms of multiple myeloma is CRAB:
  - C = Calcium (elevated),
  - R = Renal failure,
  - A = Anemia,
  - B = Bone lesions

Bone pain (back, extremities) from lytic lesions

- Weakness
- Recurrent, persistent infections (big problem)
- Renal failure (multifactorial; due in large part to toxic effect of light chains on tubular epithelium)
- Hypercalcemia (from bone resorption) can lead to neurologic changes and contribute to renal disease.

Laboratory Finding

CBC Results

1-RBC: Anemia
2-WBC: Decrease
3-PLT: Low
4-↑ESR is very high

P.B.S

1-WBC: increase
2-RBC: Rouleaux formation

Rouleaux is observed in multiple myeloma patients as a result of increased viscosity and decreased albumin/globulin ratio.
### Chemistry test

- ↑ Calcium
- ↑ Urine protein (B.J protein positive), is a urinary paraprotein composed of free light chains
- ↑ Uric acid
- Abnormal serum electrophoresis

### 2- A bone marrow biopsy

- **A bone marrow biopsy** shows presence of plasma cells. > 10 -15%
- This percentage is used in the **diagnostic criteria** for myeloma.

### Serum Electrophoresis

**Normal**

- Normal serum electrophoresis

**Abnormal**

- Serum from patient with multiple myeloma; note the monoclonal spike in the gamma region
Diagnosis
At least two of the following must present
1-The diagnostic test
Serum electrophoresis for detecting monoclonal protein spike in serum or urine
2-Bone marrow Evaluation:
greater than 10% abnormal plasma cells in bone marrow
3-lytic bone lesions seen on X-Ray skeleton

Benign monoclonal gammopathies

- Benign monoclonal gammopathies have peripheral blood findings similar to those in myeloma.

However,
- a lower concentration of monoclonal protein is usually seen.
- There are no osteolytic lesions,
- plasma cells comprise less than 10% of nucleated cells in the bone marrow.
- About 30% become malignant,

Waldenstrom’s macroglobulinemia - WM

Definition: is a rare disorder of plasma cells in which IgM is overproduced.

Characterized by
Waldenstrom’s macroglobulinemia is a malignancy of the lymphoplasmacytoid cells, which manufacture IgM.
Although the cells secrete immunoglobulin, they are not fully differentiated into plasma cells

Waldenstrom syndrome is B-cells lymphoma belong to NHL

Characterized by
the lymphoma cells are usually found in the bone marrow, lymph nodes and spleen.
1-over production of abnormal protein (macroglobulinemia)
2-hyperviscosity of the plasma OCCUR
- coagulation abnormalities (bleeding or thrombotic complications.)
- Rouleaux formation

Waldenstrom’s macroglobulinemia-Lab finding:

1-CBC
1. anemia normocytic
2. RBC: rouleux formation
3. High ESR
4. DLC: with lymphocytosis, and plasma cells
Waldenstrom macroglobulinaemia: peripheral blood film showing plasmacytoid lymphocytes (with features of both lymphocytes and plasma cells)

2-Serum Electrophoresis:
  - **Monoclonal IgM** is usually greater than 15 g/L

3-Bone marrow: bone marrow shows plasmacytoid lymphocytes

**Osteolytic lesions** indicating destruction of the bone as evidenced by radiography are seen in multiple myeloma but not in Waldenstrom’s macroglobulinemia.

In addition, Waldenstrom’s gives rise to a lymphocytosis that does not occur in multiple myeloma and differs in the morphology of the malignant cells.

What are lymphoplasmacytoid cells?
- cells have characteristics of both lymphocytes and plasma cells

**Hyperviscosity syndrome**

**Definition:** It is the case of increase of IgM paraprotein over 30 g/L in concentration, lead to large increase in viscosity of blood

**Cases related to hyperviscosity syndrome:**
- Polycythemia vera
- Multiple myeloma
- Waldenstrom’s macroglobulinemia
- acute leukemic blast crises.

**Beta2-Microglobulin**

Serum and plasma beta2 microglobulin values have emerged as

- markers for the activation of the cellular immune system,
- as well as a tumor marker in certain hematologic malignancies.
- Urine beta2 microglobulin values indicate renal filtration disorders.

Measurement of values in both serum and urine can help distinguish a problem of cellular activation from a renal disorder.
• The reference range of beta₂ microglobulin in urine samples is 0-0.3 µg/mL. In serum or plasma samples, the reference range is 0-3 µg/mL.
• It is recommended that each laboratory establish its own normal and pathological ranges of urine levels.

Diagnostic testing for multiple myeloma includes obtaining the β₂ microglobulin level, for this level is an important prognostic indicator. A patient with a level <4 mg/L is expected to have a median survival of 43 months, while one with a level >4 mg/L has a median survival of only 12 months.
**Lymphoma Introduction:**

Lymphoma is a malignant disorder of lymphocytes that starts in a lymph node.

1. Lymphoma is a cancer of the lymphatic system.,
2. They have progressive proliferation
3. Start locally at one site and after wide spread
4. In lymphoma, a tumor forms in one or more groups of lymph nodes.
5. causing enlargement of nodes and spleen ,
6. without the peripheral blood picture of leukemia
7. They reach to the bone marrow with replacement of normal hematopoiesis

**Types of lymphoma:**

- **Hodgkin lymphoma** (formerly known as Hodgkin’s disease) (HL)
- **Non-Hodgkin lymphoma** (formerly known as Non-Hodgkin’s lymphoma (NHL)

**The differences between HL and NHL**

is made upon examination of the cancerous material (from a biopsy or aspiration of the tumor tissue).

**Hodgkin’s disease (hodgkin’s lymphoma)**

**Causes:** Unknown

**Predisposing factors are:**

- Genetic
- Chemicals,Toxins
- Viruses (EBV, HTLV), HIV

**Diagnosis**

The most important diagnostic tool is:

Nodal biopsy and bone marrow biopsy for **Reed-Sternberg cell**
The Non Hodgkin Lymphoma (NHL)

Non-Hodgkin lymphomas (NHLs) are tumors originating from lymphoid tissues, mainly of lymph nodes.

These tumors may result from

1. chromosomal translocations,
2. infections,
3. environmental factors,
4. immunodeficiency states,
5. and chronic inflammation

**Diagnosis of Lymphomas**

- The main diagnostic tools is the Histology

**Specific tests:**

- Immunophenotyping
- Cytogenetics

*PBS-lymphoma cells with a high N/C ratio and inconspicuous nucleoli.*
الحمد لله رب العالمين ... ربي مني العمل ومنك الجزاء. وانت اكرم الأكرمين
اللهم تقبله مني واجعله في ميزان حسناتي وحسنات أبي وامي

اللهم اكرم ابي وامي... ربي...
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