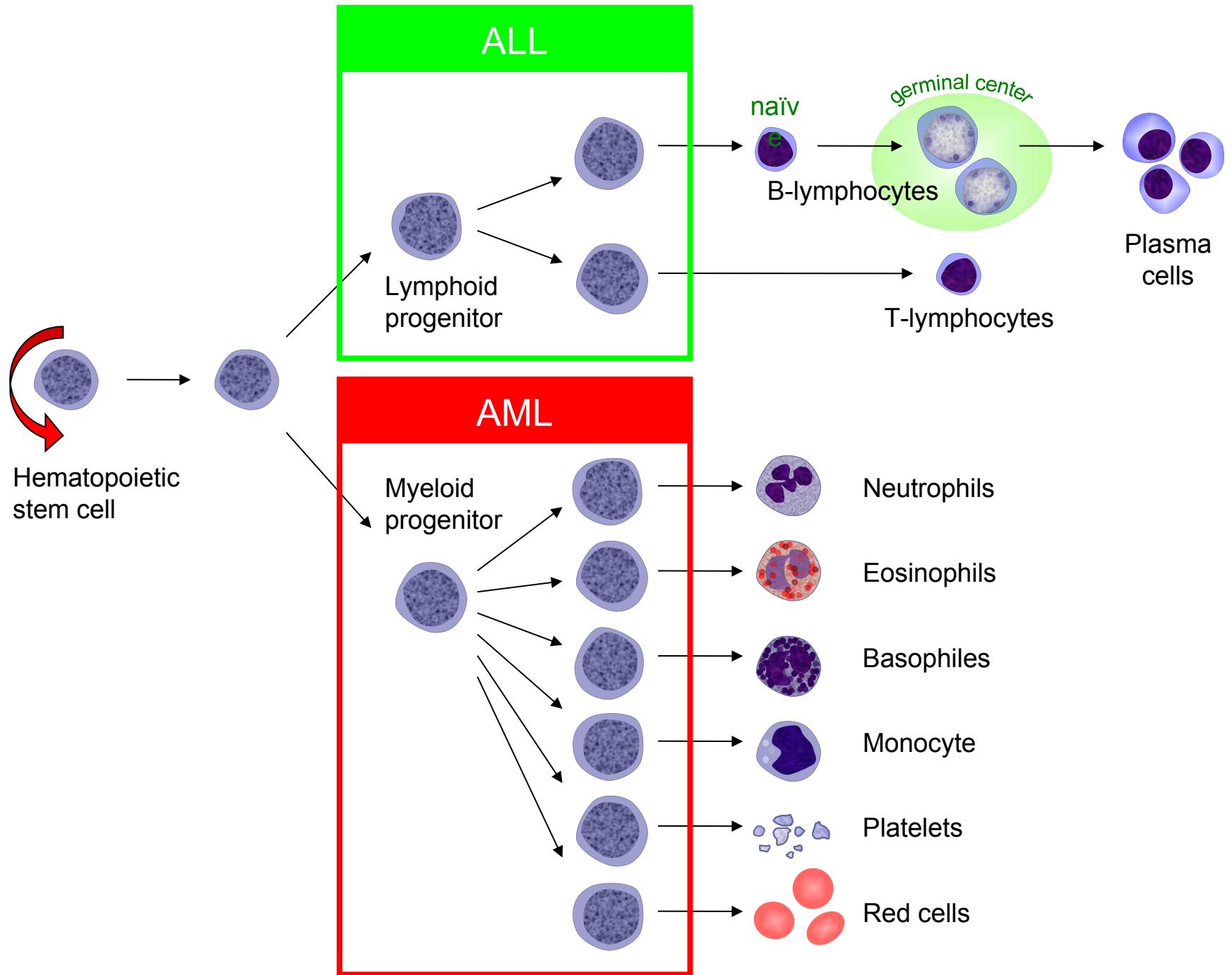


Leukaemia – Laboratory diagnosis

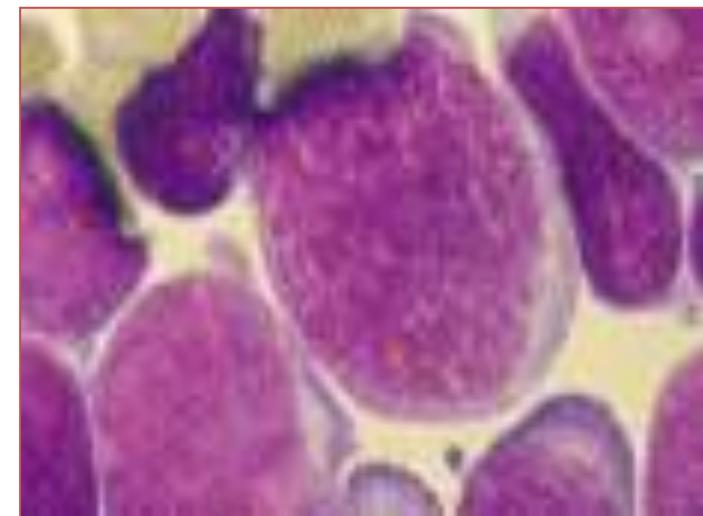
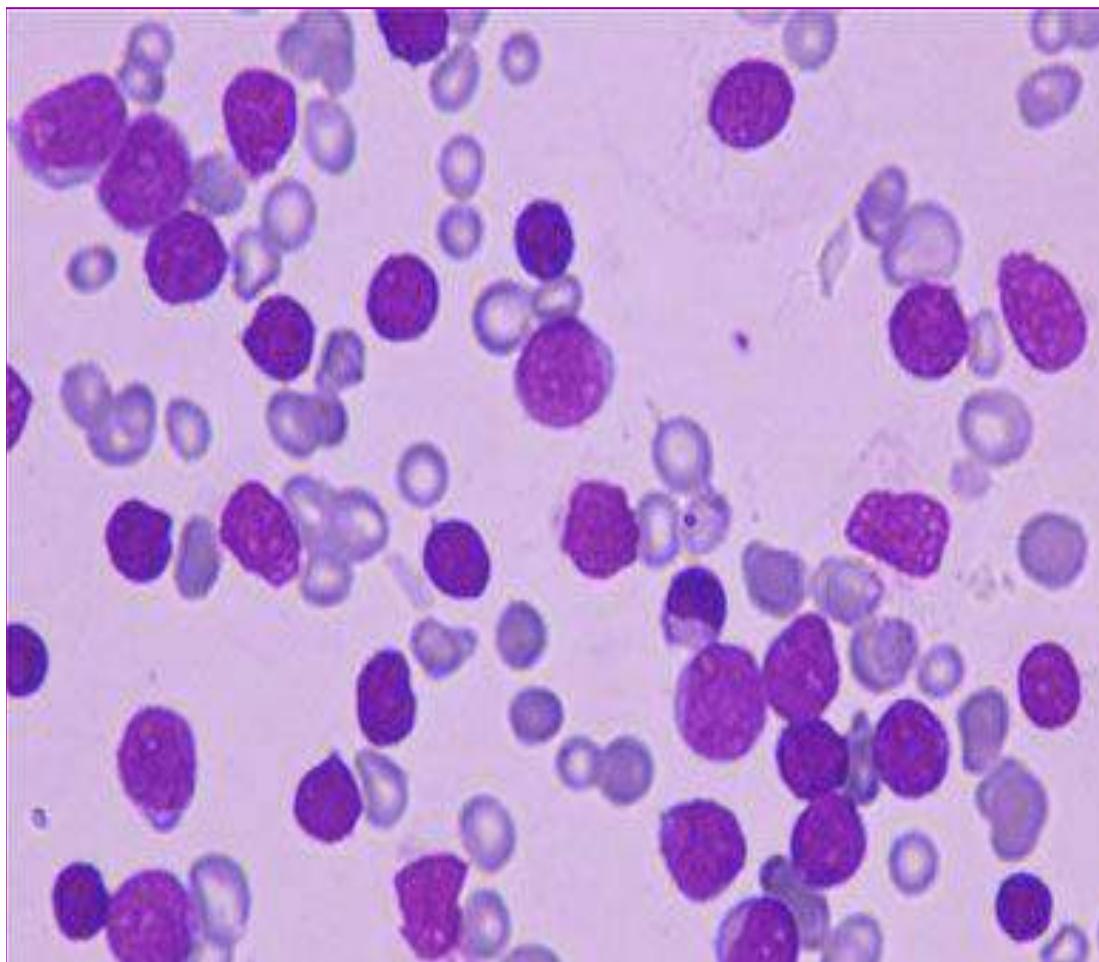
Dr. D.Aruna chaithanya,
I yr PG(Pathology),KIMS.

Leukemia

*“ Stem cell disorder characterized
by a malignant neoplastic proliferation and
accumulation of immature hematopoietic cells
in the bone marrow”*



When to suspect leukaemia



Reactive vs. leukaemic

- Normocytic normochromic blood picture
- Mature cells or reactive cells
- Platelets- normal
- Symptoms of underlying infection or disease
- Abnormality in counts comes to normal as the underlying condition is treated
- Normocytic normochromic anemia
- Immature precursor cells
- Platelet counts – increased/ decreased
- Symptoms due to leukaemic process
- Abnormality in Counts worsen with time

CRITERIA FOR CLASSIFICATION

- MORPHOLOGICAL**

- CYTOCHEMICAL**

- IMMUNOPHENOTYPING**

- CYTOGENETIC**

- MOLECULAR GENETICS**

CLASSIFICATION OF LEUKEMIAS (FAB)

ACUTE
LEUKEMIA

CHRONIC
LEUKEMIA

MYELOID

LYMPHOID

MYELOBLASTIC

LYMPHOBLASTIC

M0

L 1

M1

L 2

M2

L 3

PROMYELOCYTIC

M3

MYELOMONOCYTIC

M4

MONOCYTIC

M5

ERYTHROCYTIC

M6

MEGAKARYOCYTIC

M7

MYELOCYTIC (CML)

LYMPHOCYTIC

MYELOMONOCYTIC

PLASMACYTIC

(CMML)

HAIRY CELL
PROLYMPHOCYTIC
LARGE GRANULAR

BLAST COUNT &
AGGRESSIVENESS

MICM

Lab investigations

1. Blood count and blood film
2. Bone marrow aspirate
3. Bone marrow trephine biopsy
4. Cytochemistry
5. Flow cytometric immunophenotyping
6. Immunohistochemistry
7. Cytogenetic analysis
8. Fluorescence *in situ* hybridization
9. Molecular genetic analysis

CYTOCHEMICAL ANALYSIS

- MYELOPEROXIDASE
 - SUDAN BLACK
 - ESTERASES :
 - SPECIFIC
 - NON - SPECIFIC
 - PERIODIC ACID-SCHIFF (PAS)
 - LEUKOCYTE ALKALINE PHOSPHATASE
 - ACID PHOSPHATESE
 - TOLUDINE BLUE
 - TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE (TdT)
 - RETICULIN STAIN
 - METHYL GREEN - PYRONINE
-

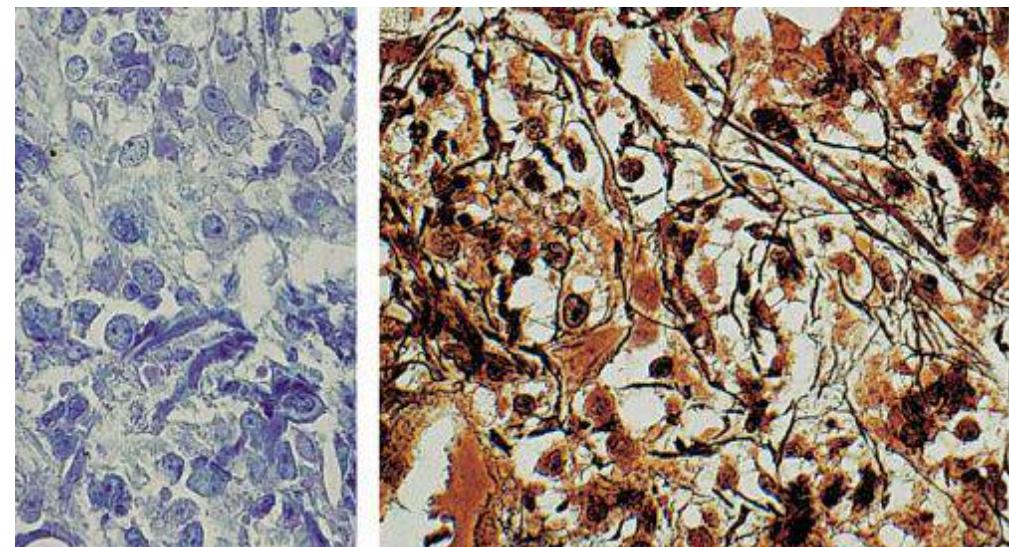
CYTOCHEMISTRY IN ACUTE LEUKEMIA

STAINS	AML	ALL
MYELOPEROXIDASE	+	--
SUDAN BLACK	+	--
PAS	+ (fine)	+ (course)
NON- SPECIFIC	+ (M4 , M5)	--
ESTERASE		
ACID PHOSPHATASE	--	-- (+ve T-ALL)
TdT	--	+

Trephine biopsy

- In cases of dry tap
- Equivocal data/insufficient cell yield in aspirate
- AML M7

blast cells (left) and increased reticulin deposition (right).



FLOW CYTOMETRY – Immunophenotyping

- Done on a peripheral blood sample / bone marrow aspirate / on a cell suspension from any infiltrated tissue.
- *Indications:*
 1. Lineage determination-myeloid/lymphoid
B cell / T cell.
 2. Aberrant expression of antigens – role in prognosis and evaluation for MRD—LAIP detects MRD.

3. Confirmation of a diagnosis of FAB M0 and acute undifferentiated leukaemias;
M7 AML(CD 61/41)
4. Identification of mixed - lineage acute leukaemia & biphenotypic leukaemias.
- 5.Taregetted therapy – CD20- rituximab
- 6.ALL (TdT+)over NHL spillover
- 7.CSF and FNAC samples for leukaemic cells.

Precursor-B ALL	CD19, CD10, CD79a, TdT, cCD22*, HLA-DR, cCD79a*
Precursor-T ALL	CD1, CD2, CD3, CD4, CD5, CD7 CD8, TdT, cCD3*
AML	CD33, CD13, CD117, CD4+CD2-, HLA-DR, cMPO*
With monoblastic differentiation	CD11b, CD16, CD14, CD64
True erythroleukemia	Glycophorin A
Acute megakaryocytic leukemia	CD41, CD61, cCD41*, cCD61*
Lineage-independent antigens	HLA-DR, CD45, CD34. CD10

- **CYTOGENETIC**- WHO classifications for acute leukemias , prognostic value in some cases
- **MOLECULAR GENETICS:**

SOUTHERN BLOT TECHNIQUE

PCR

RTPCR

Myeloid

ACUTE

CHRONIC

ACUTE MYELOBLASTIC LEUKEMIA

FAB CLASSIFICATION : AML

- M0 ACUTE MYELOBLASTIC LEUKEMIA WITH minimal differentiation
 - M1 ACUTE MYELOBLASTIC LEUKEMIA without maturation
 - M2 ACUTE MYELOBLASTIC LEUKEMIA with maturation
 - M3 PROMYELOCYTIC LEUKEMIA ,
M3v,
hypergranular
hypogranular
 - M4 ACUTE MYELOMONOCYTIC LEUKEMIA
M4eo with eosinophilia
 - M5a ACUTE MONOBLASTIC LEUKEMIA without differentiation
M5b with differentiation
 - M6 ACUTE ERYTHROLEUKEMIA
 - M7 ACUTE MEGAKARYOCYTIC LEUKEMIA
-

LABORATORY FINDINGS



PERIPHERAL BLOOD

NORMOCYTIC NORMOCHROMIC ANEMIA

NUCLEATED ERYTHROCYTES

ANISOPOIKILOCYTOSIS (VARIABLE)

THROMBOCYTOPENIA

LEUKOCYTOSIS (may be normal / decreased)

BLASTS (> 20%)

MONOCYTOSIS

NEUTROPENIA

EOSINOPHILIA & BASOPHILIA (VARIABLE)

● ***HEMOSTATIC FUNCTION TESTS :***

➤ ***Coagulation studies***

Clotting time

PT, APTT, Fibrinogen levels

★ *FDP estimation*

➤ ***Platelet studies***

Bleeding time

Platelet count

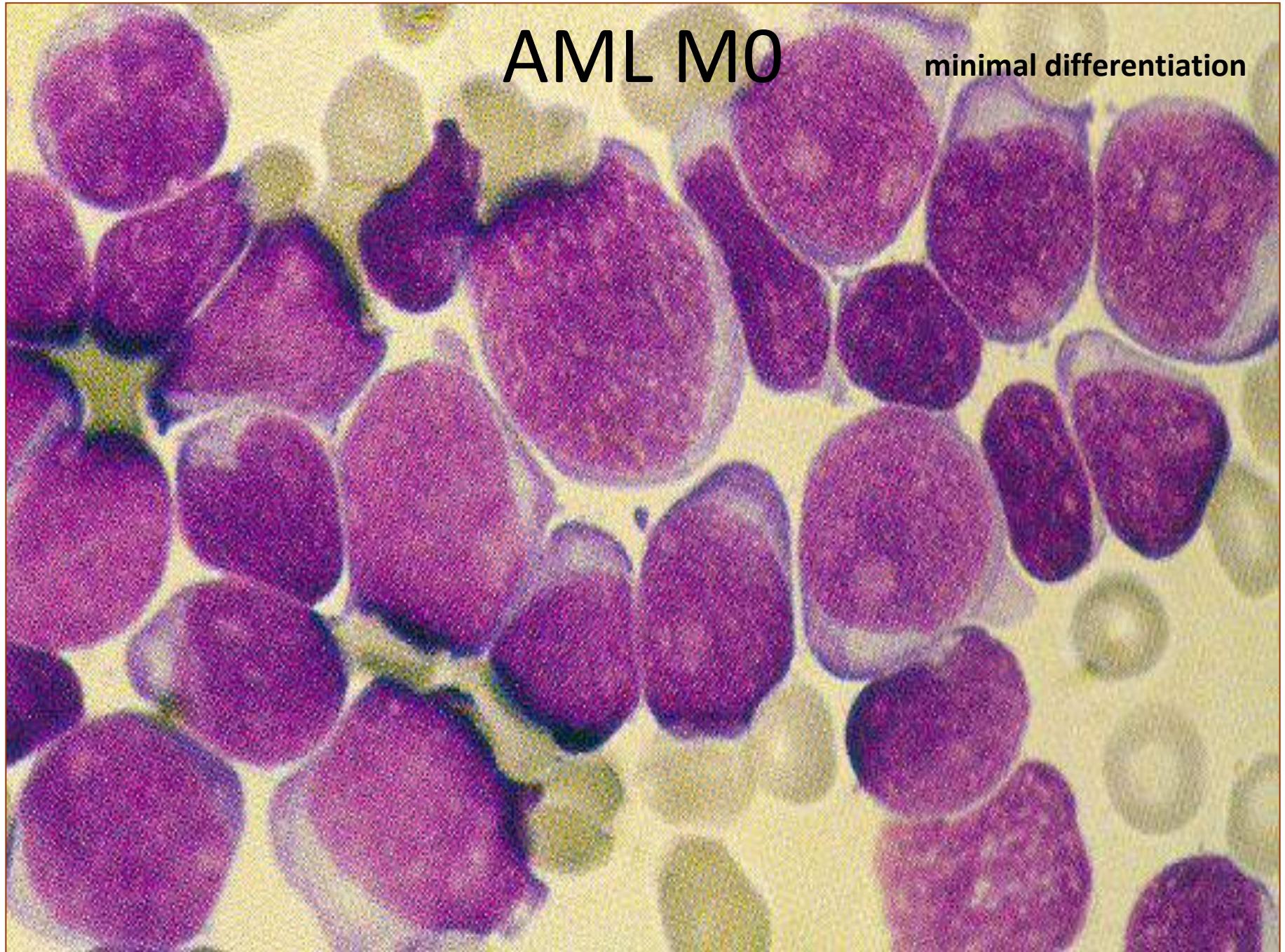
● ***PLASMA LDH***

● ***BONE MARROW***

HYPERCELLULAR ; > 30% BLASTS

CLINICAL FINDINGS

- SYMPTOMS RELATED TO
 - ANEMIA
 - NEUTROPENIA (recurrent U.Resp.T Infections)
 - THROMBOCYTOPENIA
 - BONE TENDERNESS and SOFT TISSUE NODULES
 - SPLENOmegaly (Mild to moderate)
 - HEPATOMEGALY (Mild to moderate)
 - LYMPHADENOPATHY (May or may not)
 - DIC (AML M3)
 - GINGIVAL HYPERPLASIA (AML M4)
 - DIFFUSE ERYTHEMATOUS SKIN RASH (AML M5)
-

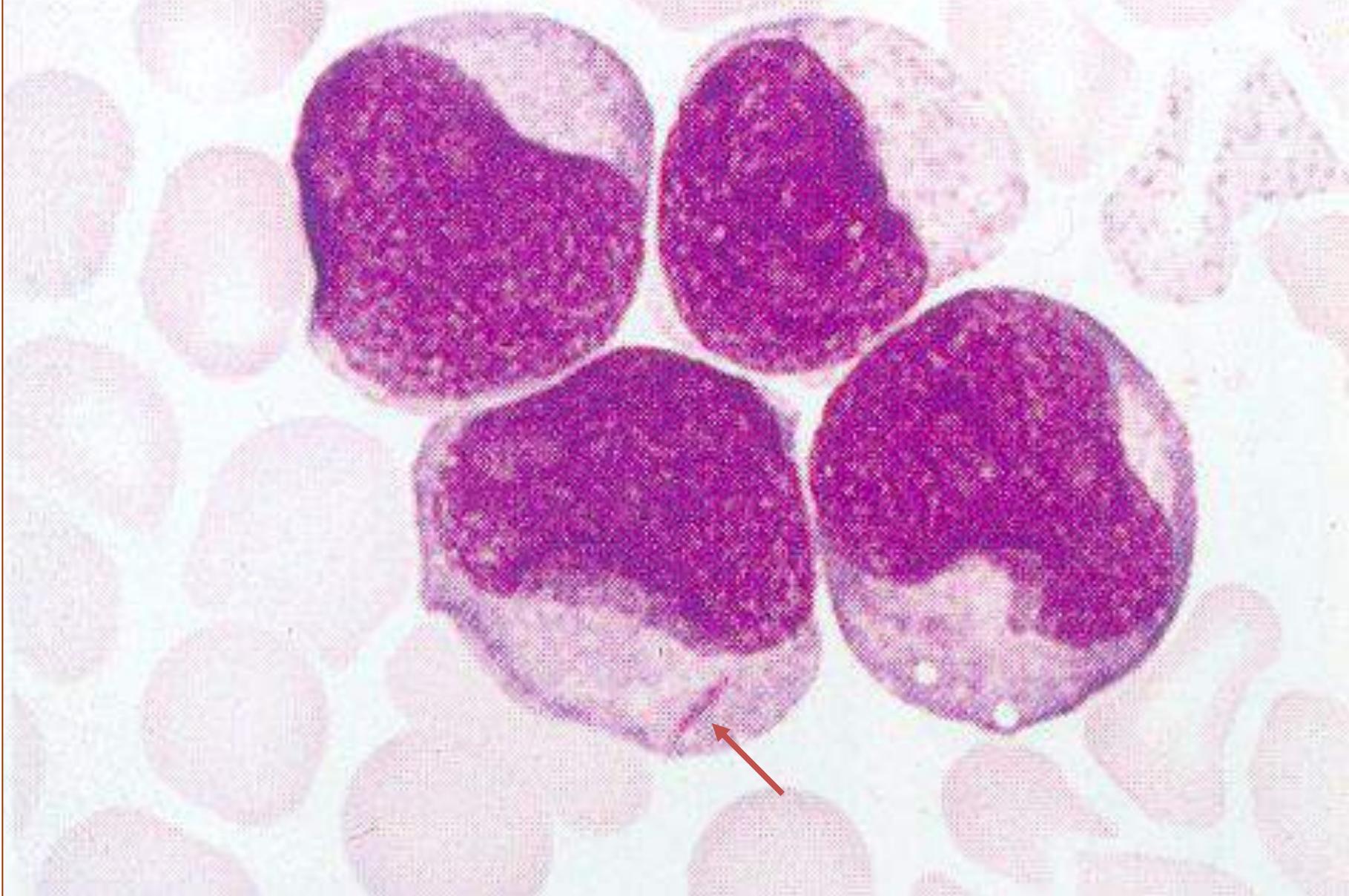


AML M0

minimal differentiation

AML M 1

without maturation



AML M2

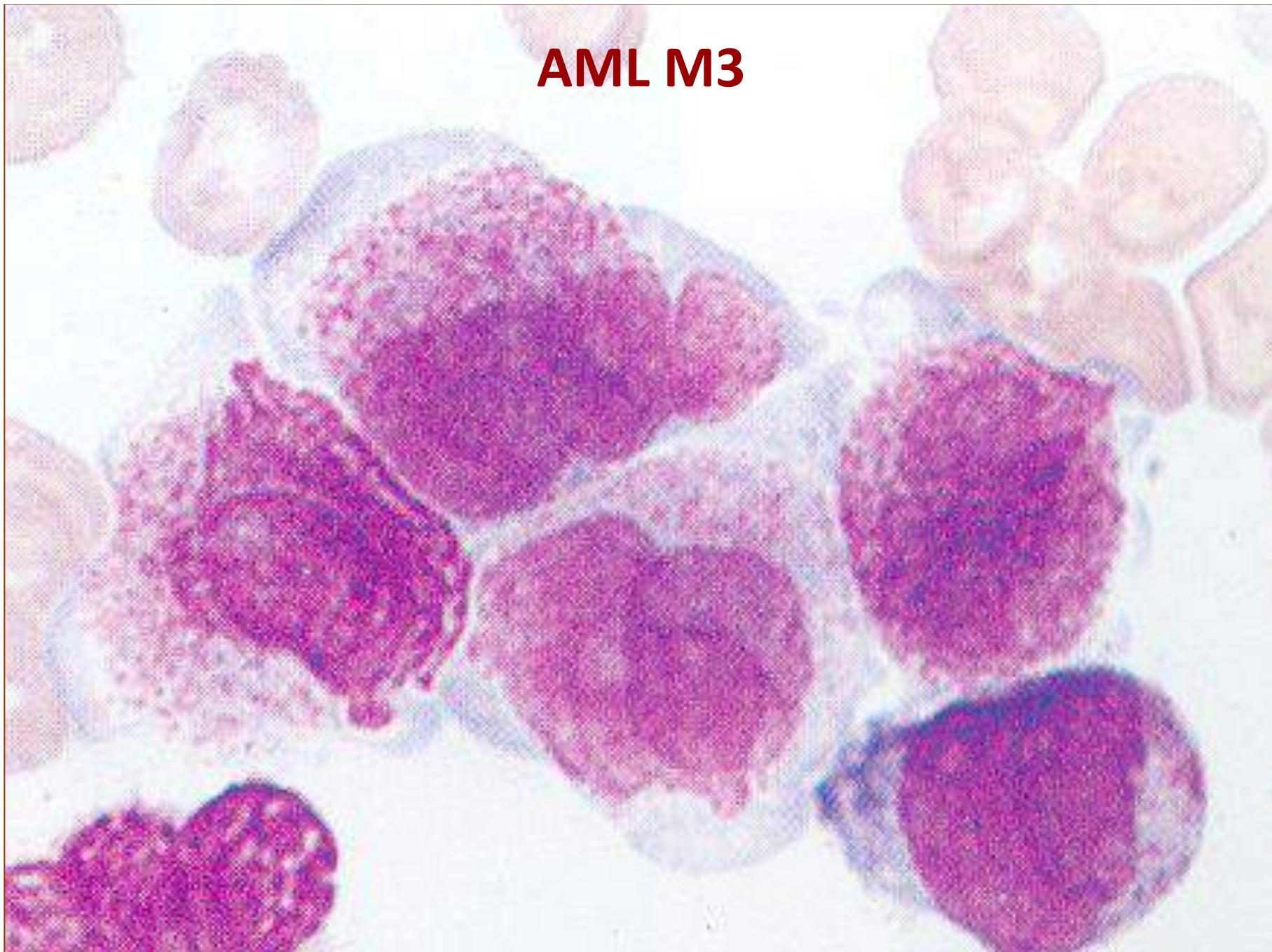
with maturation



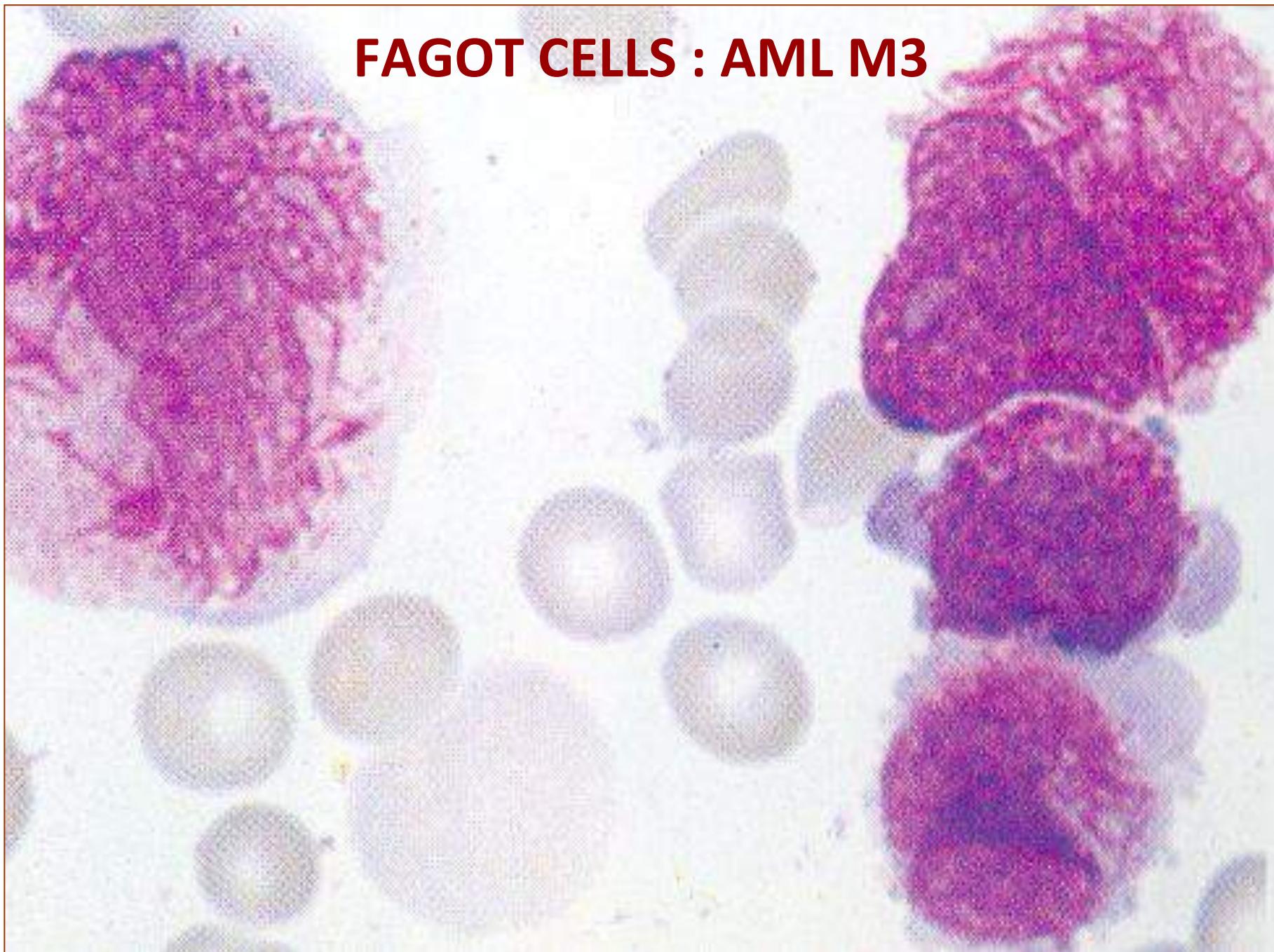
PROMYELOCYTIC LEUKEMIA

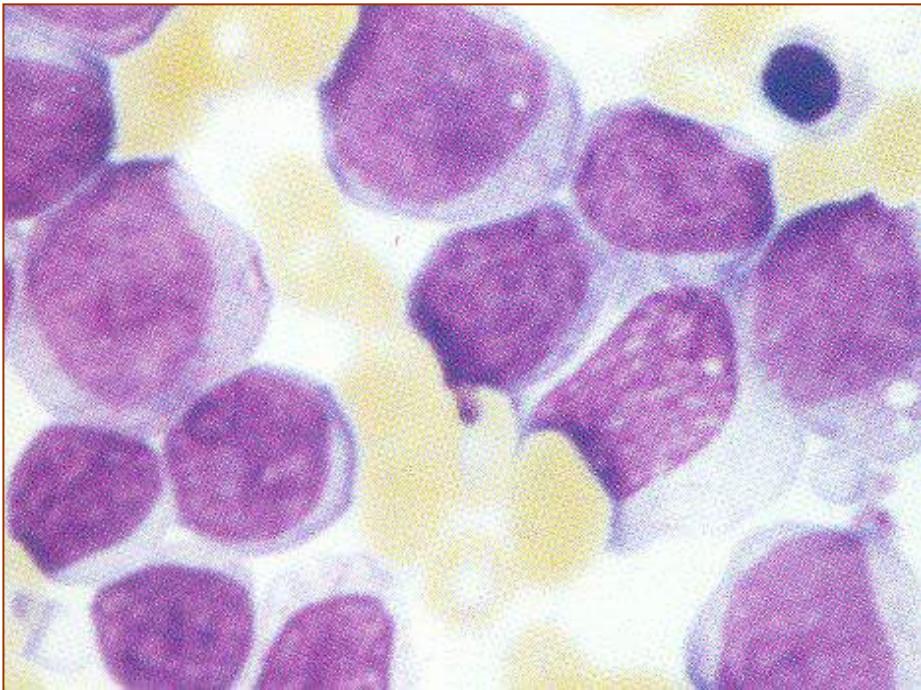
- ACCORDING TO FAB CLASSIFICATION PML - AS M3
 - TWO FORMS OF M3 (Hyper and hypogranular variants)
 - ACCOUNTS FOR 5 to 10%
 - OCCURS IN YOUNGER AGE GROUP (median age 39 yrs)
 - MORE VIRULENT
 - COMMON CLINICAL FINDING – BLEEDING
 - GRANULES CONTAIN PROCOAGULANT
 - MOST SERIOUS COMPLICATION IS DIC
 - POTENTIATES DIC DURING THERAPY
 - ADJUVANT HEPARIN THERAPY TO PREVENT DIC
-

AML M3



FAGOT CELLS : AML M3



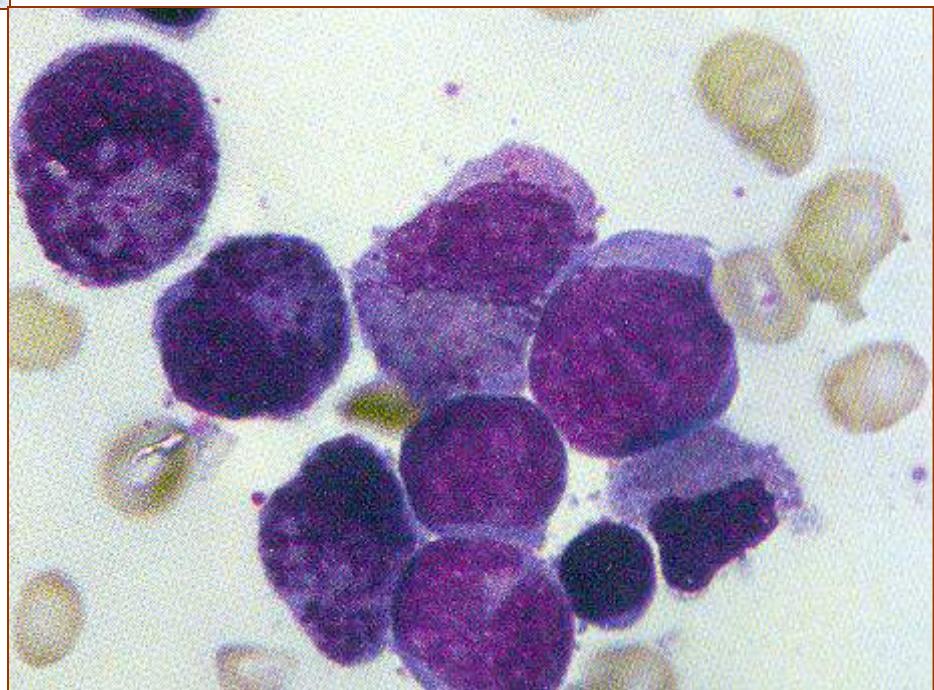


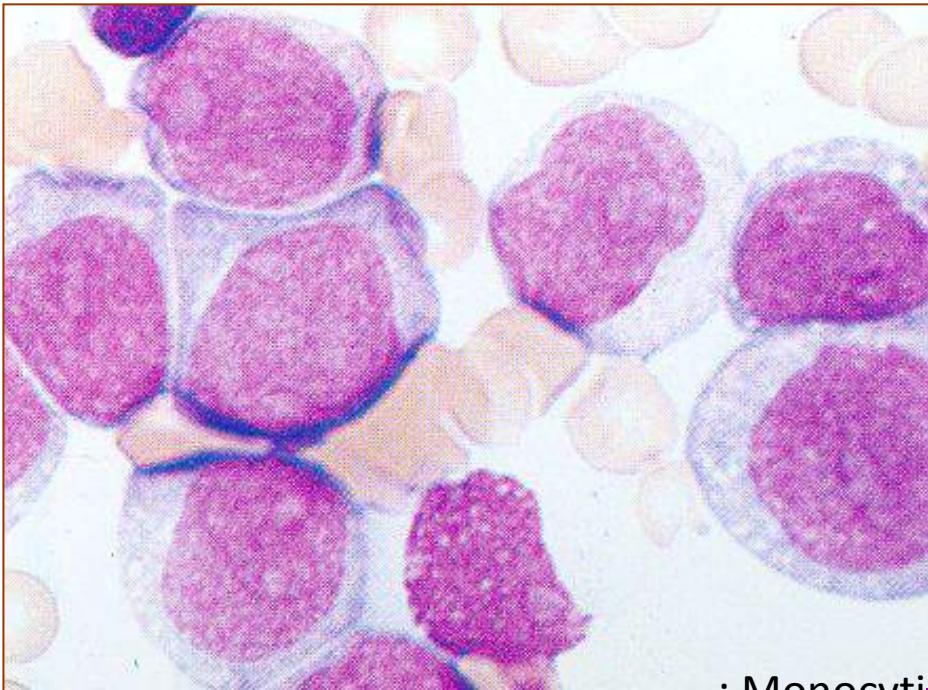
AML – M4Eo

Increase in abnormal marrow
eosinophils

AML M 4

Myelomonocytic leukemia





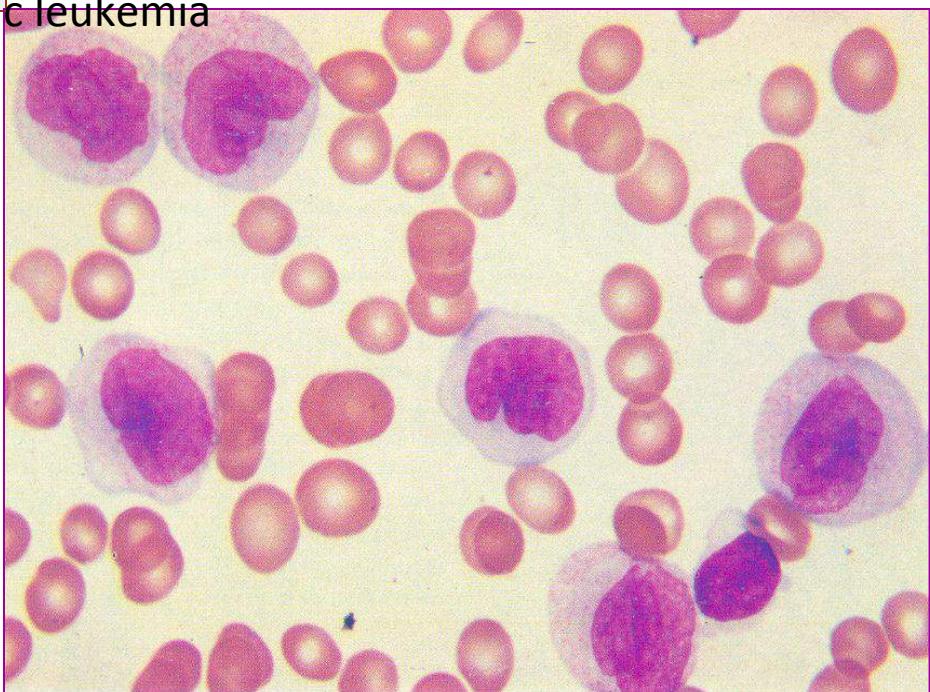
AML - M5a

>80% MONOBLASTS
<20% PROMONOCYTES AND MONOCYTES

: Monocytic leukemia

AML - M5b

<80% MONOBLASTS
>20% PROMONOCYTES AND BLASTS



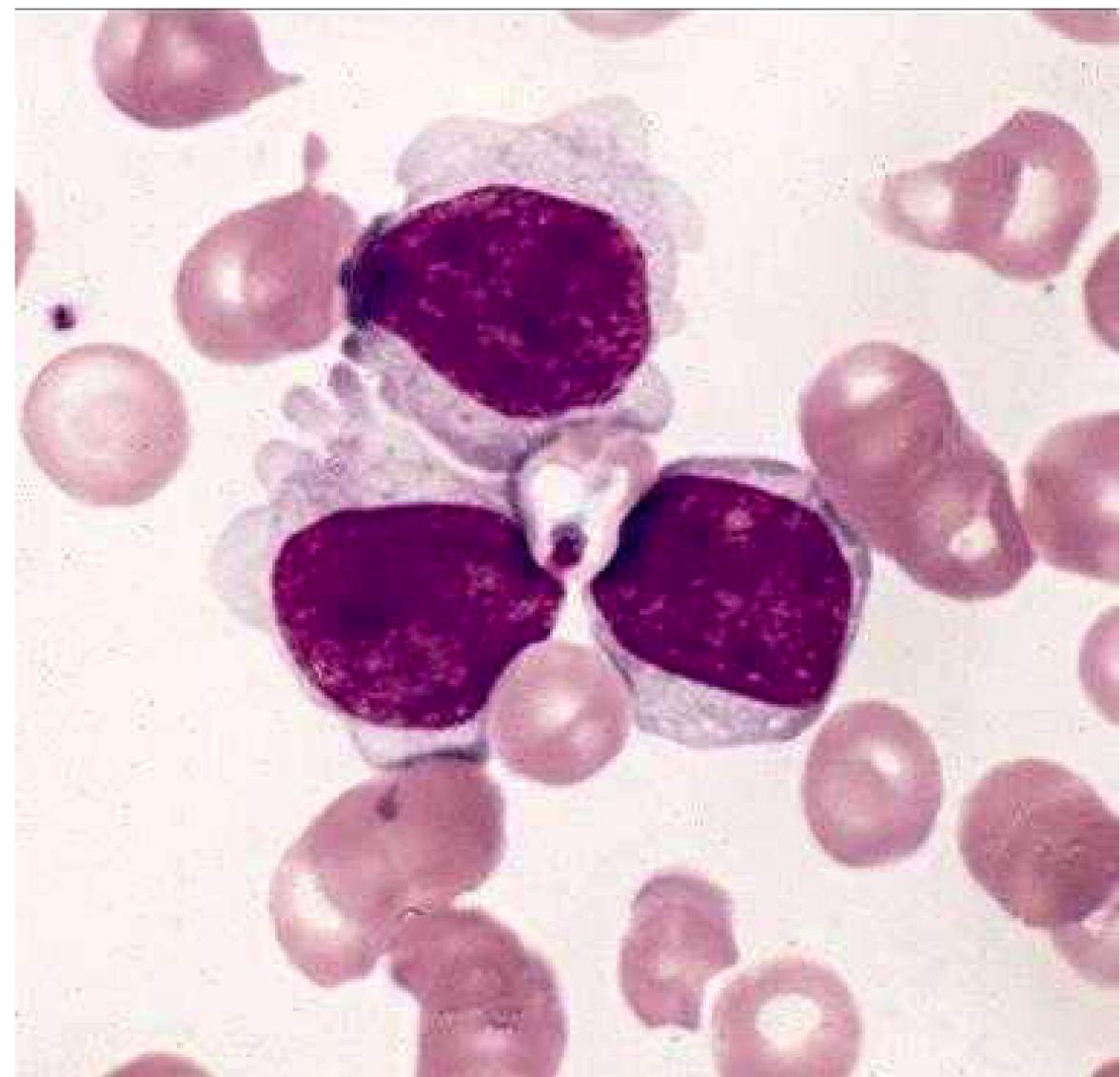
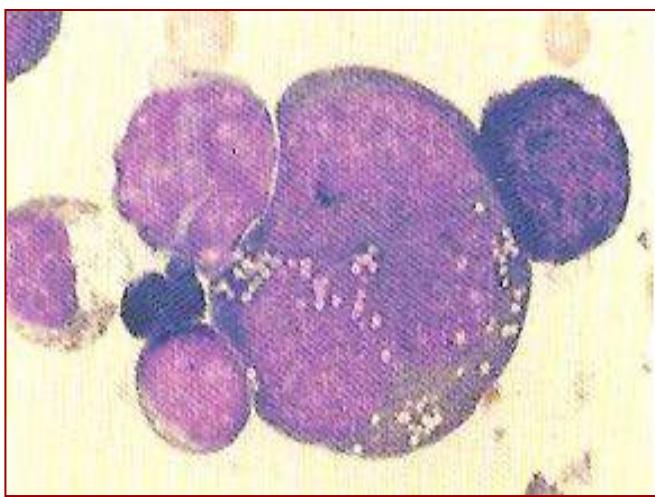
Erythroleukemia

AML – M6

Erythroid/myeloid subtype (M6a) defined by
>>50% dysplastic maturing erythroid precursors
and >20% myeloblasts; pure erythroid subtype
(M6b) defined by >80% erythroid precursors
without myeloblasts

AML-M7

: Megakaryoblastic leukemia



CYTOCHEMISTRY IN ACUTE LEUKEMIA

STAINS	AML	ALL
MYELOPEROXIDASE	+	--
SUDAN BLACK	+	--
PAS	+ (fine)	+ (course)
NON- SPECIFIC ESTERASE	+ (M4 , M5)	--
ACID PHOSPHATASE	--	-- (+ve T-ALL)
TdT	--	+

Immunophenotyping

Precursor-B ALL	CD19, CD10, CD79a, TdT, cCD22*, HLA-DR, cCD79a*
Precursor-T ALL	CD1, CD2, CD3, CD4, CD5, CD7 CD8, TdT, cCD3*
AML	CD33, CD13, CD117, CD4+CD2-, HLA-DR, cMPO*
With monoblastic differentiation	CD11b, CD16, CD14, CD64
True erythroleukemia	Glycophorin A
Acute megakaryocytic leukemia	CD41, CD61, cCD41*, cCD61*
Lineage-independent antigens	HLA-DR, CD45, CD34. CD10

CYTOGENETICS

- M 1 monosomy 5, Del (5q) , t (6;9) , t(9;22)
- M 2 t(6;9) , t(8;21) , monosomy 7
- M 3 t(15;17)
- M 4 t(6;9) ,t(8;21) , t(9;11) ,del(11) , monosomy 7
- M 4eo del(16q) ,inv(16)
- M 5 del(16q) ,inv(16) ,monosomy 7 ,del(11) ,t(9;11)
- M 6 abnormalities ch 5 & 7
- M 7 t(21;8)
-

The WHO classification of acute myeloid leukaemia (AML)-2008

1. AML with recurrent genetic abnormalities

AML with t(8;21)(q22;q22); *RUNX1 – RUNX1T1*

AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB – MYH11*

AML with t(15;17)(q22;q12); *PML – RARA*

AML with 11q23(MLL) abnormalities

2. AML with multilineage dysplasia

MDS related

denovo

3. AML and MDS therapy related- +

alkylating agent related

topoisomerase II inhibitor related ,

others

4. AML,NOS

with minimal differentiation

without maturation

with maturation

myelomonocytic

monoblastic and monocytic

erythroid

megakaryoblastic

basophilic

panmyelosis with myelofibrosis

myeloid sarcoma

CHRONIC MYELOID LEUKEMIA

DISEASE PROGRESSION

- CHRONIC PHASE
 - ACCALERATED PHASE
 - BLAST CRISIS
-

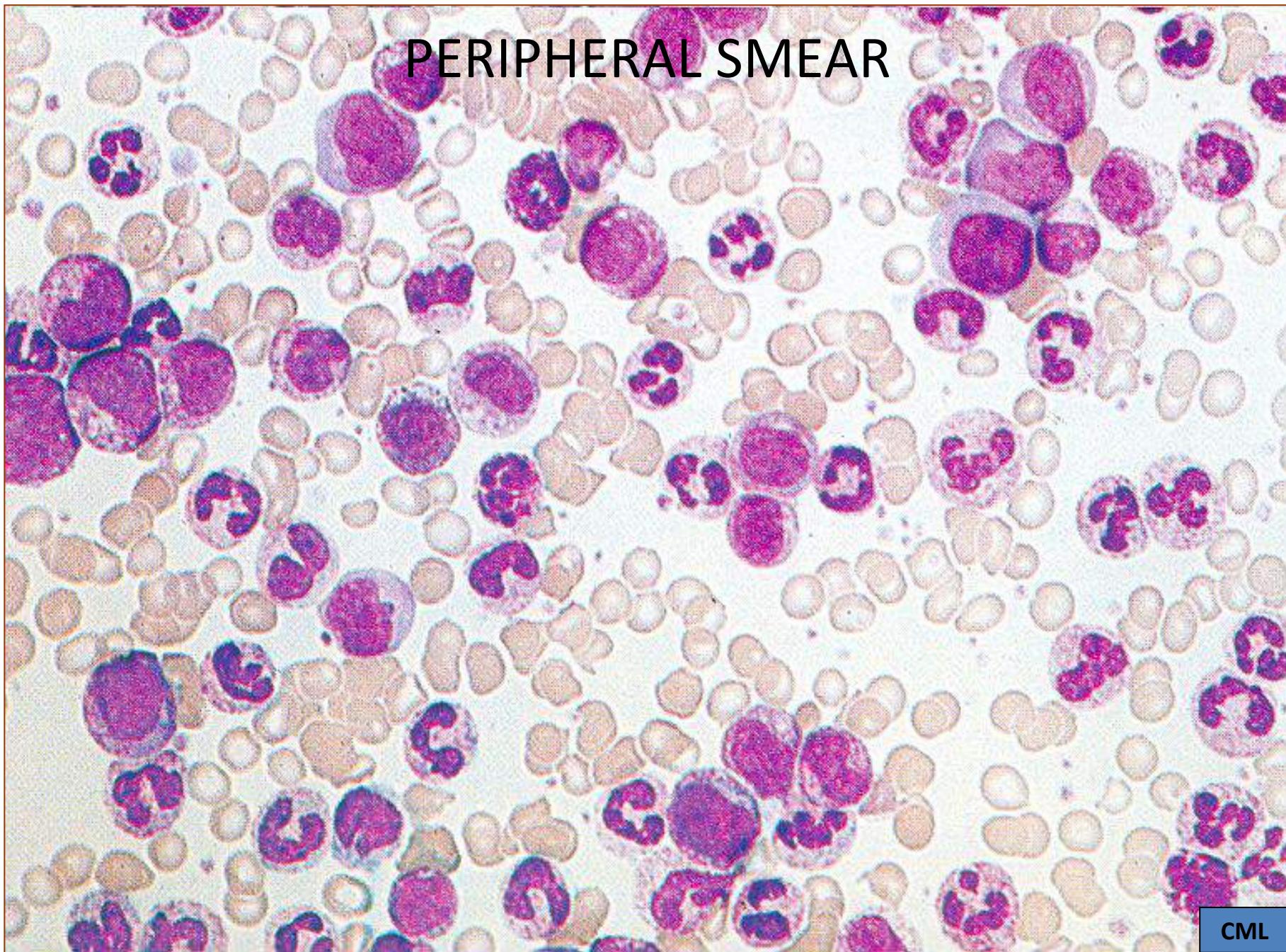
CLINICAL FINDINGS

- ***MIDDLE AGE***
 - ***INSIDIOUS ONSET***
 - ***ANEMIA SYMPTOMS***
 - ***UNEXPLAINED FEVER***
 - ***WEIGHT LOSS***
 - ***FULLNESS IN ABDOMEN (Dragging sensation in the left hypochondrial region)***
 - ***BLEEDING MANIFESTATIONS***
 - ***MASSIVE SPLENOMEGLY***
 - ***LYMPHADENOPATHY (DISEASE PROGRESSION)***
 - ***EXTRAMYELOID MASSES***
-

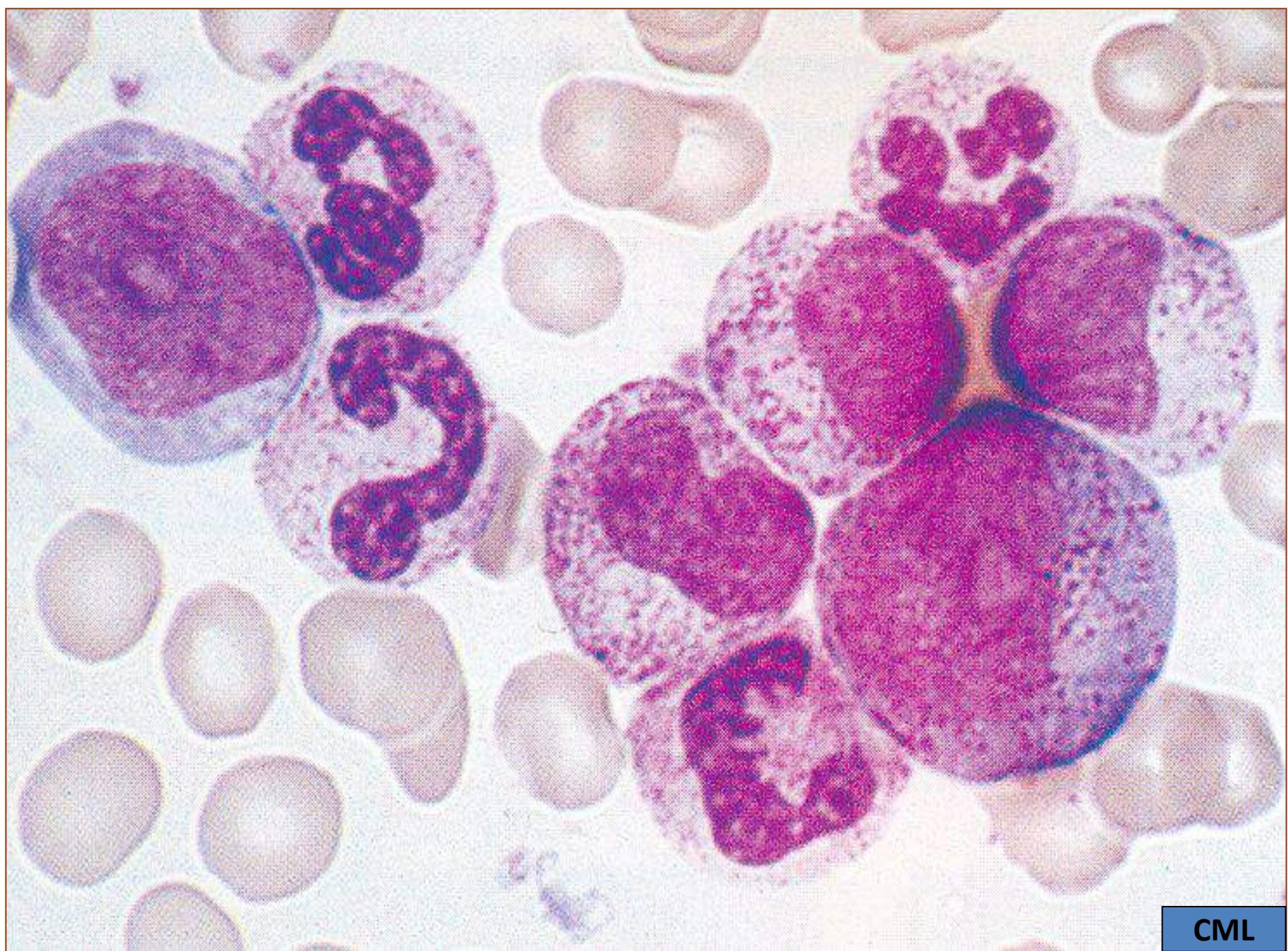
PERIPHERAL BLOOD FEATURES

- LEUKOCYTOSIS (1.0 – 5.0 laks/cu mm)
 - THROMBOCYTOSIS
 - NORMOCYTIC NORMOCHROMIC ANEMIA
 - SHIFT TO LEFT (BLAST COUNT < 20%)
 - BASOPHILIA
 - EOSINOPHILIA
 - MONOCYTOSIS
 - OCCASIONAL NRBC
 - DECREASED LAP SCORE
-

PERIPHERAL SMEAR

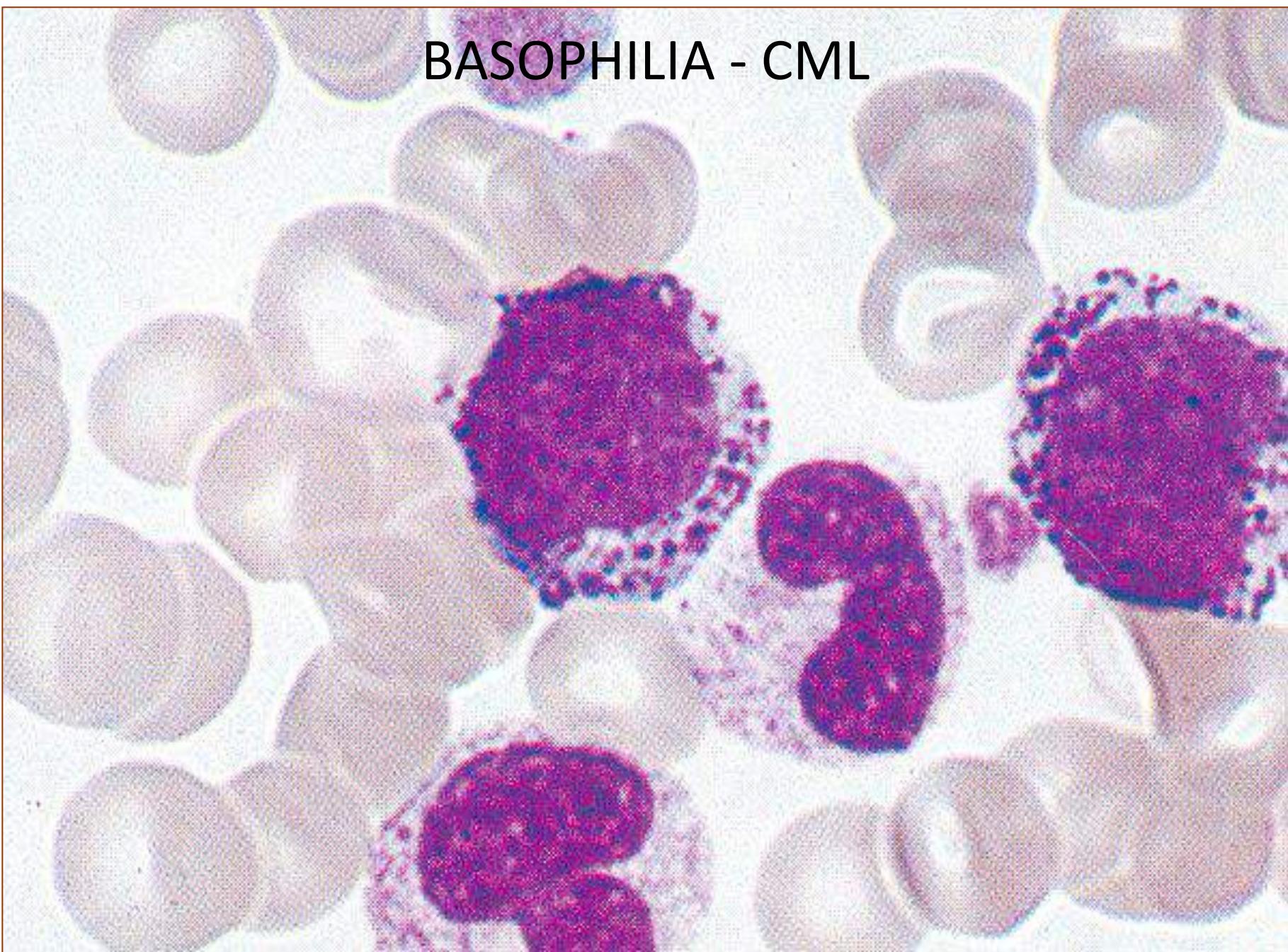


CML

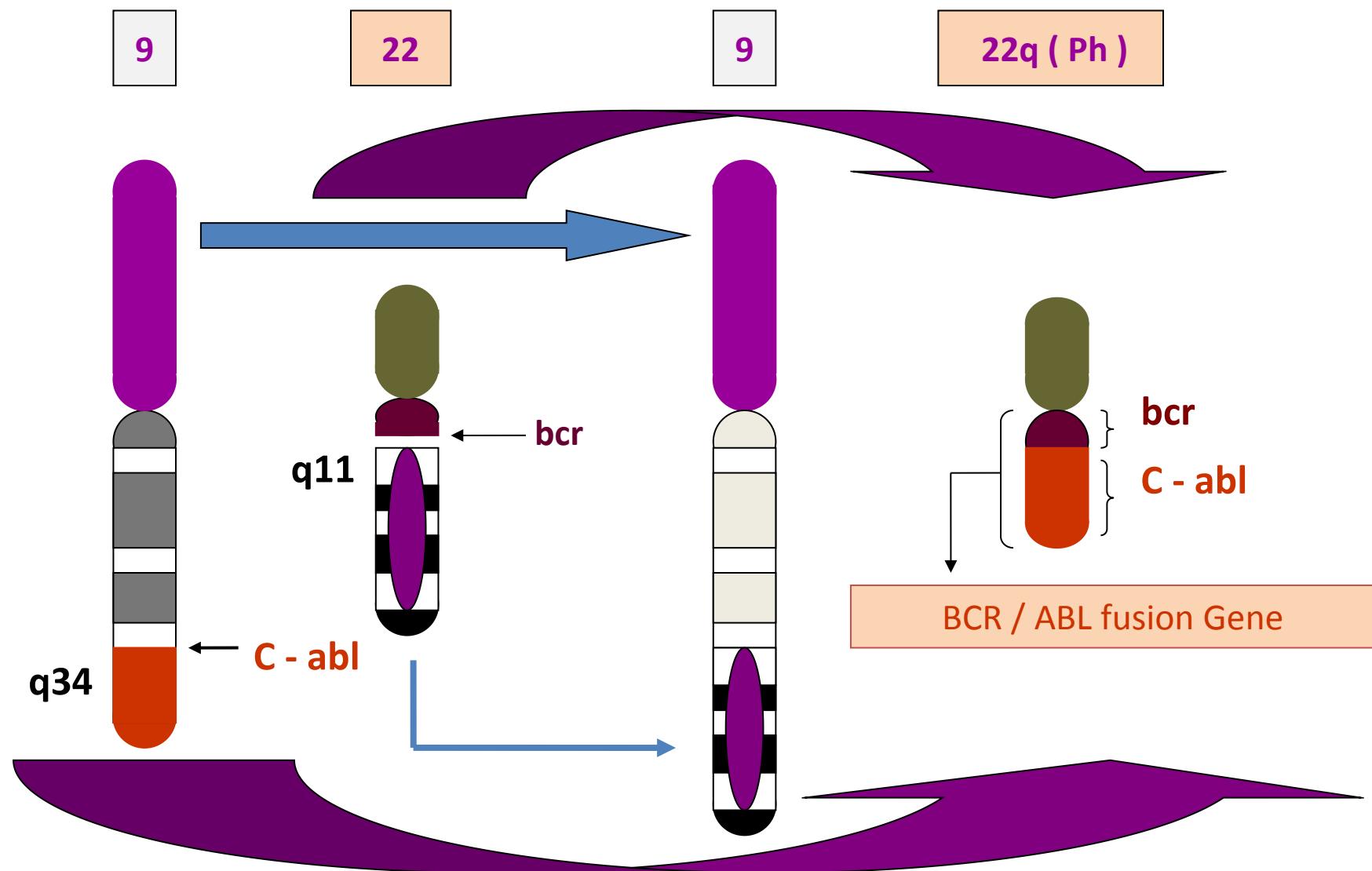


CML

BASOPHILIA - CML



(PHILADELPHIA Ch')



VARIANTS OF CML

VARIANT	AGE	LAP score	Ph chromosome
▪ TYPICAL CML	Middle age	↓	Present
▪ ATYPICAL CML	Older adults	↓	Absent
▪ JUVENILE CML			
INFANTILE VARIANT	< 5 yrs	↓	Absent
ADULT VARIANT	> 5 yrs	↓	Present
▪ CHRONIC EOSINOPHILIC LEUKEMIA	Middle age	N	Present / Absent
▪ CHRONIC BASOPHILIC LEUKEMIA	Middle age	N / ↓	Present / Absent
▪ CHRONIC NEUTROPHILIC LEUKEMIA	Over 50 yrs	↑	Absent

B) Accelerated phase

Criteria of accelerated phase

- Blasts in blood or bone marrow-10-19%
- Basophilia $\geq 20\%$
- Thrombocytopenia $<1,00,000/\text{cmm}$
- Thrombocytosis $>10,00,000/\text{cmm}$
- Additional chromosomal aberrations
- Refractory splenomegaly or refractory leucocytosis

When to suspect progression

- Decreasing platelet counts
- Increasing basophil count
- Increasing total counts
- Lymphadenopathy

c)Blast phase (blast crisis) of CML

- Criteria of blast phase
 1. Blasts $\geq 20\%$
 2. Extramedullary blast proliferation
 3. Large foci of blasts in bone marrow
- Phenotype of blasts
 1. Myeloblasts – 60-70%
 2. Lymphoblasts -10-30%
 3. MPAL– 25%
 4. Acute myelofibrosis

DIFFERENTIAL DIAGNOSIS



LEUKEMOID REACTION



**MYELOFIBROSIS WITH
MYELOID METAPLASIA**

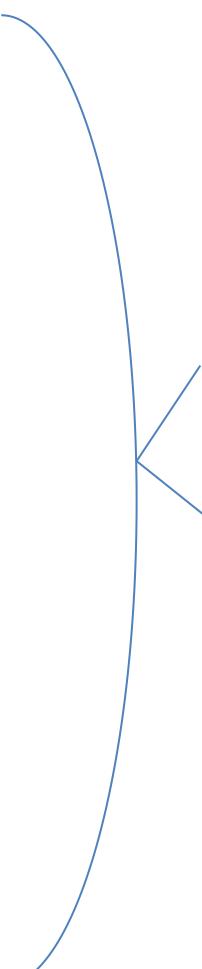
DIFFERENTIAL DIAGNOSIS

	LEUKEMOID REACTION	CML
■ LEUKOCYTE COUNT	INCREASED	INCREASED
■ DIFFERENTIAL	SHIFT TO LEFT	SHIFT TO LEFT
■ ERYTHROCYTE COUNT	NORMAL	DECREASED
■ PLATELETS	NORMAL	INCREASED / DECREASED
■ LAP SCORE	INCREASED	DECREASED
■ BASOPHILIA	NOT SEEN	PRESENT
■ PHILADELPHIA	ABSENT	PRESENT
■ CHROMOSOME		
■ COURSE	TRANSIENT	PROGRESSIVE

LYMPHOID

ACUTE

CHRONIC



ACUTE LYMPHOBLASTIC LEUKEMIA

CLINICAL FINDINGS

- ABRUPT ONSET
 - SYMPTOMS RELATED TO
 - ANEMIA
 - NEUTROPENIA
 - THROMBOCYTOPENIA
 - BONE PAIN & TENDERNESS
 - SYMPTOMS RELATED TO CNS involvement
 - LYMPHADENOPATHY ****
 - HEPATOSPLENOMEGALY **
- 
- COMMON

FAB CLASSIFICATION : ALL

ALL – L1

ALL – L2

ALL – L3

BASED ON :

MORPHOLOGY

CELL SIZE

NUCLEAR CHROMATIN

NUCLEAR SHAPE

NUCLEOLI

AMOUNT OF CYTOPLASM

CYTOPLASMIC BASOPHILIA

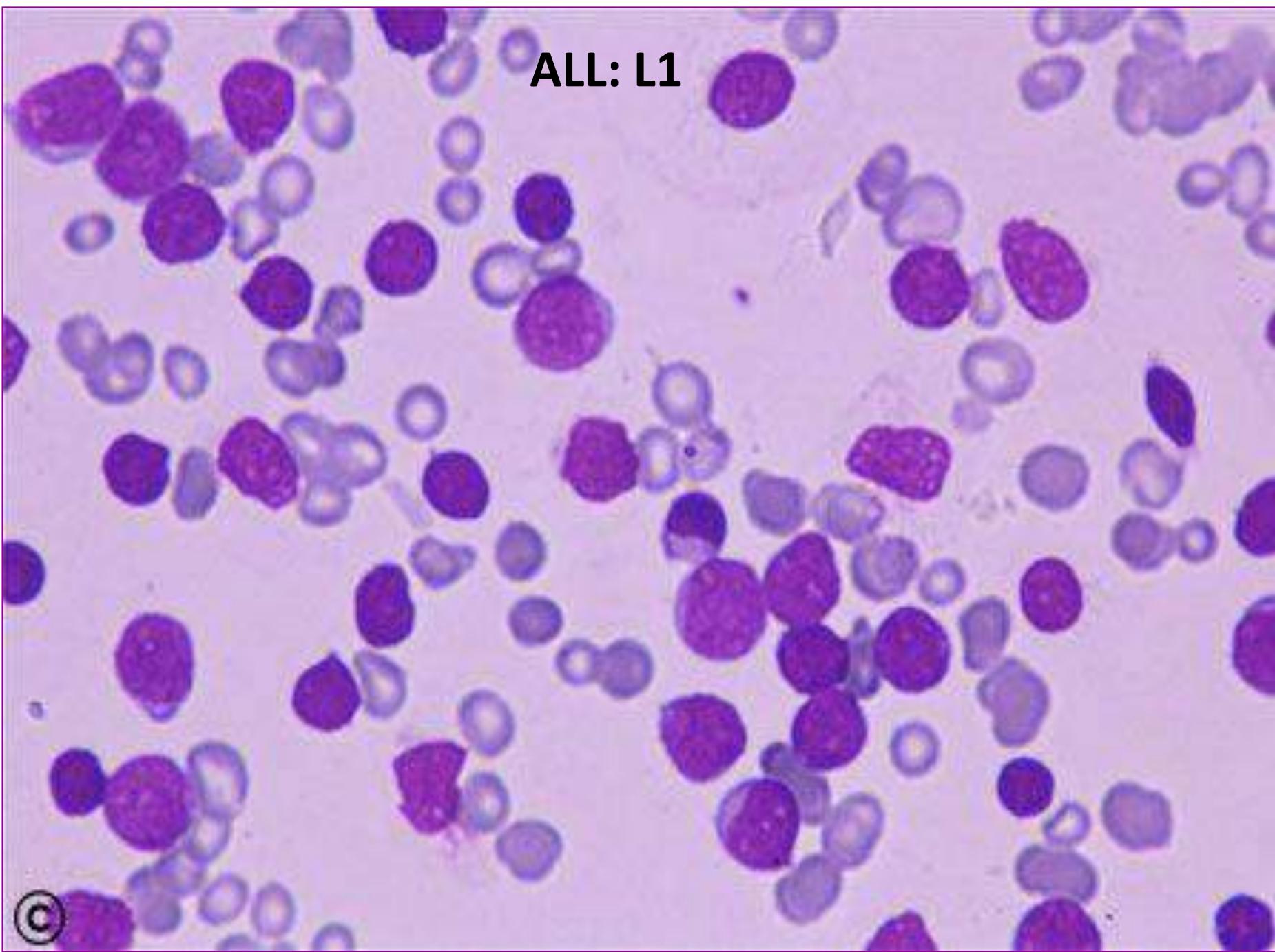
CYTOPLASMIC VACUOLATION

HETEROGENECITY

ALL : L1

- CELL SIZE : SMALL CELLS PREDOMINATE
 - NUCLEAR CHROMATIN : HOMOGENEOUS IN ANY ONE
 - NUCLEAR SHAPE : REGULAR, OCCASIONAL
CLEFTING or INDENTATION
 - NUCLEOLI : SMALL or INCONSPICUOUS
 - AMOUNT OF CYTOPLASM : SCANTY
 - CYTOPLASMIC BASOPHILIA : SLIGHT or MODERATE
 - CYTOPLASMIC VACUOLATION : VARIABLE
-

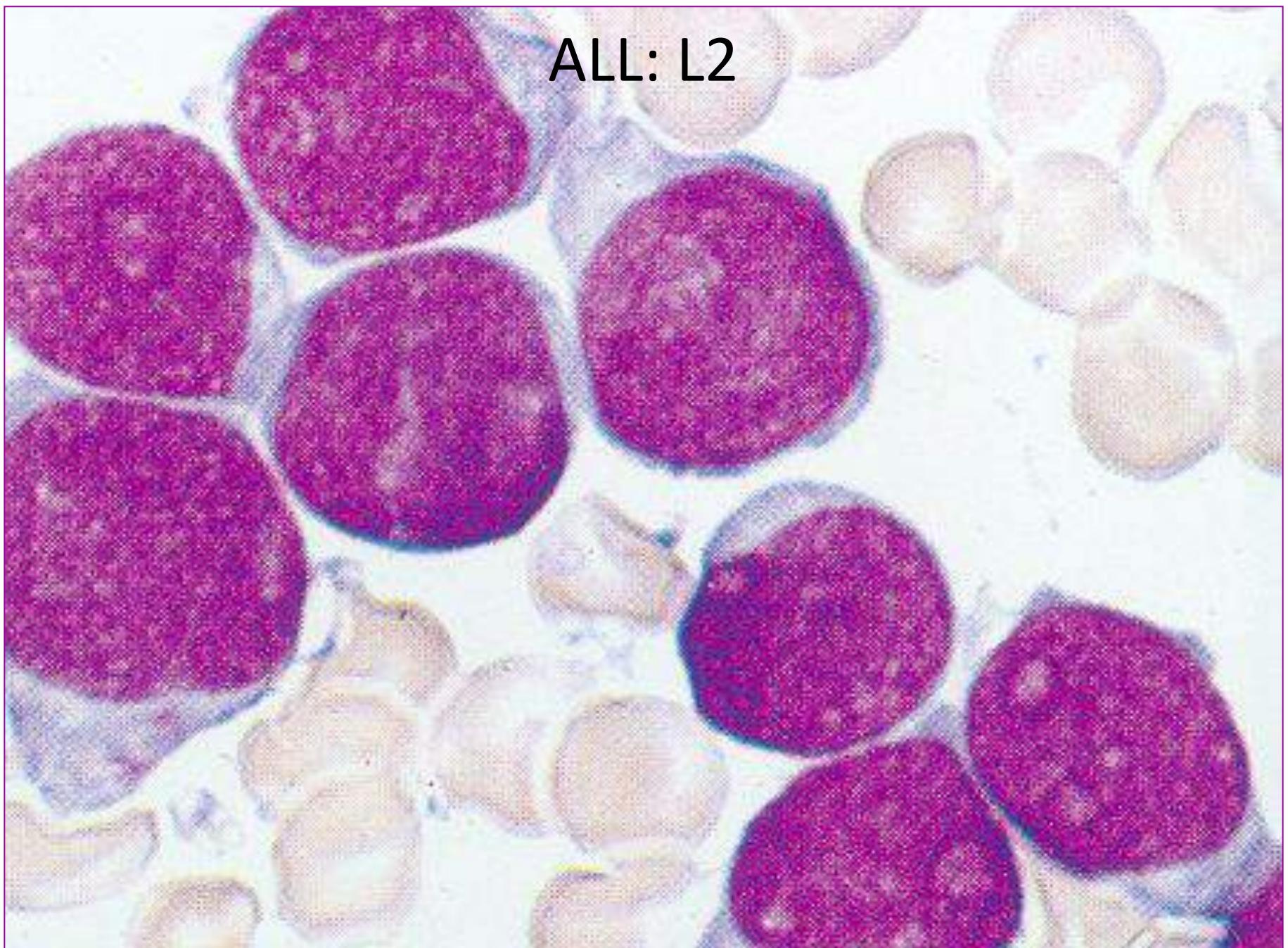
ALL: L1



ALL : L2

- CELL SIZE : LARGE, HETEROGENEOUS
 - NUCLEAR CHROMATIN : HETEROGENEOUS IN ANY ONE
 - NUCLEAR SHAPE : IRREGULAR, CLEFTING and
INDENTATION COMMON
 - NUCLEOLI : ONE or MORE PROMINENT
 - AMOUNT OF CYTOPLASM : MODERATE
 - CYTOPLASMIC BASOPHILIA : VARIABLE
 - CYTOPLASMIC VACUOLATION : VARIABLE
-

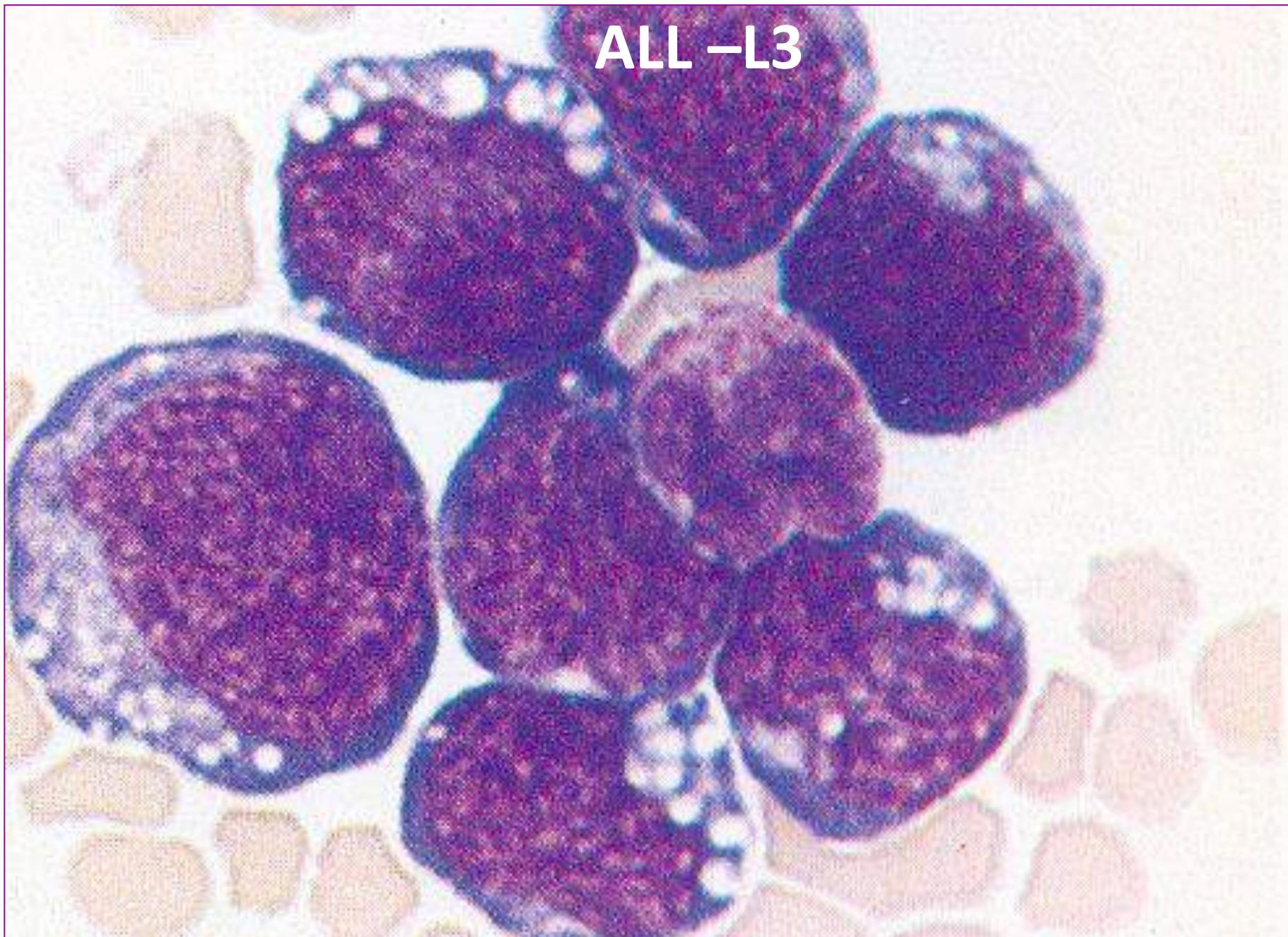
ALL: L2



ALL : L3

- CELL SIZE : LARGE and HOMOGENEOUS
 - NUCLEAR CHROMATIN : FINELY STIPPLED and HOMOGENEOUS
 - NUCLEAR SHAPE : REGULAR, OVAL to ROUND
 - NUCLEOLI : PROMINENT ONE or MORE
 - AMOUNT OF CYTOPLASM : MODERATELY ABUNDANT
 - CYTOPLASMIC BASOPHILIA : VERY DEEP
 - CYTOPLASMIC VACUOLATION : PROMINENT
-

ALL -L3



LABORATORY FINDINGS : ALL

● PERIPHERAL BLOOD

LEUKOCYTE COUNT $\uparrow / N / \downarrow$

NEUTROPENIA

LYMPHOBLASTS

NORMOCYTIC, NORMOCHROMIC ANEMIA

THROMBOCYTOPENIA

● BONE MARROW

HYPERCELLULAR

> 30% BLASTS

CYTOCHEMISTRY IN ACUTE LEUKEMIA

STAINS	AML	ALL
MYELOPEROXIDASE	+	--
SUDAN BLACK	+	--
PAS	+ (fine)	+ (course)
NON-SPECIFIC ESTERASE	+ (M4 , M5)	--
ACID PHOSPHATASE	--	-- (+ve T-ALL)
TdT	--	+

Immunophenotyping

Precursor-B ALL	CD19, CD10, CD79a, TdT, cCD22*, HLA-DR, cCD79a*
Precursor-T ALL	CD1, CD2, CD3, CD4, CD5, CD7 CD8, TdT, cCD3*
AML	*
	*
	*

CYTOGENETICS

<i>Immunophenotype</i>	<i>Chromosomal Abnormality</i>
B-Lineage ALL	t(4;11)(q21;q23) t(5;14)(q31;q32)
Pre-B cell ALL	t(1;19)(q23;p13)
B cell ALL	t(8;14)(q24;q32) t(2;8)(p11;q24) t(8;22)(q24;q11)
T cell ALL	t(11;14)(p13;q11) t(1;14)(p34;q11) t(8;14)(q24;q11) t(10;14)(q24;q11) t(1;14)(p32;q11) t(14;14)(q11;q32) t(7;9)(q35–36;q34) t(7;14)(q35–36;q11) t(7;7)(p15;q11) t(7;14)(p15;q11) inv(14)(q11;q32) inv(14)(q11;q32)
Variable	t(9;22)(q34;q11) del 9(p21–22)

The WHO classification of acute lymphoblastic leukaemia, 2008

- 1. B lymphoblastic leukaemia/lymphoma**
- 2. B lymphoblastic leukaemia/lymphoma, not otherwise specified**

B lymphoblastic leukaemia/lymphoma with recurrent genetic abnormalities++

- With t(9;22)(q34;q11.2) and *BCR – ABL1*
- With t(4;11)(q21;q23) and *MLL – MLLT2 or other 11q23 and MLL rearrangement*
- With t(12;21)(p13;q22) and *ETV6 – RUNX1 **
- With hyperdiploidy (> 50 chromosomes)
- With hypodiploidy (< 46 chromosomes)
- With t(5;14)(q31;q32) and *IL3 – IGH*
- With t(1;19)(q23;p13.3) and *TCF3 – PBX1*

- 3. T lymphoblastic leukaemia/lymphoma**

PROGNOSTIC FACTORS

Favorable factors :

- ★ 1. *L 1 subtype of ALL*
- ★ 2. *B Precursor ALL*
- ★ 3. *Leucocyte count < 10,000/ µl*
- 4. *Age between 1 – 10 years*
- 5. *Female child*
- ★ 6. *Absent or minimal extramedullary involvement*
- ★ 7. *Chromosomal translocations other than t(4;11)*
- ★ 8. *Hyperdiploid cases - t(12;21)*

Unfavorable factors :

- 1. *L 2 , L3 subtype of ALL*
- 2. *Mature B & T cell phenotype*
- 3. *Leucocyte count >50,000/ µl*
- 4. *Age < 1 year*
- 5. *Male child*
- 6. *Extensive extramedullary involvement*
- 7. *Chromosomal translocation ★ t(4;11), t(9;22)*

ALL Phenotype	Chromosome Aberration	Known Gene Loci	Prognosis
Early B cell CALLA +	★Hyperdiploid >50 Hyperdiploid 47–50 Hypodiploid del(6q) del/t 9p del/t 12p	IFNA/IFNB	good intermediate poor intermediate intermediate intermediate
eosinophilia Clg +	★t(9;22)(q34;q11) t(5;14)(q31;q32) t(1;19)(q23;p13)	ABL/BCR IL3/IGH PBL1/E2A	poor intermediate poor
Biphenotypic & CALLA-	★t(4;11)(q21;q23)		poor
B cell Clg +	★t(8;14)(q24;q32) t(2;8)(p12;q24) t(8;22)(q24;q11)	MYC/IGH IGK/MYC MYC/IGL	poor poor poor
T cell	t(11;14)(p13;q11) t(8;14)(q24;q11) inv(14)(q11q32) t(v;14)(v;q11) t(7;v)(q34–36;v) t(7;v)(p15;v)	TCL2/TCR α - δ C-MYC/TCR α TCR α /IGH/TCL1 TCR α TCR β TCR γ	poor poor poor poor poor poor

CHRONIC LYMPHOCYTIC LEUKEMIA

CLASSIFICATION : CHRONIC LYMPHOID LEUKEMIAS

- **B-CELL**

CHRONIC LYMPHOCYTIC LEUKEMIA



Peripheral B cell
neoplasm

PROLYMPHOCYTIC LEUKEMIA

HAIRY CELL LEUKEMIA

PLASMA CELL LEUKEMIA

LEUKEMIA – LYMPHOMA SYNDROMES

- **T-CELL**

LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

T-PROLYMPHOCYTIC LEUKEMIA

ADULT T-CELL LEUKEMIA-LYMPHOMA

SEZARY SYNDROME

CLINICAL FEATURES

- LYMPHADENOPATHY
 - ANEMIA
 - INFECTIONS
 - HEMORRHAGIC DIATHESIS
 - AIHA
 - SPLENOmegaly
 - SKIN INFILTRATION
-

LAB DIAGNOSIS

■ PERIPHERAL SMEAR

NORMOCYTIC NORMOCHROMIC ANEMIA

LYMPHOCYTIC LEUKOCYTOSIS

PRESENCE OF SMUDGE CELLS

NEUTROPENIA

THROMBOCYTOPENIA

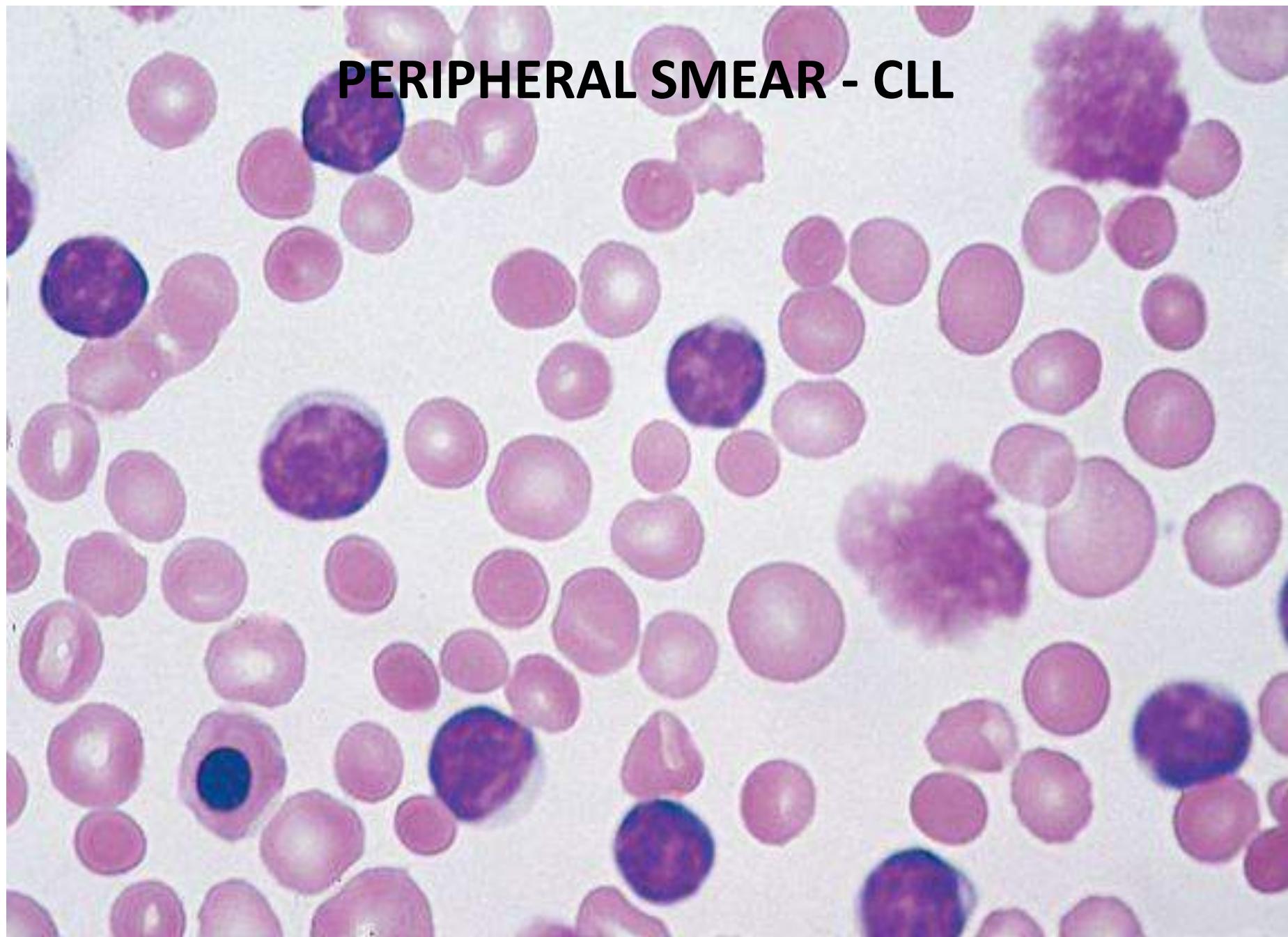
AHA BLOOD PICTURE

■ BONE MARROW

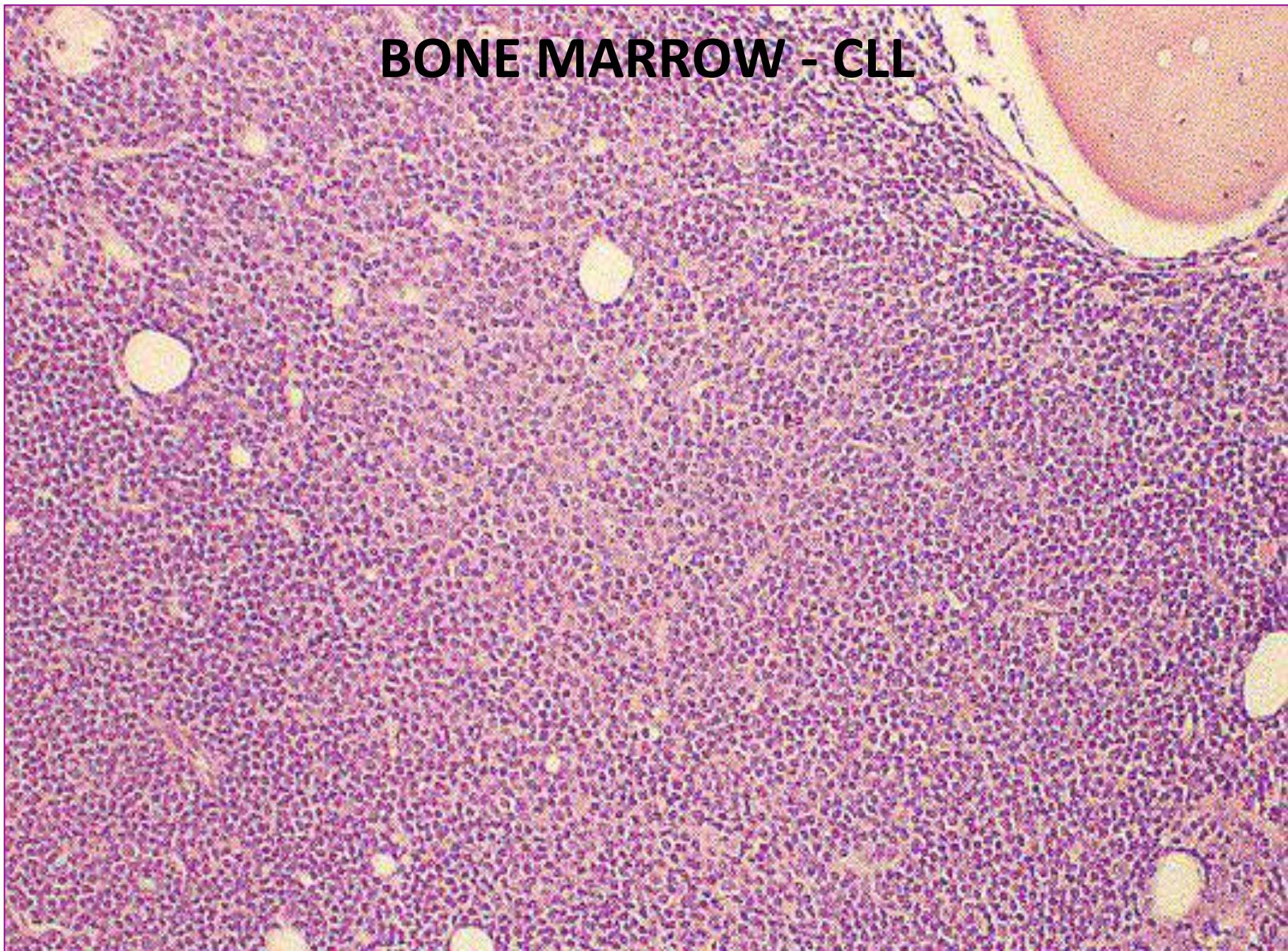
CLL - Diagnostic Criteria

- Persistent lymphocytosis.
- Absolute lymphocyte count more than 5000.
- Mature appearing B-cells with <10% of prolymphocytes
- 30% lymphoid cells in bone marrow
- Low density of surface Ig
- CD 19,20, 23*
- CD 5
- Lack of pan B cell markers other than CD5

PERIPHERAL SMEAR - CLL



BONE MARROW - CLL



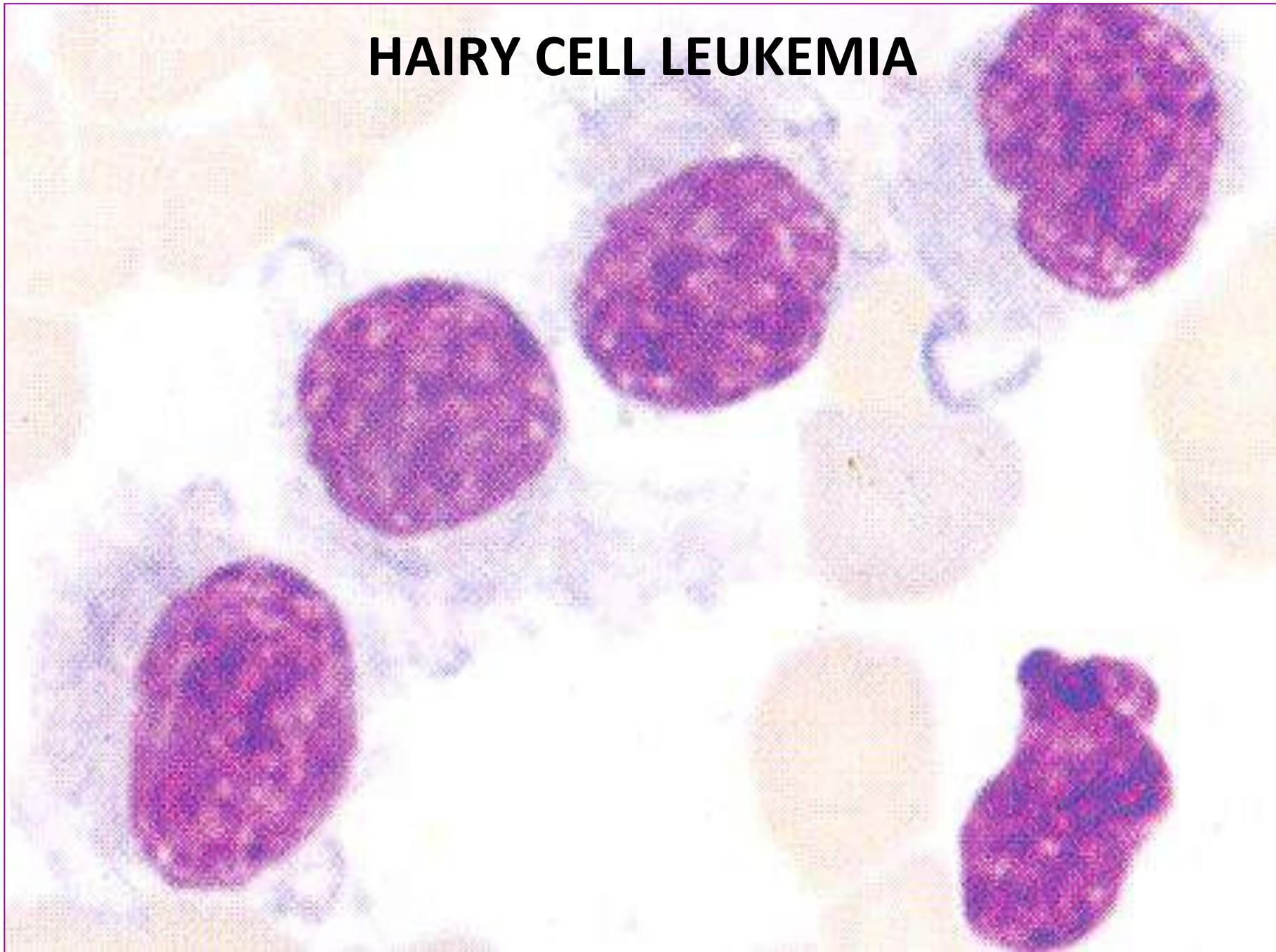
SPLEEN - CLL



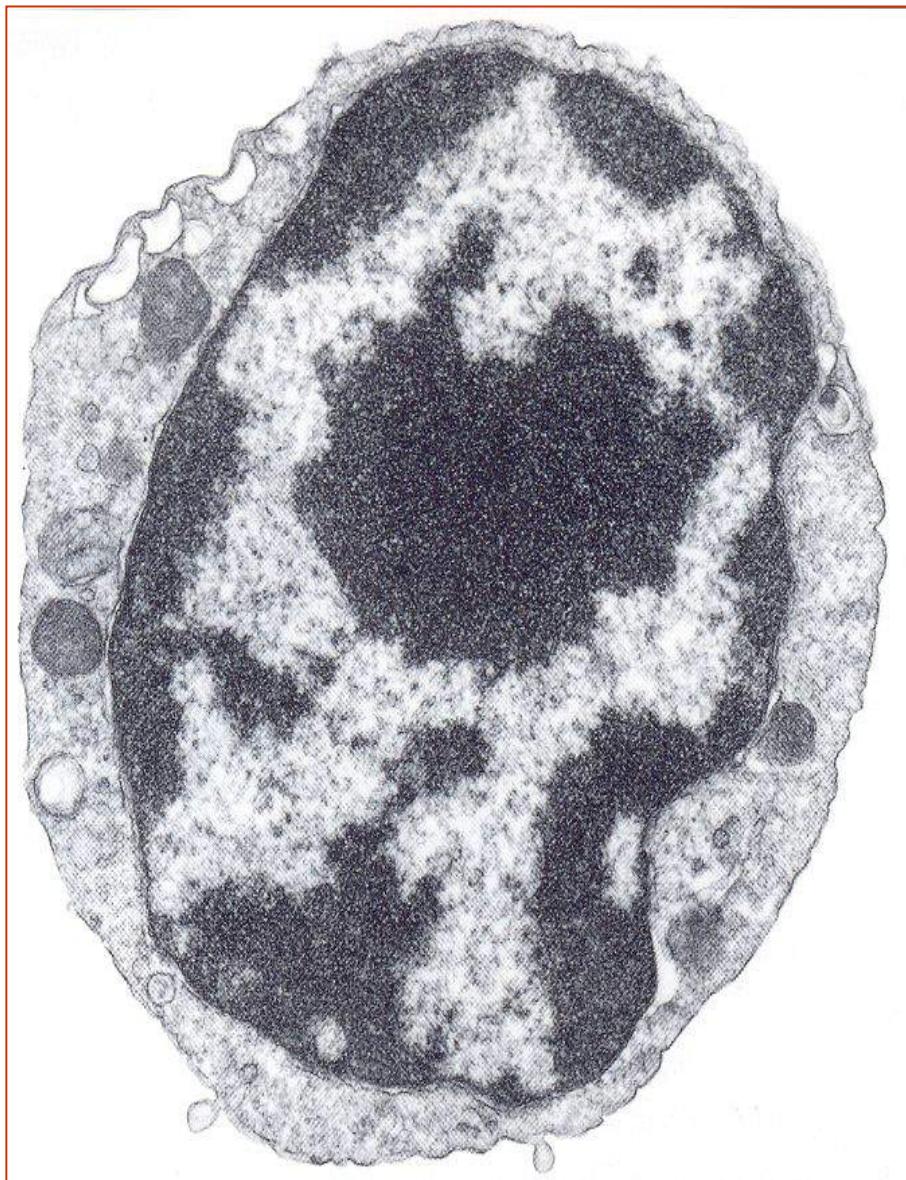
DIFFERENTIAL DIAGNOSIS

- INFECTIOUS MONONUCLEOSIS
 - WHOOPING COUGH
-

HAIRY CELL LEUKEMIA



CLL : CELL



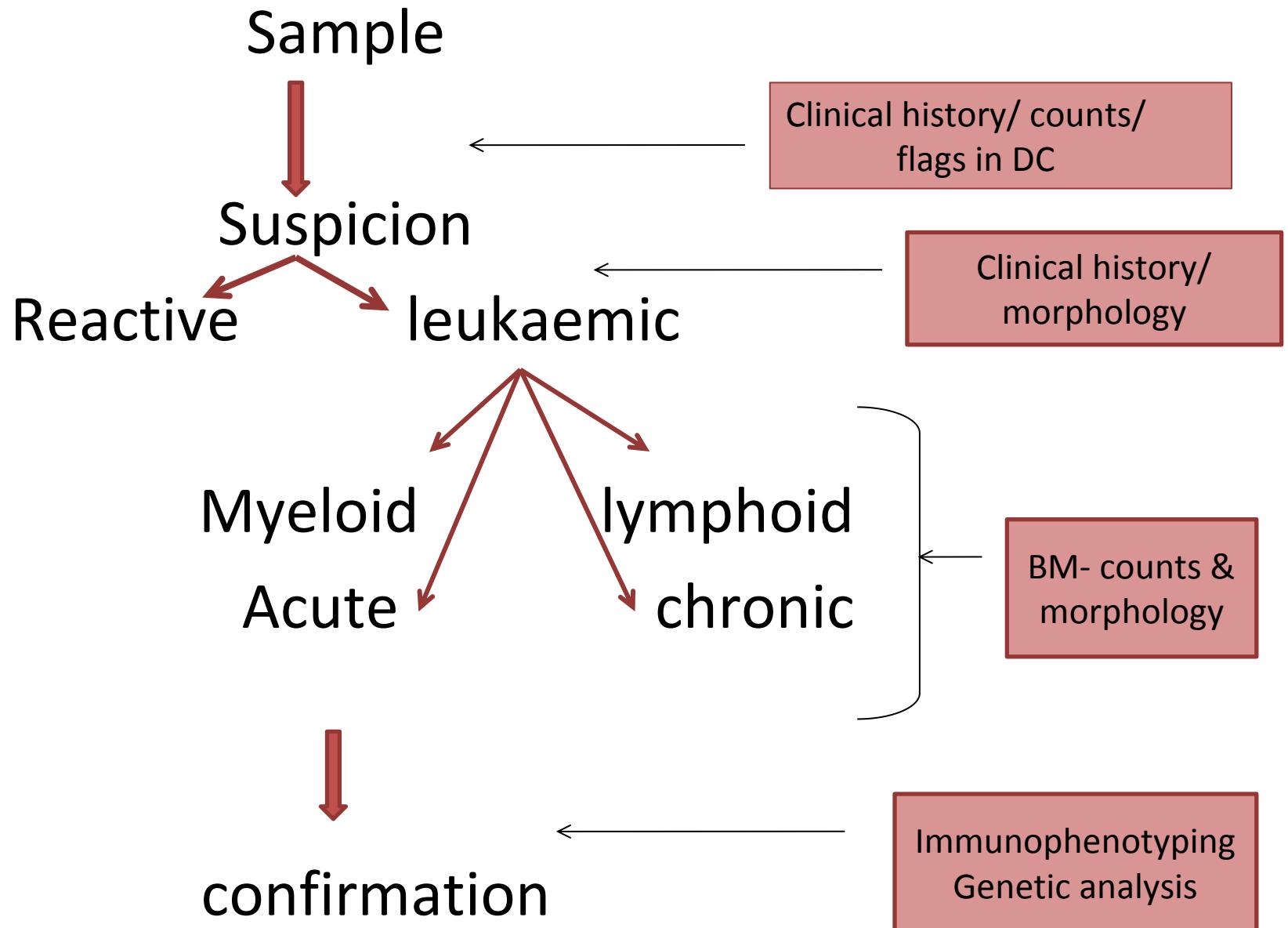
HCL : CELL



Cytogenetics

- Deletions in chromosome 13 at q 14
- Chromosome 11 at q22 or q23.
- Trisomy-12.
- Less common are deletions in chromosome 17 & 6.

TO SUMMARISE ...

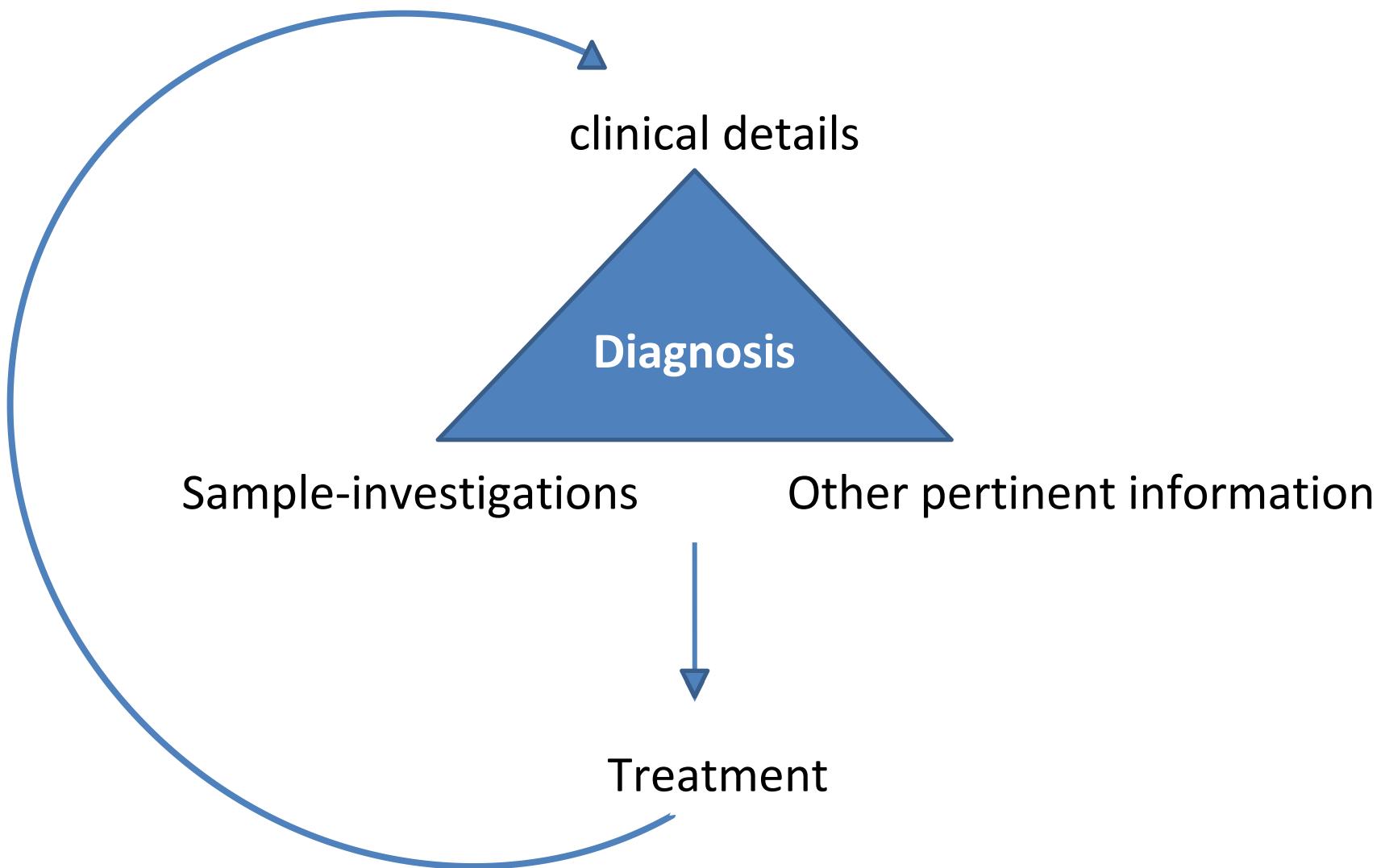


Conclusion

Three important factors contribute to accurate diagnosis:

1. The provision of clinical information;
2. The correct sample- type and time
- 3 .The integration of information across different sites dealing with diagnostic samples from a single patient.

Conclusion



References

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Thank yo

