ACUTE LEUKEMIAS

Acute Leukemia

- Definition:
- Heterogeneous group of malignant disorders which is characterized by uncontrolled clonal proliferation and accumulation of blasts cells in the bone marrow and body tissues
- Sudden onset
- If left untreated is fatal within a few weeks or months

Comparison of acute and chronic leukemias

	Acute	Chronic
Age	All ages	Usually adults
Clinical onset	Sudden	Insidious
Course (untreated)	6 mo or less	2-6 years
Leukemic cells	Immature >20% blasts	More mature cells
Anemia	Prominent	Mild
Thrombocytopenia	Prominent	Mild
WBC count	Variable	Increased
Lymphadenopathy	Mild	Present; often
Splenomegaly	Mild	Present; often

CAUSES OF ACUTE LEUKEMIA

- PRE LEUKEMIA – Myelodysplastic or myeloproliferative syndromes can evolve into AML.
- CHEMICAL EXPOSURE- Alkylating agents,benzene, aromatic organic solvents.
- RADIATION - Atomic bombings of Hiroshima and Nagasaki,X rays ,
  victims of Chernobyl nuclear reactor.
- GENETICS –Down syndrome,Ataxia telengectasia , Klinefelter's syndrome , Fanconi's anaemia.
- Naturally occuring retroviruses and the human T – cell lymphotropic viruses cause adult- ALL

PATHOPHYSIOLOGY

LEUKEMOGENESIS

- Heterogeneous, multi-step process that results in a block of differentiation, increased proliferation and inhibition of apoptosis through genetic dysregulation.

Two-hit model of leukemogenesis

Loss of function of transcription factors needed for differentiation

Gain of functional mutations of tyrosine kinases

↓ ↓

Differentiation block + Enhanced proliferation → **Acute Leukemia**

Pathophysiology

- **Acute leukemia cause morbidity and mortality through:**
  - Deficiency in blood cell number and functional neutropenia causes infections, hemorrhage due to thrombocytopenia, anaemia.
  - Invasion of vital organs- brain , lung, eyes due to increased viscosity of blood and formation of microthrombi.
  - Systemic disturbances by metabolic imbalance – hypokalemia, hyperuricemia.

SIGNS AND SYMPTOMS

- Generalised symptoms – fever fatigue,weight loss, loss of appetite.
- Enlargement of spleen, liver
- Lymph node swelling is more common in ALL
- Easy bruising, petechiae.
- Medistinal mass may be seen in ALL.

DIAGNOSIS

- BLOOD OR MARROW EXAMINED VIA LIGHT MICROSCOPY AS WELL AS FLOW CYTOMETRY
- CYTOCHEMICAL STAINS
- CYTOGENETICS AND MOLECULER TECHNIQUES TO DETECT CHROMOSOMAL TRANSLOCATIONS
- IMMUNOPHENOTYPING

Lab evaluation

1) PERIPHERAL SMEAR-

a) anaemia – normocytic , normochromic

b) leucopenia – increased blasts

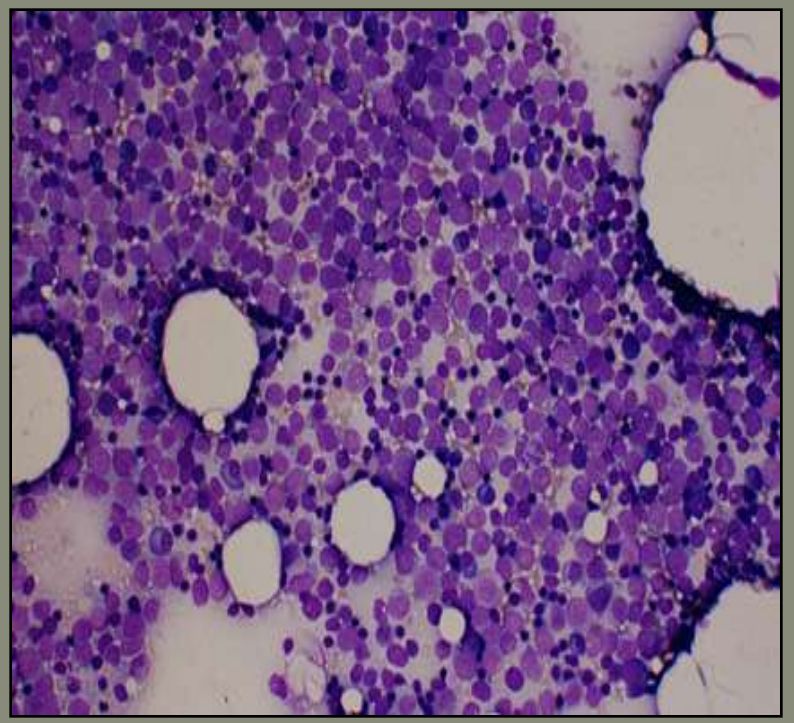
(more than 20% blasts is diagnostic of acute leukemia)

c) thrombocytopenia

2) BONE MARROW –

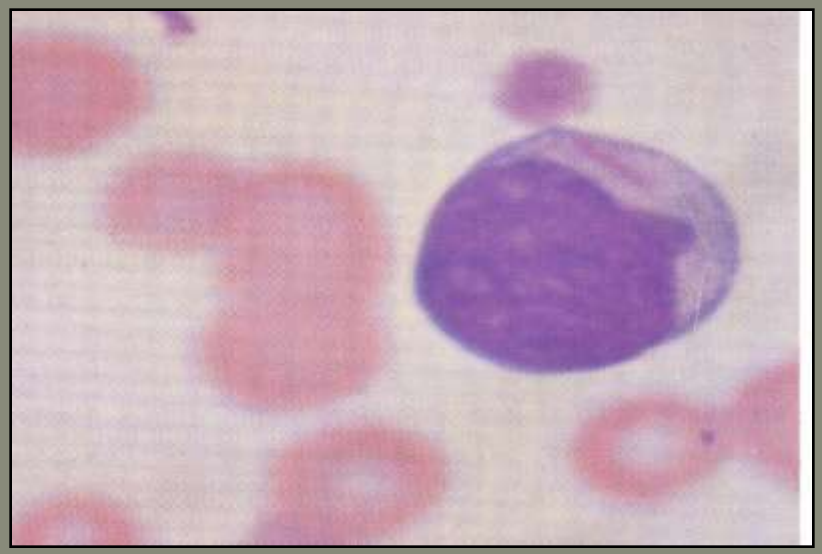
a) hypercelluler marrow

b) blasts – 20%- 90%



AML

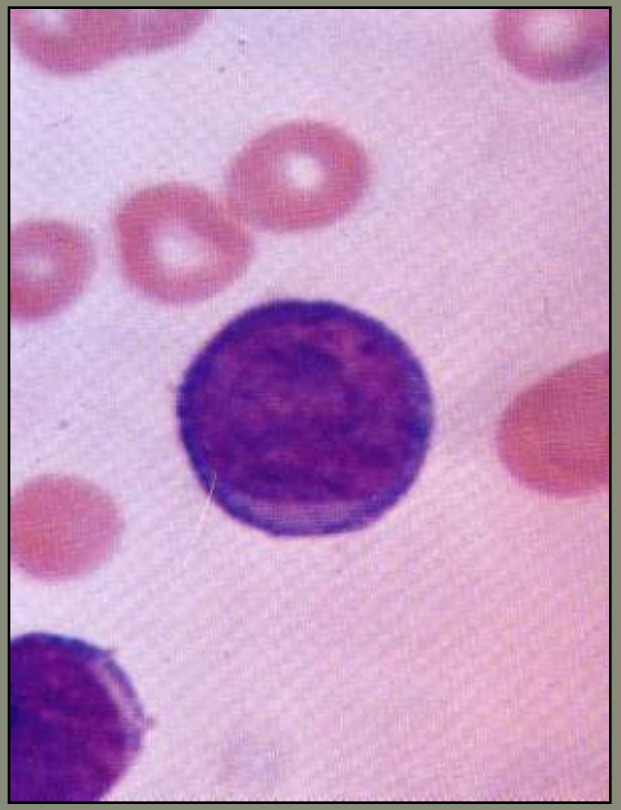
- **AML** – the **myeloblast** is a large blast with a moderate amount of cytoplasm, fine lacey chromatin, and prominent nucleoli. 10-40% of myeloblasts contain **Auer rods**.



Myeloblasts with Auer rods

ALL

- **ALL** – in contrast to the myeloblast, **the lymphoblast is a small blast** with scant cytoplasm, dense chromatin, indistinct nucleoli, and **no auer rods**



Lymphoblast

Difference between myeloblast and lymphoblast

	AML (Myeloblast)	ALL (Lymphoblast)
Blast size:	Large	Small
Cytoplasm:	Moderate	Scant
Chromatin:	Fine, Lacy	Dense
Nucleoli:	Prominent	Indistinct
Auer-rods:	Present in 50%	Never present

FAB classification of Acute myeloid leukemia (AML)

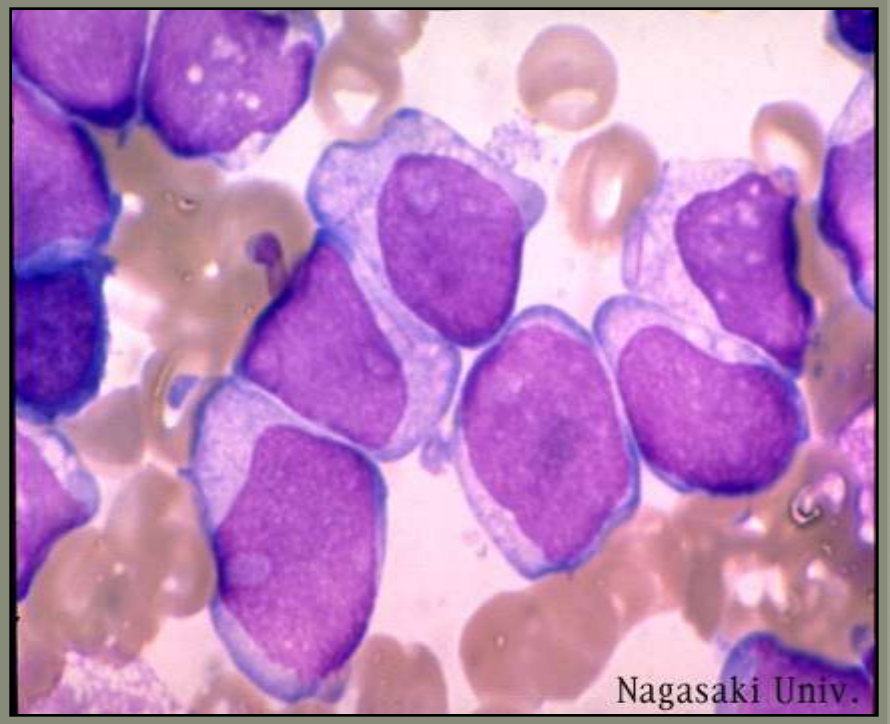
M0	AML, minimally differentiated
M1	AML, without maturation
M2	AML, with maturation
M3	Acute promyelocytic leukemia, hypergranular
M3v	Acute promyelocytic leukemia, variant, microgranular
M4	Acute myelomonocytic leukemia
M4eo	Acute myelomonocytic leukemia with eosinophilia
M5a	Acute monoblastic leukemia, poorly differentiated
M5b	Acute monoblastic leukemia, differentiated
M6	Acute erythroleukemia
M7	Acute megakaryoblastic leukemia

WHO Classification of AML

I. AML with recurrent genetic abnormalities	
AML with t(8;21)(q22;q22);	Favourable
AML with abnormal bone marrow eosinophils [inv(16)(p13q22) or *CBFB/MYH11*]*b*	Favourable
Acute promyelocytic leukemia [AML with t(15;17)(q22;q12) (*PML/RAR*) and variants]*b*	Intermediate
AML with 11q23 (*MLL*) abnormalities	poor
II. AML with multilineage dysplasia	
With prior MDS	Very poor
Without prior MDS	Poor
III. AML and myelodysplastic syndromes, therapy-related	
Alkylating agent–related	Very poor
Topoisomerase type II inhibitor–related	Very poor
IV. AML not otherwise categorized	intermediate

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, CD11b and HLA DR positive.

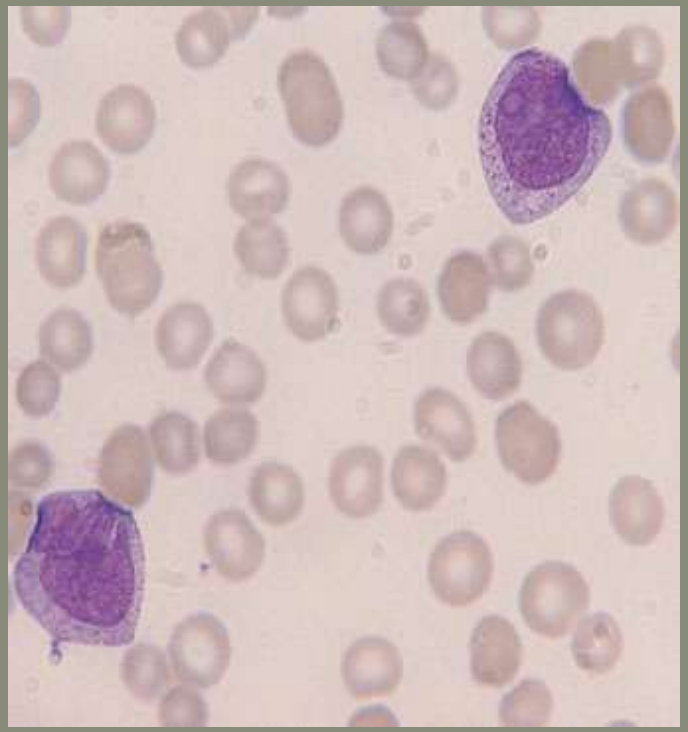


M1 - Acute Myeloblastic Leukemia without maturation

- Minimal maturation of marrow nonerythroid cells is present.
- Most of the blasts are agranular with high N/Cratio
- Auer rods are infrequent.
- **Staining:** Relatively few blasts (5-10%) are MPO+ve.
- A minimum of 3% MPO positive blasts are required for diagnosis.
- NSE and PAS negative.
- **Immunophenotype:** Variably positive for CD13, CD14, CD11b, CD33, and HLA-DR.
- **Chromosome Abnormalities:** t(9;22) Philadelphia chromosome, 8+, -5, and -7.

M2 – Myeloblastic with maturation

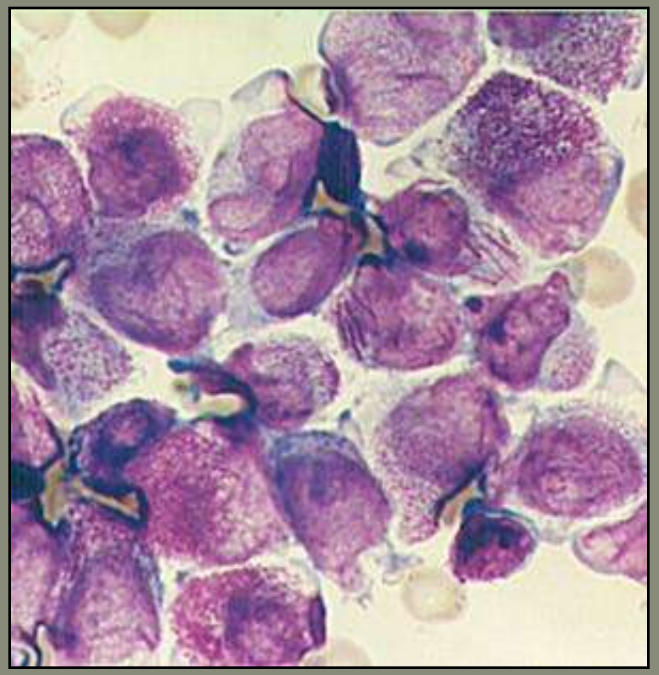
- M2 is the **most common** (20-40%).
- **Maturation**: Auer rods are frequent
- **Staining**: The blasts are largely MPO +VE NSE and PAS are generally-ve
- **Immunophenotype**: Variable positivity for CD13, CD33, and HLA-DR, but are –ve for CD14 and CD11b.
- **Chromosome Abnormalities**: t(8;21), 8+, -5, and -7.
- **Cell Morphology**:In M2 AML promyelocytes are also present
- They have more numerous granules than myeloblasts and mayhave an eccentric nucleus and a Golgi zone. This is characterized by an **8,21 chromosomal translocation**



M2 subtype showing two promyelocytes.

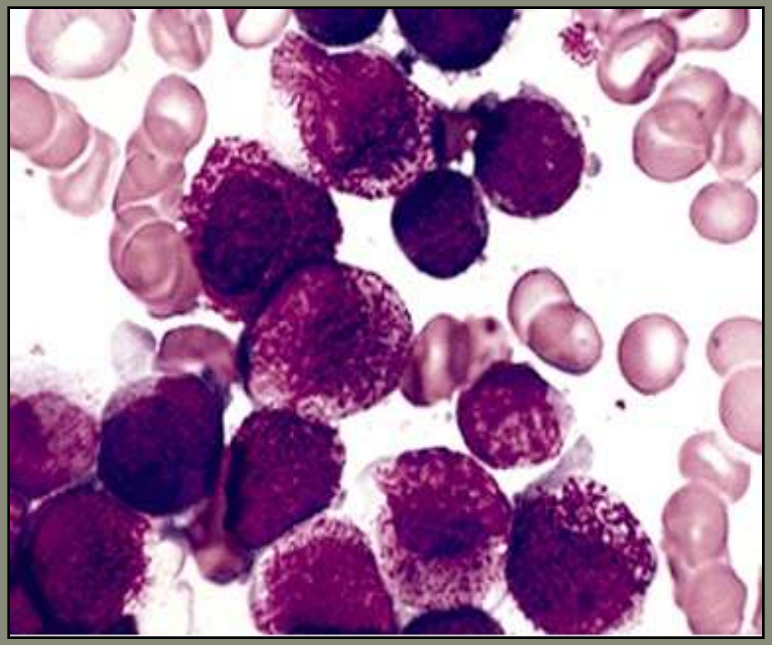
M3 Acute Promyelocytic Leukemia (APML)

- 10-15%
- Marrow cells hypergranular promyelocytes
- Auer rods may be seen
- **Classical - Hypergranular**, 80% leukopenic
- **Variant - Hypogranular,** leukocytosis
- Granules contain procoagulants (thromboplastin-like) - massive DIC
  t(15:17) is diagnostic



M3 – Hypergranular promyelocytic

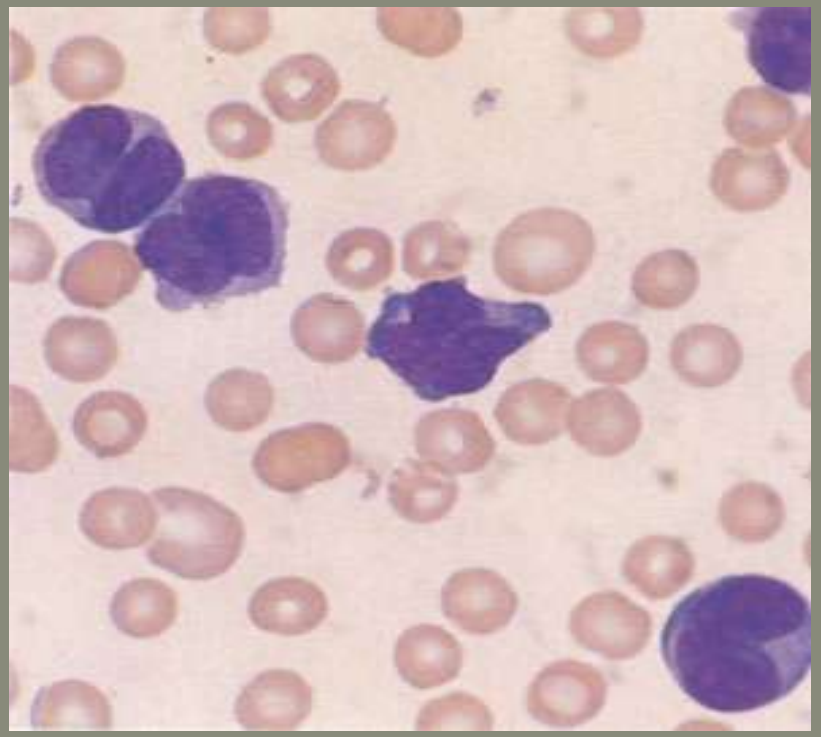
- This form of AML has a bone marrow with >30% blasts
- Is more virulent than other forms
- Occurs with a medium age of 39
- The WBC count is decreased
- Treatment causes a release of the granules and may send the patient into disseminated intravascular coagulation and subsequent bleeding
- It is characterized by a 15,17 chromosomal translocation



Note hypergranular promyelocytes

M3m – Hypogranular promyelocytic

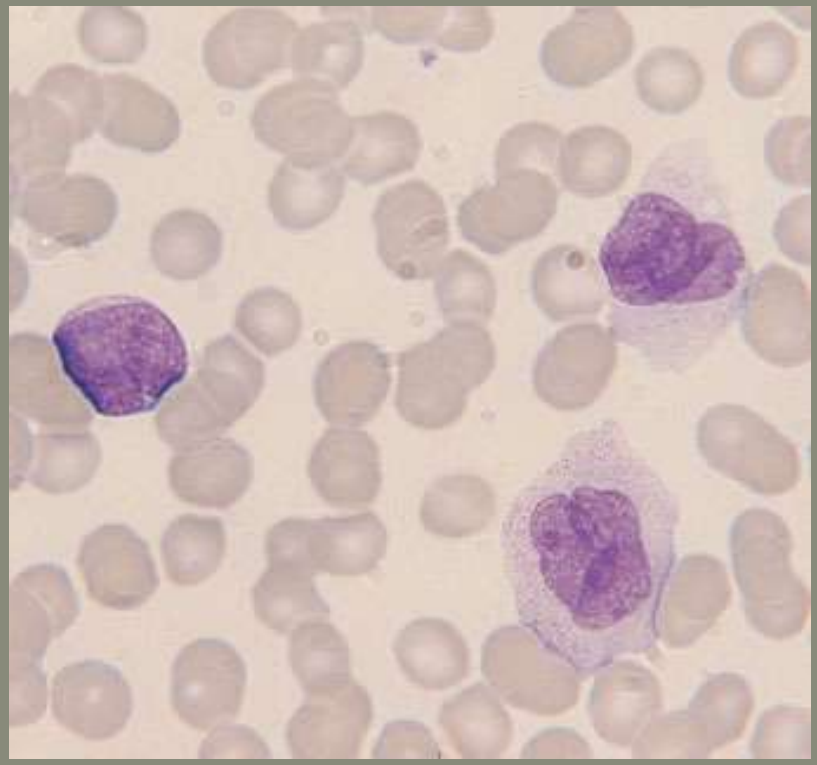
- The bone marrow has > 30% blasts
- The WBC count is increased.
- Like the M3 type, treatment causes a release of the granules and may send the patient into disseminated intravascular coagulation and subsequent bleeding and
- It is characterized by a 15,17 translocation



Note hypogranular promyelocytes

M4 – Acute Myelomonocytic Leukemia

- 10-15%
- Both myeloblasts and monoblasts are present
- Monoblasts positive for NSE
- Subset asscociated with inv(16)
- Increased incidence CNS involvement
- Monocytes and promonocytes 20% - 80%



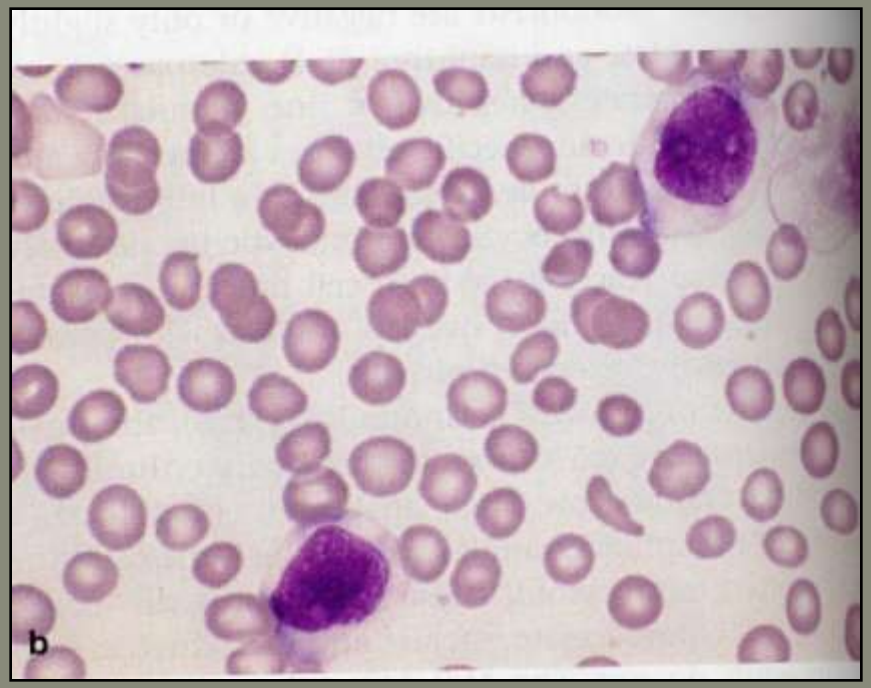
M4 subtype showing a myeloblast (left) and two monoblasts (right).

Monoblasts vs. Myeloblasts

Monoblasts	Myeloblasts
More cytoplasm which is often basophilic	scanty cytplasm
Cytoplasmic vacuolation seen	Cytoplasmic vacuolation not seen
Fewer granules	More granules
Occasionally will see slight nuclear fold	Typically round nucleus
Few, large nucleoli	More nucleoli

M5a - Acute Monoblastic Leukemia

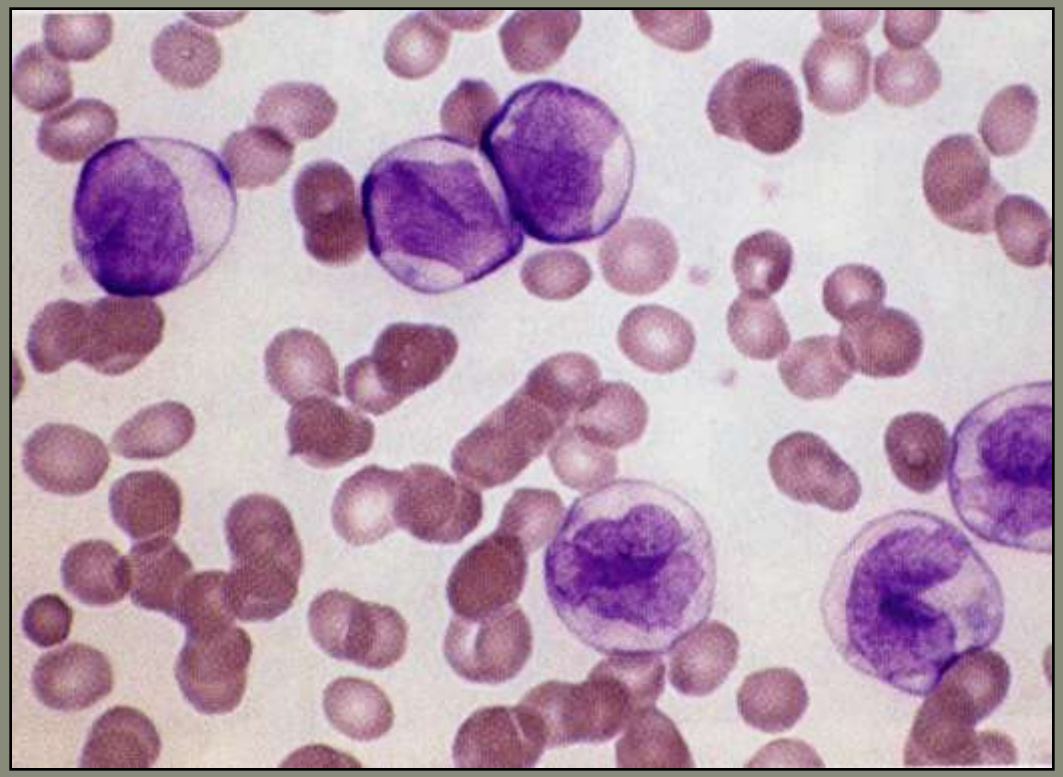
- 10-15%
- Poorly differentiated
- >80% monoblasts
- Monoblasts(peroxidase-neg, NSE-pos) & promonocytes predominate in marrow & blood



Monoblasts

M5b

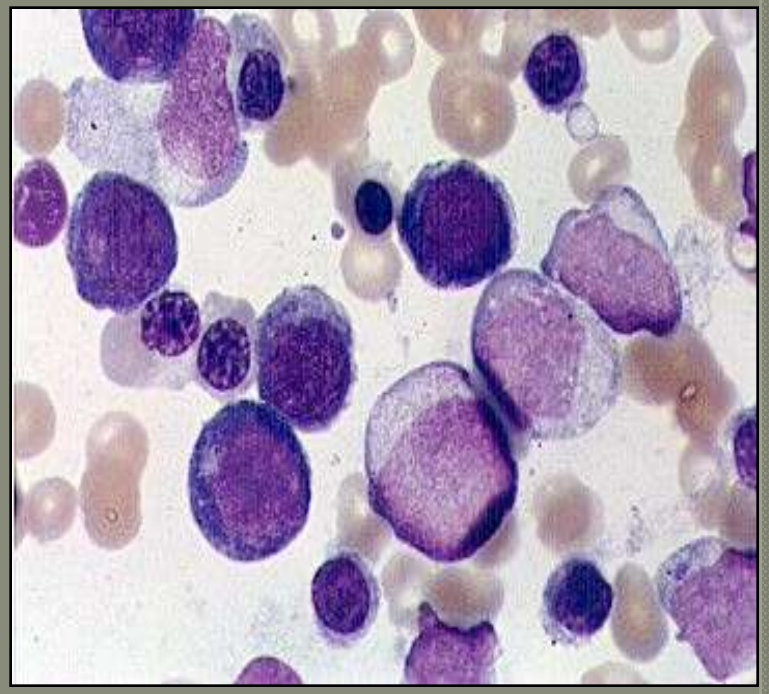
- <5%
- Well differentiated
- <80% monoblasts
- Mature monocytes predominate in blood.
- Promyelocytes are also +nt
- Often asso with infiltration into gums ,CNS, lymph nodes & extramedullary sites
- Weakness, bleeding and diffuse erythematous skin rash



Note monoblasts, promonocytes, and monocytes.

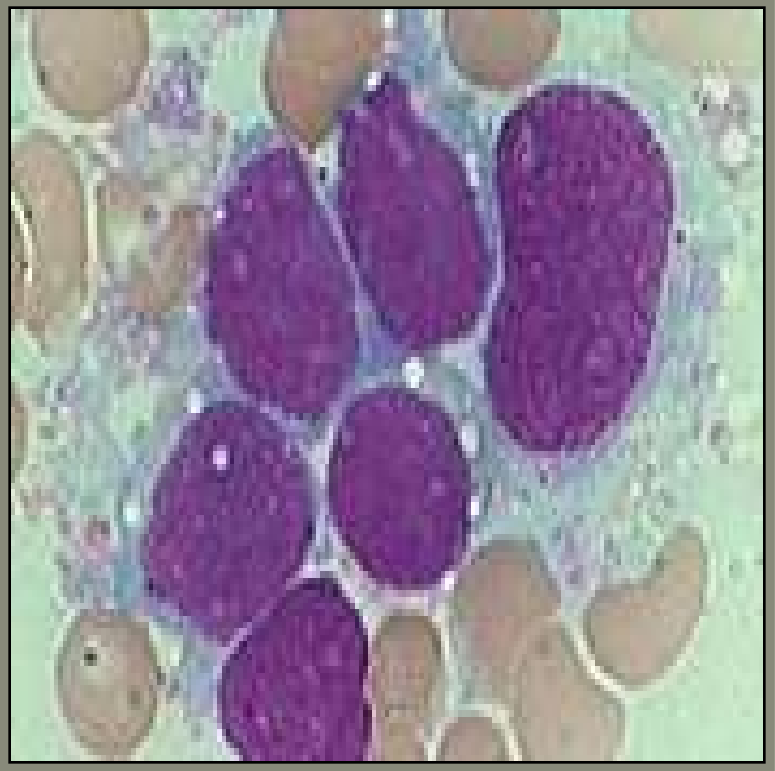
M6 - Acute Erythroblastic Leukemia

- >50% of the nucleated marrow cells are abnormal nucleated RBCs
- **Morphology**: The leukemic red cells are frequently bizarre with extreme dysplastic features including: giant forms, multinucleation, cytoplasmic vacuolization, cytoplasmic buds, and megaloblastoid changes.
- **Staining:** The blasts are MPO-ve, but often +ve for NSE. The malignant red cells are PAS positive,
- **Immunophenotype** **+ve** for glycophorin A.
- **Chromosome Abnormalities**: 8+, -5, del(5q), and -7.

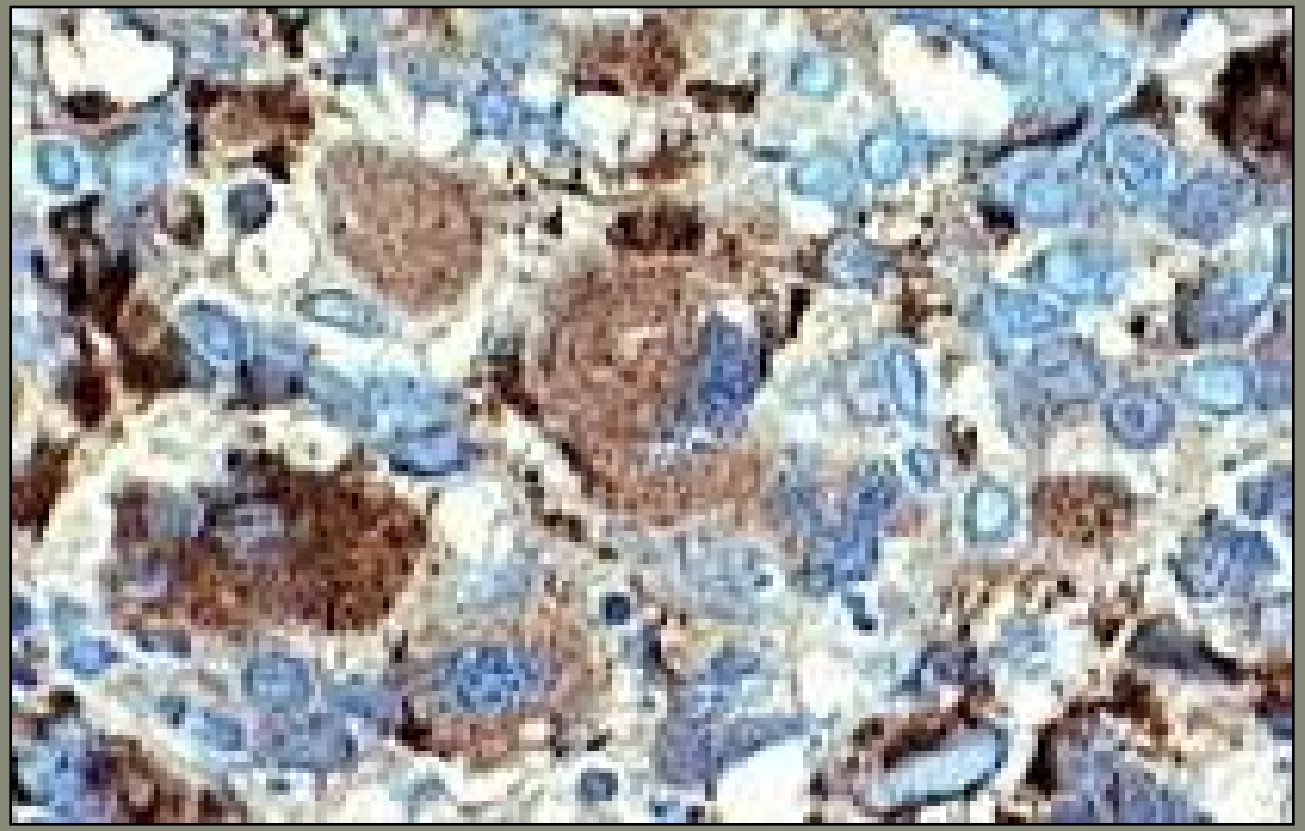


M7 - Acute megkaryoblastic leukemia

- May be accompanied by atypical megakaryocytes.
- The marrow is often fibrotic.
- **Staining**: M7 blasts are MPO negative and variably positive for PAS and NSE.
- Immunophenotypic studies of M7 are positive for glycoproteins GP Ib andGP IIb/IIIa.
- Factor VIII related protein is usually found in the megakaryoblast cytoplasm.
- **Chromosome Abnormalities**: t(1;22), have been associated with M7 in infants.
- M7 blasts may have granular cytoplasm and shed 'platelets'.



M7 - Acute megkaryoblastic leukemia



Immunoperoxidase staining (brown) for Factor VIII related protein identifies the blasts as being of megakaryocyte lineage.

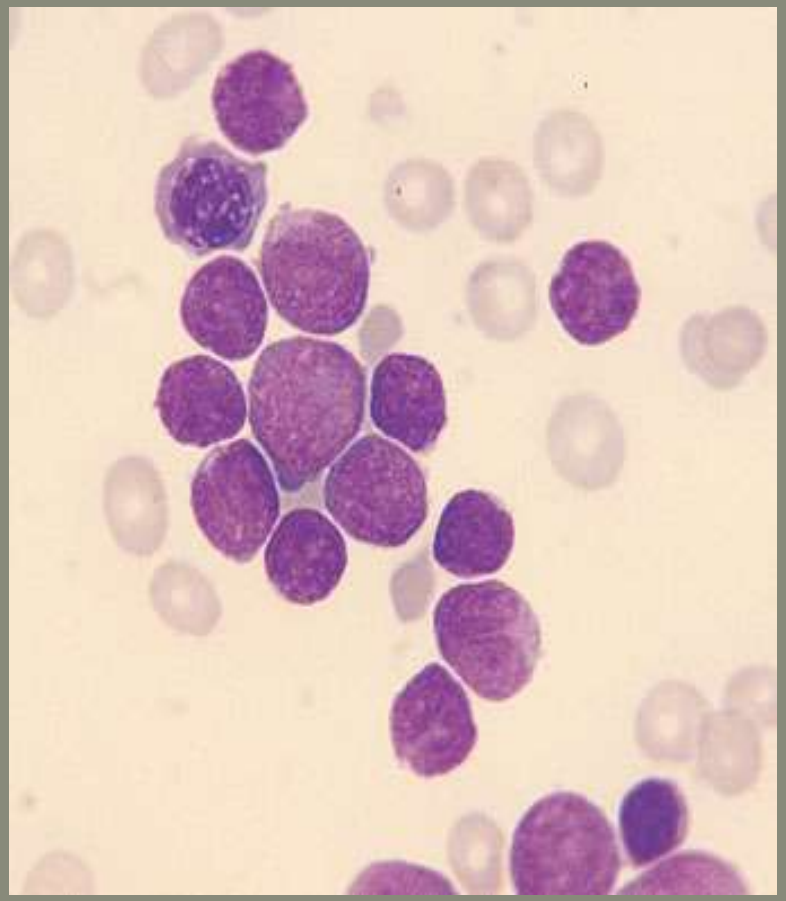
Morphologic subtypes of acute lymphoblastic leukemias (FAB classification)

Subtype	Morphology	Occurrence (%)
L1	Small round blasts, clumped chromatin	75
L2	Pleomorphic larger blasts, clefted nuclei, fine chromatin	20
L3	Large blasts, nucleoli, vacuolated cytoplasm	5

Acute Lymphoblastic Leukemia

L1 - most common found in children

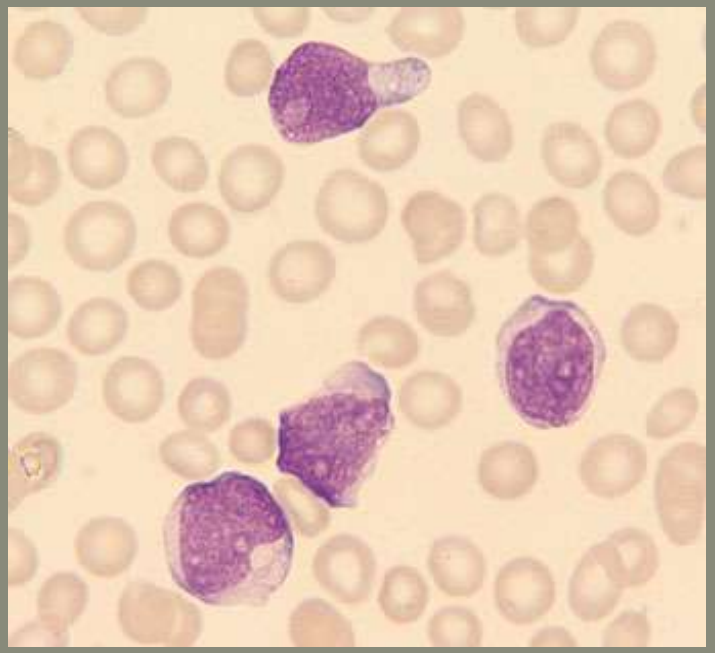
- Best prognosis.
- The cell size is small with fine or clumped homogenous nuclear chromatin and absent or indistinct nucleoli.
- The nuclear shape is regular, occasionally clefting or indented.
- The cytoplasm is scant, with slight to moderate basophilia and variable vacuoles.



Acute Lymphoblastic Leukemia

L2 – most frequent ALL found in adults.

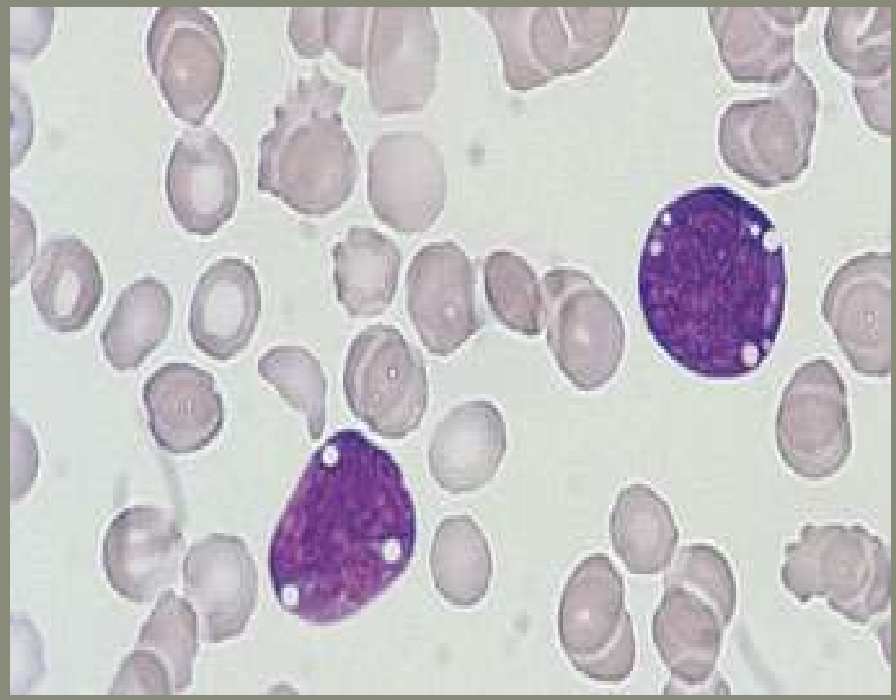
- The cell size is large and heterogenous with variable nuclear chromatin and prominent nucleoli.
- The nucleus is irregular, clefting and indented.
- The cytoplasm is variable and often moderate to abundant with variable basophilia and variable vacuoles.



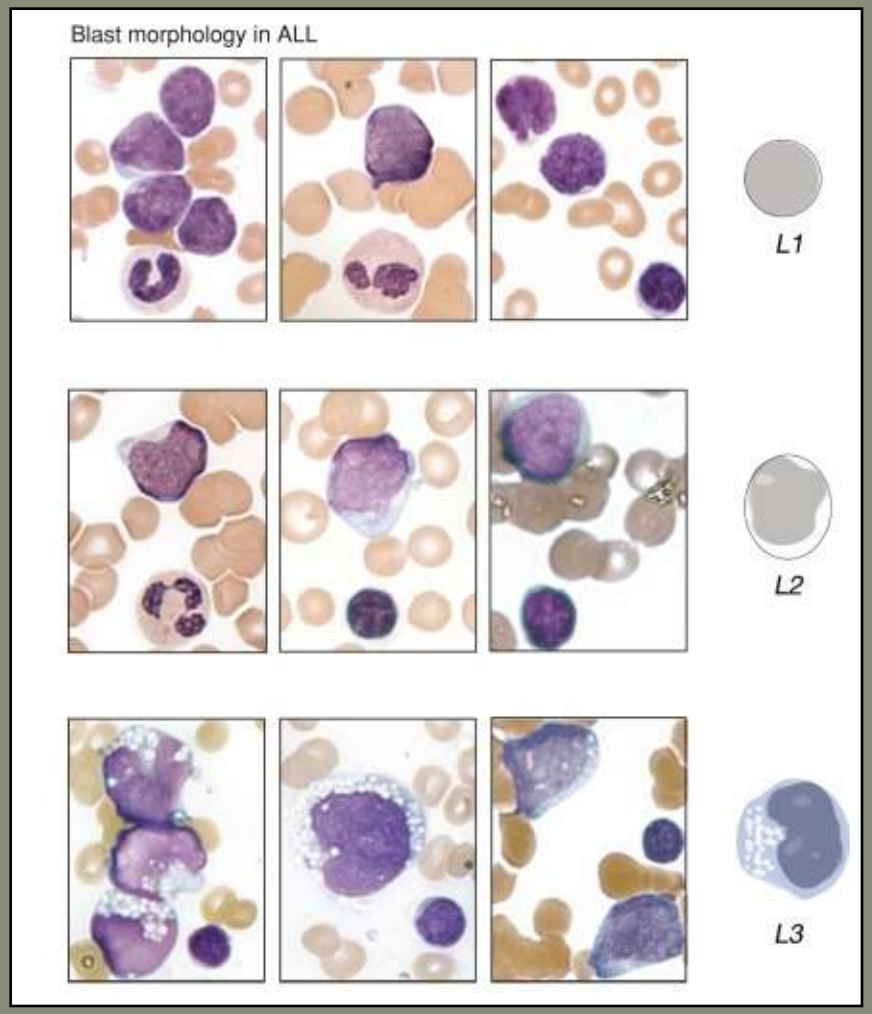
Acute Lymphoblastic Leukemia

L3 – This is the rarest form of ALL

- The cell size is large, with fine, homogenous nuclear chromatin containing prominent nucleoli.
- The nucleus is regular oval to round.
- The cytoplasm is moderately abundant and is deeply basophilic and vacuolated.



Acute Lymphoblastic Leukemia



Acute leukemias with mixed lineage

Occasionally there are acute leukemias that are biphenotypic and display phenotypes for two different lineages

- B lymphoid/myeloid
- T lymphoid/myeloid
- B/T lymphoid
- Myeloid/Natural killer
- A rare trilineage leukemia has also been seen (was B/T lymphoid/myeloid)

CYTOGENETICS

Cytogenetics and molecular techniques used in study of acute leukemia

- SOUTHERN BLOT
- PCR & RT-PCR
- FISH
- CGH (Comparative genomic hybridisation)
- IN-SITU HYBRIDISATION

Examples of translocations

A.INVOLVING TRANSCRIPTION REGULATION

- Retinoic acid receptor translocation: AML M3
- Core binding factor translocation: AML M2,M4
- 11q23 translocation :Mixed lineage leukemia
- E2A translocation : childhood B-ALL

B.INVOLVING TYROSINE KINASES

- BCR-ABL fusion (resistance to apoptosis)
- FLT3 mutations (loss of autoinhibition & constitutive tyrosine kinase activity)

CYTO-CHEMISTRY

CYTO-CHEMISTRY

Use of special stains to differentiate the types of blasts as:

- Myeloid
- Lymphoid
- Monocytoid

Commonly employed stains in leukemia diagnosis

Stain	Comments
Sudan black B	**Myelomonocytic cells**
Myeoperoxidase	**Myelomonocytic cells**
Chloroacetate esterase	**Granulocyte blasts**
Alpha naphthylbutyrate esterase	**Monocytic cells**
Periodic acid- Schiff	**Lymphoblast, erythroblasts - block positivity** **Myeloblast-speckle pattern**

Diagnostic markers of AML

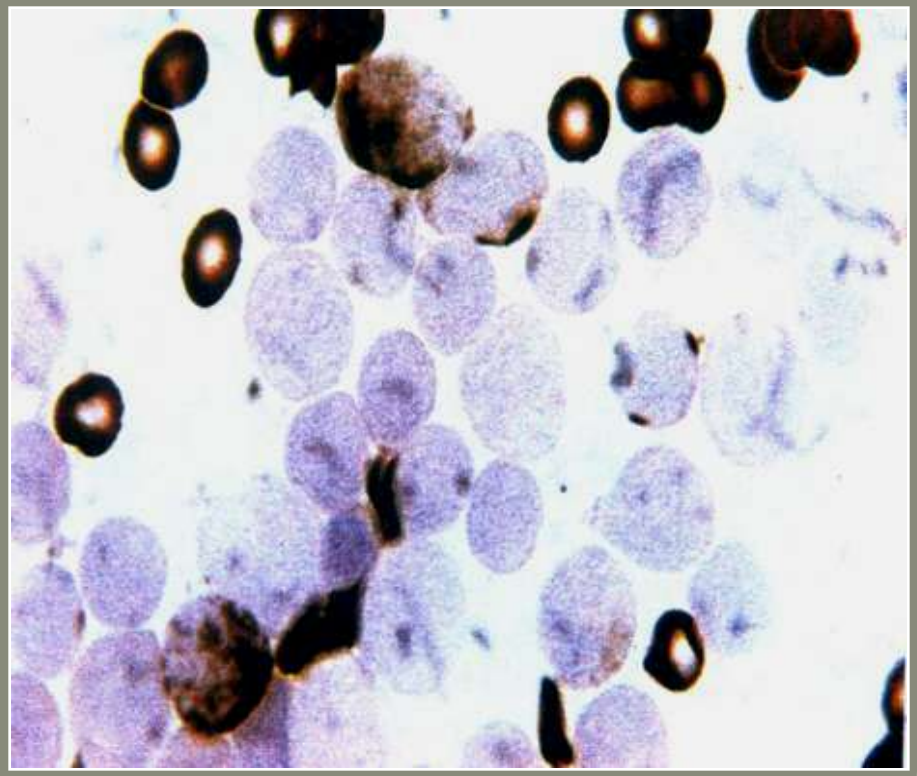
FAB classification	Cytochemistry		
	MPO	PAS	Esterase
Mo undifferentiate	-	-	-
M1 (myeloid)	+	-	-
M2 (myeloid with differentiation)	+	-	-
M3 (APL)	++	-	-
M4 (AMMoL)	+	-	+
M5 (AMoL)	-	+ block	++
M6 (erythroid)	-	++	-
M7 (megakaryocytic)	-	±	-

Acute lymphoblastic leukemias - reactivity with special stains

Subtype	Peroxidase or Sudan black	Non-specific esterase	Periodic acid-Schiff
L1	-	-	+++
L2	-	-	+++
L3	-	-	+++

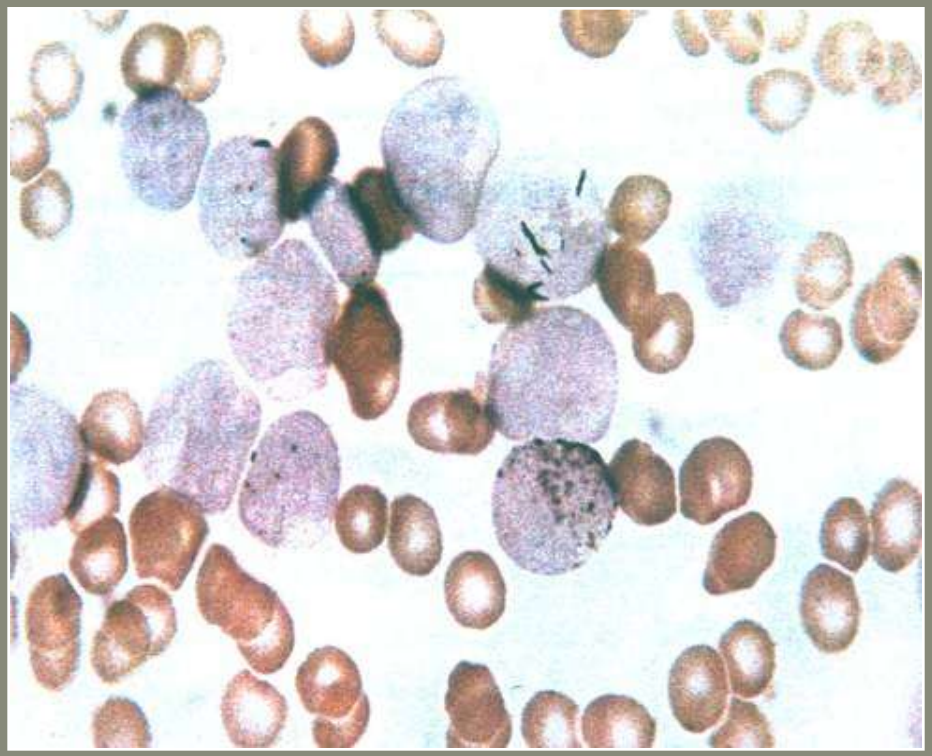
MYELOPEROXIDASE

- SPECIFICITY:
- Stains-
  - Primary & secondary granules of neutrophils, eosinophils & monocytes(weak)
  - Auer rods
- Doesn't stain-
  - Lymphoid & erythroid precursors.



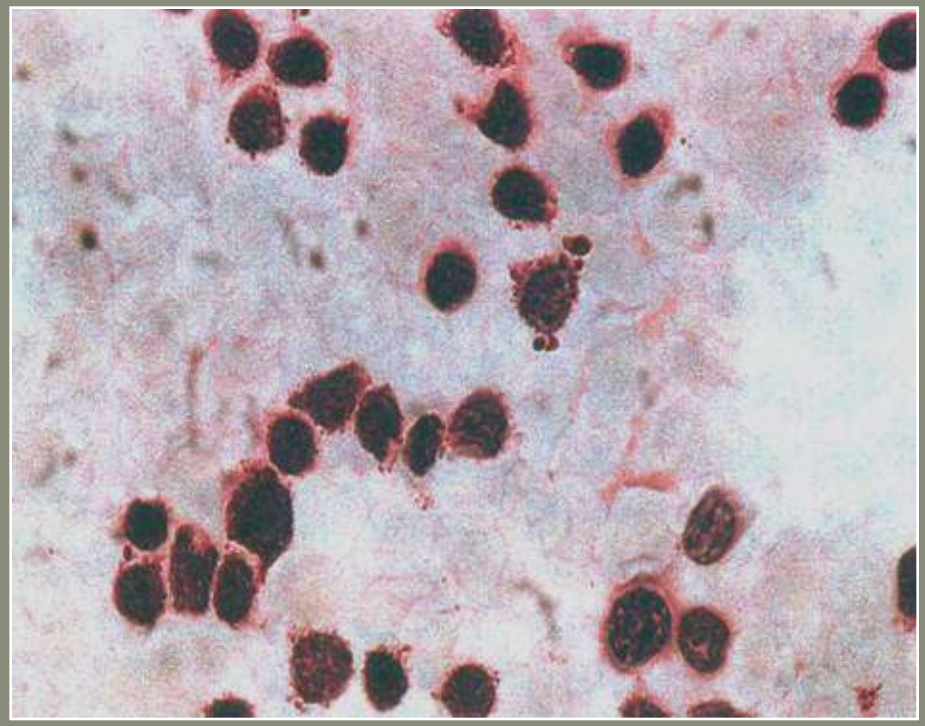
SUDAN BLACK-B

- SPECIFICITY:
- Stains-
  - Primary & secondary granules of neutrophils, eosinophils, monocytes.
  - Auer rods
- Doesn`t stain-
  - Lymphoid & erythroid precursors



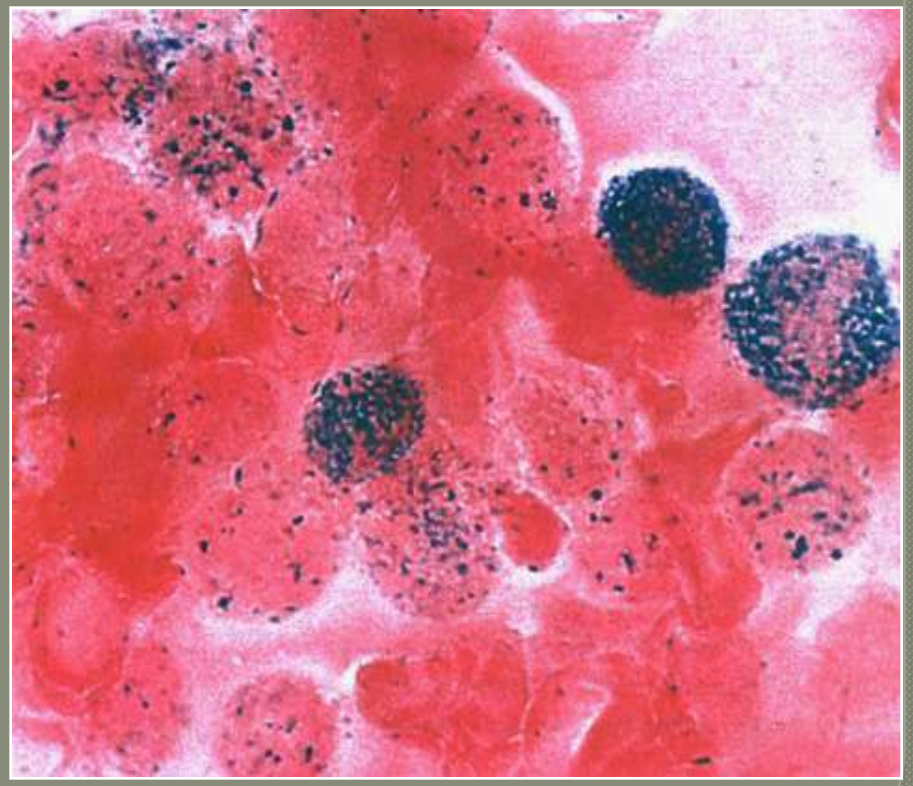
PAS

- SPECIFICITY: Primarily reacts with glycogen
- Stains-
- neutrophils granules(PAS positivity increases with maturation)
- eosinophil cytoplasm
- monocytes
- some T & B lymphocytes & many leukaemic blasts



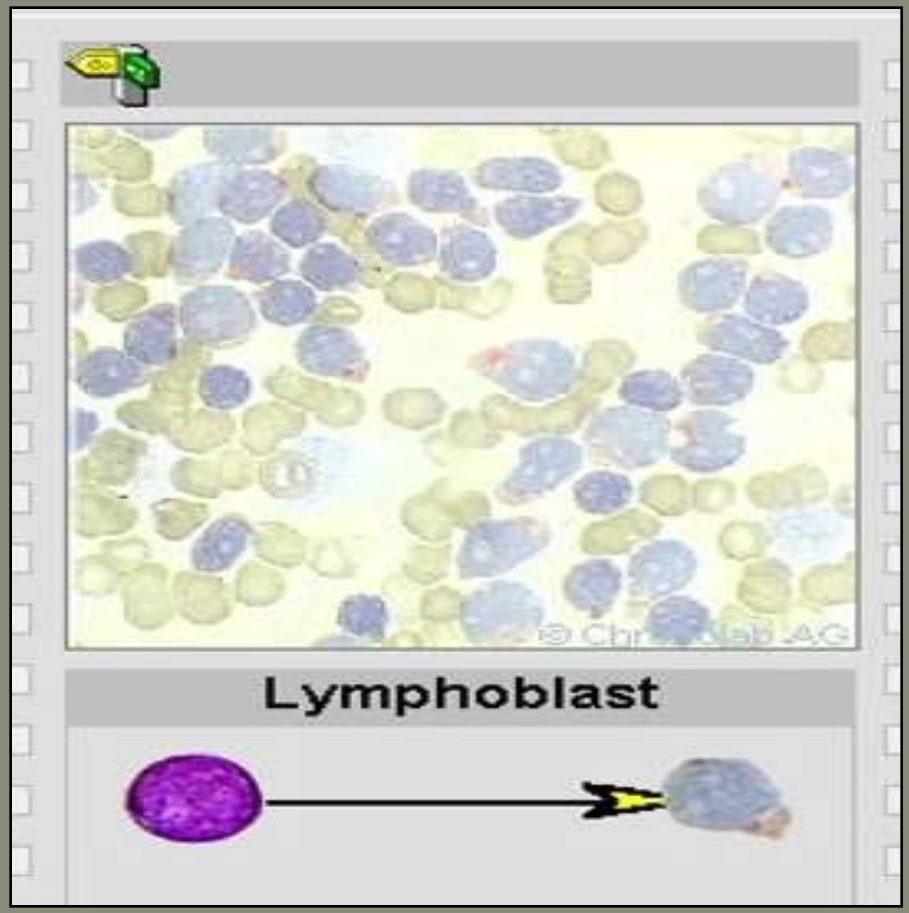
NSE (non-specific esterase)

- SPECIFICITY: an enzyme whose activity is found in monocytes.
- Two types of substrates are commonly used—
  - alpha-naphthyl-butyrate
  - alpha-naphthyl-acetate
- The former is specific for monocytes whereas the latter also reacts with NSE of megakaryocytes (NaF inhibits NSE of monocytes only).



Acid phosphatase

- Acid phosphatase may be found in myeloblasts and lymphoblasts.
- T lymphocytes have a high level of acid phosphatase and this can be used to help make a diagnosis of acute T-lymphocytic leukemia



Leukocyte Alkaline phosphatase

- Leukocyte alkaline phosphatase – is located in the tertiary granules of segmented neutrophils, bands and metamyelocytes.
- The LAP score is determined by counting 100 mature neutrophils Each cell is graded from 0 to 4
- The total LAP score is calculated by adding up the scores for each cell.
- Normal SCORE - 0-400/100 cells.

LAP SCORE

0	Negative, no granules
1	Occ. granules scattered in cytoplasm
2	Moderate no. of granules
3	Numerous granules
4	Heavy positivity with numerous coarse granules crowding cytoplasm, freq.overlying the nucleus

IMMUNO-PHENOTYPING (IMMUNOLOGIC MARKERS)

IMMUNOPHENOTYPING

1. Identify antigens present on the blast cells
2. Differentiate T-ALL and B-ALL
3. Determine whether the leukemia is lymphoid or myeloid (especially important when cyto-chemical markers are negative or equivocal. E.g : AML-MO)
4. Certain antigens have prognostic significance
5. Rare cases of biphenotypic where both myeloid and lymphoid antigens are expressed.
6. Able to identify the subtype of leukemia. E.g.: AML-M7 has a specific surface marker of CD 61 etc.

References

- Wintrobes clinical hematology
- Pathologic basis of disease- Robbins and Cotran
- Practical haematology- Dacie and Lewis
- Harrisons 17th edition
- Internet search