National University-Sudan

Faculty of Medical Laboratory Sciences

Student Practical Manual-
Haematology and Immunohaematology Department

Fourth Year, Semester (7)
Leukemia's and Lymphomas Investigations
(MLS-HAEM-412)

Student Name:……...

ID: ……**Batch**…….....…...

Instructions

- Wear lab coat
- Wear Gloves
- Avoid swallow any chemical
- Follow the procedures provided
- Write your results in this manual

Content

White blood cell count:

Blood cells enumeration:

1. Automated instrument
2. Manual method:

   This procedure consist of diluting the blood with special diluent, transferring small volume of diluting blood onto a ruled glass platform (a hemcytometer) and counting the cells under a microscope We report the final result as the number of cells per cubic mm.

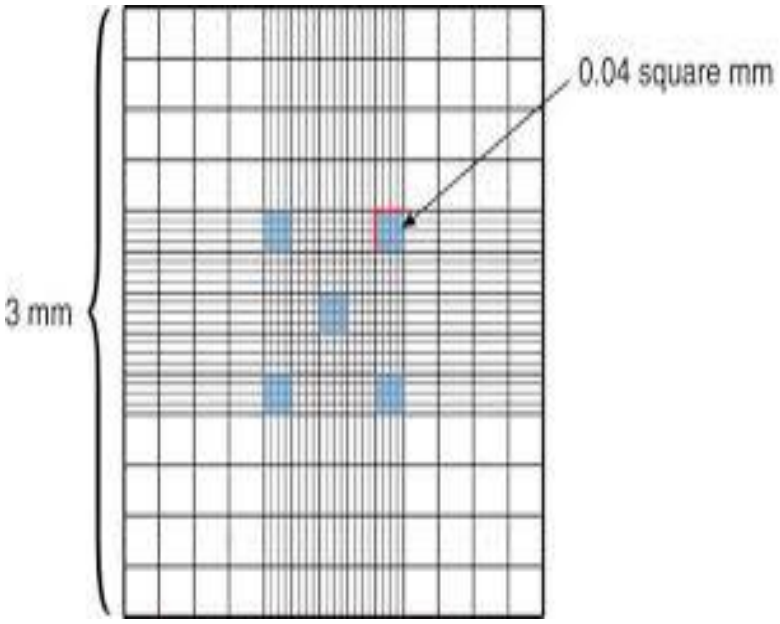
Haemocytometer:

It comprised tow identical ruled glass platforms squared by an H shaped moat.

The four corner squares (labeled W) are each subdivided into 16 squares, these are used for counting white blood cells WBC).

We use the large square in the center which is subdivided into 25 squares each one divided into 16 squares fir counting red blood cells (RBC) and platelets.

Haemocytometer contain toe raised ridges on which the cover glass rest, there is a distance 0.1 mm between the cover glass and the counting area.



Enumerating Leukocytes (WBC):

Principle:

To count WBCs, whole blood is diluted 1:20 in a weak acid (glacial acetic acid), which lyses (rupture) the red blood cells

After the hemacytometers is charged with the diluted blood, cells are counted microscopically in the four large squares labeled (W).

Cells should be counting in the tow glass platform, and the difference between them should be less than 10, and the difference between the highest and lowest counts should be less than 15

Requirements:

1. glacial acetic acid 2 %
2. counting chamber and cover glass
3. 1 ml pipette
4. 0.02 ml pipette
5. test tubes
6. microscope
7. gauze
8. Blood sample

Method:

1. Add 0.02 ml blood sample to 0.38 ml of diluting fluid
2. Mix well for at least 2 min
3. Prepare the counting chamber
4. Smoothly fill the chamber
5. Leave the chamber on bench for at least 2 min
6. Using power 10 objective, count the white cells in the specific area in the chamber

Counting formula:

Cell count (per cubic mm) = number of cells counted * dilution actor\ area count * depth (total volume)

Reference value:

$4 – 11 * 10^{9}$\L

Student's findings (measurements or observations):

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comments and interpretation:

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Evaluation (carried out by the instructor):

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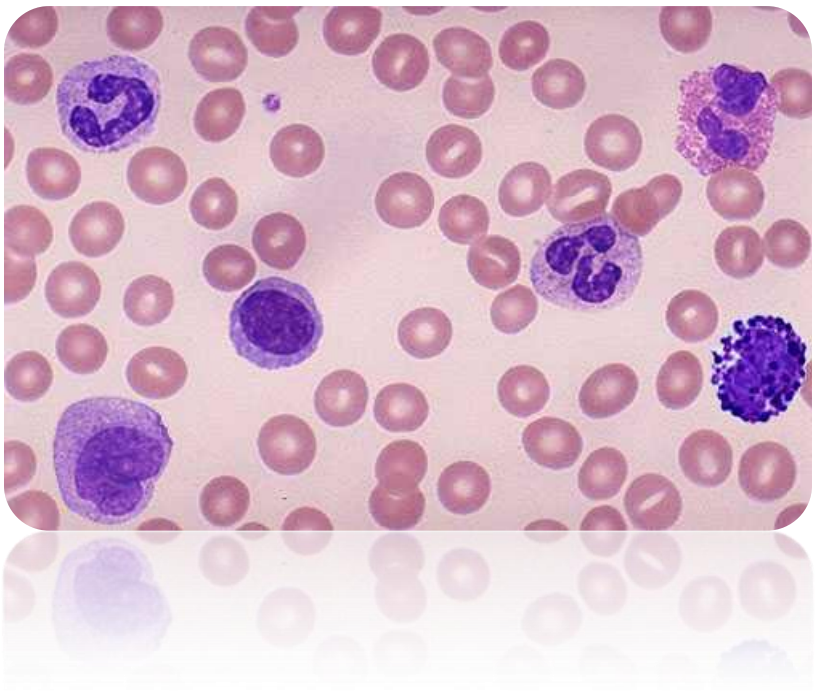
Laboratory diagnosis of benign WBCs disorders

Evaluation of white cells:

An important part of examination white cells is to assess the frequency of morphological evaluation include the appearance of:

1. The nucleus
2. The nature of granules or inclusion in the cytoplasm
3. The presence of immature forms
4. The various types of granulocytic, lymphoid and monocytic cells



Benign disorders can be classified into:

1. Quantitative disorders
2. Qualitative disorders

Quantitative disorders:

1. Leukocytosis:

An increased of circulating leukocytes above 11 * 10^{9}\L. based on the type of cell increased it divided to (Neutrophilia, Lymphocytosis Monocytosis, Eosinophilia, and Basophilia)

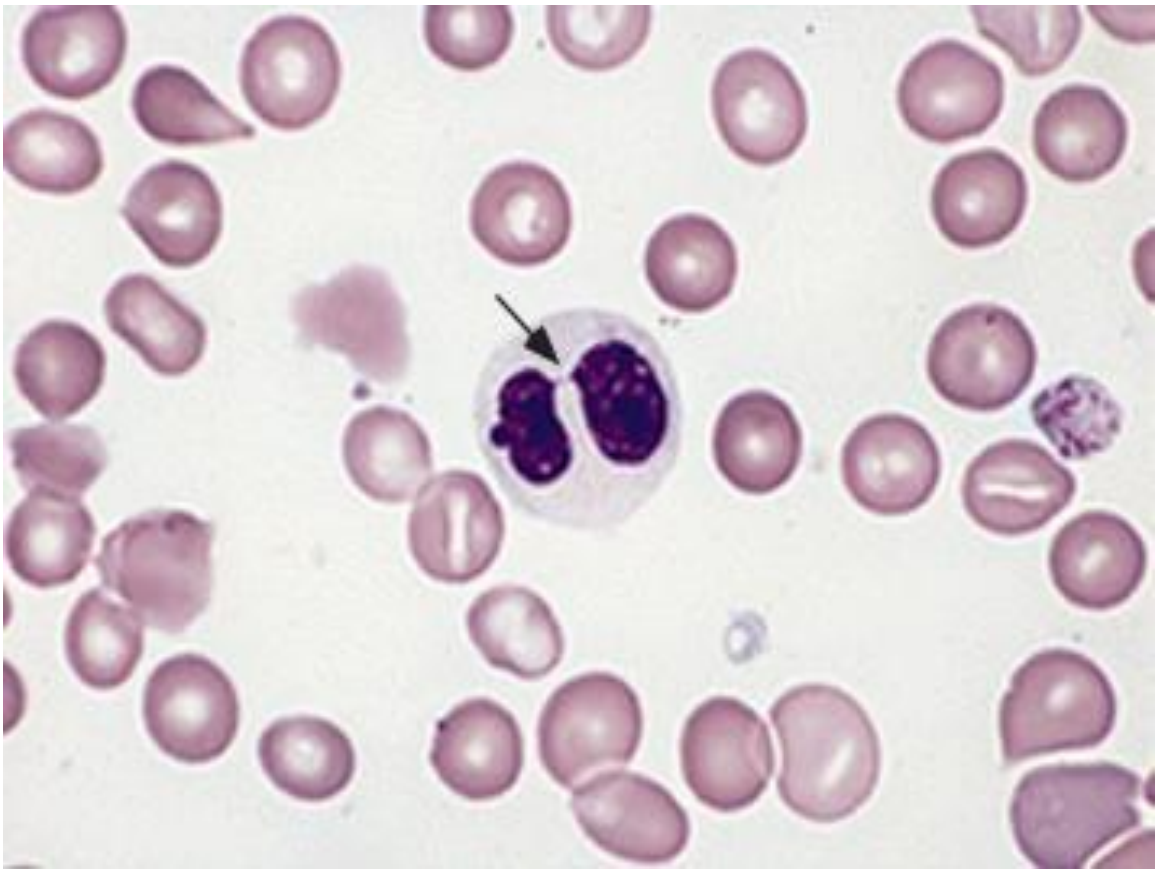
2. Leukopenia:

Also Known as leukocytopenia is a decreased of white cells lower than $4 * 10^9$\L.

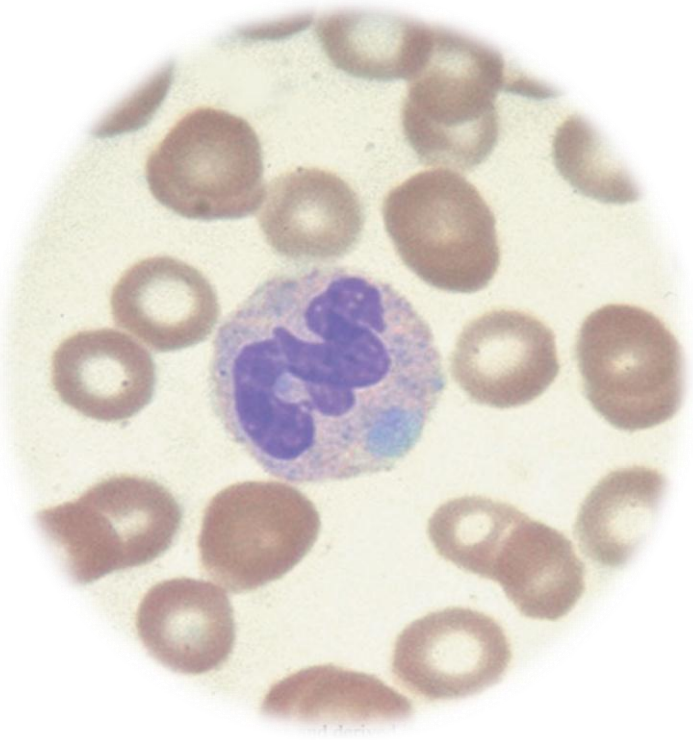
3. Neutrophilia:
   Increased the neutrophil count more than $7.5 * 10^9$\L. occasionally marked up to $30 * 10^9$\L. Characterized by shift to left (Metamyelocytes, and band forms)
4. Neutropenia:
   Decreased or ineffective production, characterized by low number of neutrophils
5. Lymphocytosis:
   Increased lymphocyte count > $5 * 10^9$\L in adult
   Or > $7.2 * 10^9$L in children
   Or > $9 * 10^9$\L in infants
6. Lymphocytopenia:
   Decreased lymphocyte < $2.5 * 10^9$\L
7. Monocytosis:
   Increased monocytes count above $0.8 * 10^9$\L
8. Monocytopenia:
   Is a form of leukopenia associated with a deficiency of monocytes
9. Eosinophilia:
   An increased eosinophil count above than $0.5 * 10^9$\L
10. Basophile:
    Is condition characterized by increased basophil count more than $0.1 * 10^9$\L

Qualitative disorders:

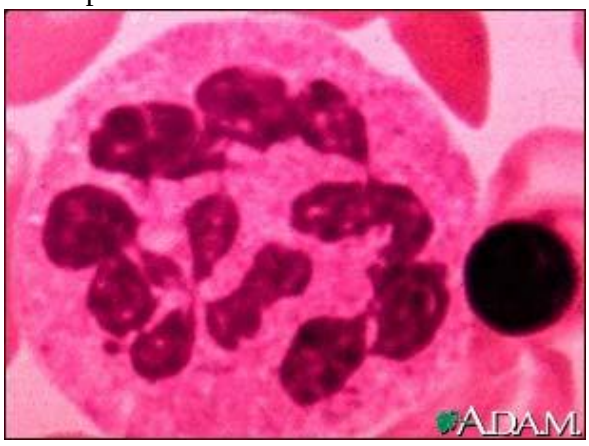
1. Pelger – Huet anomaly:
   Its bilobed neutrophil hypo segmentation neutrophils are normal in function



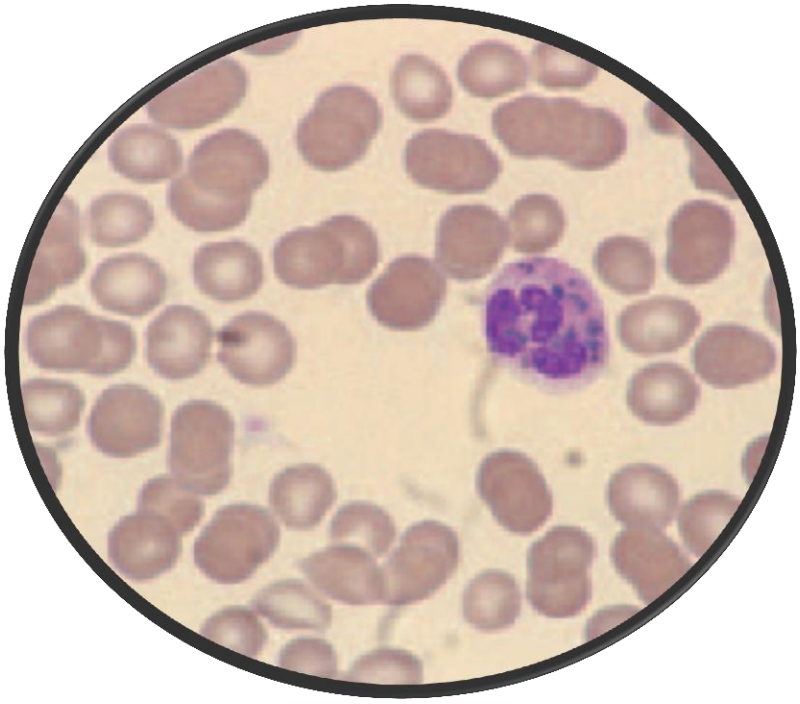
2. May- Hegglin anomaly:
   Abnormal condensation of RNA appears as basophilic inclusion in the cytoplasm of neutrophil known as Dohle's body.



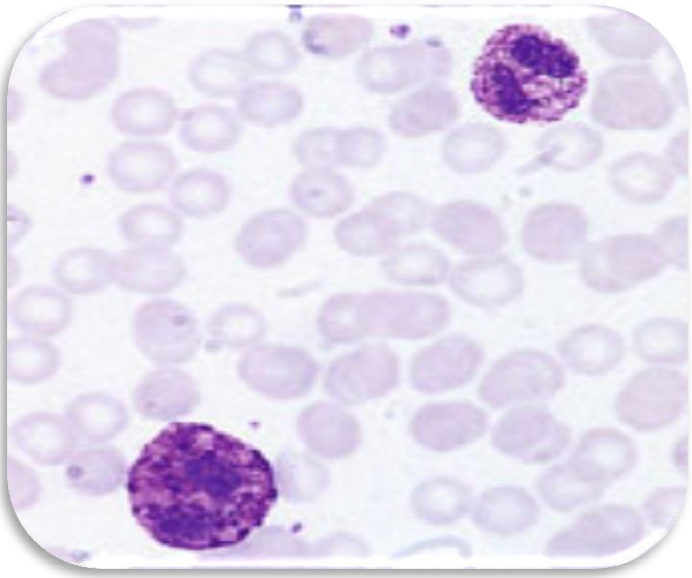
3. Hyper segmented neutrophil:
   Inherited as autosomal dominant, or in case of megaloblastic anaemia. The neutrophil normal in function.



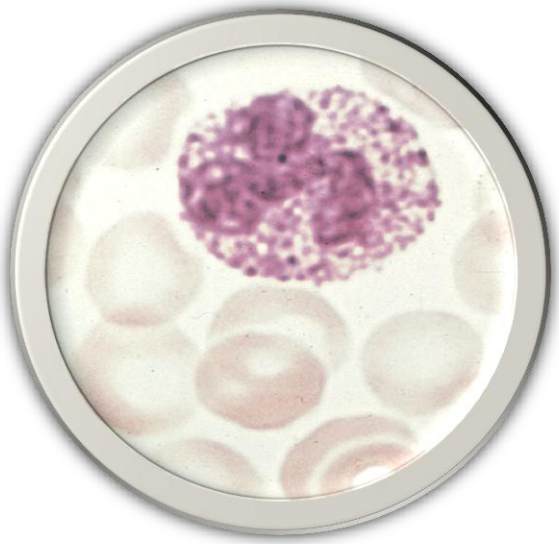
4. Chediak – Higashi syndrome:
   Giant neutrophil granules in children who have poor resistance to infections. The secondary granules of neutrophil are morphologically normal.



5. Alder- Reilly anomaly:
   Neutrophil granules take on deep purple color with Romanowisky stains.
   Present in all types of granulocytes as well as in lymphocytes and monocytes.



6. Toxic granulation:



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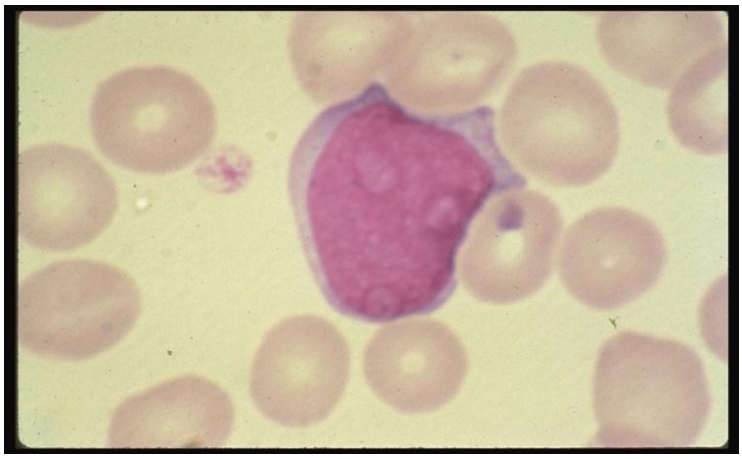
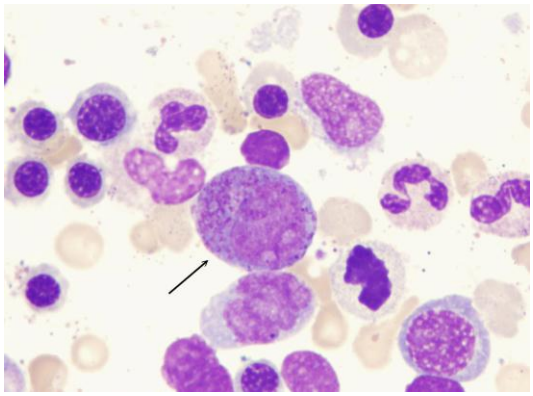
Immature white blood cells:

- **<u>Myeloblast;</u>**
- **This is the first cell in the granulocytic series.**
- **- Round, 15-20 um in diameter (two to three times of mature RBCs).**
- **- Scanty, deep blue cytoplasm.** No Granules
- **-Nucleus is centrally placed, round in shape, fine chromatin, 2-5 nucleoli.**
- N/C ratio 6/1
- ~ 2% of normal marrow

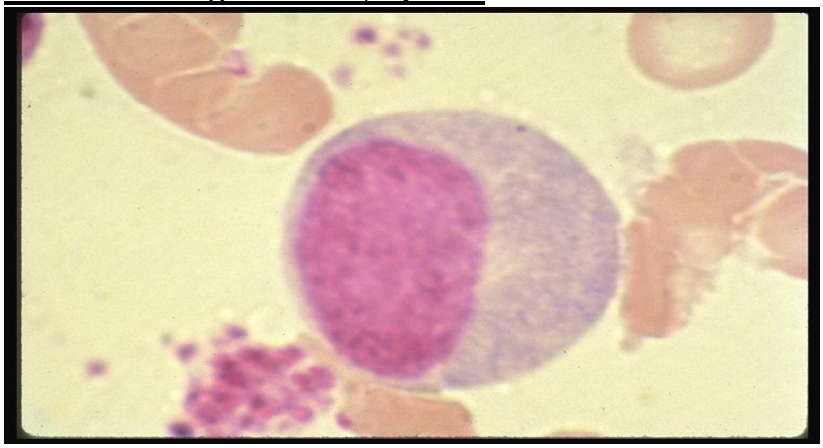


PROMYELOCYTE:

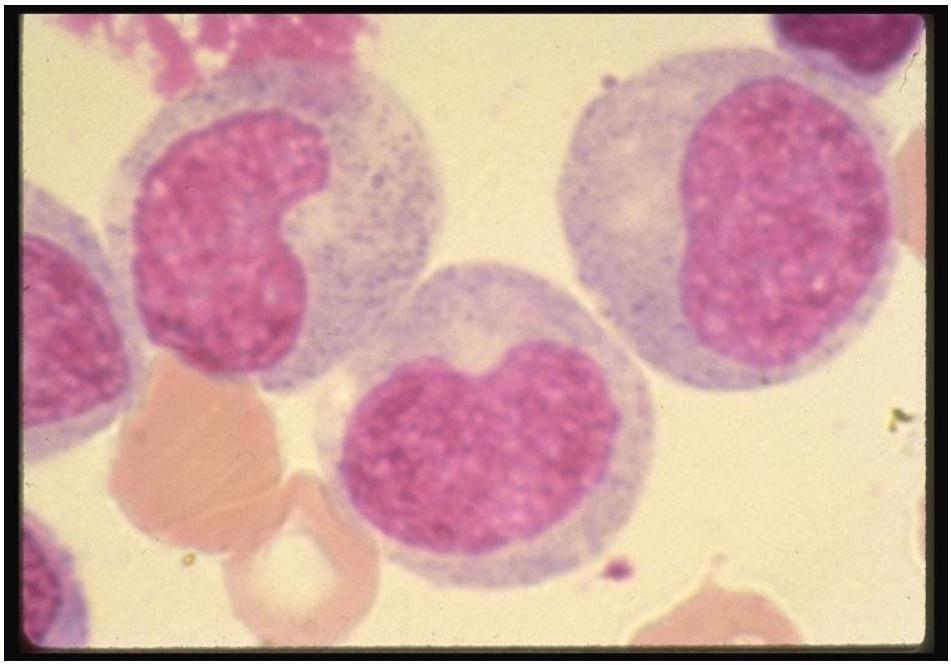
- **Usually larger than MB!**
  Cytoplasm stains less basophilic than myeloblast
  Cytoplasm contains primary granules(azurophilic granules)(large prominent, reddish purple granules)
  <u>Nucleus:</u>
  shape :oval or round
  Location: central or eccentric
- **N/C ratio is 4/1**



MYELOCYTE:
Size: 12-18 um
Nucleus:
shape :oval or round or flattened on one side
Color :dark purple
chromatin : coarser chromatin pattern, invisible nucleoli
Cytoplasm:
color: pinkish-blue
content: variable granules ,small pinkish to reddish specific granules first appearing next to the nucleus
and then throughout the cytoplasm



META-MYELOCYTE:
Post mitotic pool
: Metamyelocyte:
10-15 um in diameter, round in shape, **cytoplasm** is **pink-blue** in color with numerous secondary granules.
The primary granules still present but not seen.
Nucleus: Kidney-shaped nucleus. **Nucleoli : none**



STAB or BAND FORM:

- **Round in shape, about 12 um in diameter, cytoplasm is pink- blue color**
- **Primary granules are not present but there are few specific granules.**
- **Nucleolus is band U-S or crescent in shape Chromatin is coarse and clumped**
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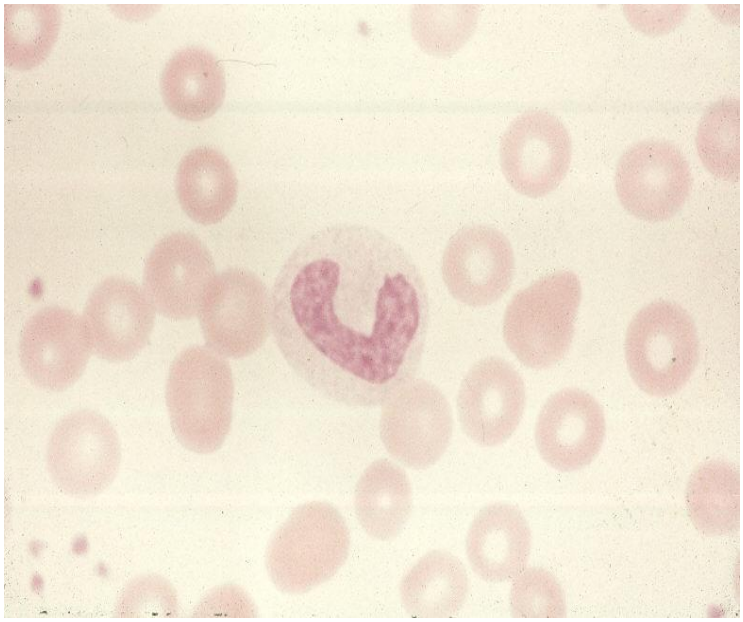

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Laboratory diagnosis of acute leukemia:

Acute leukemia is defined as the presence of over 30 % of blast cells in the bone marrow at clinical presentation. It is further subdivided into acute myeloid leukemia (AML), and acute lymphoblastic leukemia (ALL).
Myoblasts may be distinguish from lymphoblasts by three ways:

1. Morphology (presence of Auer rods)
2. Reactivity with cytochemical stain
3. Reactivity with cell surface markers

Complete blood count (CBC) of acute leukemia:

1. Low RBC count, Hb, and PCV
2. Incased WBC count
3. MCV, MCH, MCHC are normal
4. Low platelets count
5. High red cell distribution width (RDW)

Peripheral blood picture (PBP)

RBCs: Normocytic normochromic cells

WBCs: Immature white cells (BLAST)

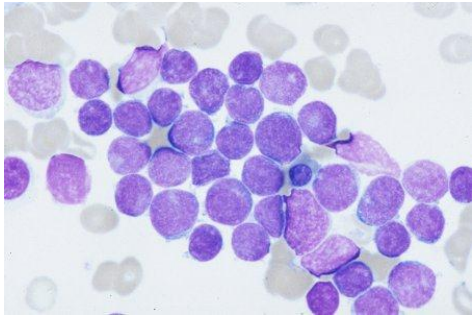
PLT: Thrombocytopenia

Bone marrow aspirate:

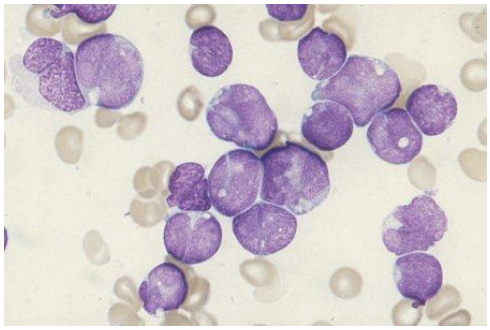
Percentage of cells in e bone marrow is blasts are particularly important, a diagnosis of acute leukemia nearly required that at least 20 % to 30 % of the cells in the bone marrow are blast.

Morphological features of ALL (fAB classification):

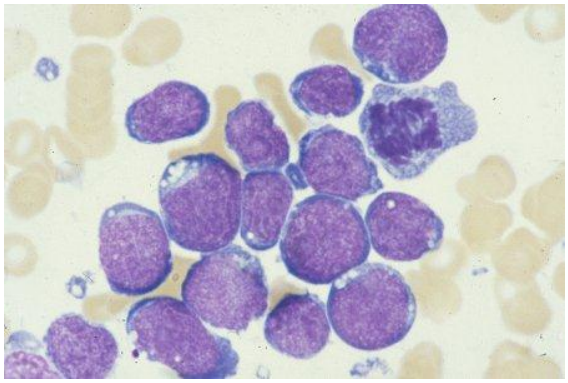
L-1 85%, small uniform cells



L-2 14%, large heterogonous cells



L-3 (Burkett's) 1% childhood, vacuolated cytoplasm



Acute myeloid leukemia (fAB classification) :

M0 Minimally differentiated AML 5% - 10% Negative or < 3% blasts stain for MPO ,PAS and NSE

blasts are negative for B and T lymphoid antigens, platelet glycoproteins and erythroid glycophorin A.

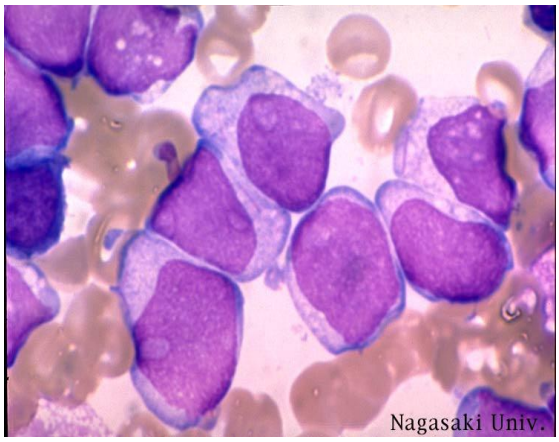
Myeloid antigens : CD13, CD33 and CD11b are positive.

M1 Myeloblastic without maturation 10 - 20%

>90% cells are myeloblasts

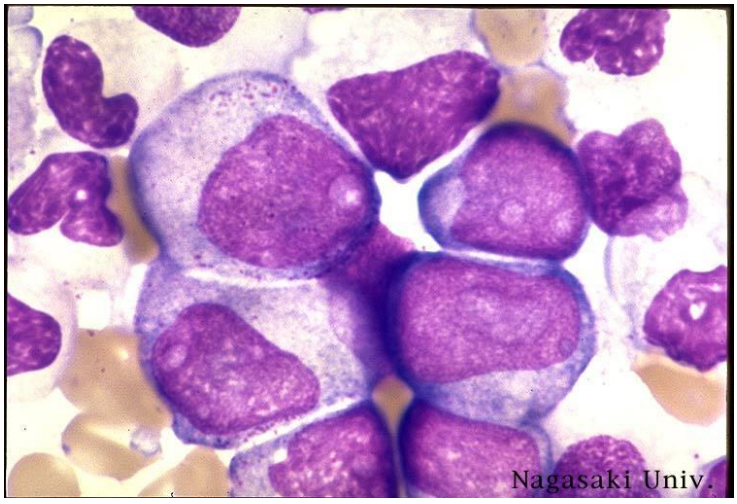
3% of blasts stain for MPO

+8 frequently seen



M2 AML with maturation 30 - 40%

30% - 90% are myeloblasts ~ 15% with t(8:21)



M3 Acute Promyelocytic Leukemia (APML) 10-15%

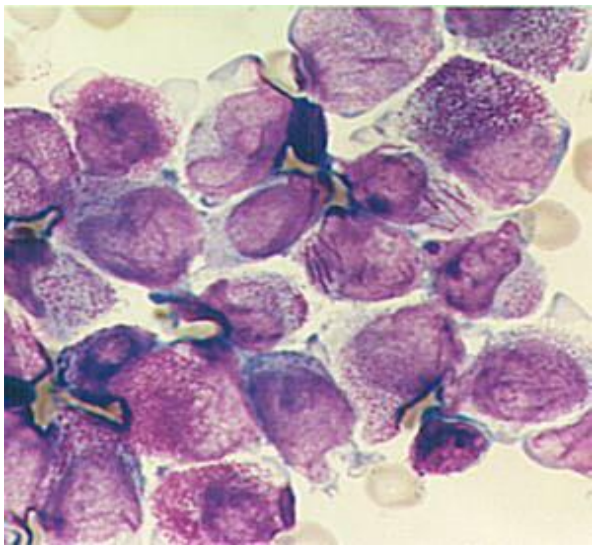
marrow cells hypergranul promeyelocytes

Auer rods/ faggot cells may be seen

Classical-Hypergranular, 80% leukopaenic

Variant-Hypogranular, leukocytosis

Granules contain procoagulants (thromboplastin-like) - massive DIC
t(15:17) is diagnostic



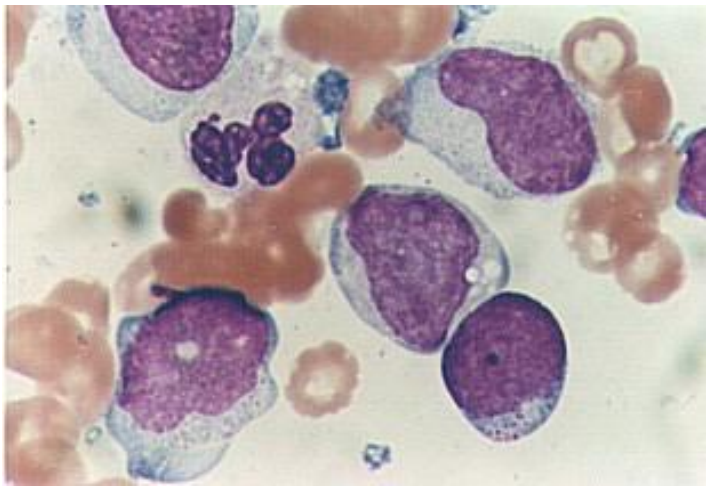
M4 Acute Myelomonocytic Leukemia 10-15%

Incresed incidence CNS involvement

Monocytes and promonocytes 20% - 80%

M4 with eosinophilia ((M4-Eo), assoc with del/inv 16q

– marrow eosinophil from 6% - 35%,

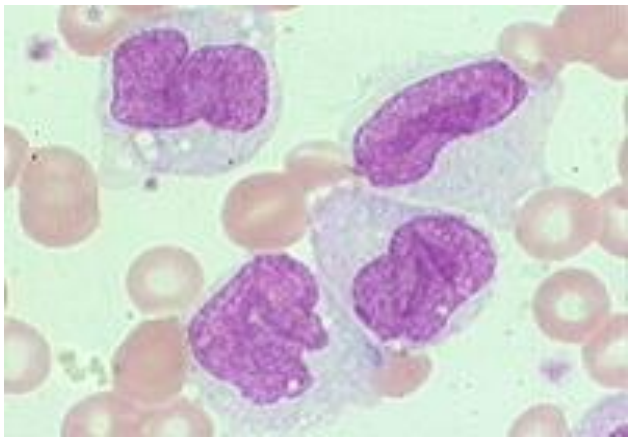


M5a Acute Monoblastic Leukemia 10-15%

M5b AMoL with differentiation <5%

Often asso with infiltration into gums/skin

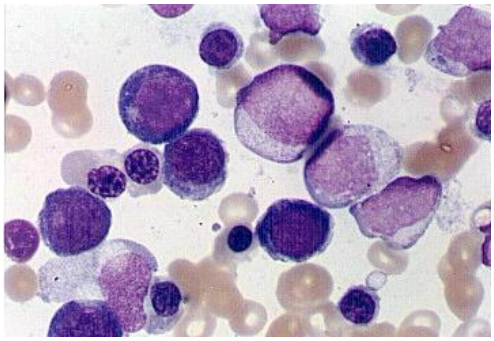
Weakness, bleeding and diffuse erythematous skin rash



M6 Erythroleukemia (Di Guglielmo) <5%

50% or more of all nucleated marrow cells are erythroid precursors,

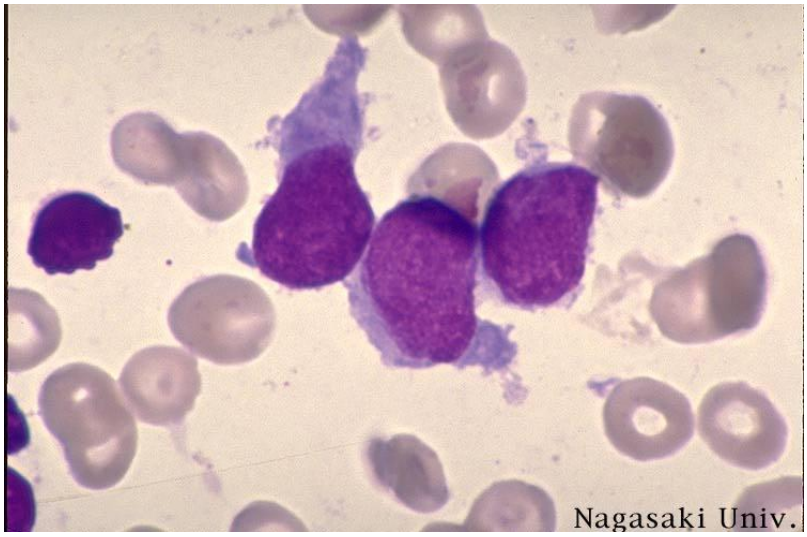
and 30% or more of the remaining nonerythroid cells are myeloblasts (if <30% then myelodysplasia



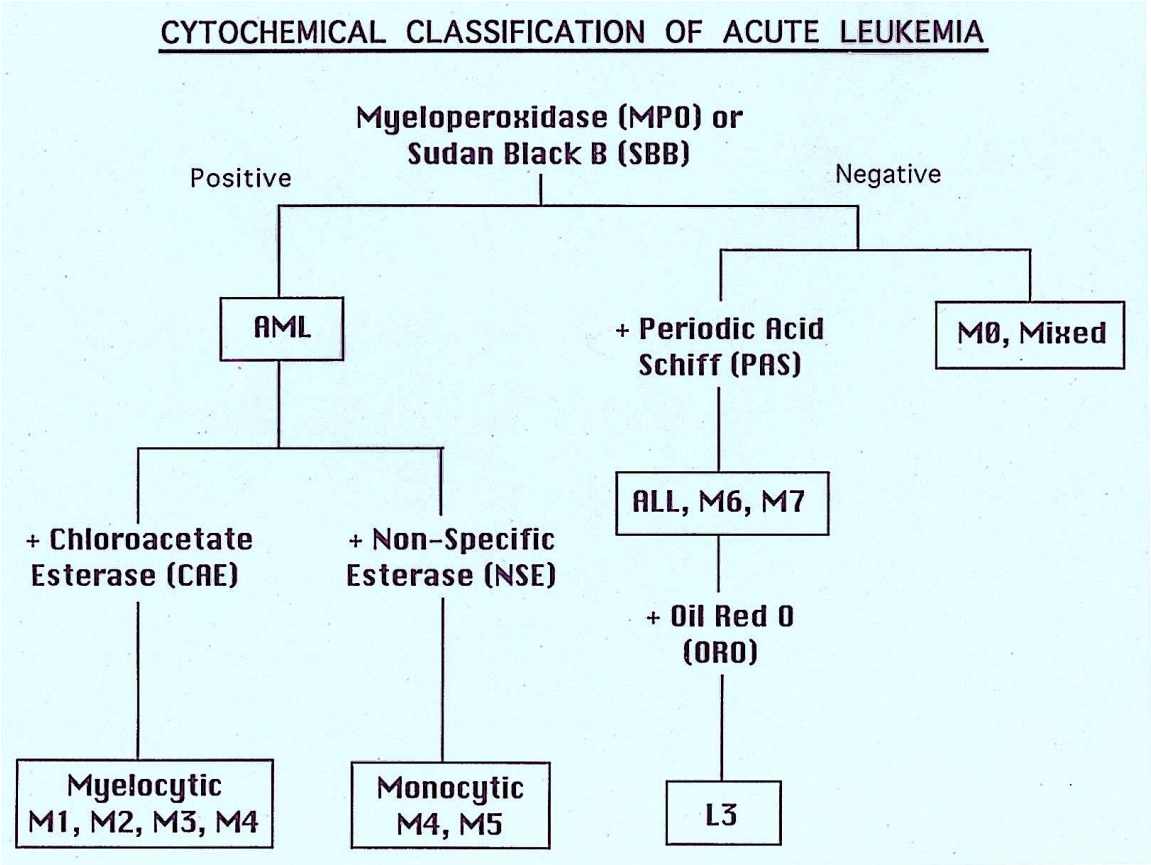
M7 Acute Megakaryoblastic Leukemia <5%

Assoc with fibrosis

(confirm origin with platelet peroxidase + electron microscopy or MAb to vWF or glycoproteins



Cytochemial stains:



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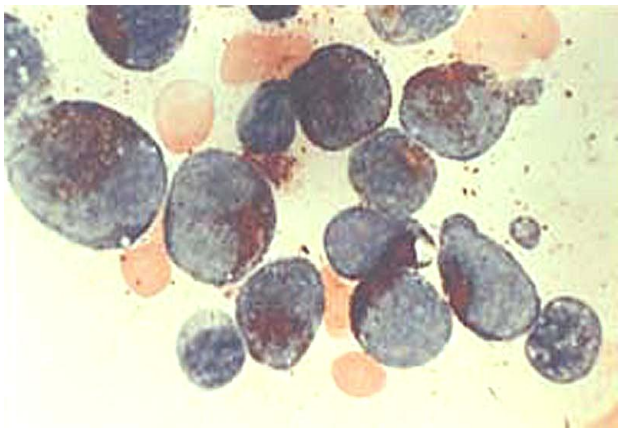
Cytochemical Stains:

Myeloperoxidase (MPO):

Principle:

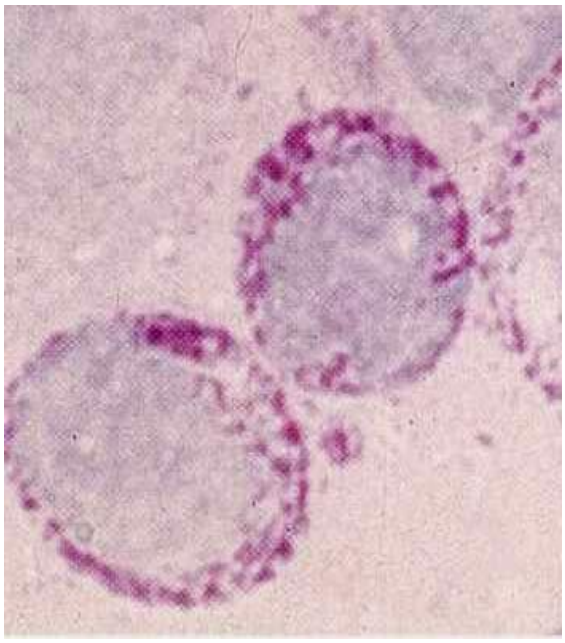
p-Phenylene diamine + Catecol + H_2O_2 $\xrightarrow{\text{MPO}}$ Brown black deposits



Periodic Acid Schiff:

Principle:

Periodic acid + Glycogen $\xrightarrow{\text{oxidation}}$ Aldehyde + Schiff reagent (para-rosaniline, Na metabisulfite) ——> Red deposit

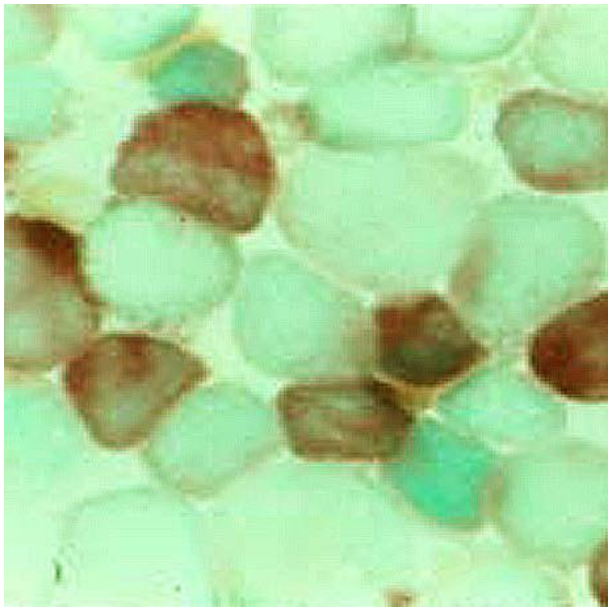


Non-Specific Esterase (Monocytic Line):

Principle:

a Naphthyl acetate —> Free naphthyl compounds

+Stable diazonium salt (eg, Fast blue RR)————> Brown deposits



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Loaoratory diagnosis of chronic myeloid leukemia (CML):

Complete blood count:

1. Hb: slight decreased
2. TWBCs: marked increased (50 – 300 * 10^9\L
3. Platelet count: increased

Peripheral blood picture:

RBCs: Normocytic normochromic

WBCs: full spectrum of myeloid stages (Blast, Promyelocytes, Myeloytes, Metamyelocytes) with mature cells.

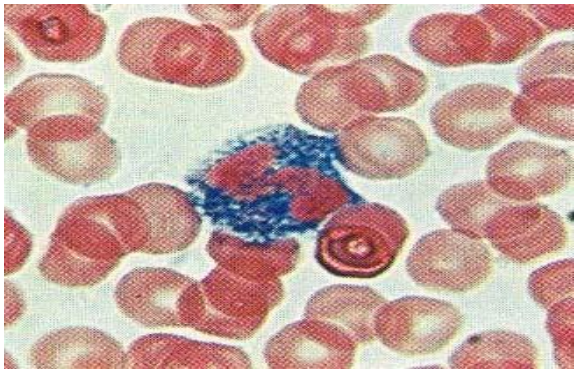
PLT: Thrombocytosis

Bone marrow aspiration:

1. Hypercellular: (E:G ratio 1:10)
2. Myeloctic hyperplasia
3. Increased megakaryocytes
4. Variable fibrosis

Special studies:

1. LAP score (leukocyte alkane phosphatase)
2. Count 100 consecutive segmented and bands
3. Score:
   0 = no granules
   1+ = occasional diffuse granules
   2+ = moderate number of granules
   3+ = many strongly positive granules
   4+ = confluent strongly positive granules

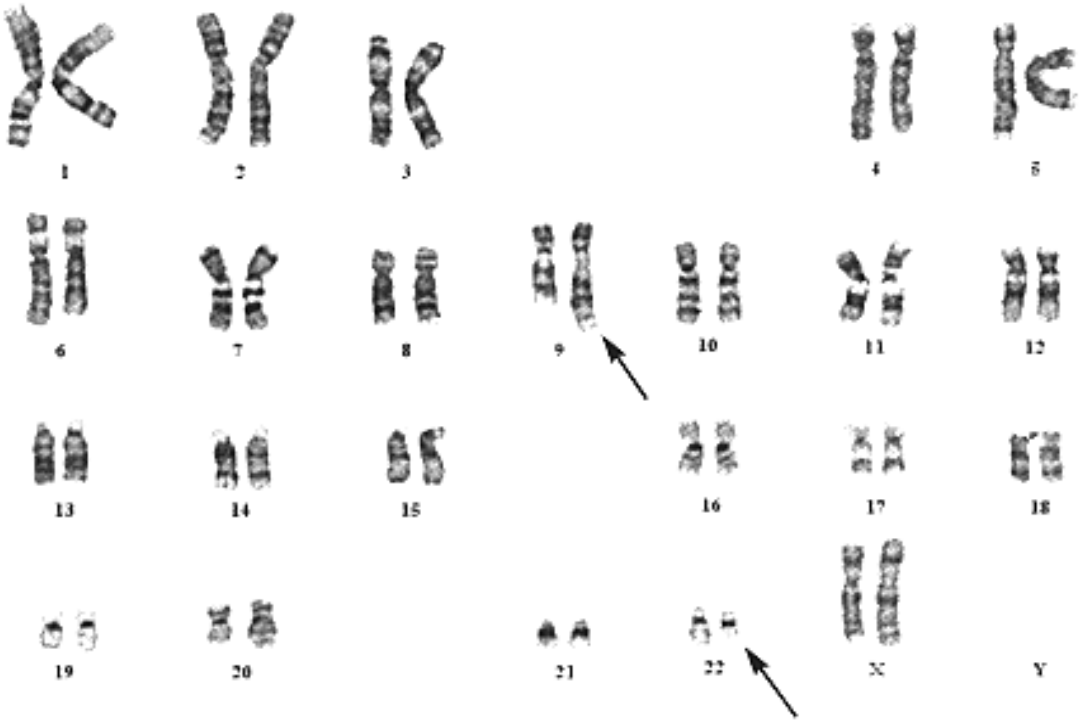


Exemple:

0	x	35 cells	= 0
1+	x	30 cells	= 30
2+	x	20 cells	= 40
3+	x	10 cells	= 30
4+	x	5 cells	= 20

120 LAP Score

3. Cytogenetic to determine the Philadelphia chromosome :
4.



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Laboratory diagnosis of CLL:

Complete blood count:

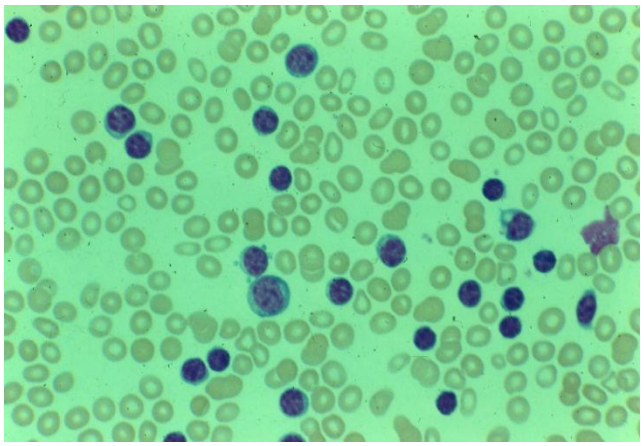
1. Hb, TRBCs, and PCV are low
2. Increased TWBCs and persistent lymphocytosis (have more than 10.000 lymphocytes\mm^3
3. Platelet: decreased

Peripheral blood film:

RBCs: Normocytic normochromic

WBCs: persistent lymphocytosis and smudge cells

PLT: Thrombocytopenia



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Laboratory diagnosis of Multiple Myeloma

- Also called plasma cell myeloma
- Is a cancer of plasma cells, can form a mass in the bone marrow and soft tissues called plasmacytoma .
- **Clinical features:**

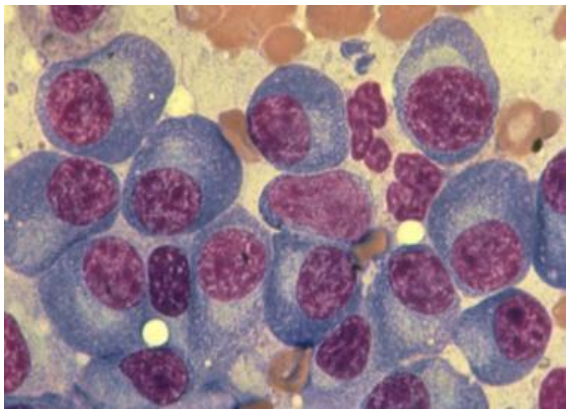
1. Often no symptoms
2. Bone pain
3. Bleeding
4. Infection
5. Anaemia

Laboratory diagnosis:

1. CBC: anaemia, thrombocytopenia
2. ESR: HIGH
3. Renal dysfunction
4. High serum protein (beta 2 micro globulin)
5. High serum calcium
6. Bence jones protein in urine

Confirmatory tests:

1. Protein electrophoresis of blood or urine to detect a par protein (M protein)
2. Bone marrow examination to detect the accumulation of the plasma cells
3. Immunophenotyping (CD 56, CD 38 , CD 138, CD 319)
4. Cytogenetic
5. Molecular analysis



Student's findings (measurements or observations):

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comments and interpretation:

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Evaluation (carried out by the instructor):

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Name and signature of the instructor:

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Date: …….**.....****.....**

Lymphoma:

- Is a group of blood cell tumors that develop from lymphocytes.
- **Clinical features:**

1. Enlarged lymph nodes
2. Fever
3. Weight loss
4. Night sweats

Classification:

1. Hodgkin's lymphoma (HL)
2. Non Hodgkin's lymphoma (NHL)

Differentiate between them by diagnostic cell called reed Sternberg cell which found in HL

Other types of lymphoma:

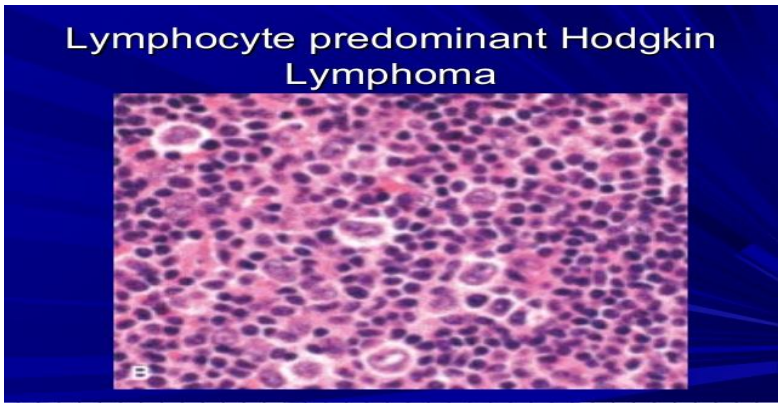
1. T cell lymphoma
2. B cell lymphoma
3. Burkett's lymphoma
4. Follicular lymphoma
5. Diffuse large B cell lymphoma
6. Mantle cell lymphoma

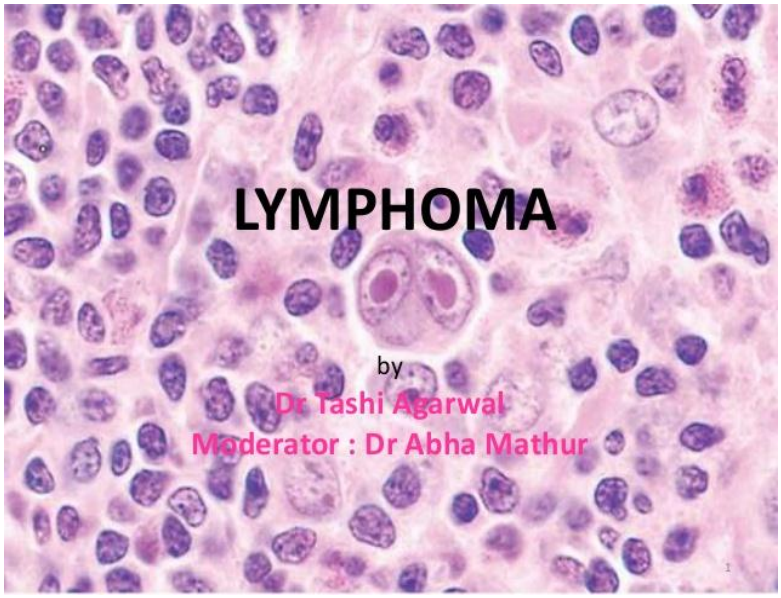
Diagnosis:

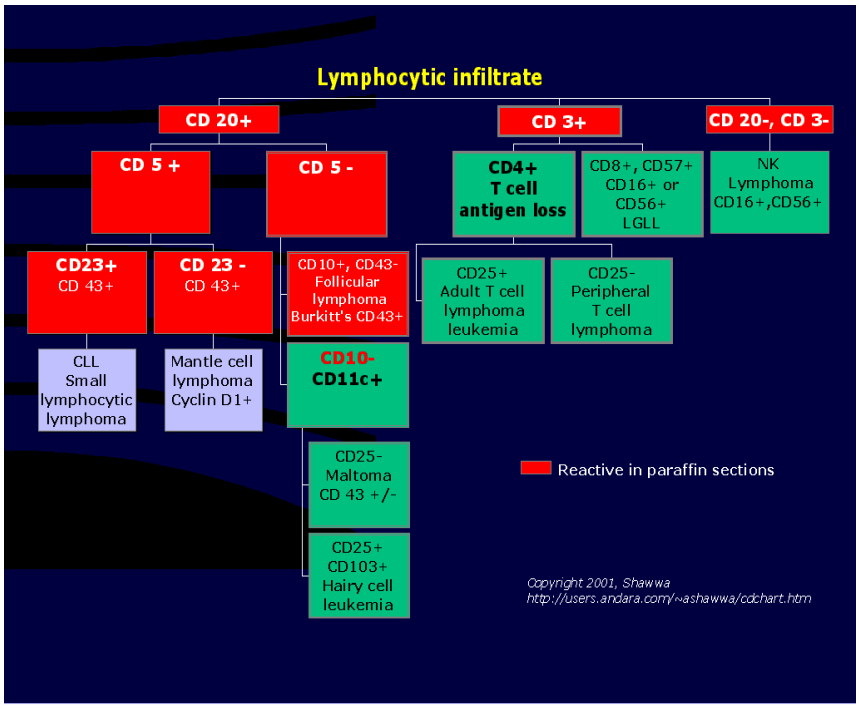
- biopsy (histological technique)
- CBC also useful in diagnosis
- PBP
- Bone marrow aspiration

Confirmatory tests:

1. immunofluorescent, immunohisto chemistry)
2. Cytogenetic (fluorescent in situ hybridization (FISH))
3. Molecular analysis


Lymphocyte predominant Hodgkin Lymphoma


LYMPHOMA
by
Dr Tashi Agarwal
Moderator : Dr Abha Mathur



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Evaluation (carried out by the instructor):

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Name and signature of the instructor:

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