Payvand

Clinical Specialty Lab.

Islamic Azad University

Hematology Workshop

Peripheral Blood Smear in Leukemia Diagnosis

Behzad Poopak, DCLS PhD.

Associate Professor of Hematology

9.3.1395

bpoopak@gmail.com

Maturation Sequences: Myeloid Series

- ✓Cell size
- ✓Cytoplasm color, volume, granulation
- ✓Nucleus size, color, chromatin pattern
- ✓Nucleoli presence

Myeloblast

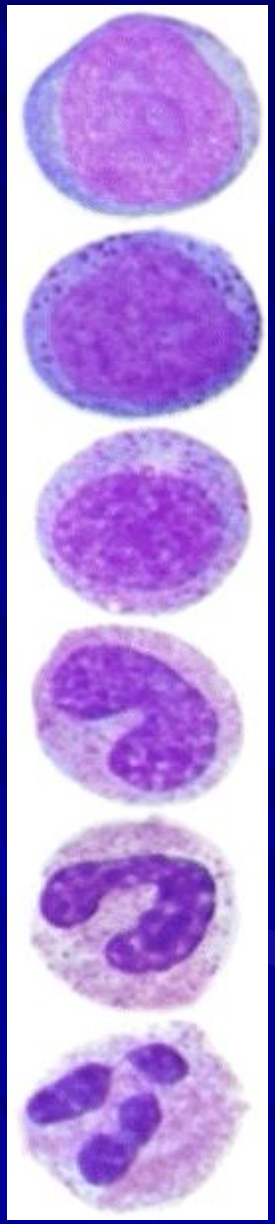
Promyelocyte

Myelocyte

Metamyelocyte

Band

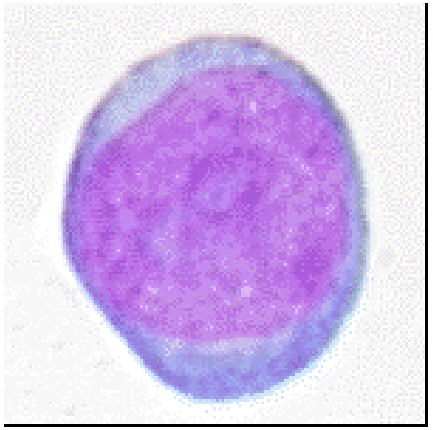
Neutrophil



Normal myeloid development & morphology

Myeloblast

- Blast cells in normal myeloid maturation have:
- Diameter of 12–20 µl &
- A relatively large round or oval
- Nucleus with a fine chromatin pattern
- One or more distinct nucleoli
- The cytoplasm is basophilic with an absent Golgi zone &
- Granules may or may not be present.

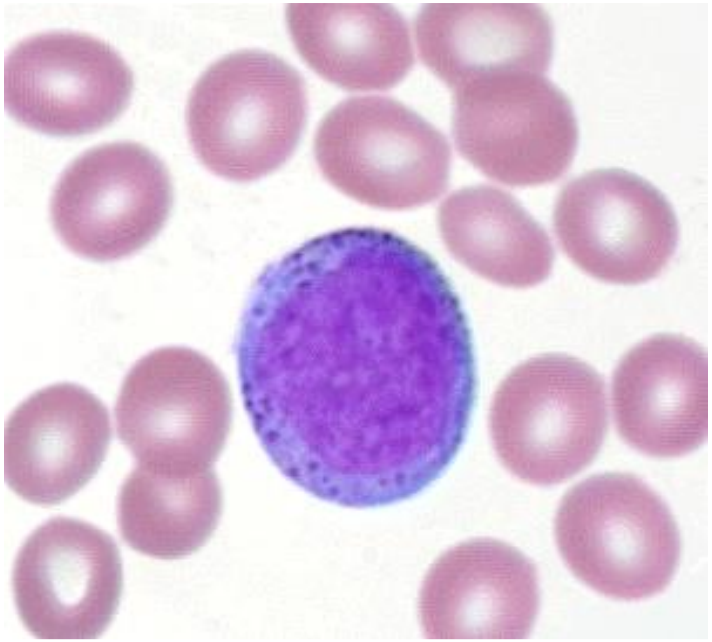


0-1% BM

Normal myeloid development & morphology

ProMyelocyte

- Normal promyelocytes are 15–25 µl in diameter,
- Have an oval or round nucleus
- Fine/intermediate chromatin
- A usually visible and prominent nucleolus.
- The cytoplasm is basophilic
- Contains blue-violet Red (primary) granules.
- A pale area equating to the Golgi zone is present adjacent to the nucleus.

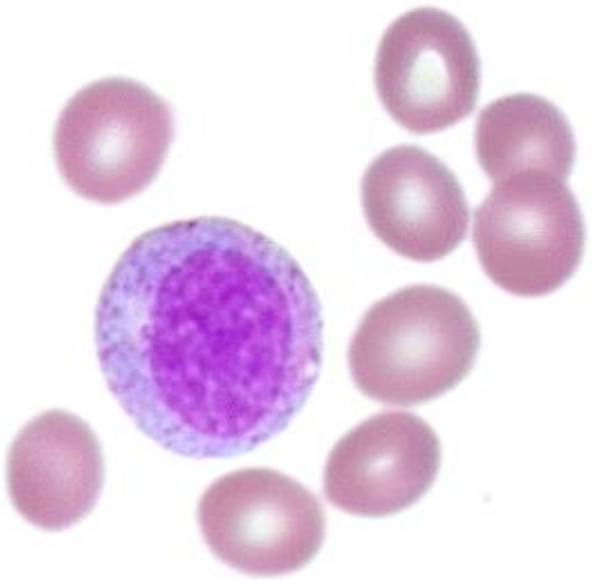


2-5% BM

Normal myeloid development & morphology

Myelocyte

- The myelocyte is slightly smaller than the promyelocyte (10–18 µl)
- Round or oval nucleus which may be eccentrically placed.
- The nuclear chromatin shows a moderate degree of coarse clumping
- Nucleoli are not seen.
- There is a moderate amount of blue-pink cytoplasm which contains numerous red-violet granules.
- As the myelocyte matures, the secondary granules develop definite neutrophilic, eosinophilic or basophilic characteristics.

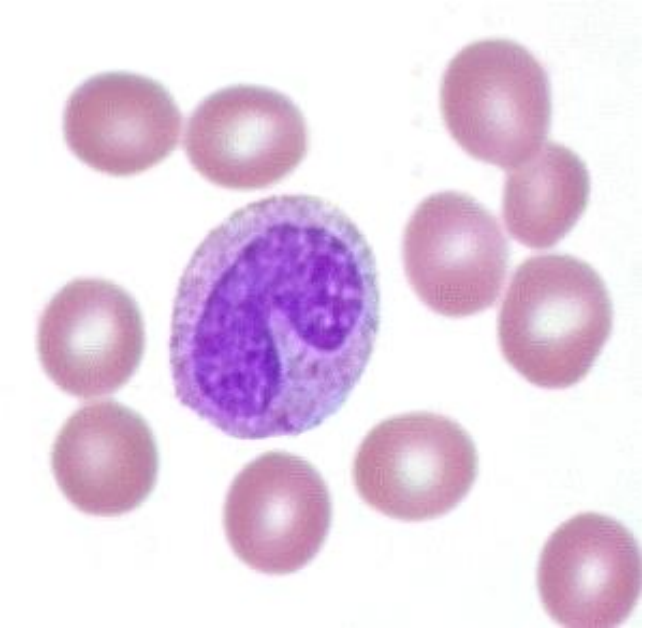


10-20% BM

Normal myeloid development & morphology

MetaMyelocyte

- The metamyelocyte is smaller than the myelocyte
- Indented or kidney-shaped nucleus.
- Nucleoli are not observed.
- The cytoplasm is usually clearly pink
- Contains granules that are clearly differentiated as neutrophilic, eosinophilic or basophilic.
- N.B. Immature granulocytes (promyelocytes, myelocytes and metamyelocytes) are not usually seen in normal peripheral blood.

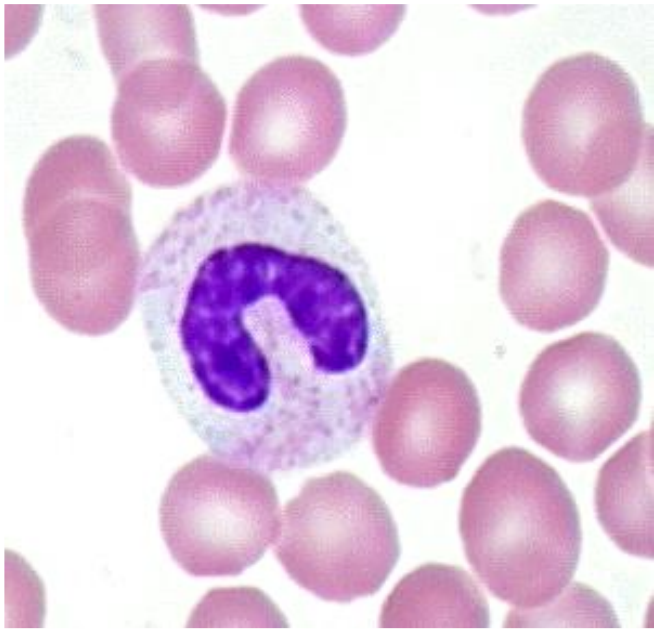


15-30% BM

Normal myeloid development & morphology

Band neutrophil

- Band neutrophils are 10–14µl in diameter
- A nucleus that is non-segmented or has rudimentary lobes that are connected by a thick band rather than a thread.
- Cytoplasm is abundant, pink contains many small violet-pink neutrophilic or secondary granules distributed evenly throughout the cell.
- ***Many laboratories do not report band neutrophils on adult patients or children due to inter-observer variation in band neutrophil classification; this is a well recognized and acceptable practice.***
- ***It is recommended that band neutrophils be counted as segmented neutrophils in the differential.***
- Appropriate comments may be made if increased numbers are seen in the blood film.

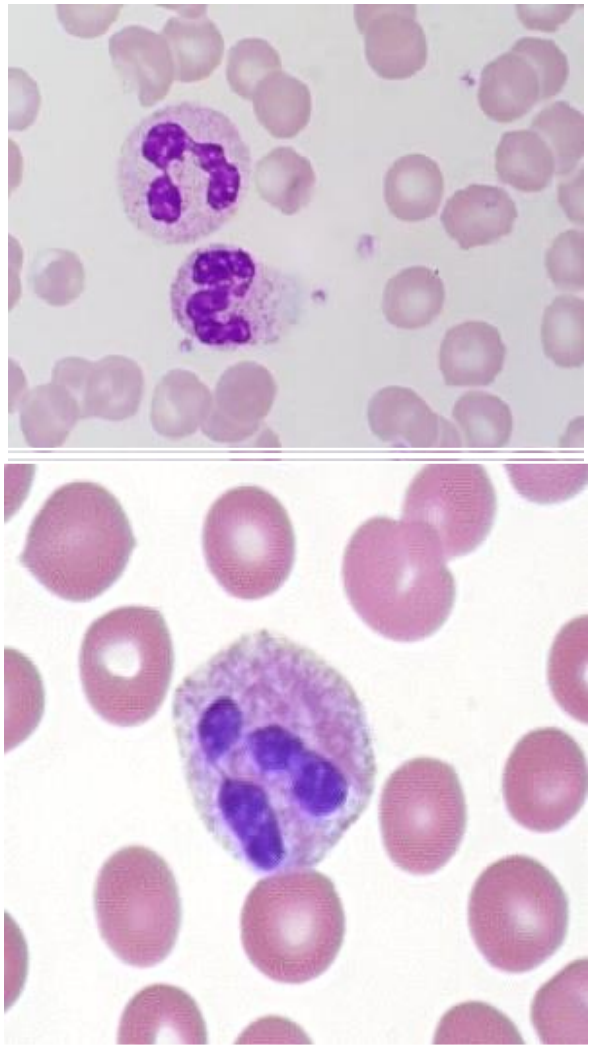


10-40% BM

Normal myeloid development & morphology

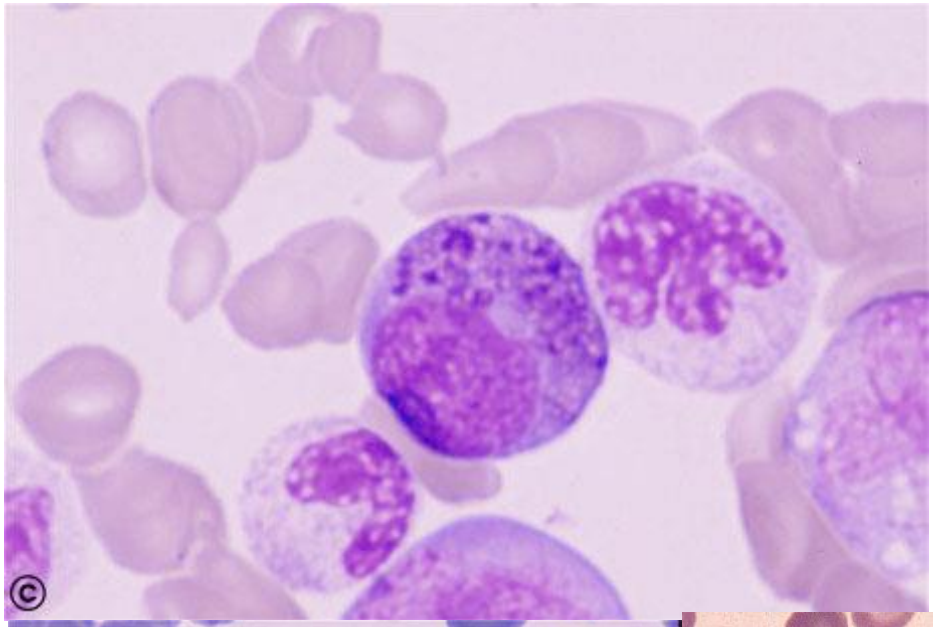
Segmented neutrophil

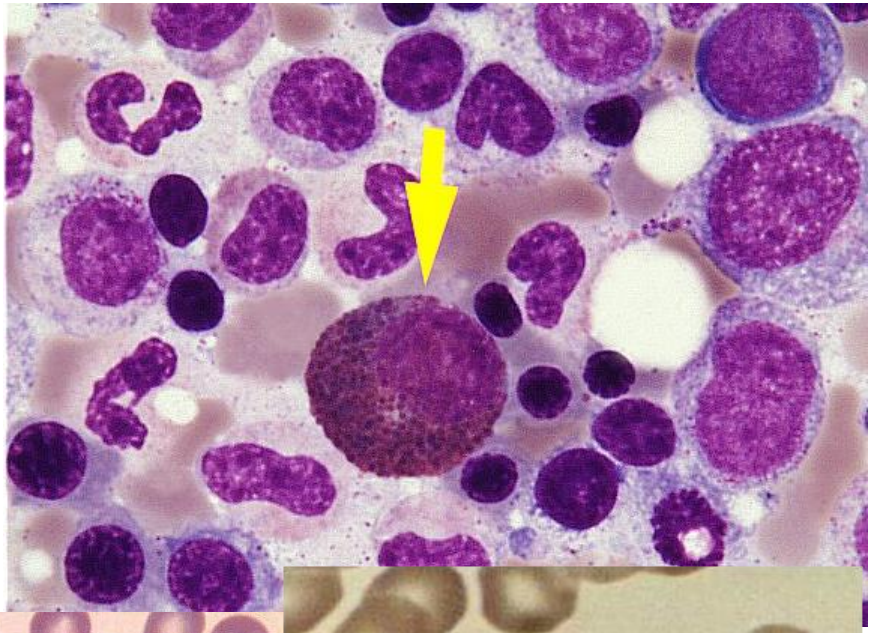
- A granulocyte that is 10–14 µl in diameter
- lobulated nucleus (usually 3–4 lobes, but small numbers of 2 and 5 lobed neutrophils may also be seen) connected by a thin thread of chromatin.
- The chromatin is coarse, stains violet and is arranged in clumps.
- Small nuclear appendages may be seen.
- There is abundant pink cytoplasm with many small secondary granules.

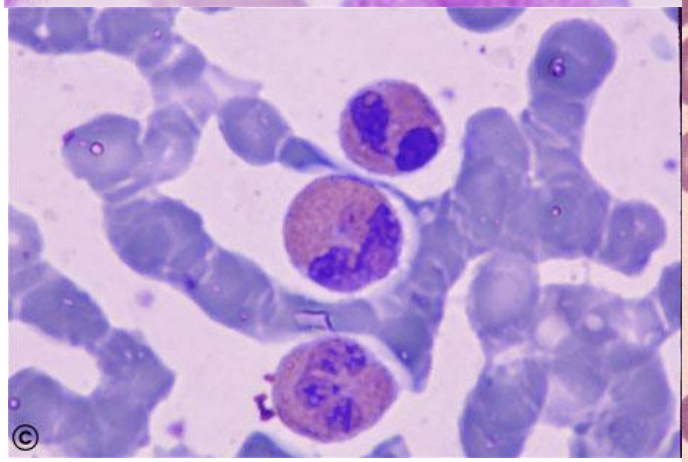


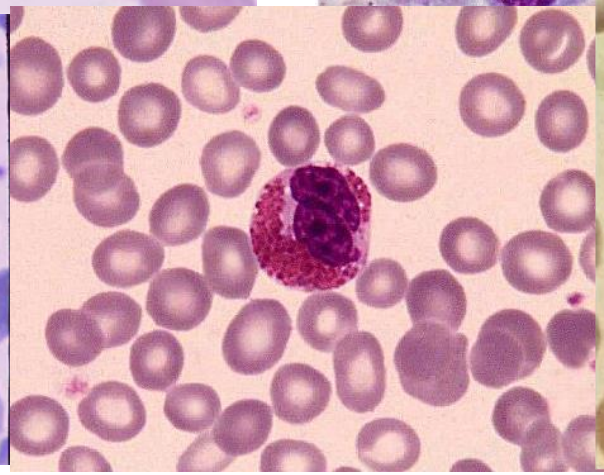
10-30% BM

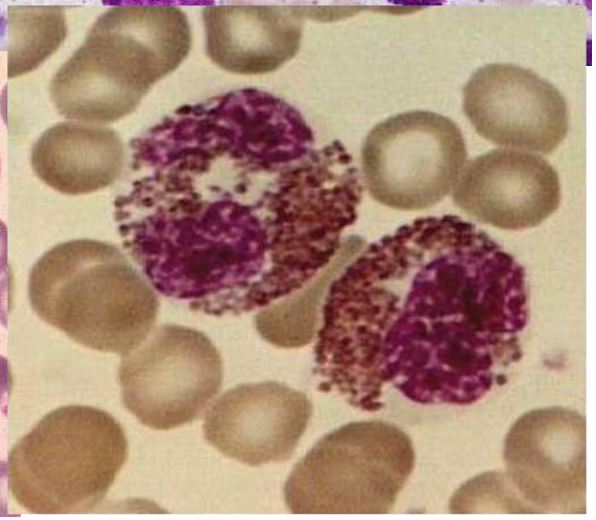
Eosinophil Precursors



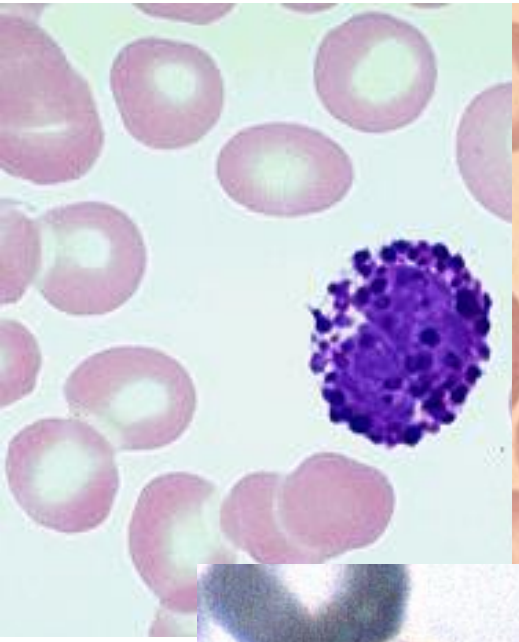


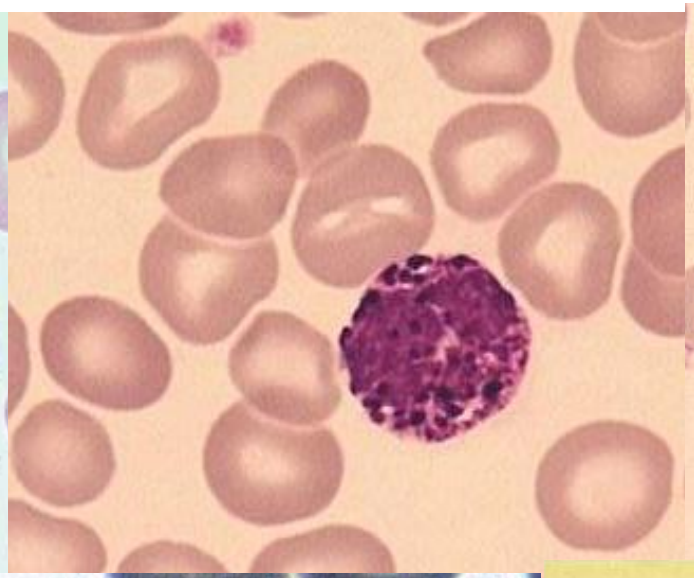


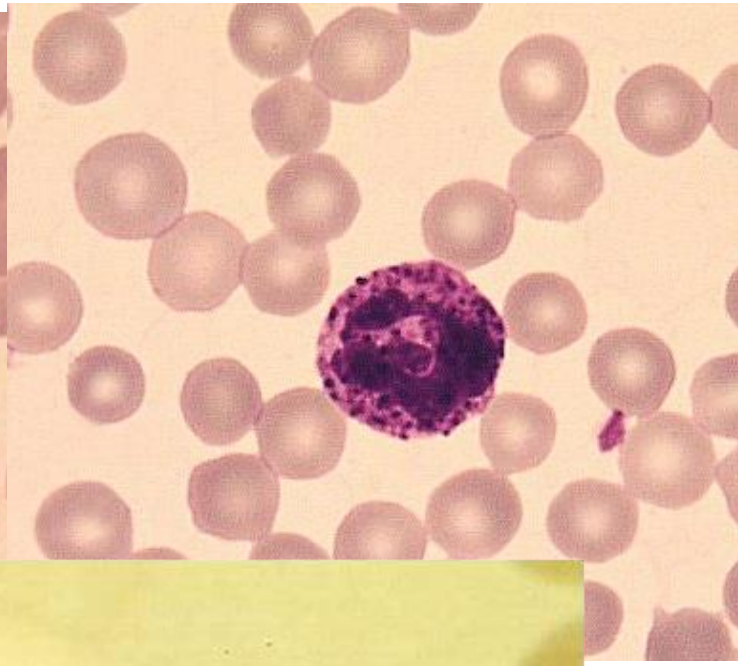


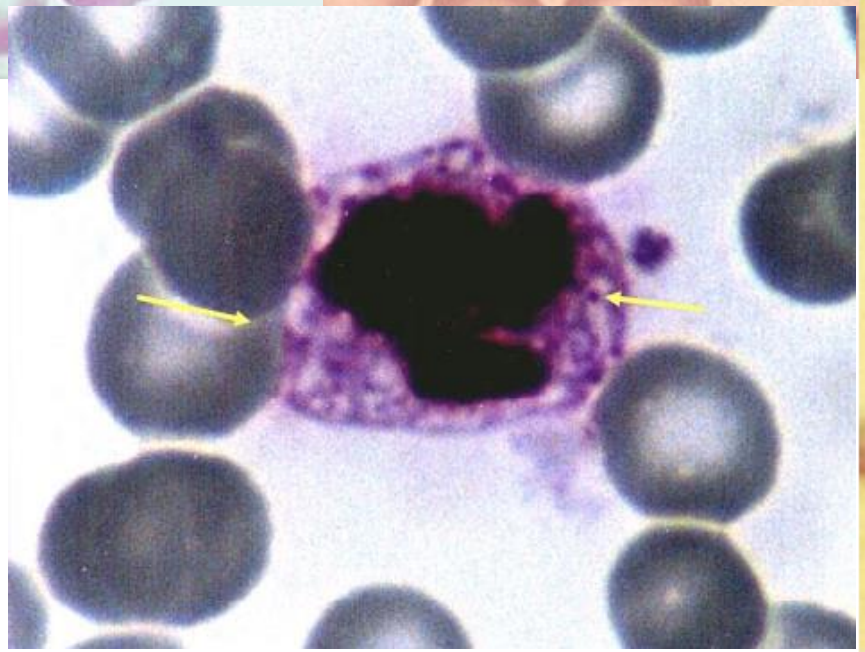


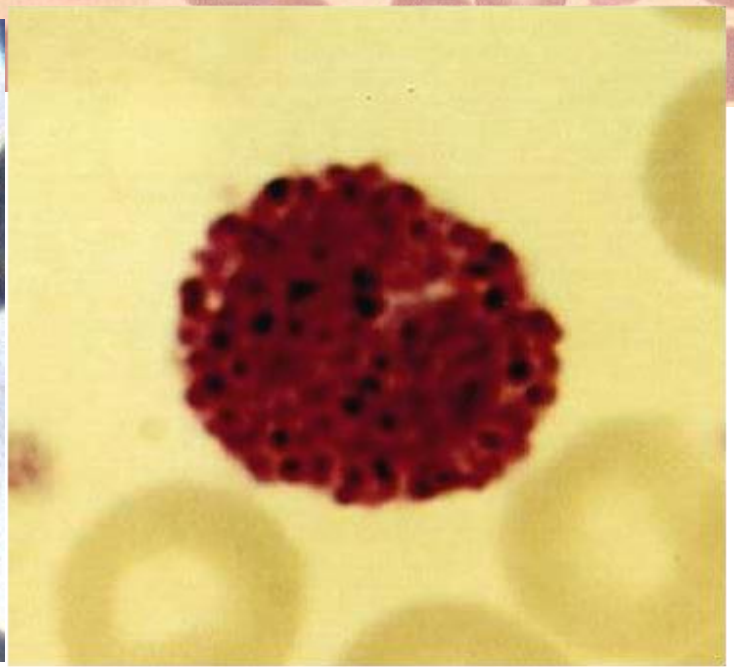
Basophil Precursors



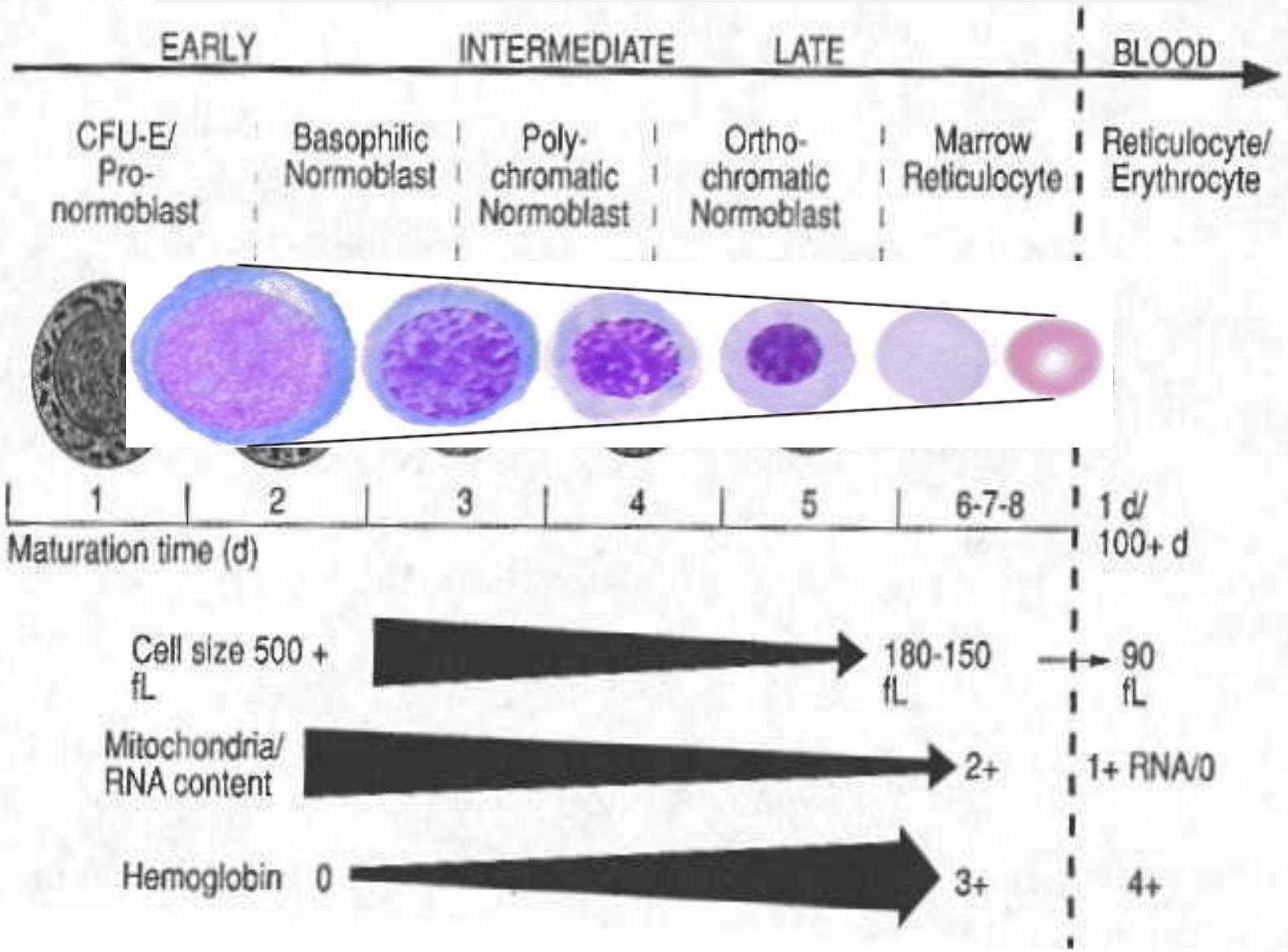




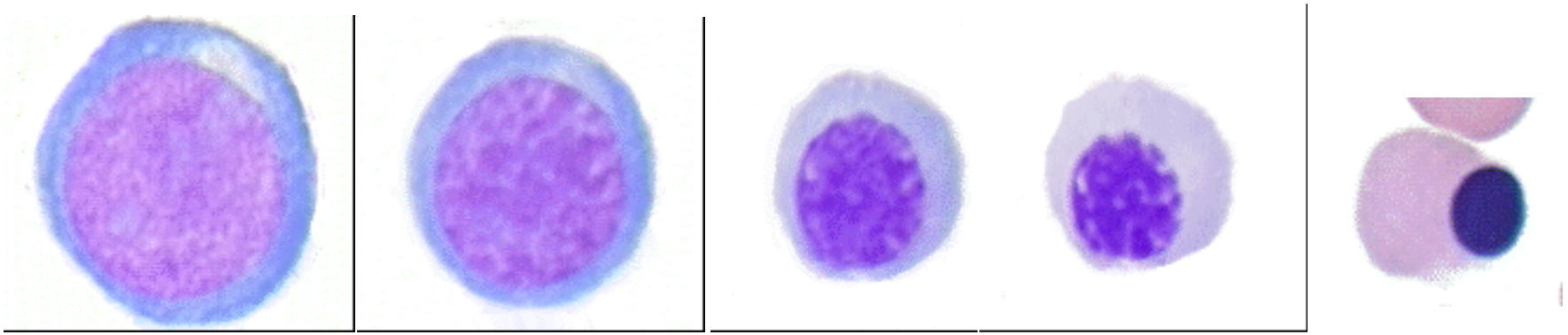




Bone Marrow Erythropoiesis

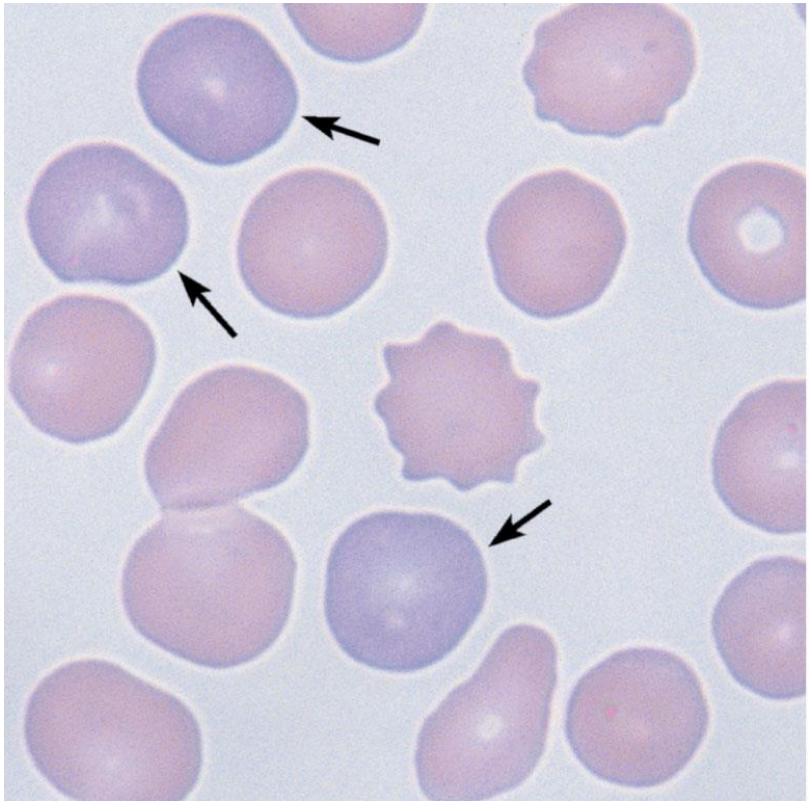


Stages in Red cell (erythroid) Maturation

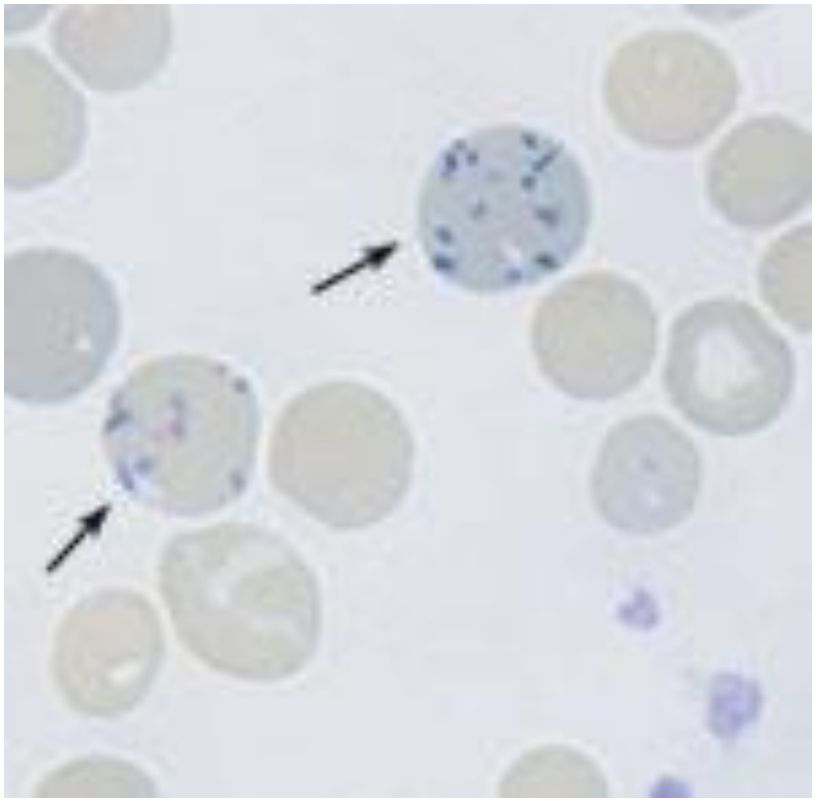


Proerythroblast | Basophilic erythroblast | Polychromatic erythroblast (two examples) | Orthochromic erythroblast

Polychromasia vs. Reticulocytes



Wright Staining



Vital Staining (BCB)

Normal lymphocyte development and morphology

Lymphoblast & Prolymphocyte

- The lymphoblast: 8–20 µl.
- The nucleus is round or oval with fine granular chromatin
- One or more indistinct nucleoli.
- The cytoplasm is scanty and basophilic, and cytoplasmic granules are absent.
- It cannot be reliably distinguished from some types of undifferentiated or minimally differentiated myeloblasts and therefore should be counted as a blast cell.

Prolymphocyte

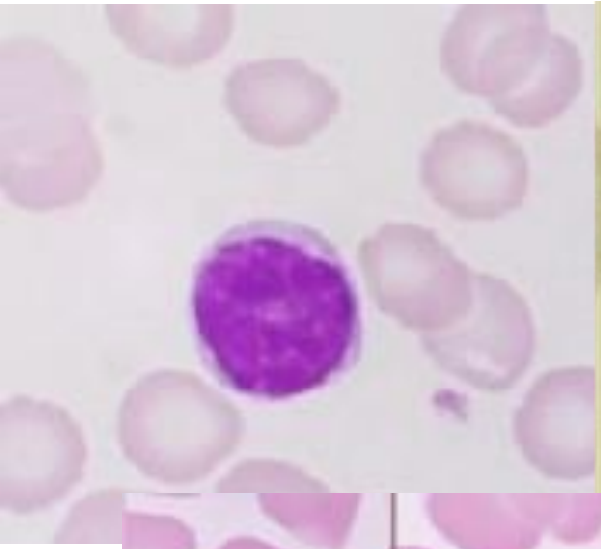
- The nucleus is round
- Contains a single prominent nucleolus.
- More cytoplasm than a lymphoblast
- The chromatin is more condensed.

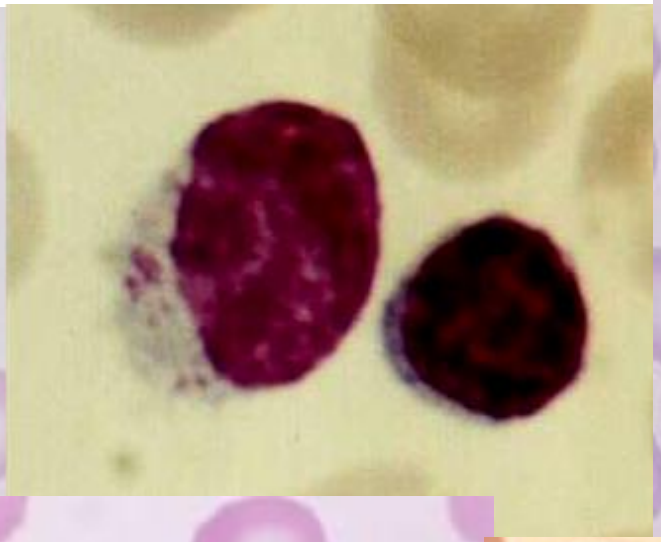
N.B. Lymphoblasts and prolymphocytes are not usually seen in the normal peripheral blood.

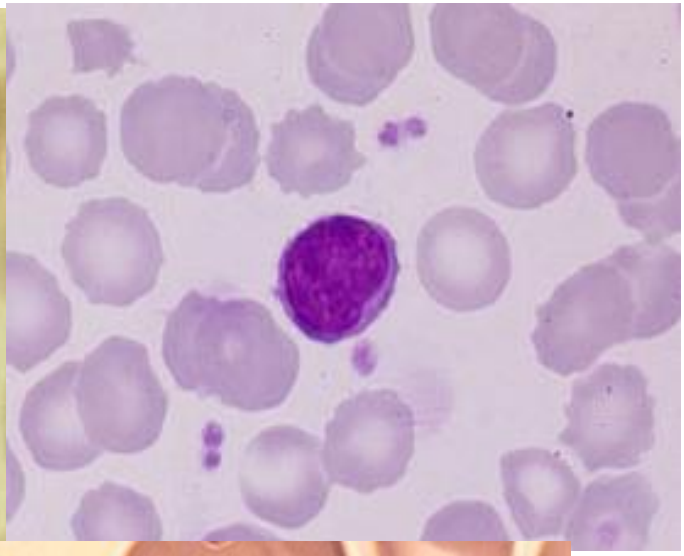
Lymphocyte:

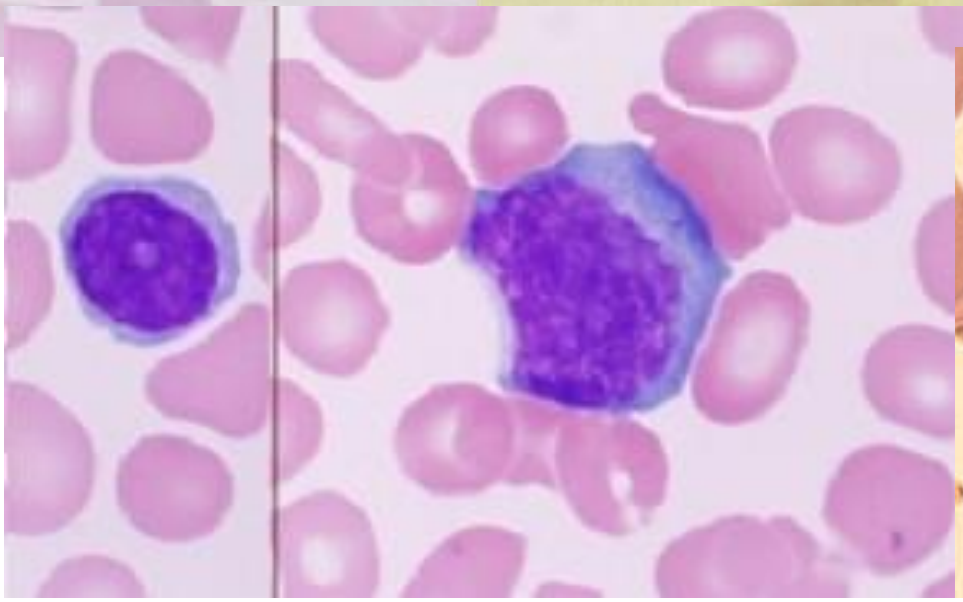
Lymphocytes seen in the peripheral blood are predominantly **small (10–12 µl),** or, less frequently **large (12–16 µl).**

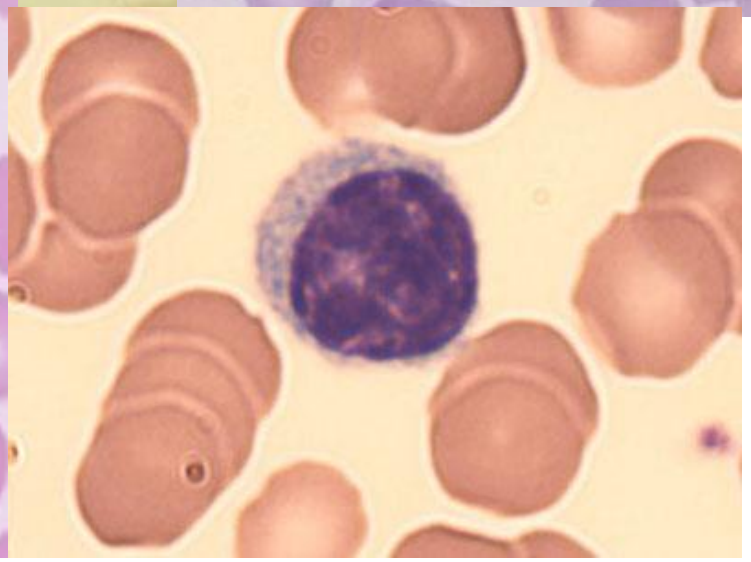
Normal lymphocyte morphology











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2015 John Wiley & Sons Ltd, *Int. Jnl. Lab. Hem.* 2015, **37**, 287–303

ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features

L. PALMER*, C. BRIGGS†, S. MCFADDEN‡, G. ZINI§, J. BURTHEM¶, G. ROZENBERG**, M. PROYTCHEVA††, S. J. MACHIN†

- It is recommended that ***reactive lymphocyte*** is used to describe lymphocytes with a ***benign etiology*** and
- ***Abnormal lymphocyte*** with an accompanying description of the cells is used to describe lymphocytes with a ***suspected malignant*** or ***clonal etiology***.

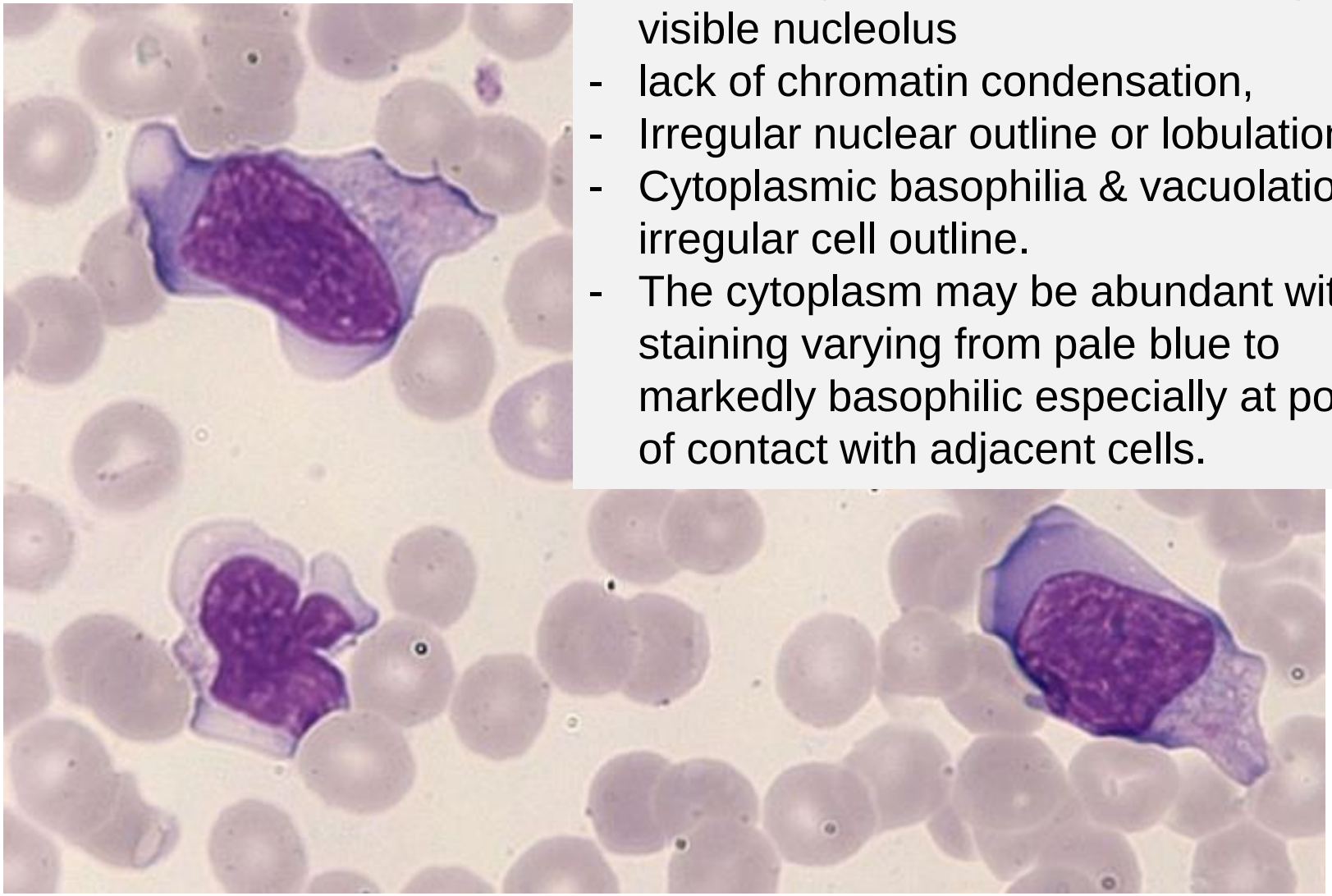
Reactive Lymphocyte

Infectious mononucleosis –

flowing basophilic cytoplasm

Abnormalities of reactive lymphocytes include:

- Increased cell size,
- Immaturity of the nucleus including a visible nucleolus
- lack of chromatin condensation,
- Irregular nuclear outline or lobulation,
- Cytoplasmic basophilia & vacuolation & irregular cell outline.
- The cytoplasm may be abundant with staining varying from pale blue to markedly basophilic especially at points of contact with adjacent cells.



The "WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues"

- As underlined by the Editors of 4th edition of the monograph,

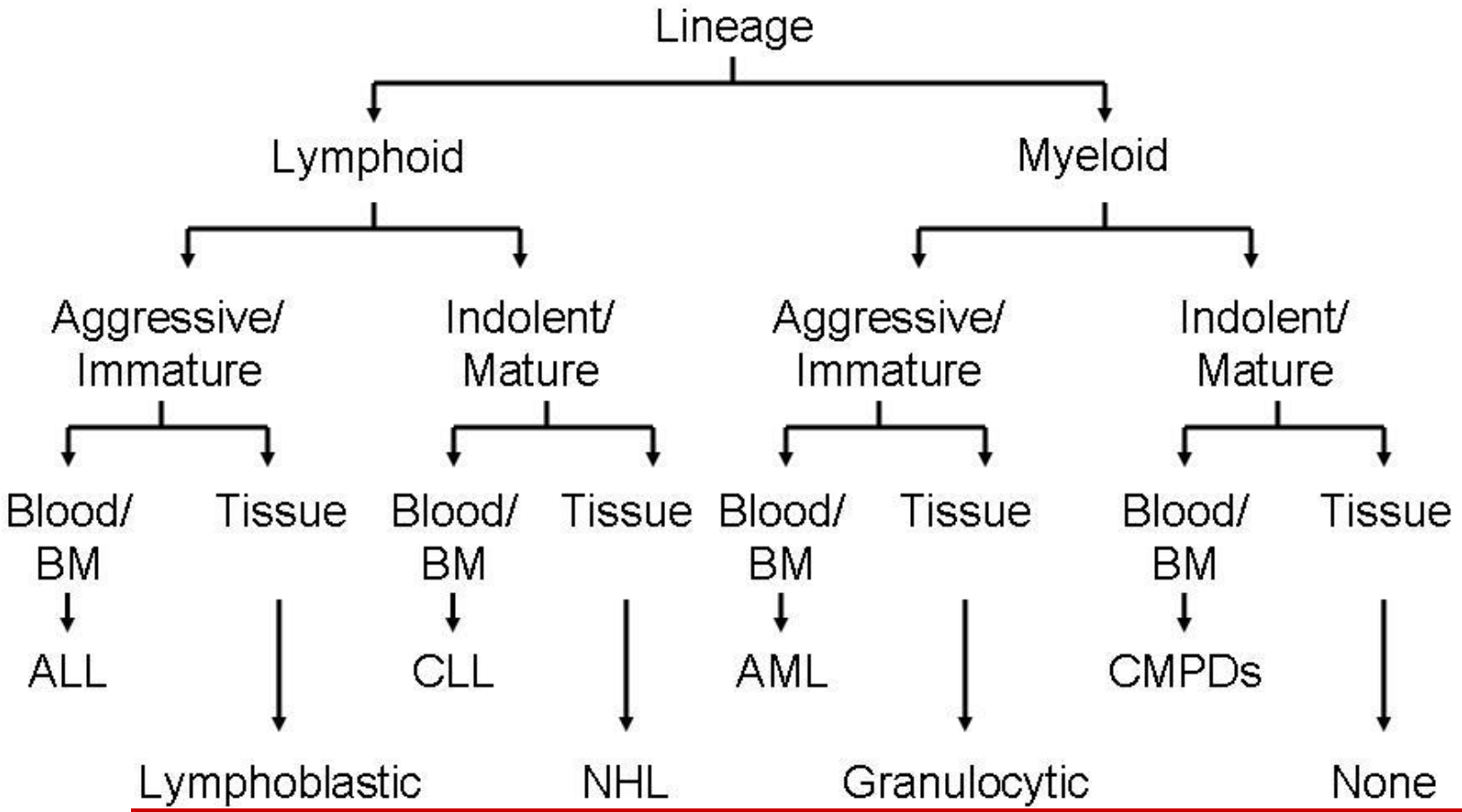
"classification is the language of medicine: diseases must be described, defined and named before they can be diagnosed, treated and studied.

A consensus on definitions and terminology is essential for both clinical practice and investigations."

Hematopoietic Neoplasms

- The majority of hematopoietic neoplasms can be classified and characterized according to **three characteristics:**
- **Lineage:** ***Myeloid (myelogenous)*** versus ***Lymphoid***
- **Survival:** ***Acute*** versus ***Chronic***
- **Predominant Sites of Involvement:** ***Blood and Bone Marrow*** versus ***Tissue.***

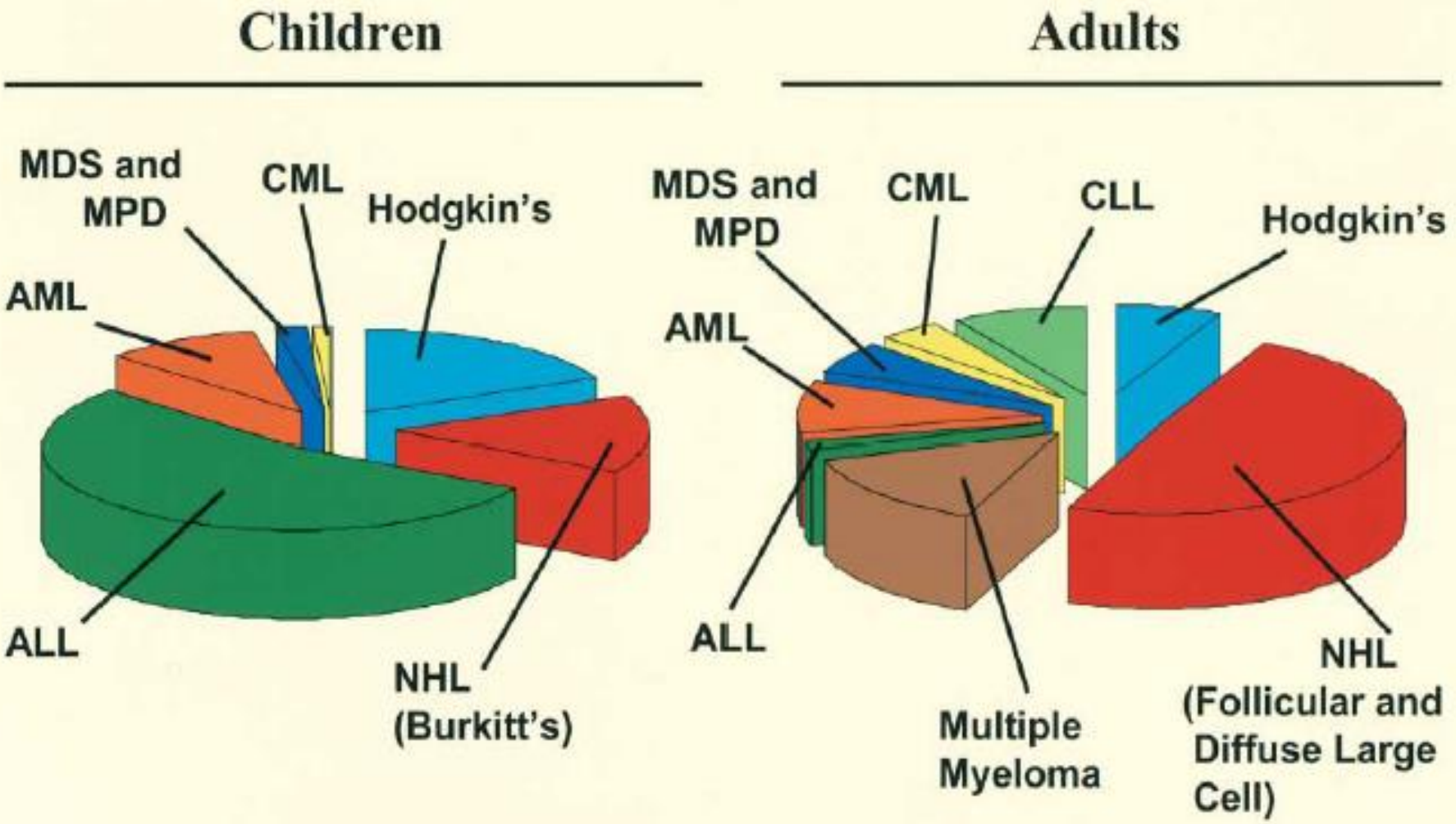
Classification of Hematopoietic Neoplasms



CMPD: Chonic Myeloproliferative Disorders

MPN: Myeloproliferative Neoplasms

Hematopoietic Malignancies



James and Shannon (2002) Cancer Cell 2:437

Background of the WHO Classification/Revision

The classification uses all available information :

- Morphology,
- Cytochemistry,
- Immunophenotype,
- Genetics
- Clinical features
- It is a consensus classification, Nearly 30 clinicians and clinical scientists from around the world members of the Myeloid and Acute Leukemia **Clinical Advisory Committee** (**CAC**)

Clinical manifestations

- Symptoms due to:
  - **Marrow infiltration**
    - Anemia: fatigue, pallor, weakness
    - Neutropenia: infection, fever
    - Thrombocytopenia: bleeding, bruising, petechia
  - Tissue infiltration: LAP, Splenomegaly, Leukemia cutis, L. meningitis
  - Leukostasis
  - Constitutional symptoms
  - Other (DIC)
- Usually short duration of symptoms

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clinical practice guidelines

Annals of Oncology 00: 1–14, 2016
doi:10.1093/annonc/mdw025

Acute lymphoblastic leukaemia in adult patients: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†

D. Hoelzer[1], R. Bassan[2], H. Dombret[3], A. Fielding[4], J. M. Ribera[5] & C. Buske[6] on behalf of the ESMO Guidelines Committee*

[1]ONKOLOGIKUM Frankfurt am Museumsufer, Frankfurt, Germany; [2]Hematology Unit, Ospedale dell'Angelo e Ospedale SS. Giovanni e Paolo, Mestre-Venezia, Italy; [3]Institut Universitaire d'Hematologie Hopital St Louis, Paris, France; [4]Cancer Institute, University College London, London, UK; [5]Department of Clinical Hematology, ICO-Hospital Germans Trias i Pujol, Jose Carreras Research Institute, Universitat Autonoma de Barcelona, Barcelona, Spain; [6]CCC Ulm, Institut für Experimentelle Tumorforschung, Universitätsklinikum Ulm, Ulm, Germany

Diagnostic work-up in adult ALL

- ***The initial diagnostic work-up must be carried out expeditiously and before any chemotherapy (within 1–2 working days) to:***
- Confirm ALL diagnosis,
- Distinguish B-cell precursor (BCP) ALL from T-cell ALL (T-ALL),
- Distinguish Burkitt leukaemia (B-ALL) from BCP-ALL (different treatment required),
- Distinguish Philadelphia (Ph) chromosome-positive (Ph+) ALL from Ph-negative (Ph−) ALL (different treatment required), and
- Shorten time to treatment start.

Diagnostic step	Results/ALL subsets	Recommendations
Morphology		
– Bone marrow and peripheral blood	– Lymphoid/undifferentiated blasts (≥20% bone marrow involvement)	Mandatory
– Cerebro-spinal fluid	– FAB L3 morphology in Burkitt leukaemia	Recommended
	– CNS involvement	Mandatory
Immunophenotype		
– MPO (differential diagnosis versus AML)	– MPO negative; B/T markers >20% (CD3, CD79a >10%)	Mandatory
– B-lineage markers: CD19, CD79a, cCD22 (at least 2); others: TdT, CD10, CD20, CD24, cIgM, sIg (kappa or lambda)	– B-lineage ALL: Pro-B/B-I (CD19/CD79a/cCD22+) Common/B-II (CD10+/cIgM−) Pre-B/B-III (cIgM+/sIg−) Mature-B/B-IV (sIg+)	Mandatory
– T-lineage markers: cCD3; others: TdT, CD1a, CD2, CD5, CD7 CD4, CD8, TCR α/β or γ/δ	– T-lineage ALL: Pro-T/T-I (cCD3/CD7+) Pre-T/T-II (CD2/CD5) Cortical-T/T-III (CD1a+) Mature-T/T-IV (CD3+/CD1a−)	Mandatory
– Stem/myeloid cell markers (variable): CD34, CD13, CD33, CD117		

Cytogenetics/genetics		
– Cytogenetics/FISH/RT-PCR	– ALL with adverse clinico-biological features: Ph+ ALL (rapid detection, to TKI therapy) t(4;11)+ ALL t(1;19)+ ALL other high-risk cytogenetics	Mandatory
– CGH/SNP/GEP/NGS	– ALL with adverse clinico-biological features: Ph-like ALL ETP ALL *NOTCH1/FBW7*-unmutated/*RAS/PTEN*-altered T-ALL *IKZF1*, CLRF2, *MLL*, *TP53*, *CREBBP*, *RAS* alterations	Recommended for new clinical trials
MRD study		
– MRD marker(s): LAIP (immunophenotype)/molecular probe (PCR)	– MRD-based risk classification	Mandatory
Storage of diagnostic material		
– Cell banking/storage of DNA/RNA/protein lysates	– Additional/future studies	Highly recommende
HLA typing		
– Patient/siblings	– Early application of SCT if required	Recommended

clinical practice guidelines

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Acute myeloblastic leukaemias in adult patients: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†

M. F. Fey[1] & C. Buske[2], on behalf of the ESMO Guidelines Working Group*

[1]*Department of Medical Oncology, Inselspital and University of Bern, Bern, Switzerland;* [2]*Comprehensive Cancer Center Ulm, Institute of Experimental Cancer Research, University Hospital Ulm, Ulm, Germany*

These Clinical Practice Guidelines are endorsed by the Japanese Society of Medical Oncology (JSMO)

Diagnostic work-up in AML

- Diagnostic work-up of AML must in include **morphology** of PB & BM, **cytogenetics** & **molecular genetics** assessed before start of therapy

- Bone marrow aspirate and biopsy as well as peripheral blood films
- Immunophenotyping of peripheral blood and bone marrow aspirates
- Cytogenetics and molecular genetics (PCR and FISH techniques)
- Routine chemistry including liver and kidney parameters
- Coagulation profile
- Blood group and HLA typing of patient and family members
- Radiology to include dental survey as well as CT scan of chest and abdomen (or chest X-ray and abdominal ultrasound)
- Sperm preservation in men (according to patient preference)
- Serum pregnancy test in female patients

What is the first step in suspected patients to Acute Leukemia?

Requesting Lab. Tests

CBC & PBS

The First CBC in Diagnosis of childhood acute lymphoblastic leukemia

Article · January 2014

Moussavi F [1] , Hosseini SN [2,*] ,Saket S [3] , Derakhshanfar H [4]

Int J Med Invest 2014; vol 3; num 1; 9-12 http://www.intjmi.com

- ALL is primarily detected by PB which is a sign of BM defect
- The importance of CBC test for leukemias detection (especially ALL):
  - CBC is common,
  - available,
  - reasonable, and
  - the specimens can be easily taken

Accurate & Precise evaluation of the CBC & its result is necessary

CBC finding in ALL,

n:97;Age: 1mon.-14y/o; F:49(50.5%), M:48(49.5%)

- ***96.9% of the cases had abnormal CBC results and only 3 cases (3.1%) had normal CBC results***
- 91% neutropenia, 90% Low PLT, 90% anemia, 77% pancytopenia.
- Leukocytosis in 39%, blast in 25%, eosinophilia 4%, and NRBC in 3% of the participants.
- 3 patients (3 %) were detected with normal CBC and

7 patients (7 %) were detected with abnormality in one type of cell.

Systemic approach: CBC finding at presentation

- **AML:** Hematopoietic failure (markedly reduced RBC, absolute neutrophil and platelet counts)
- **MDS:** Cytopenias key Virtually never have leukocytosis at presentation
- **MDS/MPN:** Hybrid blood picture

At least one elevated and one reduced HP lineage

- **MPN:** At least one elevated linage (cytosis)

No cytopenias in stable phase

Cell Counters

- **8-9 Parameters Cell Counters:**

  WBC, RBC, Hb, Hct, Plt, MCV, MCH, MCHC, RDW

- **Partial Differential Cell Counters:** 9 parameters

  + % / # Granulocytes, Lymphocytes, **Mixed(Mid)** cells (Eos, Baso, Mono,Lymph Variant, Blast)

- **Full Diff Cell Counters:** 9 parameters + % / # :

  Neutrophils, Eosin., Baso., Lymph., Mono.

Flags, Histo & Cytograms, Specific Parameters (LUC, MPXI,..)

CBC IN ACUTE LEUKEMIA

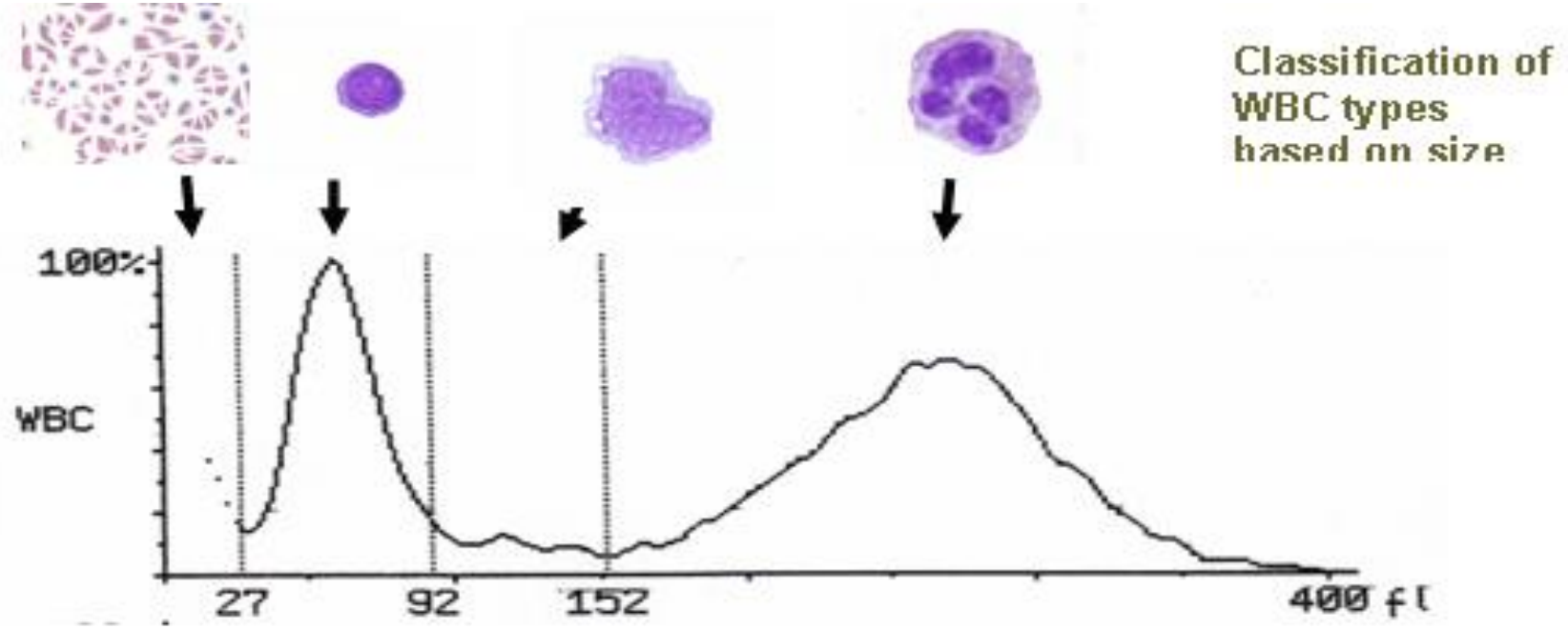
- ***Reduced Hb , Hct , RBC***
- ***WBC (leukopenia, normal, leukocytosis & hyperleukocytosis)***
- ***Platelet***
- ***RBC indices***
- ***WBC differential by automation***
- ***Peripheral blood smear***

PJMS- Volume 2 Number 2: July- December 2012
Original Article

White blood cell suspect flags and white blood cell histogram pattern in acute leukemias

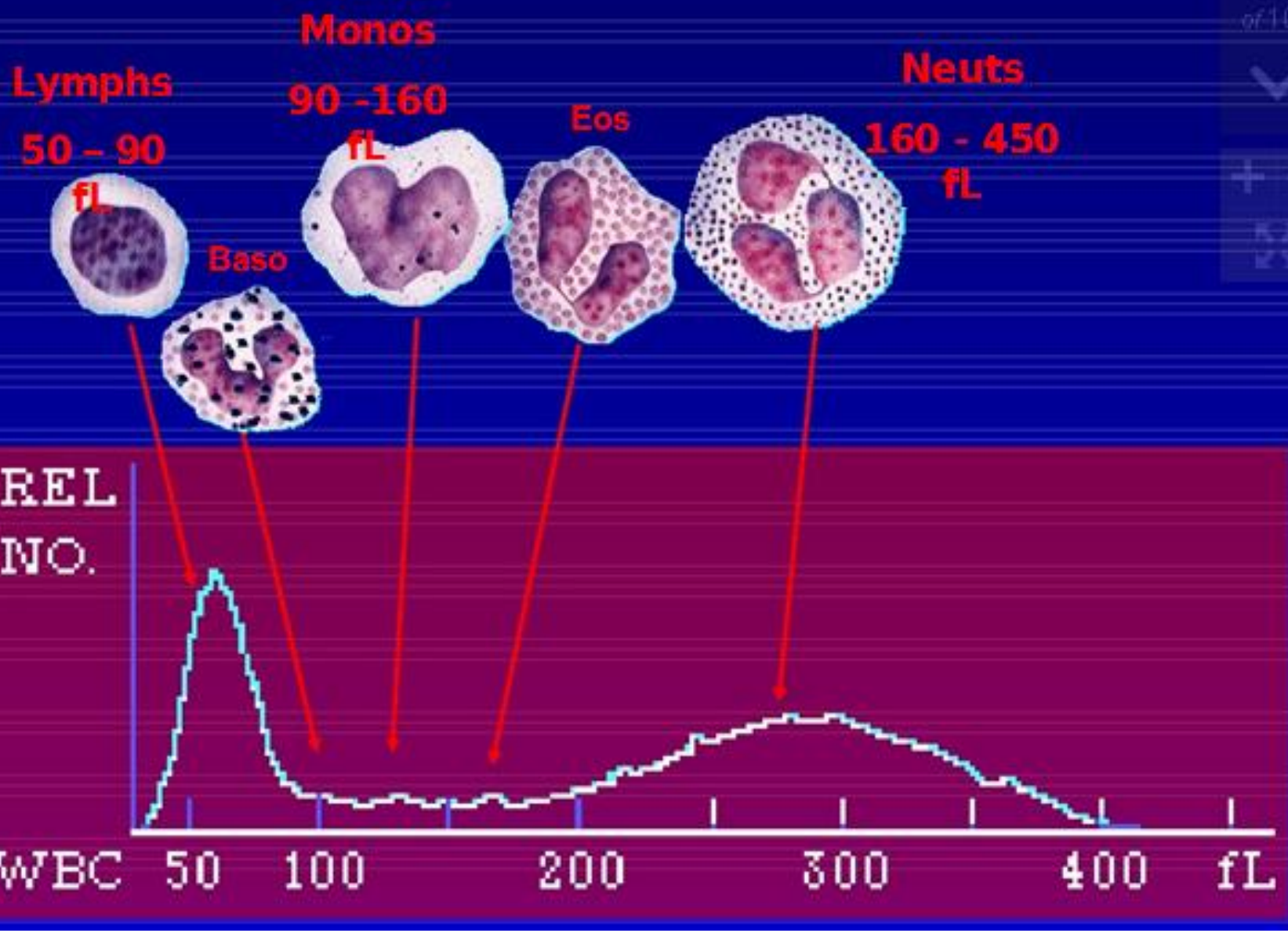
Ostwal Kunal[1], Wilkinson Anne[2]

- ***The histogram is a representation of the sizing of the leukocytes. The differentiation is as follows:***



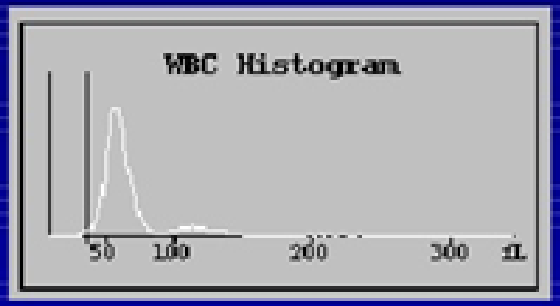
Lymphocytes: 35-92 fL
Mid or Mixed Cells: 92-152 fL
Granulocytes: 152-450 fL

Leukocyte Histogram

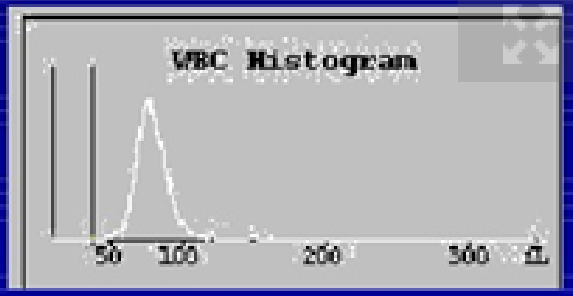


WBC HISTOGRAMS

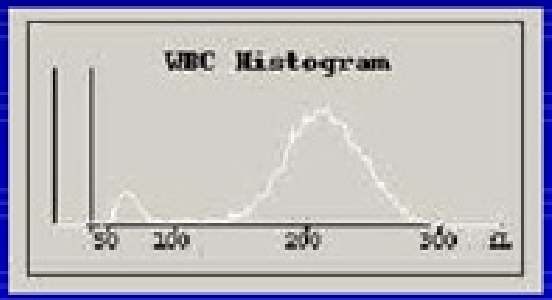
ImmNE1 & ImmNE2



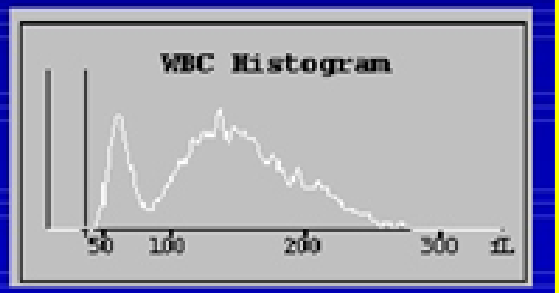
Lymphocytosis



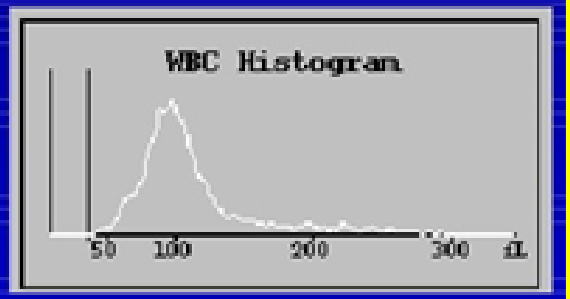
Variant Lymph



ImmNE2

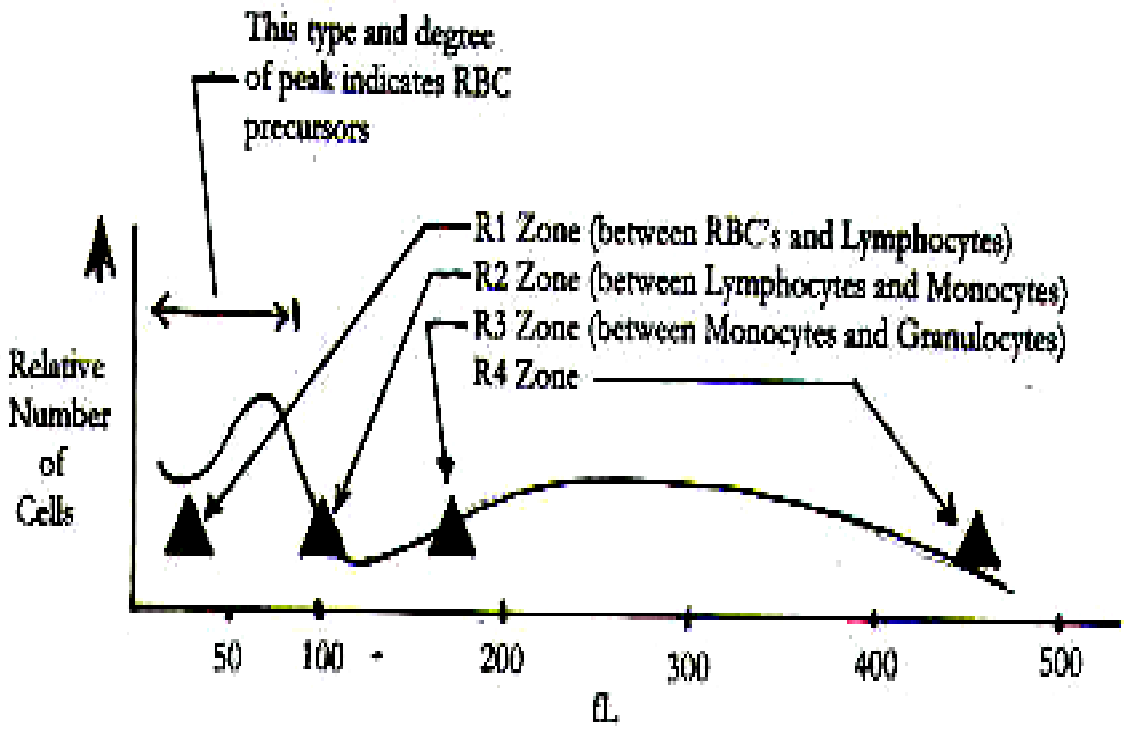


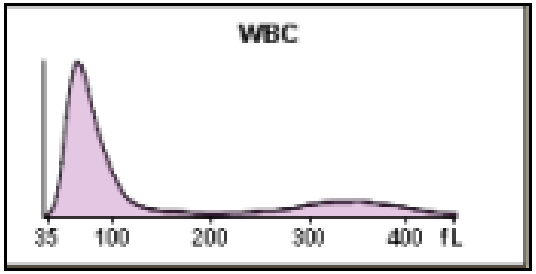
Eosinophilia



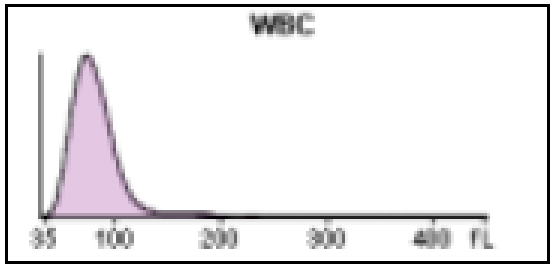
Blasts

Flags In Partial Diff Cell Counters

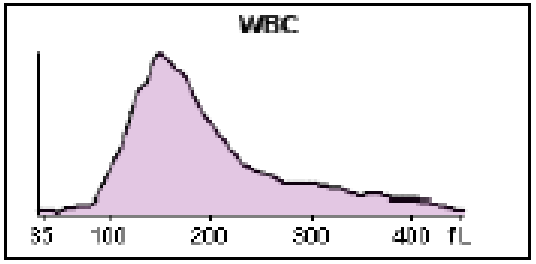




Lymphocytosis



Variant Lymph



Blasts

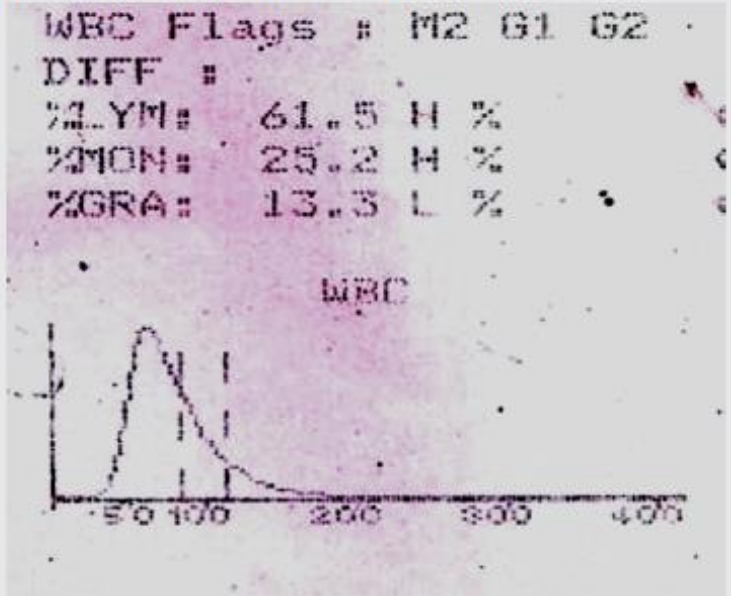
The Following Table Lists The Region (R) Flags And The Abnormalities They May Represent:

Abnormality	Region	R Flag
Erythrocyte precursors (NRBCs) Nonlysed erythrocytes Giant and/or clumped platelets Heinz body Malaria	Far left(<35fL)	**R1**
Blasts Basophilia Eosinophilia Plasma cells Abnormal/variant lymphs	**Between lymphs and monos**	**R2**
Abnormal cell populations Eosinophilia Immature granulocytes	Between mons and granulocytes	**R3**
Increased absolute granulocytes	Far right(>450fL)	**R4**
Multiple flags		**RM**

Histogram of ALL vs. AML & their flags

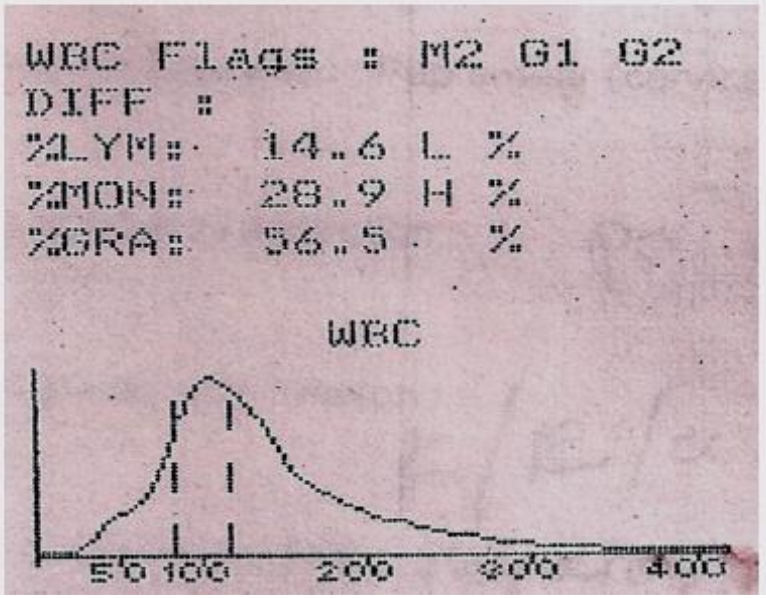
ALL

Figure 1:
Histogram of ALL showing M2 G1 G2 flags and a high differential count in the lymphocyte region



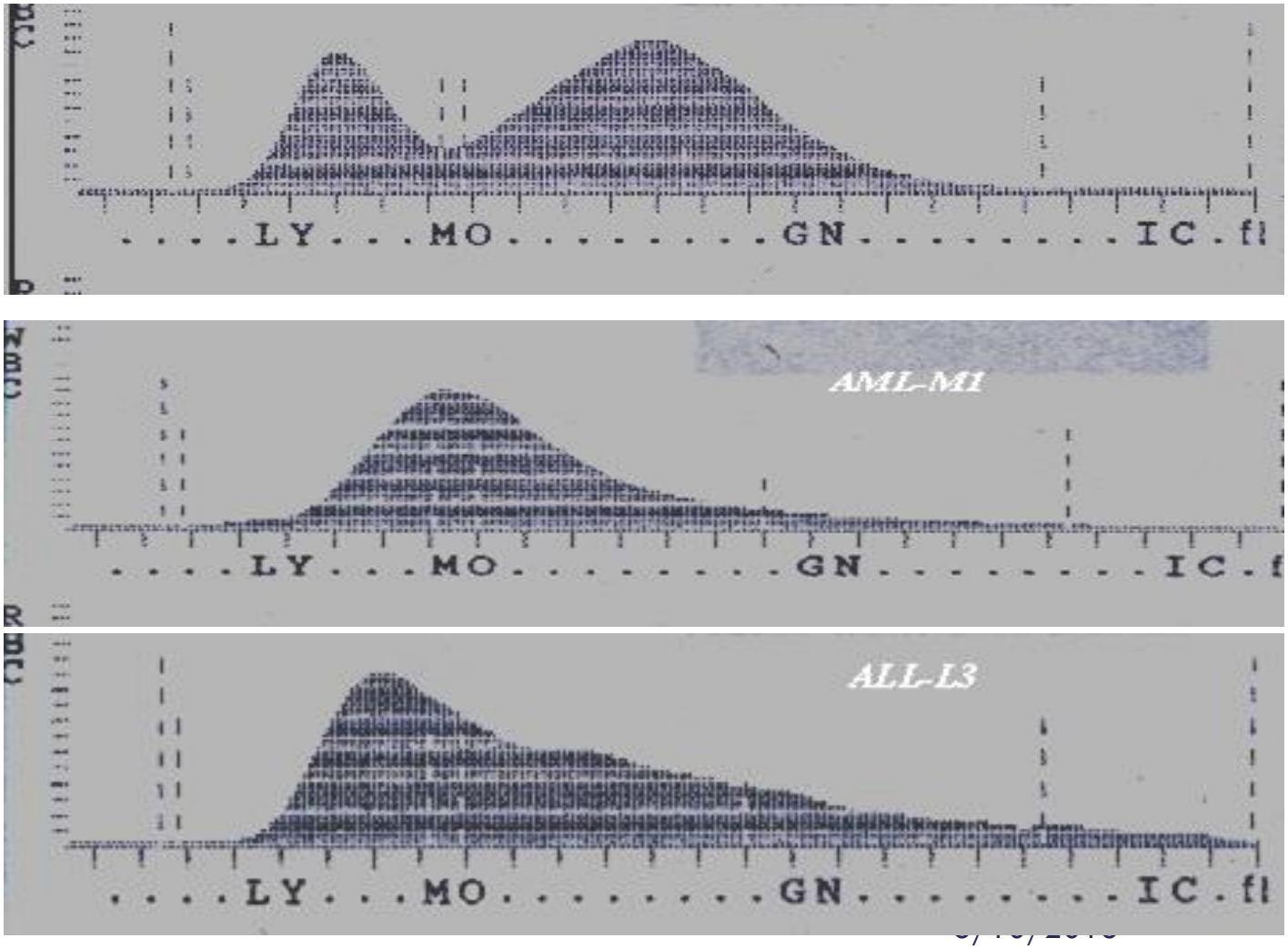
AML

Figure 2:
Histogram of AML showing M2 G1 G2 flags and a high differential count in the monocyte and granulocyte region



Partial Diff Cell Counter

LEUKOCYTE CYTOGRAM, NORMAL vs AML-M1 & ALL-L3



Full Diff Cell Counters

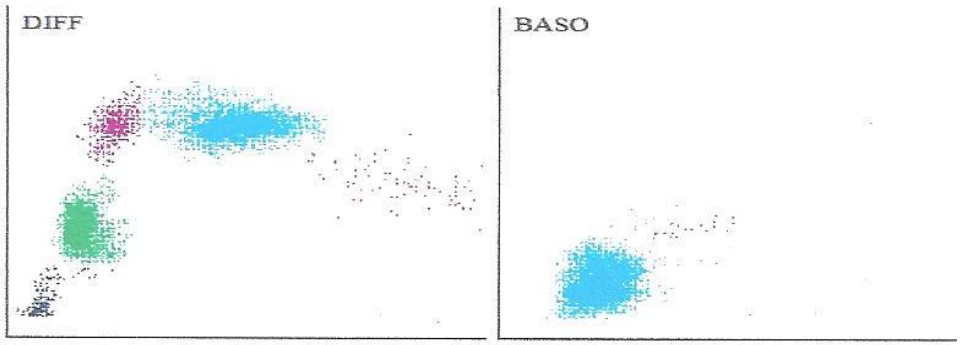
Normal CBC

Name: Age: Year

Test Time: 02/14/2012 08:24 AM Gender: Sample ID: 748

Parameter	Result	Unit	Ref.range
WBC	5.76	10^3/uL	4.00-11.00
Neu#	3.27	10^3/uL	1.80-7.00
Lym#	1.90	10^3/uL	1.50-4.00
Mon#	0.46	10^3/uL	0.12-0.80
Eos#	0.11	10^3/uL	0.02-0.35
Bas#	0.02	10^3/uL	0.00-0.20
Neu%	56.9	%	50.0-70.0
Lym%	33.0	%	20.0-40.0
Mon%	7.9	%	3.0-12.0
Eos%	1.9	%	0.5-5.0
Bas%	0.3	%	0.0-1.0

Histogram and Flags



Pink: Mono. *Red: Eos.* *Blue: Neu+Lym+Mon+Eos*

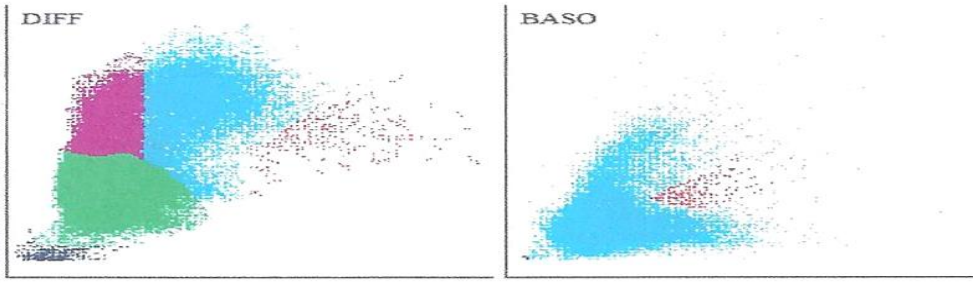
Green: Lym. *Blue: Neut. + Baso* *Red: Baso.*

Full Diff Cell Counters

Normal vs. Acute Leukemia

Parameter		Result	Unit	Ref.range
WBC	H	92.66	10^3/uL	4.00-11.00
Neu#	H	14.27	10^3/uL	1.80-7.00
Lym#	H	66.99	10^3/uL	1.50-4.00
Mon#	H	10.66	10^3/uL	0.12-0.80
Eos#	H	0.46	10^3/uL	0.02-0.35
Bas#	H	0.28	10^3/uL	0.00-0.20
Neu%	L	15.4	%	50.0-70.0
Lym%	H	72.3	%	20.0-40.0
Mon%		11.5	%	3.0-12.0
Eos%		0.5	%	0.5-5.0
Bas%		0.3	%	0.0-1.0

Histogram and Flags



RBC

Comments

Manual Diff Cmt

Neut: 13%

Band: 4%

Lym: 43%

Mon: 3%

Eo: 1%

Blast: 35%

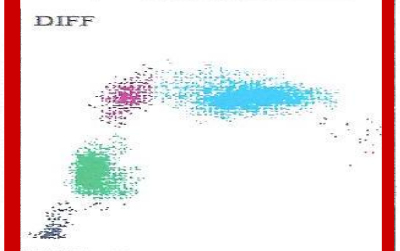
Myelocyte: 1%

2nRBC/100WBC

Age:

Samp

His



RBC

WBC Flag
Abn./Atypical Lym?
Neutrophilia
Lymphocytosis
Monocytosis
Basophilia
Leucocytosis

Full Diff Cell Counters

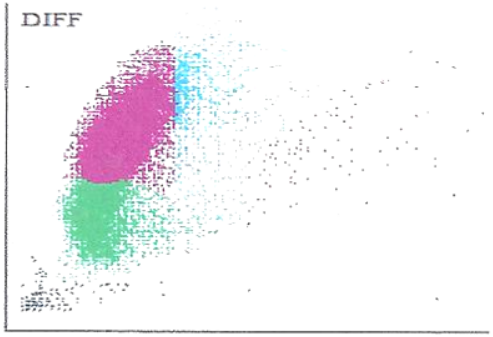
Normal vs. Acute Leukemia

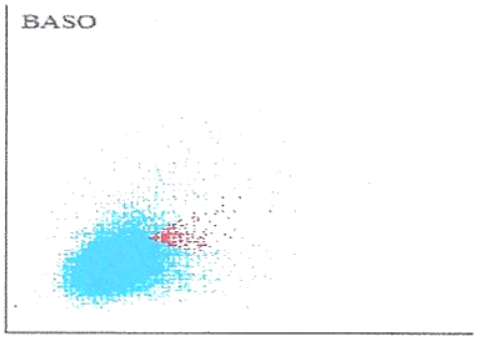
WBC	H	17.09	10^3/uL	4.00-11.00
Neu#		2.17	10^3/uL	1.80-7.00
Lym#	H	4.60	10^3/uL	1.50-4.00
Mon#	H	9.98	10^3/uL	0.12-0.80
Eos#		0.12	10^3/uL	0.02-0.35
Bas#	H	0.22	10^3/uL	0.00-0.20
Neu%	L	12.7	%	50.0-70.0
Lym%		26.9	%	20.0-40.0
Mon%	H	58.4	%	3.0-12.0
Eos%		0.7	%	0.5-5.0
Bas%	H	1.3	%	0.0-1.0

Blasts with gran

Comments

Manual Diff Count.
Lymph. 20%
Monocytic cells 27%
Blasts 28%
Promyelocyte 2%
Myelocyte 7%





Pink: Mono. *Red: Eos.* *Blue: Neu+Lym+Mon+Eos*

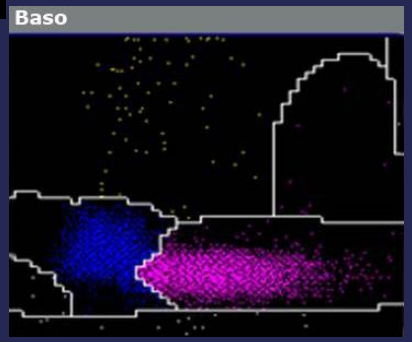
Green: Lym. *Blue: Neut. + Baso* *Red: Baso.*

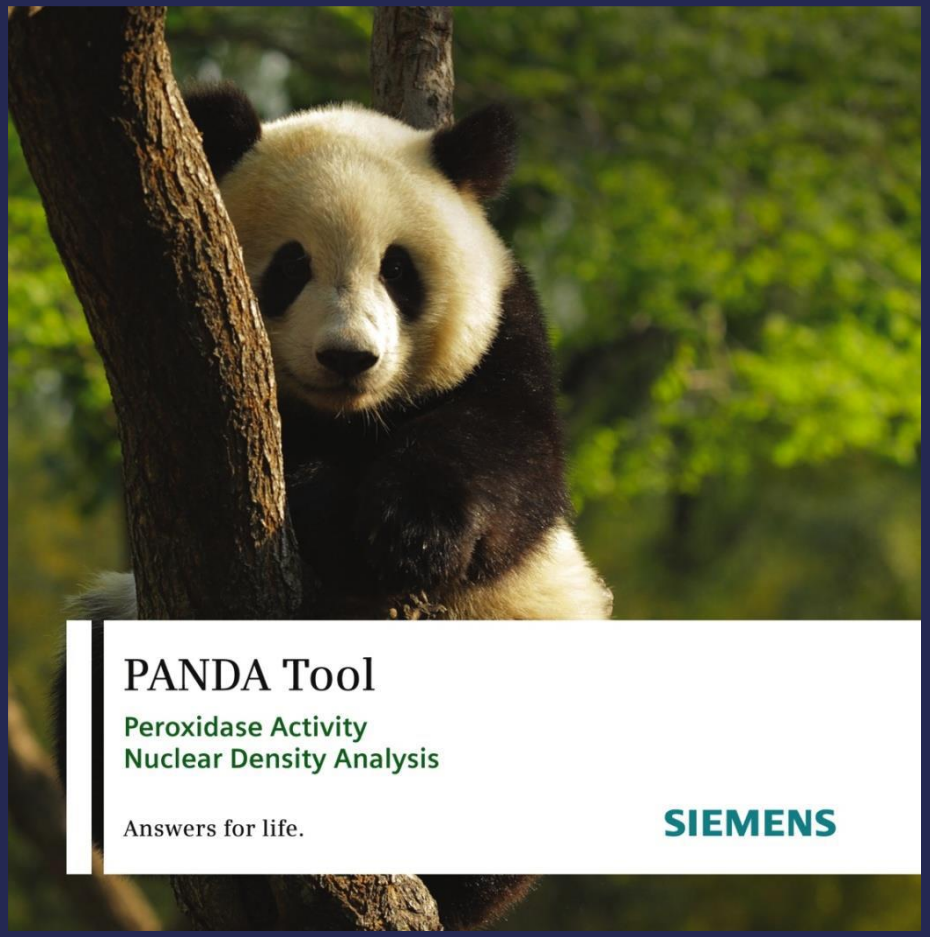
WBC Flag
Abn. /Atypical Lym?
Lymphocytosis
Monocytosis
Basophilia

RBC Flag
RBC Abn Distribution
Anisocytosis
Anemia

PLT Flag
Thrombocytopenia

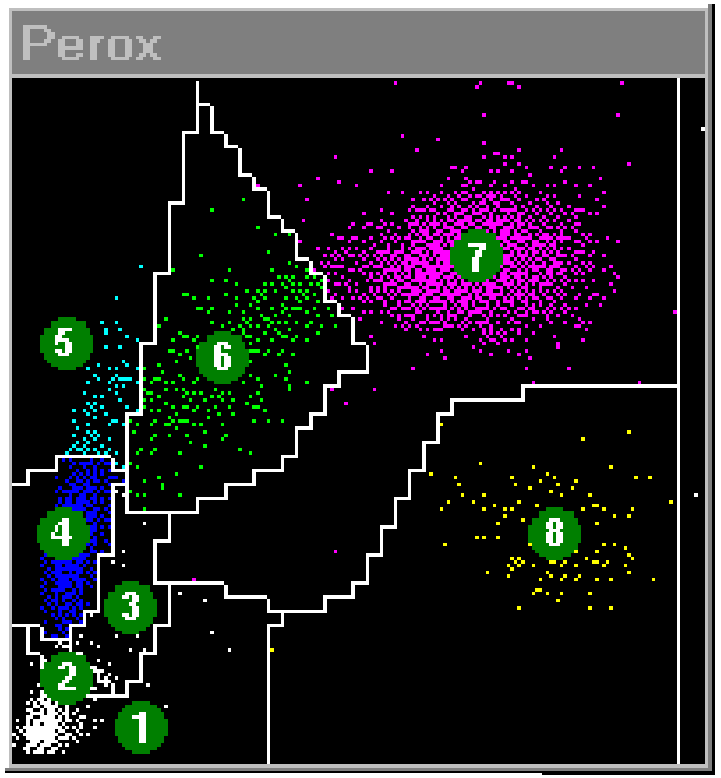
PANDA Peroxidase Activity and Nuclear Density Analysis

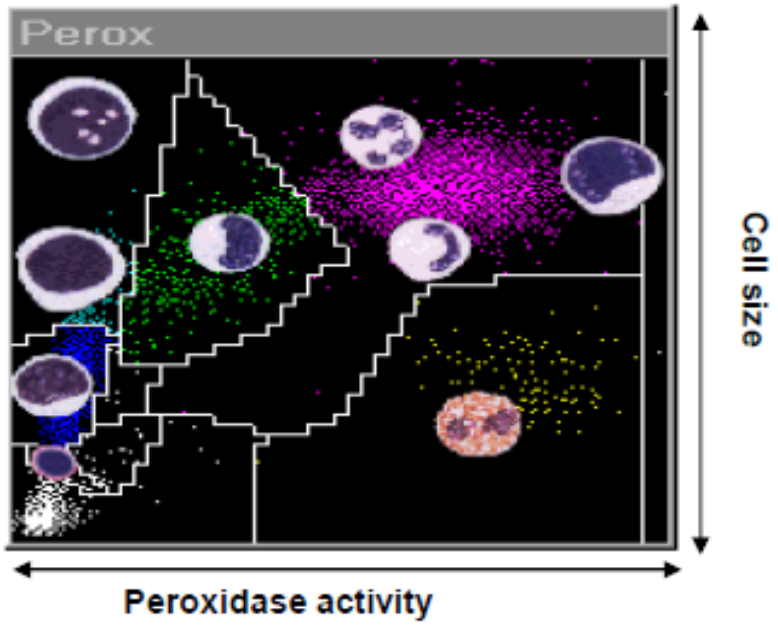




The peroxidase channel

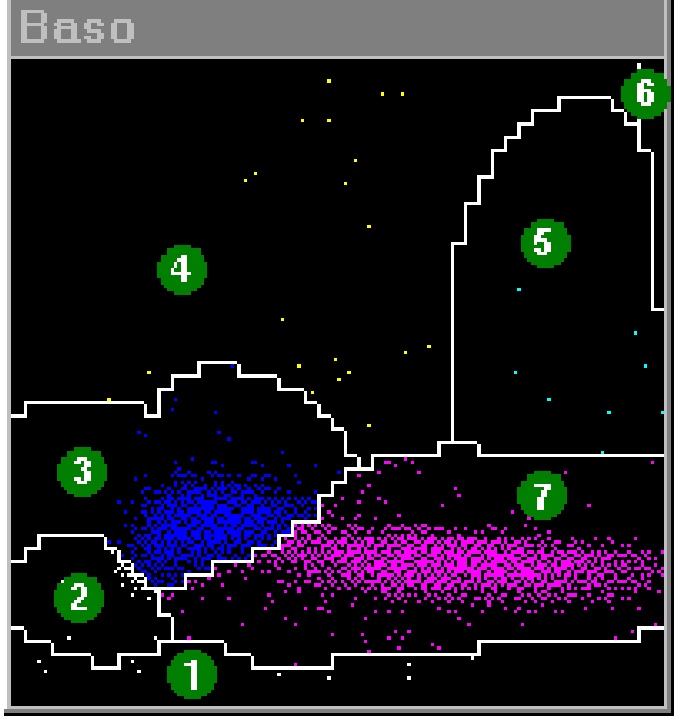
1. Noise
2. Nucleated RBCs
3. Platelet clumps
4. Lymphs and basos
5. LUC
6. Mono
7. Neutrophils
8. EOS

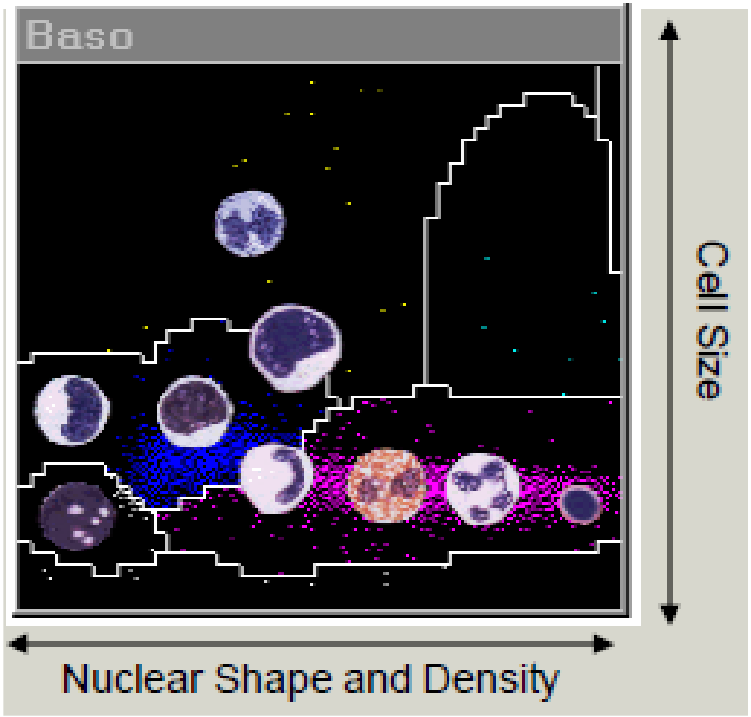




Basophil / nuclear lobularity channel

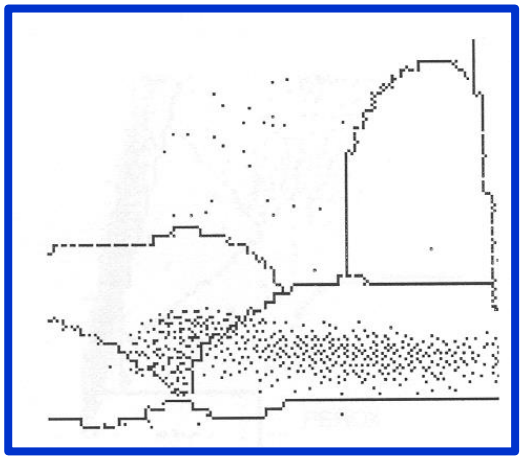
1. Noise
2. Blast cell nuclei
3. Mononuclear WBCs
4. Basophils
5. Baso Suspect
6. Saturation
7. Polymorphonuclear WBCs



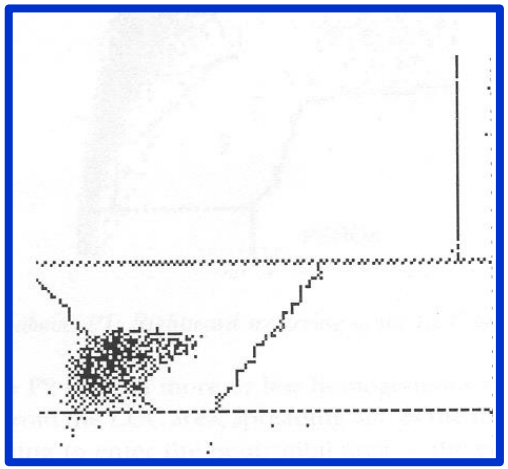


“PANDA” in Haematopoietic Malignancies

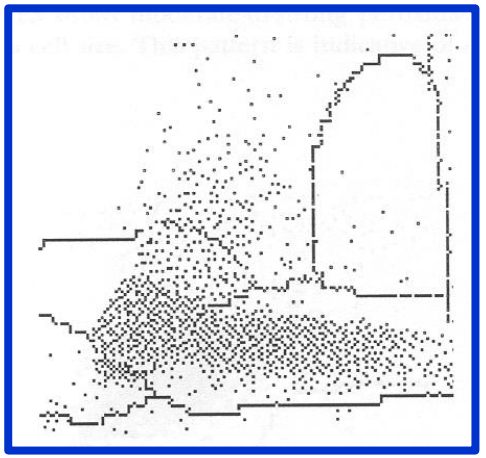
- Peroxidase Activity & Nuclear Density Analysis
- Distinct classified cytogram pattern:
- Basophil channel: D0 -D2



D0



D1



D2

THE BIOMEDICAL SCIENTIST
FEBRUARY 2012

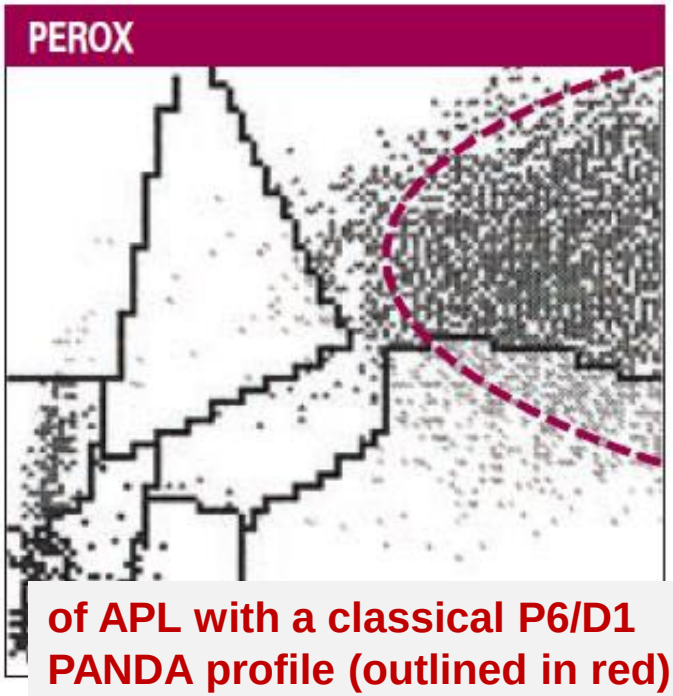
Diagnosis of acute promyelocytic leukaemia: the PANDA approach

- **APL**, once an almost uniformly fatal disease, is now treatable and curable. However, ***early identification*** is key to achieving a satisfactory outcome for patients.
- **EARLY DEATH RATE**
- As bleeding is a major cause of early death, ***APL is a medical emergency*** that demands early recognition and immediate treatment.

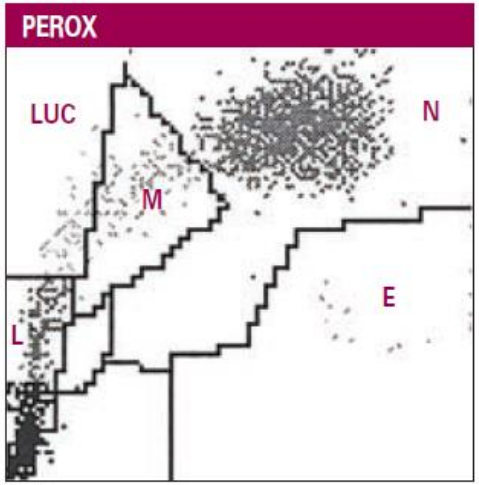
EARLY DEATH RATE

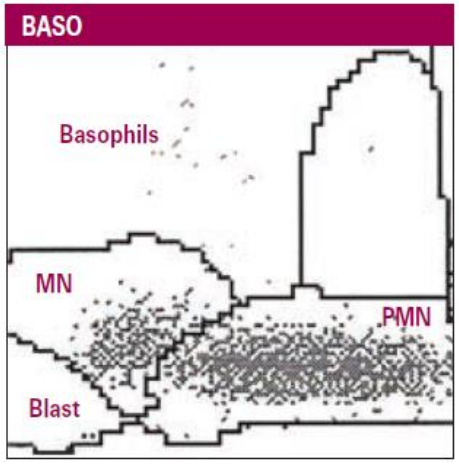
- Previous data: early death rate of approximately **5–10%** within the **1st month** of starting treatment with ATRA.
- Alarmingly, the current study showed an overall early death rate of **17.3%.**
- Furthermore, this figure rose significantly with increasing age to **24.2%** for patients aged **≥55 years** compared with **12.3%** among patients aged **≤34 years**.
- ***The study concluded with a call for the need to educate clinicians and allied healthcare professionals in the recognition of the disease to ensure early treatment and thus reduce early death and improve cure rates in APL.***

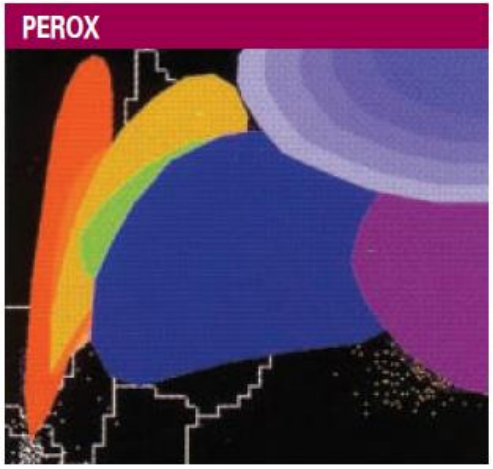
Normal Peroxidase & Basophil Cytogram

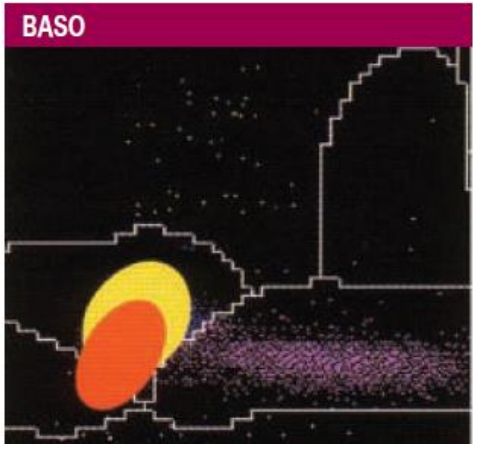


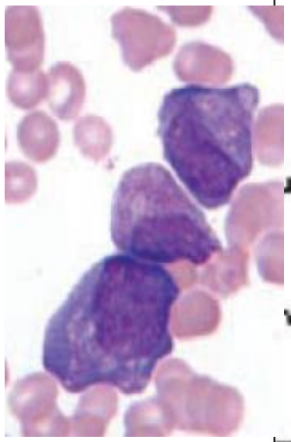
of APL with a classical P6/D1 PANDA profile (outlined in red).

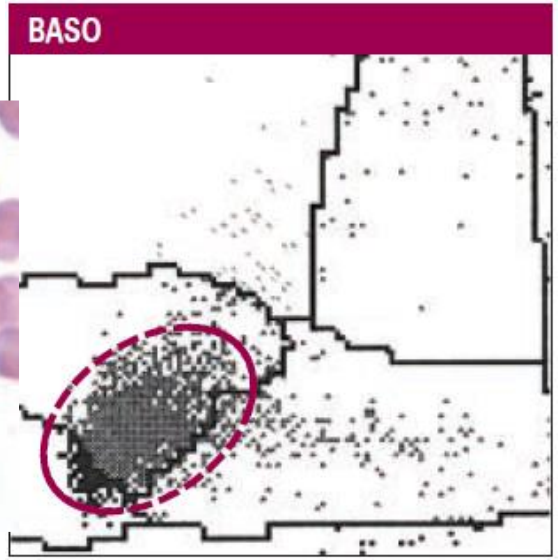










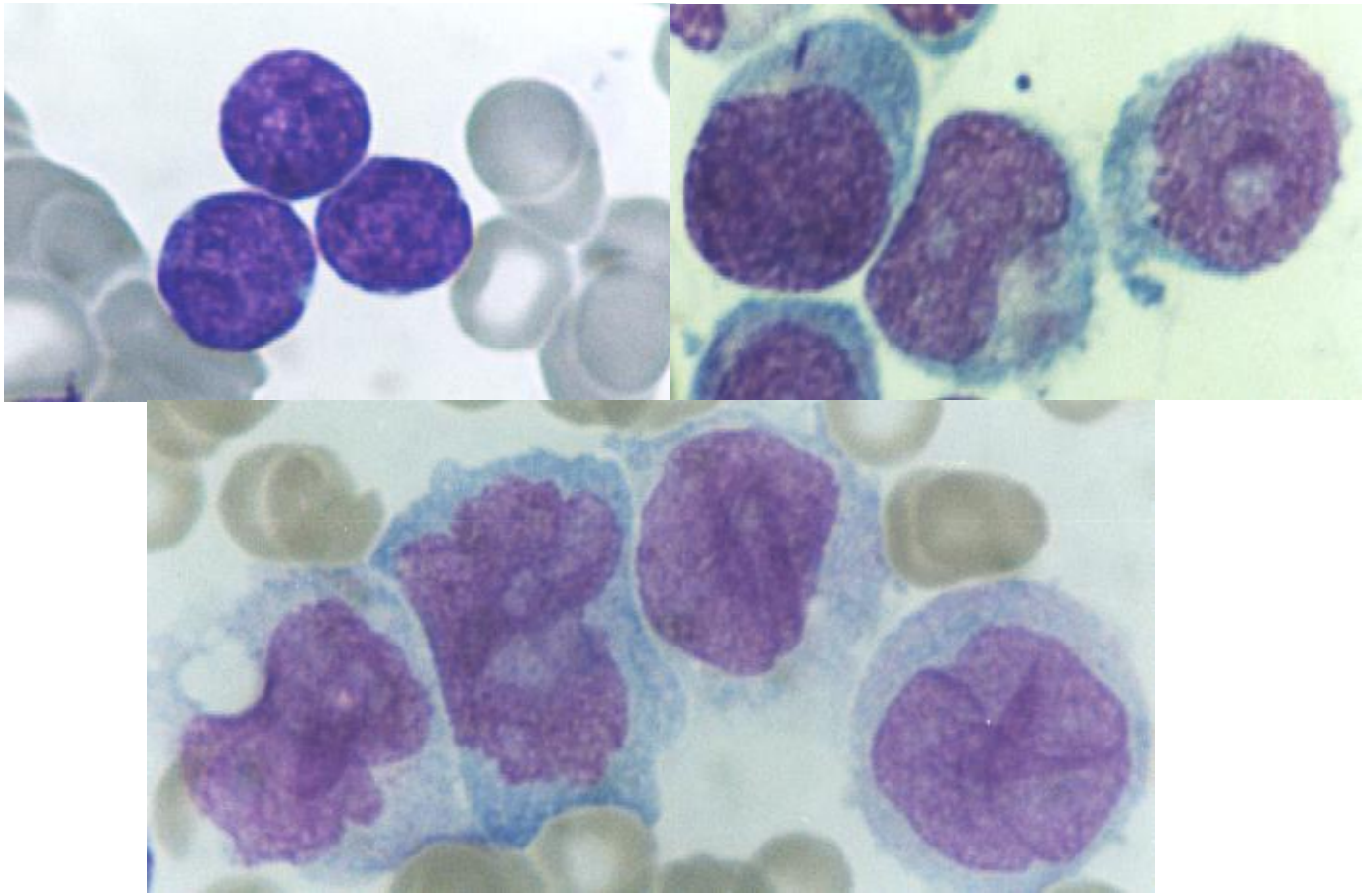


The PANDA system. Peroxidase activity is graded as P0 (orange), P1 (pale orange), P2 (yellow), P3 (green), P4 (dark blue), P5 (light blue) or P6 (purple). Nuclear density is graded as D0 (yellow) or D1 (orange).

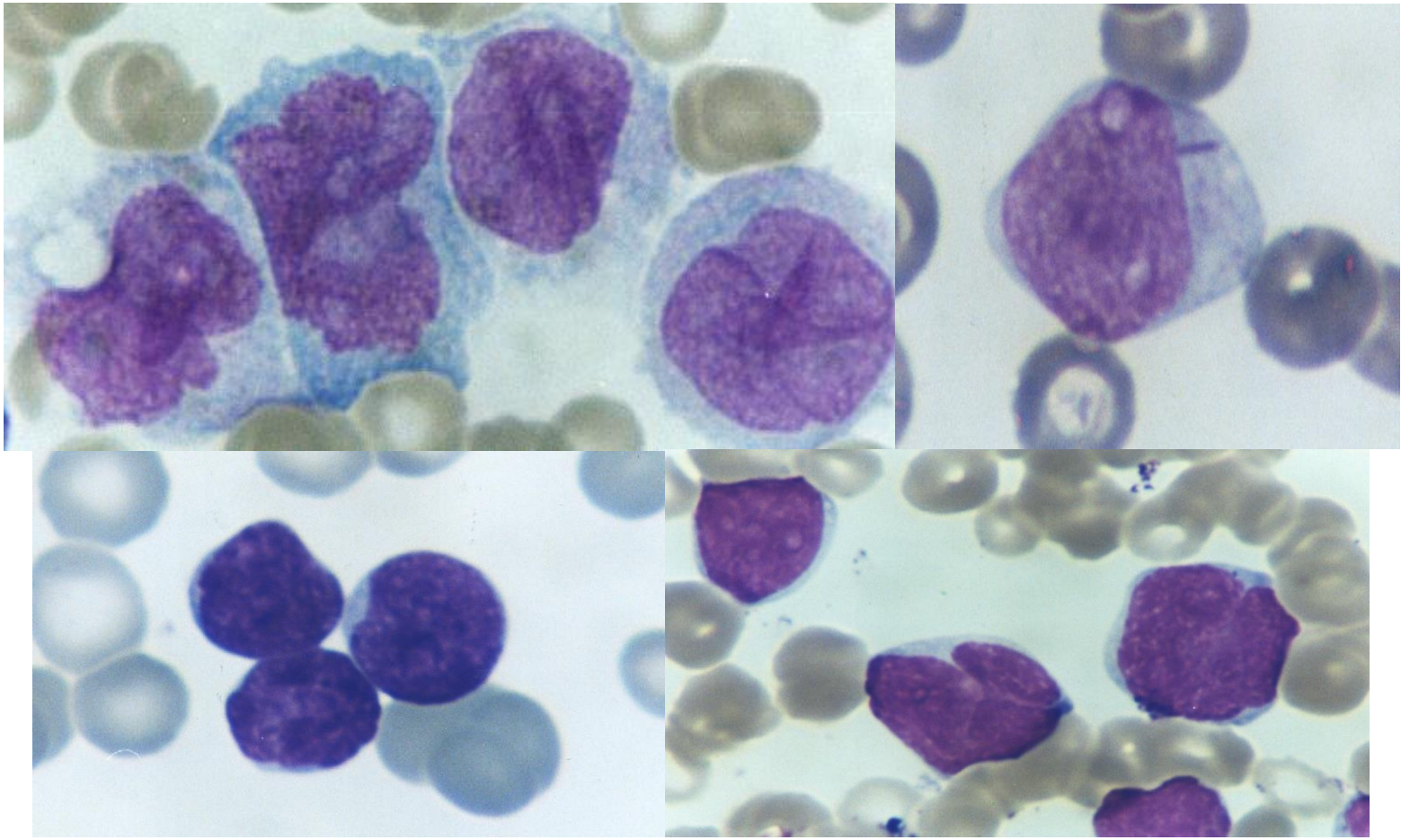
GENERAL MORPHOLOGY OF LEUKEMIC BLASTS

- *Cell size*
- *Nuclear cytoplasmic ratio*
- *Nuclear shape*
- *Nuclear chromatin pattern*
- *Nucleoli*
- *Cytoplasm*
- *Granules*
- *Auer Rods*
- *Vacuoles*

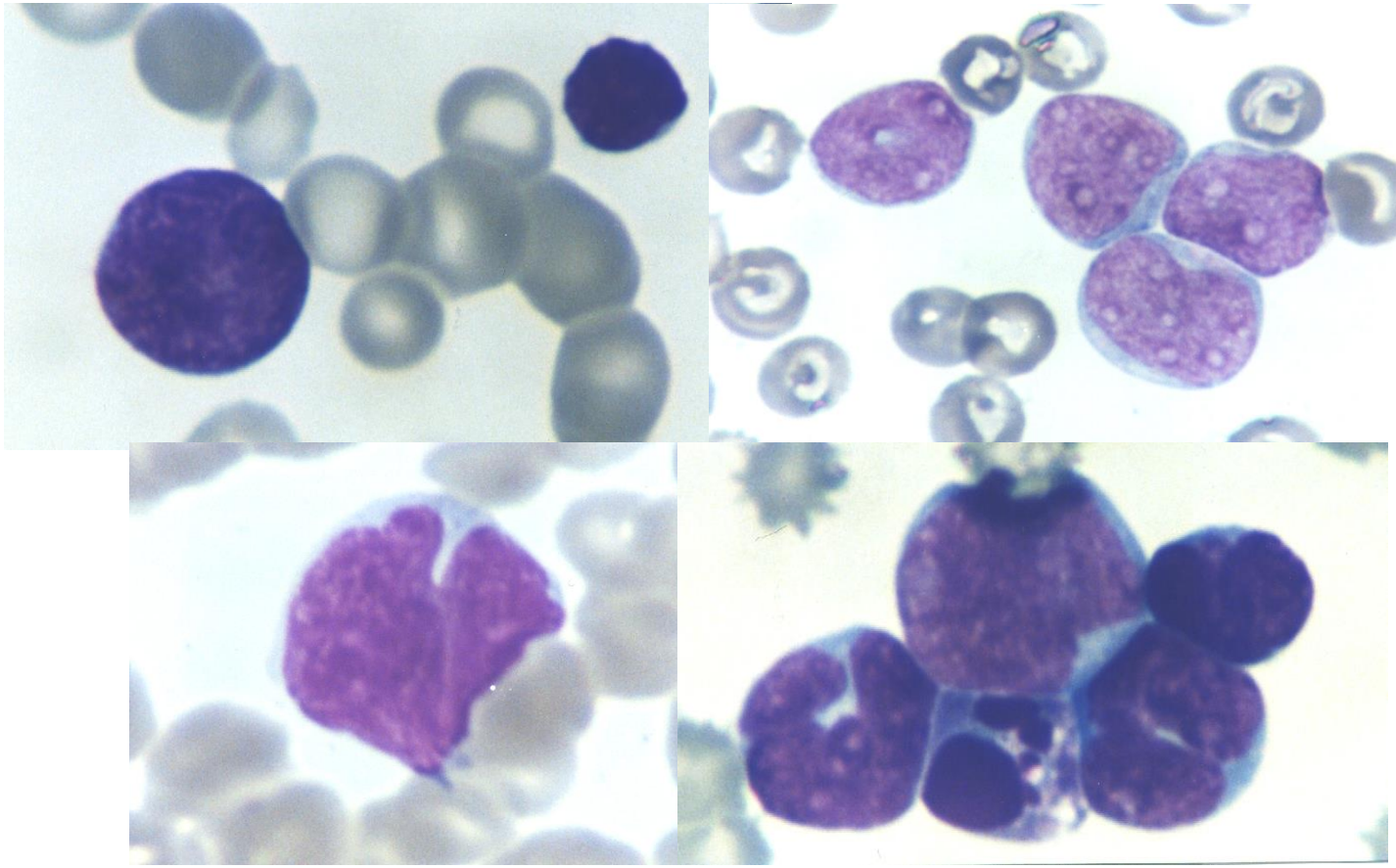
SIZE OF BLASTS



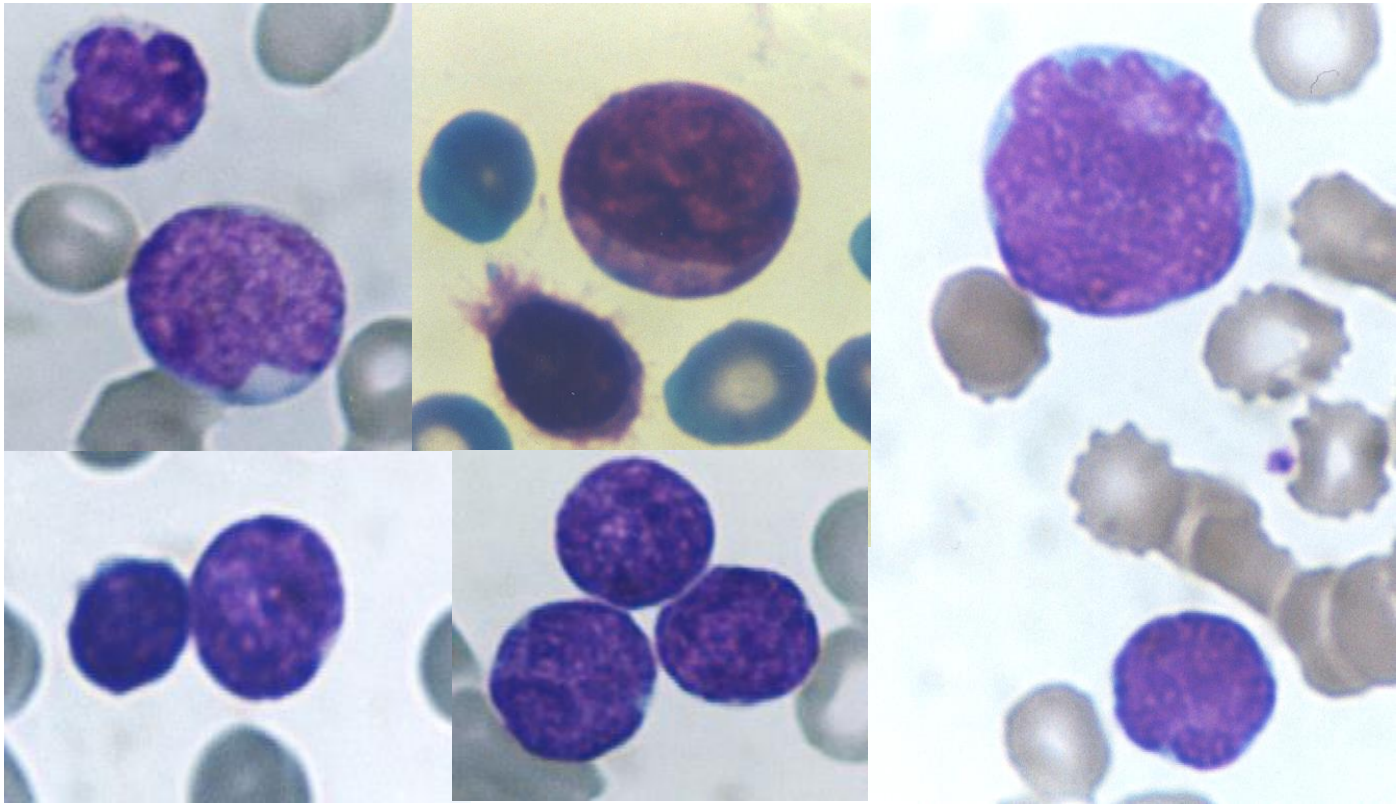
NUCLEAR CYTOPLASMIC RATIO



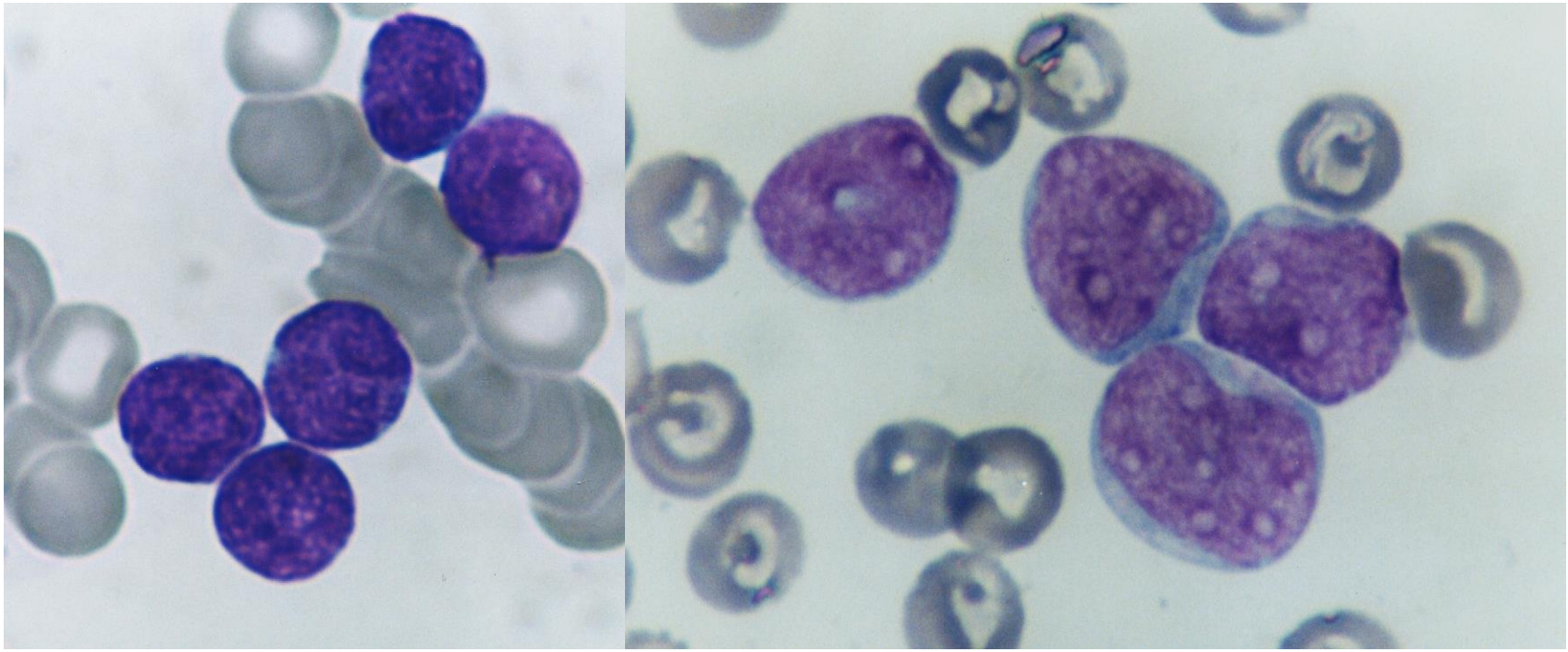
NUCLEAR SHAPE



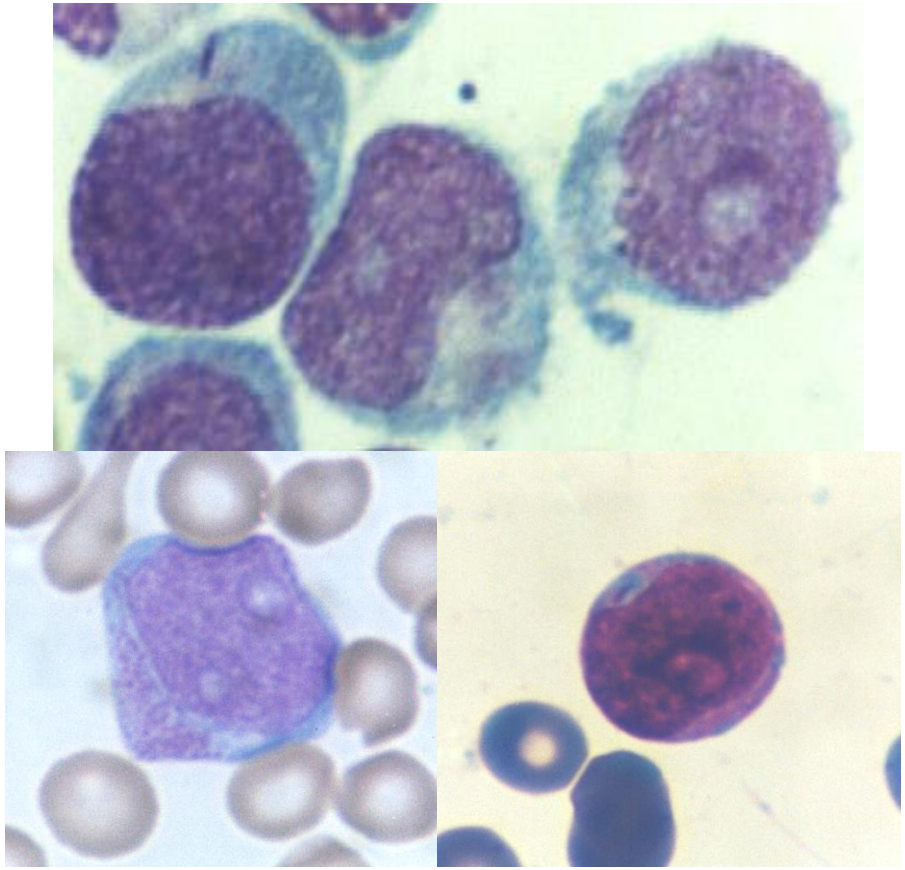
NUCLEAR CHROMATIN PATTERN



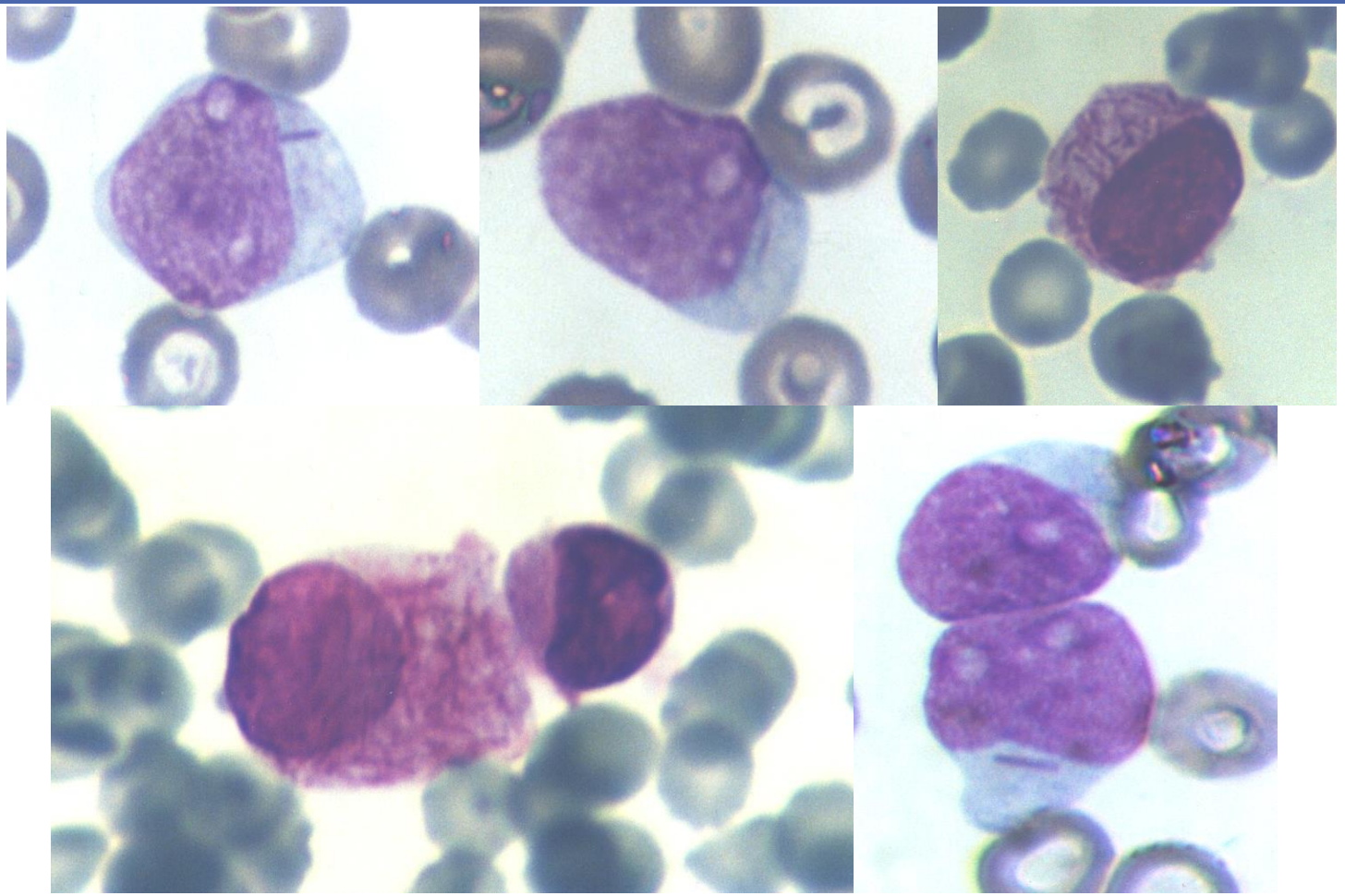
NUCLEOLI



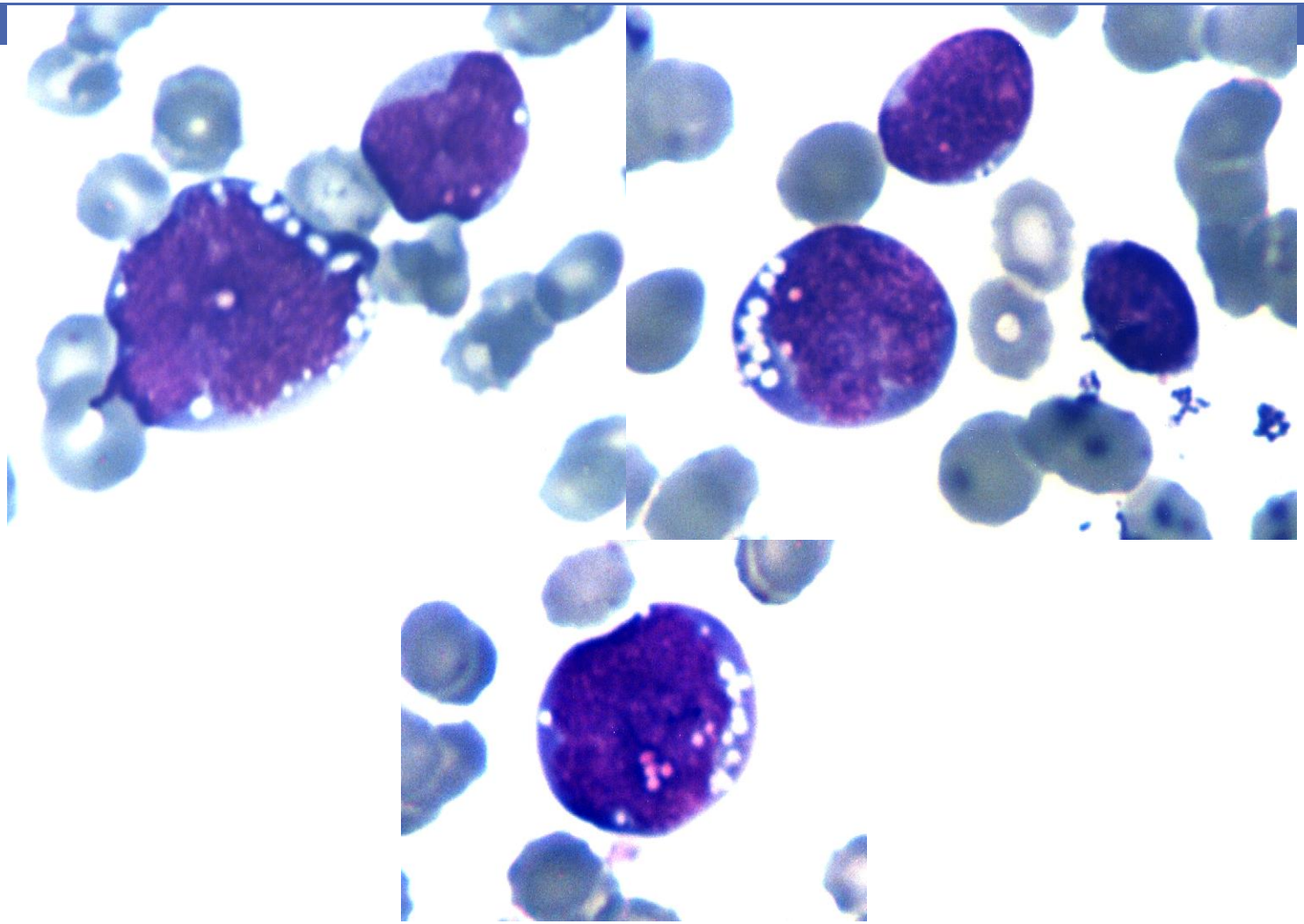
CYTOPLASMIC GRANULES



AUER RODS



VACOULES IN BLASTS



BLASTS IN ALL vs. IN AML

- *The chromatin in lymphoblasts is more clumped & irregularly distributed*
- *In AML blasts, the chromatin is fine, delicately stippled or lacy, & evenly distributed*

BLASTS IN ALL vs. IN AML

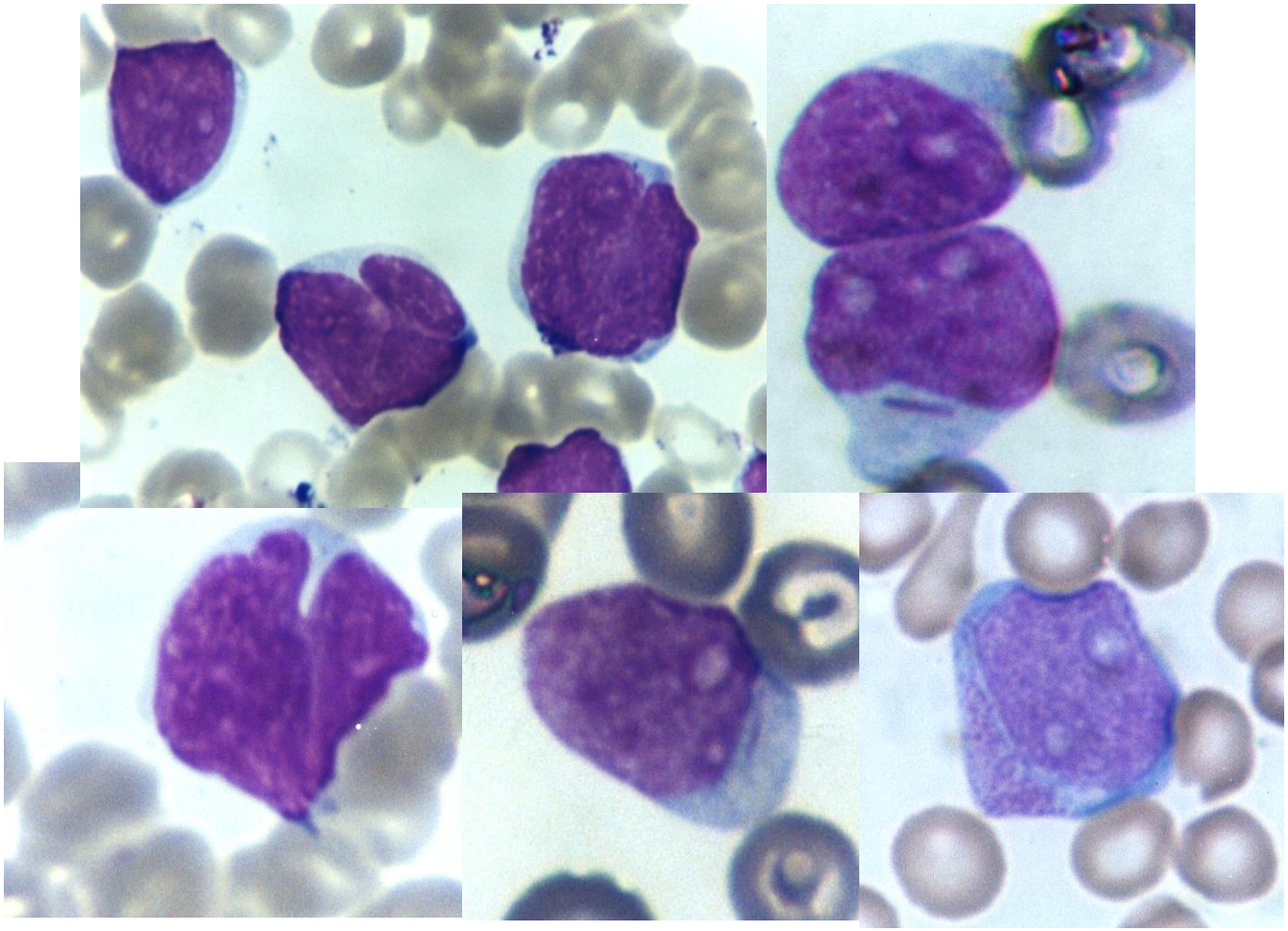
- *In ALL, nucleoli may be indistinct or appear prominent because of chromatin condensation along the nucleolar & nuclear membrane*

- *In AML, nucleoli are single or multiple & are usually prominent; nuclear & nucleolar membrane are indistinct*

BLASTS IN ALL vs. IN AML

- *Lymphoblasts, nuclei are folded or convoluted, & the cytoplasm is scant & without granules*
- *Myeloblasts have more abundant cytoplasm which may contain fine granules*
- *Auer rod is diagnostic of non-lymphoid lineage*

BLASTS in ALL vs. in AML



Mediterr J Hematol Infect Dis 2014; 6; Open Journal System

Review Article

Diagnosis and Subclassification of Acute Lymphoblastic Leukemia

Sabina Chiaretti,[1] Gina Zini[2] and Renato Bassan[3]

[1] Division of Hematology, Department of Cellular Biotechnologies and Hematology, "Sapienza" University of Rome, Rome, Italy
[2] Hematology, Catholic University Sacred Heart Policlinico Gemelli, Rome, Italy
[3] Hematology and Bone Marrow Transplant Unit, Ospedale dell'Angelo e SS. Giovanni e Paolo, Mestre-Venezia, Italy

Morphological characteristics of blasts cells in ALL vs. AML

(adapted from Morphology of Blood Disorders, 2nd Edition. d'Onofrio G, Zini G, Bain B.J. 2014.)

	Lymphoblasts	**Myeloblasts**
General characteristics	Blast population tends to be homogeneous	Blast population tends to be heterogeneous, with the exception of the undifferentiated form
Size	Variable, mainly small	Variable, mainly large
Nucleus	Central, mainly round; sometimes indented, particularly in the form in adults Nucleocytoplasmic ratio very high in the form that occurs in children Nucleocytoplasmic ratio lower in the form that occurs in adults	Tending to be eccentric, round, oval or angulated; sometimes convoluted, particularly in the form with a monocytic component Nucleocytoplasmic ratio high in undifferentiated blast cells and in some megakaryoblasts Nucleocytoplasmic ratio mainly low in the form with differentiation
Chromatin	Fine, with dispersed condensation Very condensed in small lymphoblasts	Fine, granular, delicately dispersed
Nucleoli	Absent in small lymphoblasts Sometimes indistinct	Almost always present, often large and prominent, double or triple
Cytoplasm	Scanty, basophilic Sometimes with a single long projection ('hand-mirror cell')	Variable Abundant in monoblasts With protrusions in erythroblasts and megakaryoblasts
Granules	Rarely present, azurophilic and always negative for peroxidase, esterases and toluidine blue	Present in forms with differentiation and positive with cytochemical stains – peroxidase in the neutrophil and esoinophil lineages –nonspecific esterase in the monocyte lineage –toluidine blue in the basophil lineage
Auer rods	Always absent	Can be present Typically present in the hypergranular promyelocytic form
Vacuolation	Can be present	Can be present Almost always present in forms with a monocytic component

Morphology

Advantages:

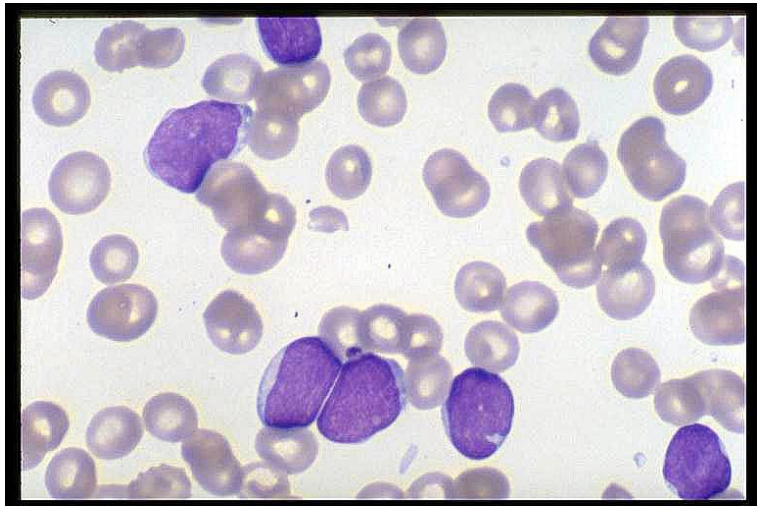
- Simple method,
- Available
- Rapid diagnosis
- Guide for requesting other tests
- Save money

Disadvantages:

- Need Good prep, Fix , staining
- Need professional trained personnel
- Negative impact of automation
- Different format of report
- Non specific names are common: atypical cells, immature cells,…
- Consultation is not defined

Morphology
>20% blasts

AML



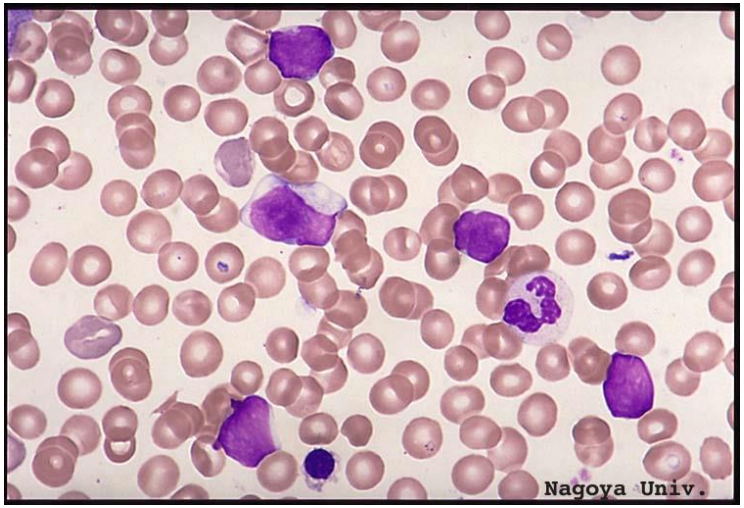
Larger

Slightly more cytoplasm, may be granular

AUER ROD

Larger more open nuclei with prominent nucleoli

ALL

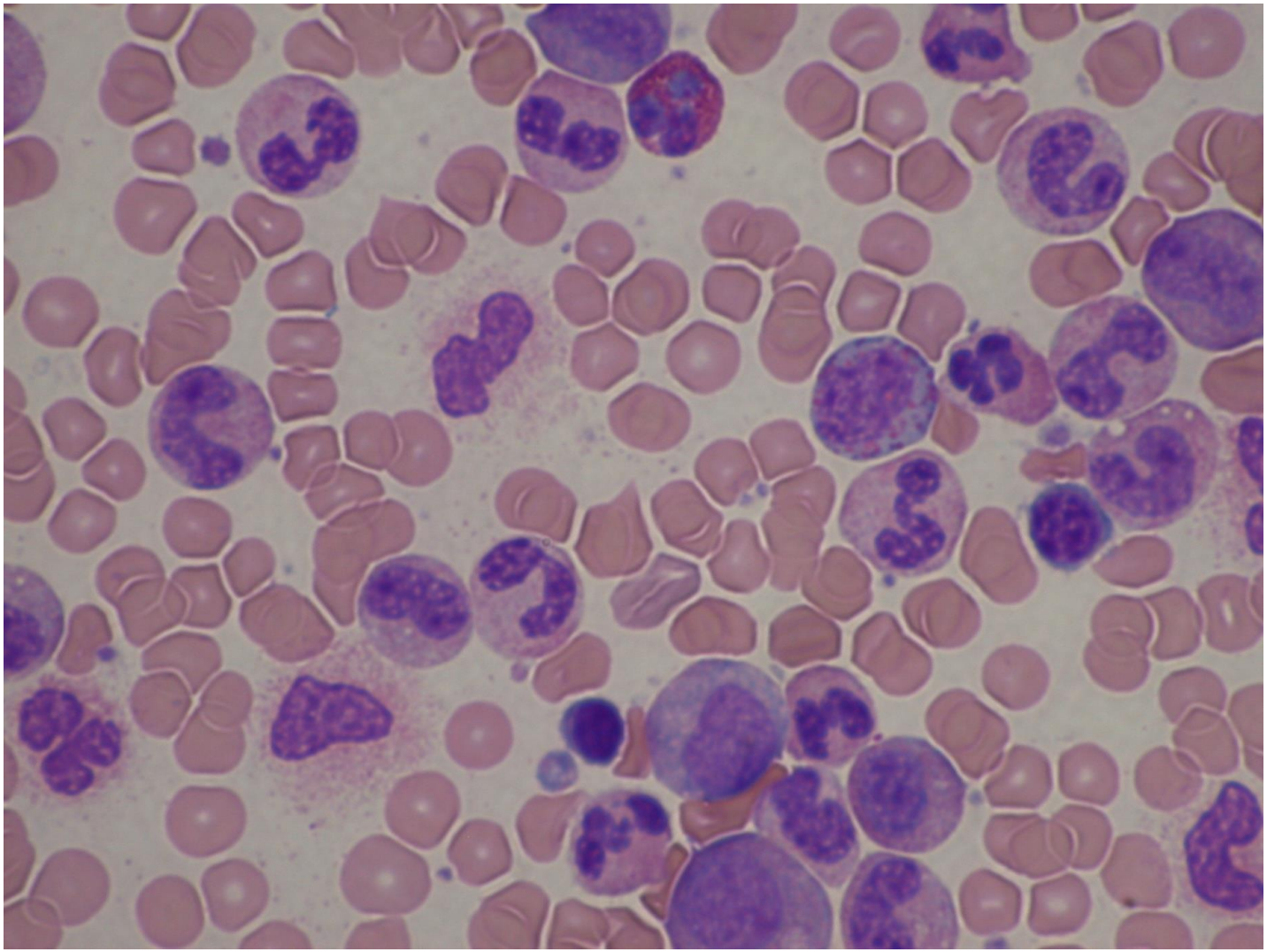


Smaller

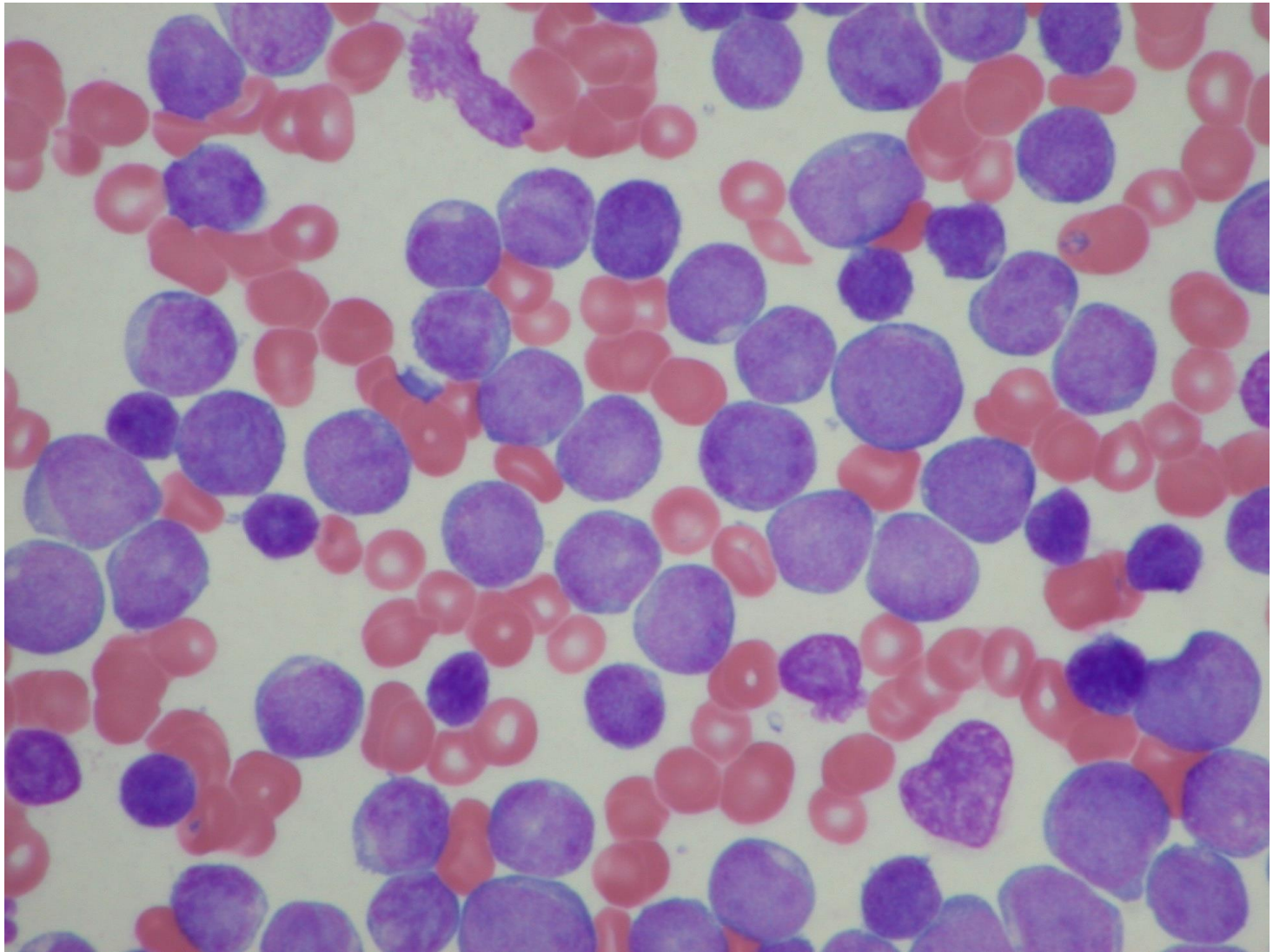
High NC ratio, usually cytoplasm lacks granules

Smaller nuclei, less open chromatin with indistinct nucleoli

Normal Bone Marrow Aspirate



Bone Marrow Aspirate of ALL



Guidelines for using the WHO classification of myeloid neoplasms

- In the WHO classification the term "***myeloid***" includes all cells belonging to the granulocytic (neutrophil, eosinophil, basophil), monocytic/macrophage, erythroid, megakaryocytic and mast cell lineages.

Guidelines for using the WHO classification of myeloid neoplasms

- Blast percentages should be derived, when possible, from **200**-cell leukocyte differential counts of the **PB smear** and **500**-cell differential counts of all nucleated BM cells on cellular **marrow aspirate smears** stained with Wright-Giemsa

Guidelines for using the WHO classification of myeloid neoplasms

Specimen requirements:

- PB and BM specimens ***prior to any definitive therapy***
- PB and cellular BM aspirate smears and/or touch preparations stained with Wright-Giemsa or similar stain
- BM biopsy, at least 1.5 cm in length and at right angles to the cortical bone, recommended for all cases if feasible

Table 2. Collection and processing of bone marrow aspirate and core biopsy specimens

Specimen	Test	Anticoagulant or media	Fixative	Further processing	Staining (no. of slides or sections)*
Aspirate	Smear (6 slides)	None/EDTA	Air dry, methanol-fix	–	MGG or Wright stain (2 slides), Prussian Blue (1 slide), cytochemistry
Aspirate	Squash (≥2 slides)	None/EDTA	Air dry, methanol-fix	–	MGG or Wright stain (1 slide), Prussian Blue (1 slide)
Aspirate	Particle clot	–	NBF, AZF, B5, Bouin's etc	Paraffin embed, cut sections	H&E (3 sections), Giemsa, IHC, histochemistry, FISH
Aspirate	Flow cytometry	Heparin	Further processing according to specific protocols		
Aspirate	Molecular genetics	EDTA			
Aspirate	Cytogenetics, FISH	Sterile tissue culture media e.g. RPMI with 10% bovine fetal serum			
Aspirate	Microbiology	Sterile plain or heparinized tubes, lysis centrifugation tubes or culture media			
Core biopsy	Histology	–	NBF, AZF, B5, Bouin's, etc.	Decalcify, paraffin embed, cut sections	H&E (2–4 sections), reticulin (1 section), Giemsa, IHC, histochemistry, FISH
Core biopsy	Touch imprint (≥2 slides)	–	Air dry, methanol-fix	–	MGG or Wright stain (1 slide), cytochemistry

*Several smears and imprints should be left unstained for possible immunostains, cytochemical stains, FISH or DNA extraction. Additional sections of particle clots and BM biopsy specimens should be cut as required.

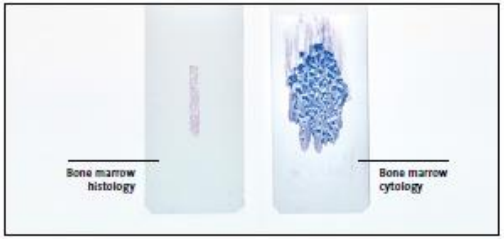
AZF, acetic acid–zinc–formalin; B5, mercuric chloride, sodium acetate and formalin; EDTA, ethylenediamine tetra-acetic acid; FISH, fluorescent *in-situ* hybridization; H&E, haematoxylin and eosin; IHC, immunohistochemistry; MGG, May–Grünwald Giemsa; NBF, neutral buffered formalin.

BLOOD SMEAR

ASPIRATE SMEARS

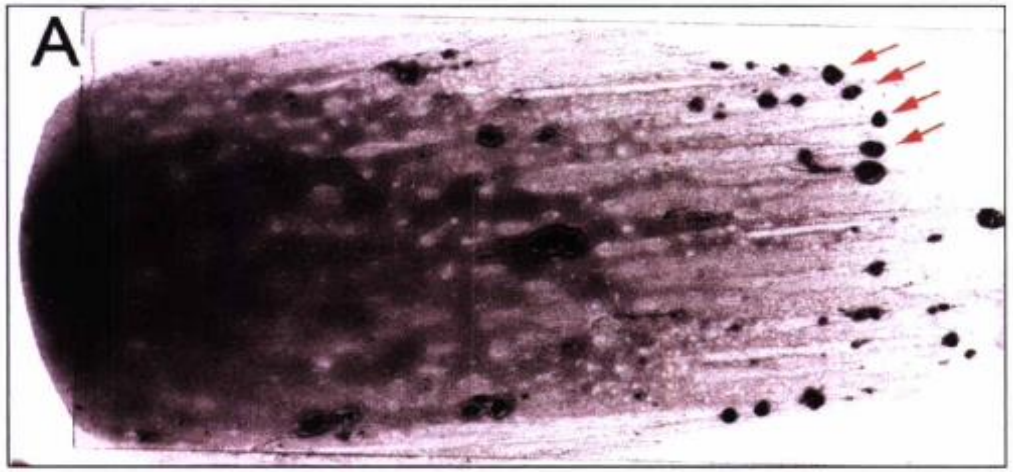
CRUSH PREP

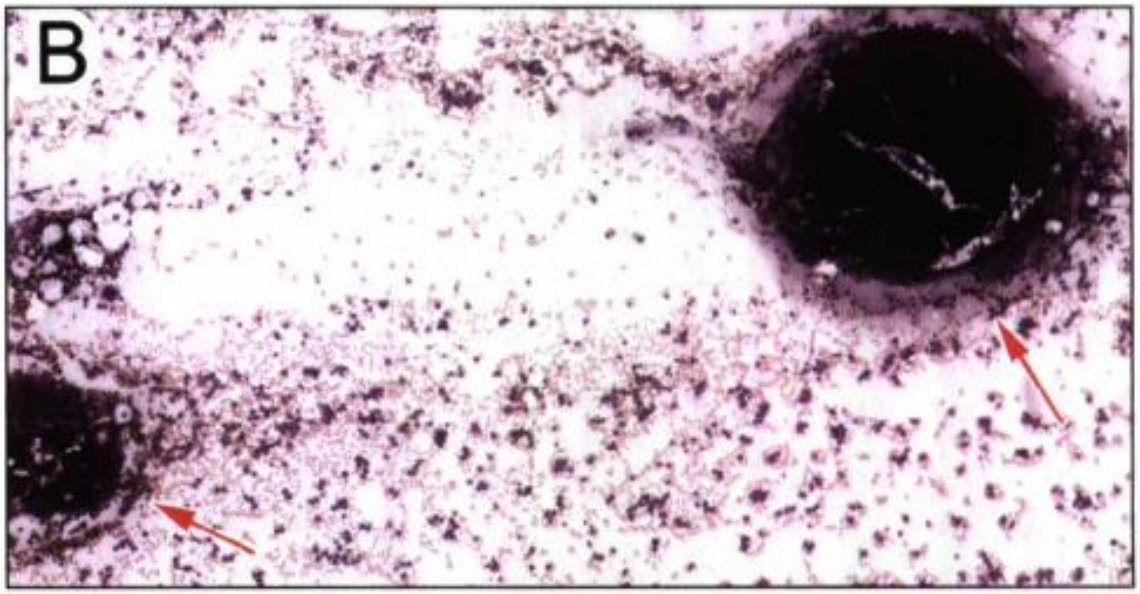
TOUCH PREP



Wright-Giemsa stain

Prussian Blue


A


B

Squash or crushed smear

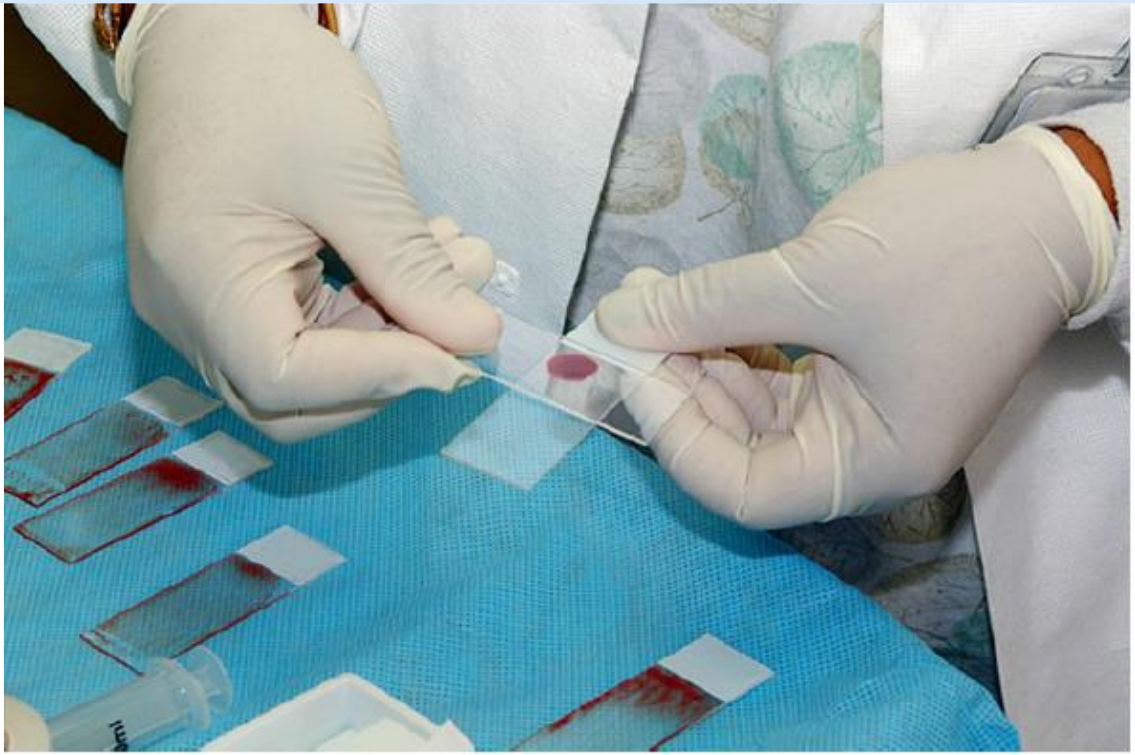


Fig 8. Preparing aspiration smears. An experienced medical technologist is preparing smears from small drops of the bone marrow aspirate placed on glass microscope slides.

Ann Hematol (2012) 91:497–505
DOI 10.1007/s00277-011-1347-4

ORIGINAL ARTICLE

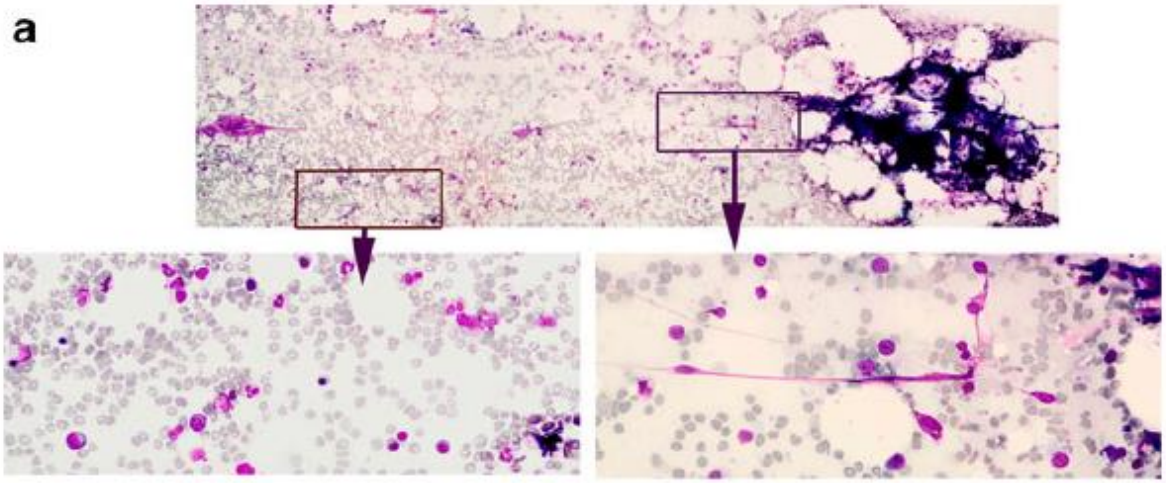
Microscopic examination of bone marrow aspirates in malignant disorders of haematopoiesis—a comparison of two slide preparation techniques

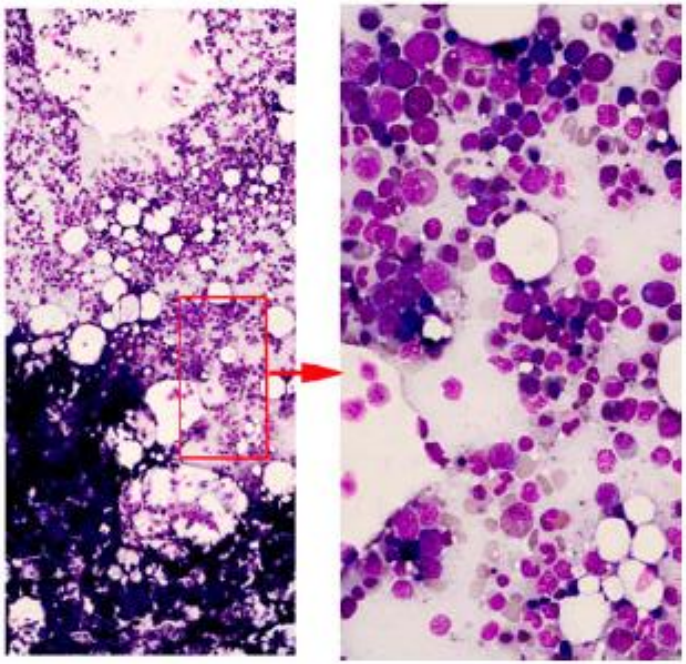
Krzysztof Lewandowski · Agnieszka Complak · Andrzej Hellmann

BMA Smear Prepared by Two Method

Bone marrow smear prepared using technique 2

Bone marrow smear prepared using technique 1





Pros & cons

- Many of the significant symptoms, easily seen in slides prepared using technique 2 (i.e**. focal lymphocytic or mast cell infiltrations**) might not be observed in slides prepared using technique 1.
- In some specific situations (i.e. **presence of villous lymphatic cells**), technique 1 could better preserve single cell morphology.
- Furthermore, the results obtained using the slides prepared by ***technique 2 correlated better with the clinical picture*** and trephine biopsy examination results.
- **Therefore, recommend the use of technique 2 as the primary method for establishing a diagnosis or for making therapeutic decisions**
  - **But with accordance to ICSH, both techniques should be used.**

Guidelines for using the WHO classification of myeloid neoplasms

Assessment of blasts

- Determine ***blast percentage*** in PB and BM by visual inspection
- Count **myeloblasts**, **monoblasts**, **promonocytes**, **megakaryoblasts** (but not **dysplastic megakaryocytes**) **as blasts** when summing blast percentage for diagnosis of AML or blast transformation;
- Count **abnormal promyelocytes** as "***blast equivalents***" in **APL**

TYPES OF LEUKEMIC BLASTS
WHO 2016

Blast enumeration:

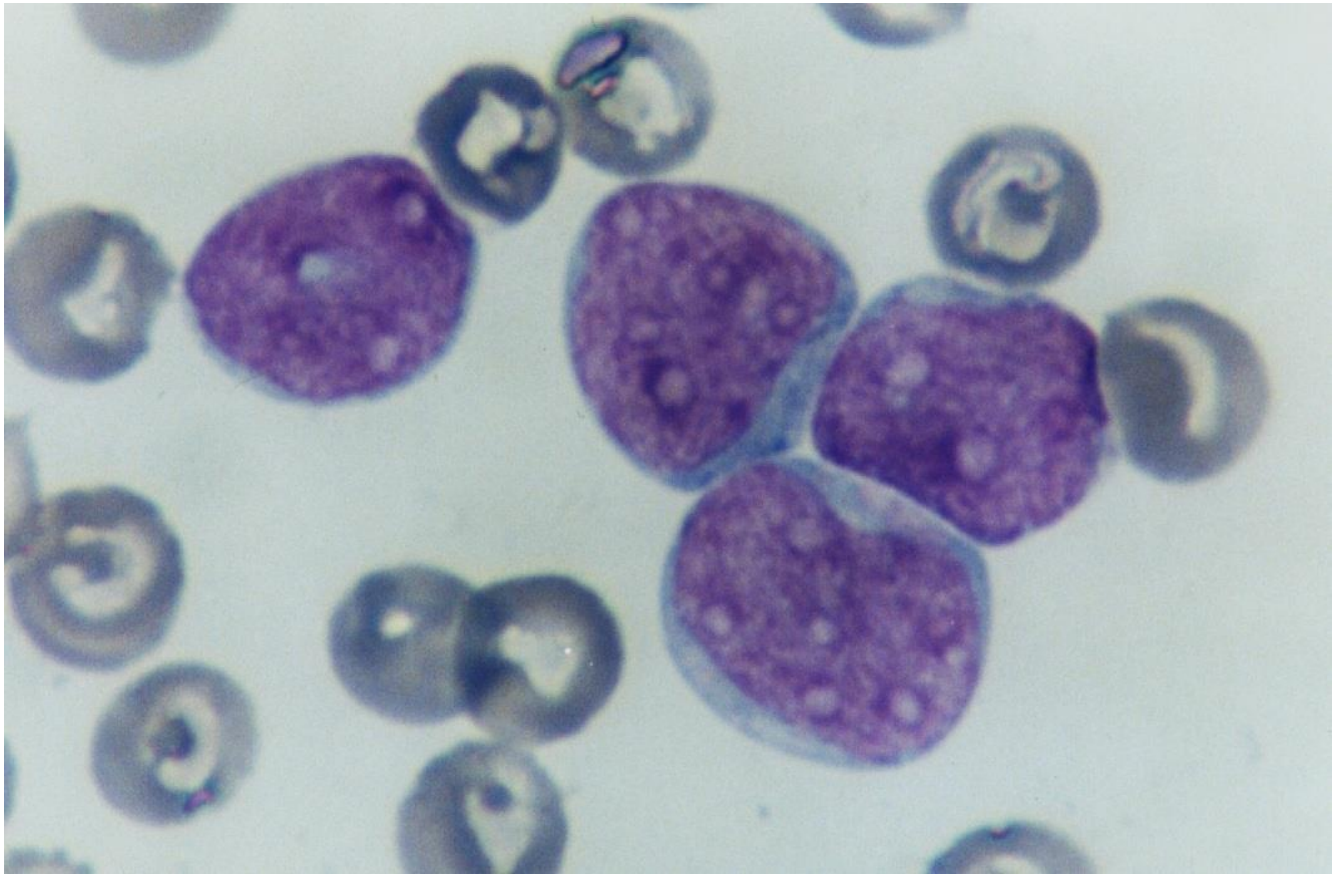
I. Blasts and Immature Myeloid Elements

- **Myeloblasts**
- **Promyelocytes***
- **Monoblasts**
- **Promonocytes**
- **Erythroblasts†**
- **Megakaryoblasts**

II. Systematic Approach: Blast ID and Enumeration

- Recognize blasts and blast equivalents
- Promonocytes always included in blast percentage
- Promyelocytes only included in blast percentage in APL
- Blast percentage based on total BM cells for all AML subtypes (revised erythroleukemia criteria)
- Blast enumeration based on morphologic differential cell count (not flow cytometry percents)

Definitions of blast cells?



Problem ?

- It is often assumed that definitions of blast cells are applied ***uniformly*** by hematologists/pathologists worldwide, and
- That blast cells could be identified and counted very easily.
- ***Unfortunately this is not so.***

Definition of myeloblasts

Myeloblasts were defined in terms of :

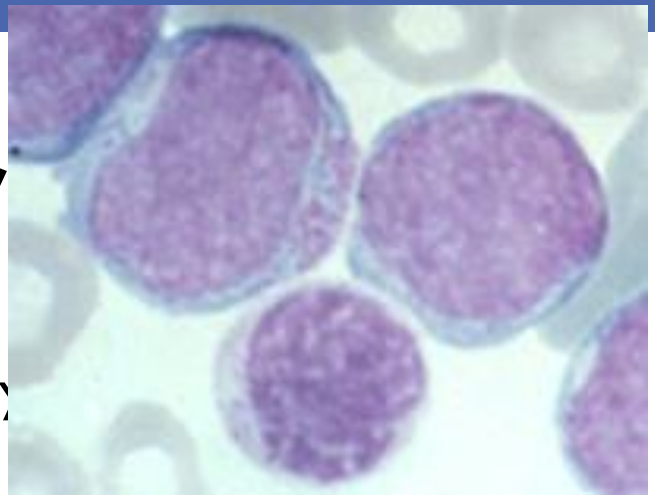
A. nuclear characteristics:

1. A high nuclear/cytoplasmic ratio,
2. Easily visible nucleoli and
3. Usually, but not invariably, fine nuclear chromatin.
4. Nuclear shape is variable.

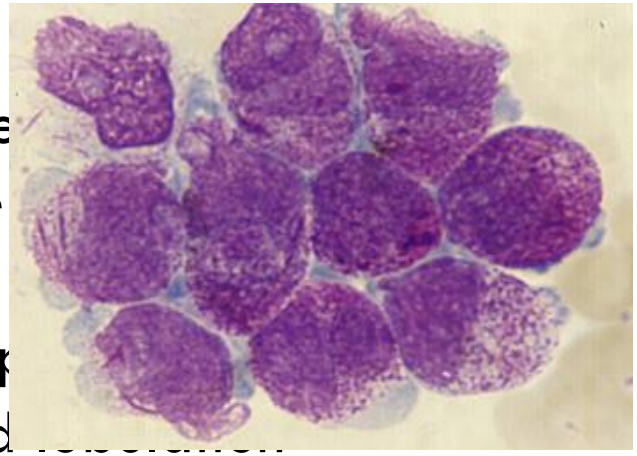
B. Cytoplasmic characteristics include:

1. variable cytoplasmic basophilia;
2. There may or may not be granules or Auer rods but no Golgi zone is detected .

Morphologic Features of Blasts and Other Immature Cells (Blast Equivalents)



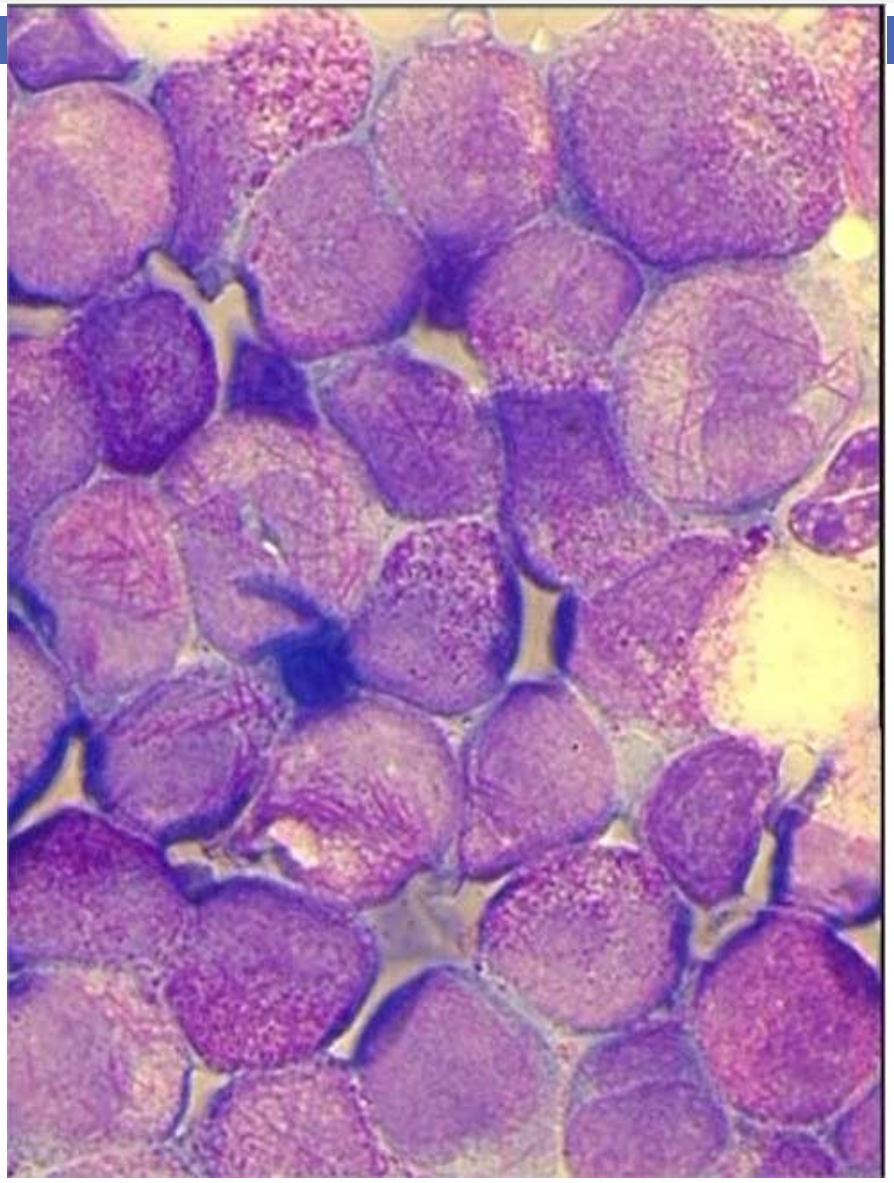
- **Myeloblast**
- Large nucleus with finely dispersed chromatin, nucleoli
- Relatively high nuclear/cytoplasmic ratio
- Variable number of cytoplasmic granules, may limited portion of cytoplasm



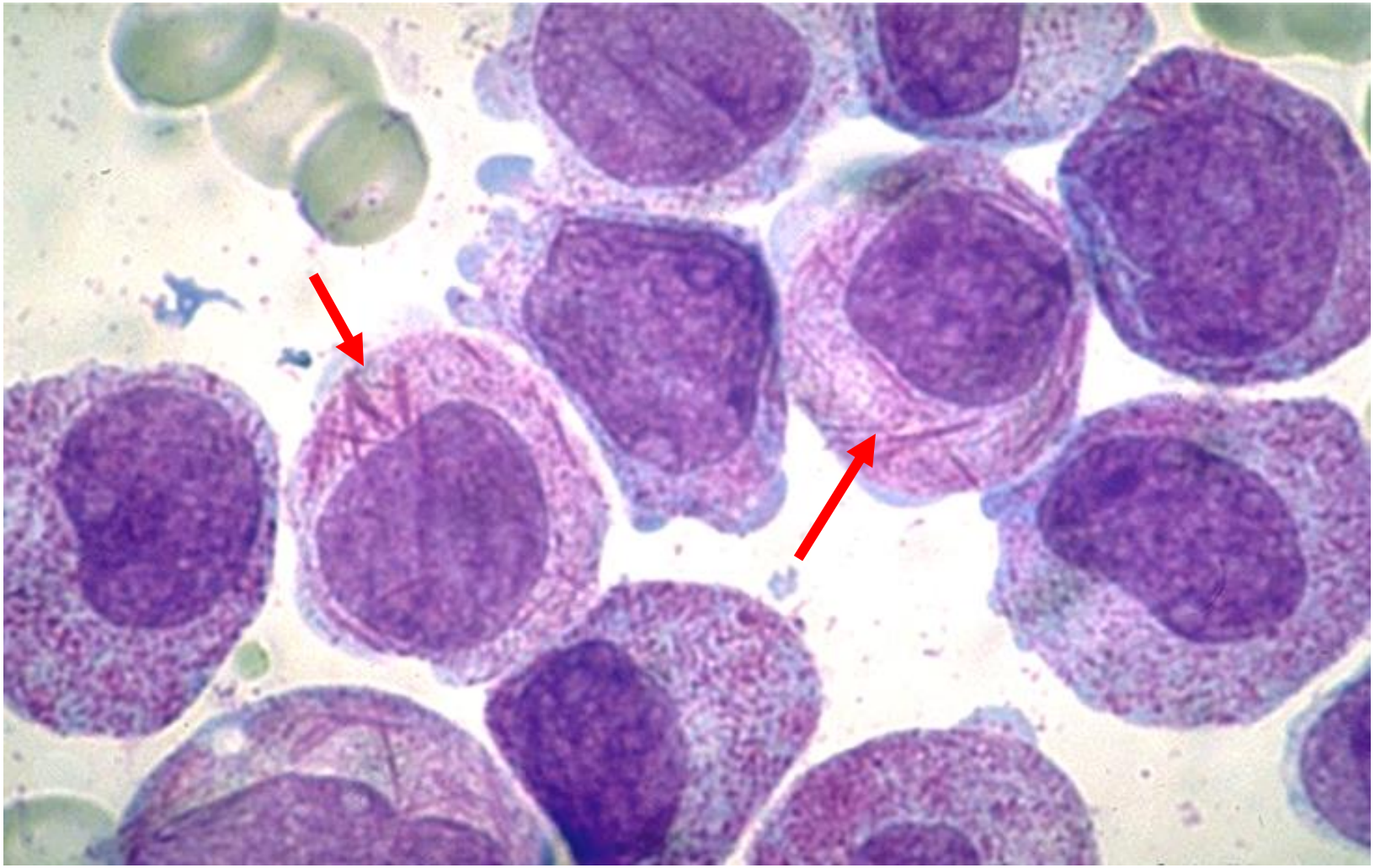
- **Promyelocyte (Blast equivalent in APL)**
- Nuclear chromatin slightly condensed; nucleoli nucleus often eccentric and Golgi zone may be
- Numerous cytoplasmic granules--may be more cytoplasm
- In APL intense cytoplasmic granularity usually configuration variable, but nuclear folding and characteristic of microgranular variant of APL

FAB classification of AML-M3

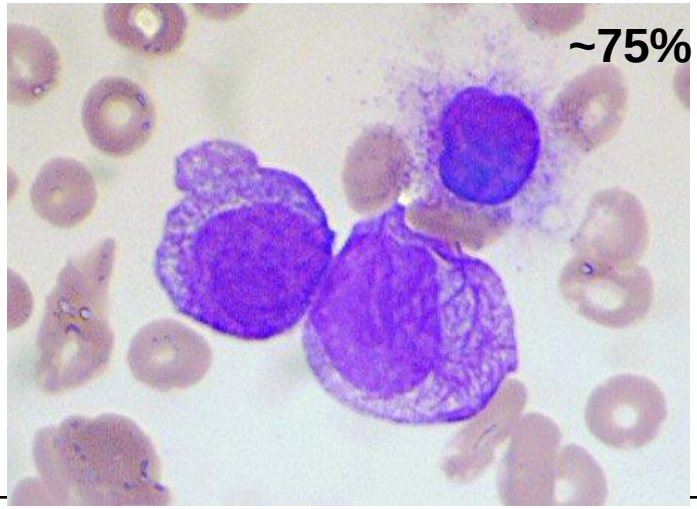
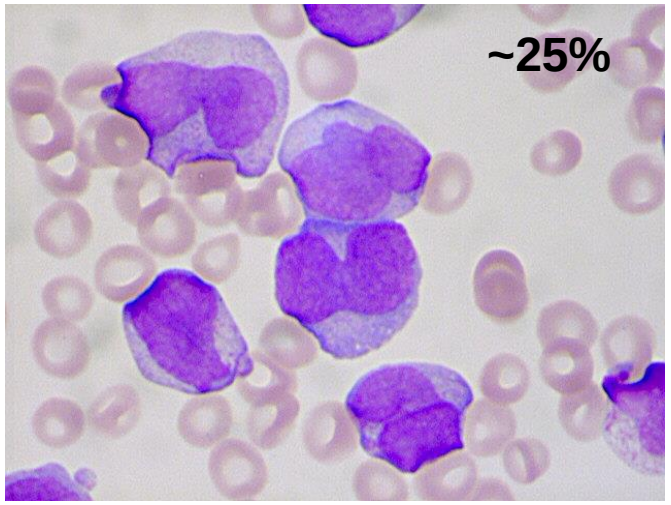
- **AML-M3** **(5-8%)**
  - Acute Promyelocytic Leukemia, APL
  - Maturation arrest at the stage of promyelocytes
    - ≥ 30% promyelocytes and blasts
  - Clinical--DIC
  - Genetics t(15;17)
    - PML-RARα
  - Treatment--ATRA



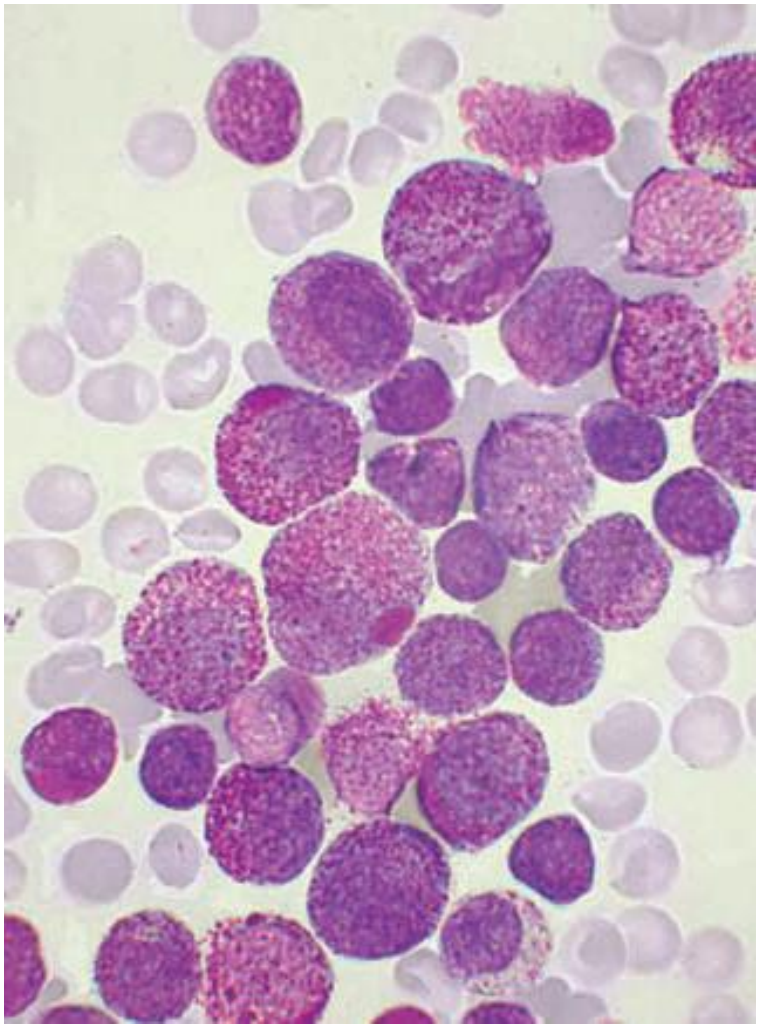
Auer rods in AML-M3: Faggot Cells

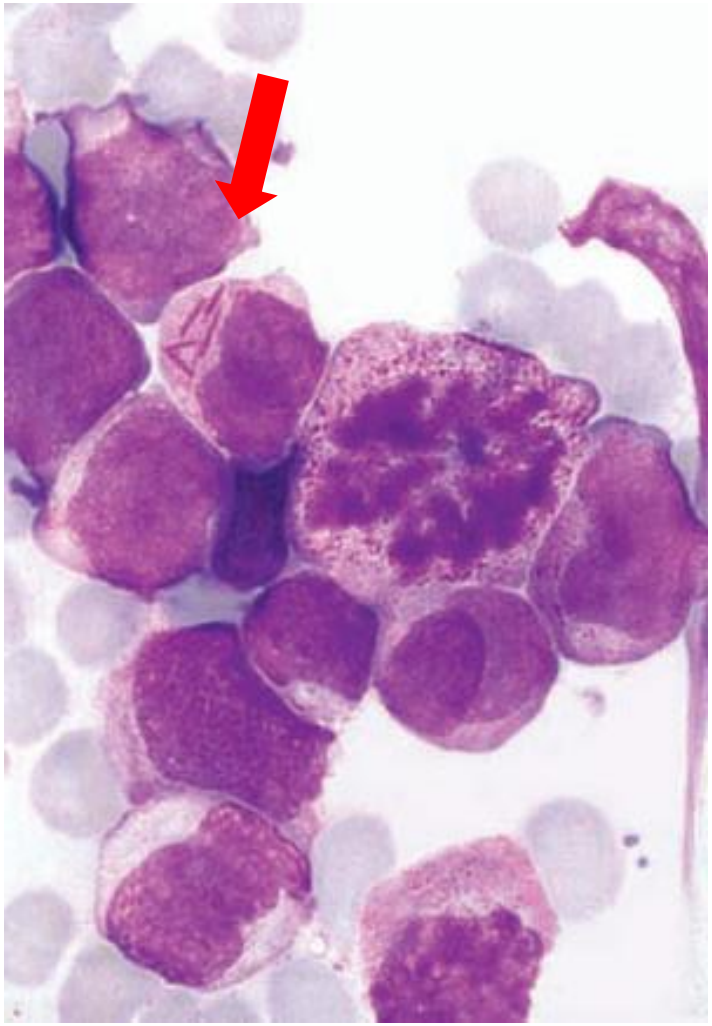


Morphology

Hypergranular type	Micrograniular type
	
• Cytoplasm is packed with densely packed, sometimes coalescent large granules • Auer rods (often large, sometimes in bundles)	• Bilobed nuclei, apparent paucity of granules (submicroscopic granules) • May be mistaken for monoblasts • Auer rods may be present
Low WBC	High WBC 50,000 to 200,000/µL
Strongly MPO+	Strongly MPO+

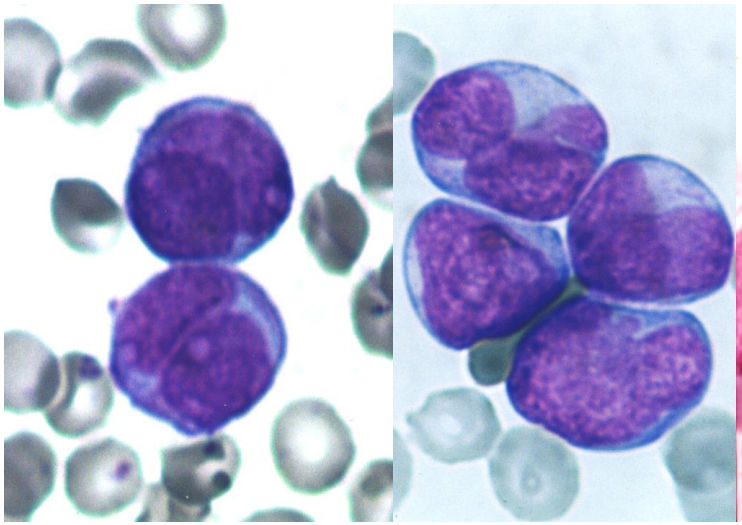
AML-M3 Classic Type ,Faggot Cells





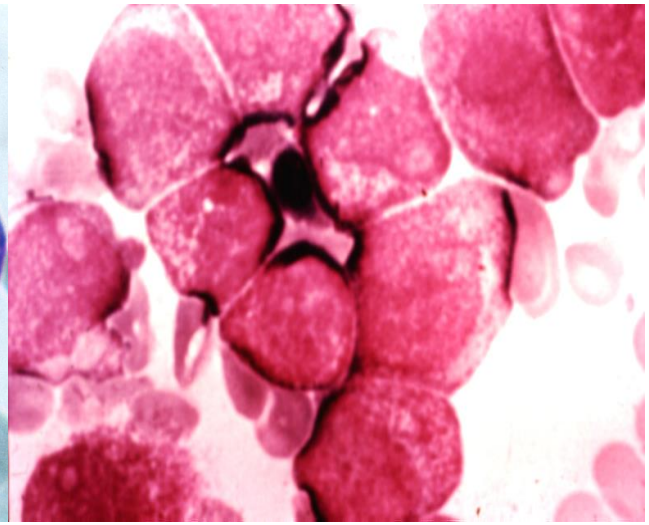
PROMYELOCYTES IN CLASSIC M3 VS M3V

Microgranular type

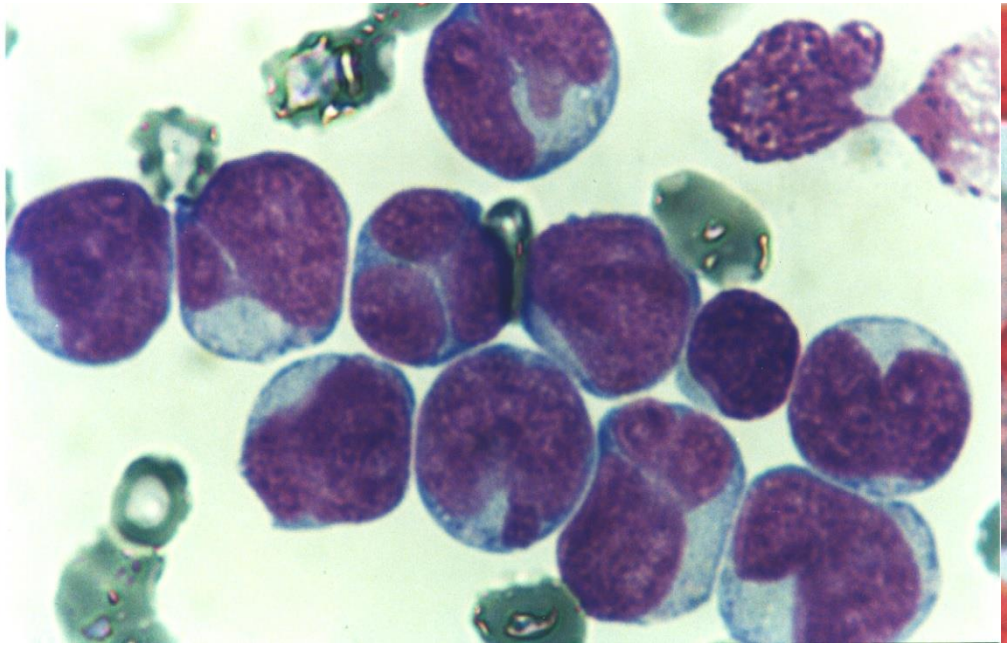


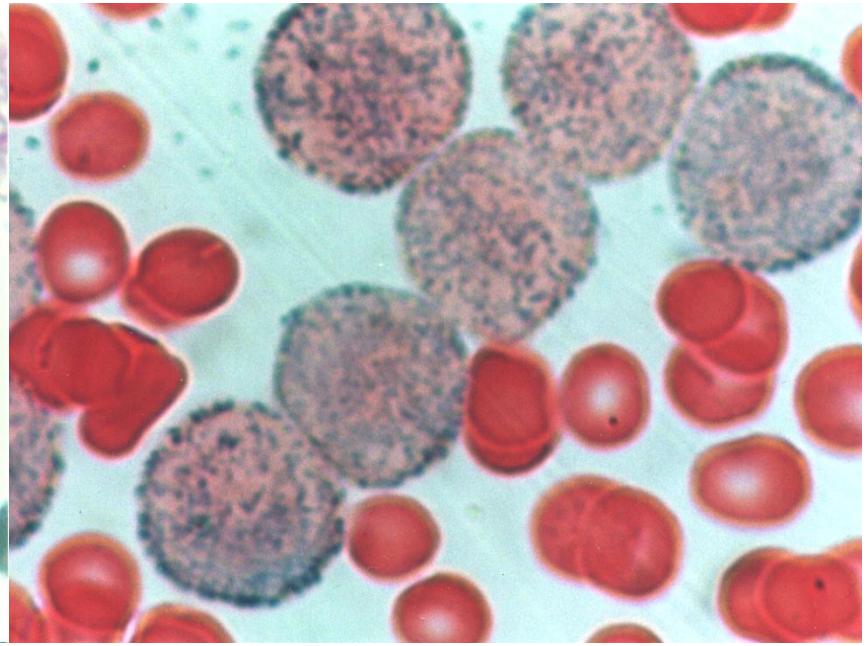
Bilobed nuclei & Vermiforms

Hypergranular type



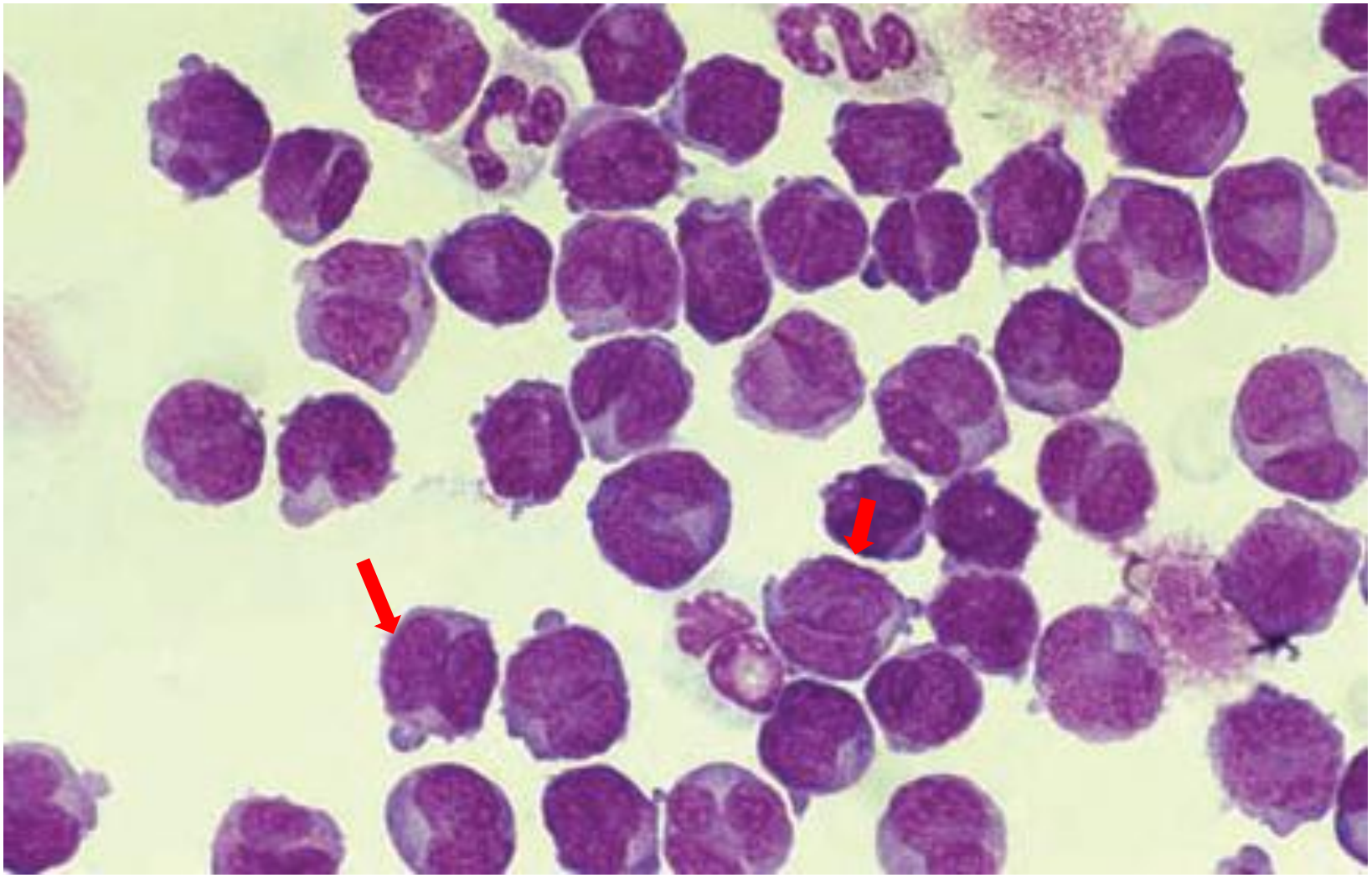
PROMYELOCYTES IN M3V





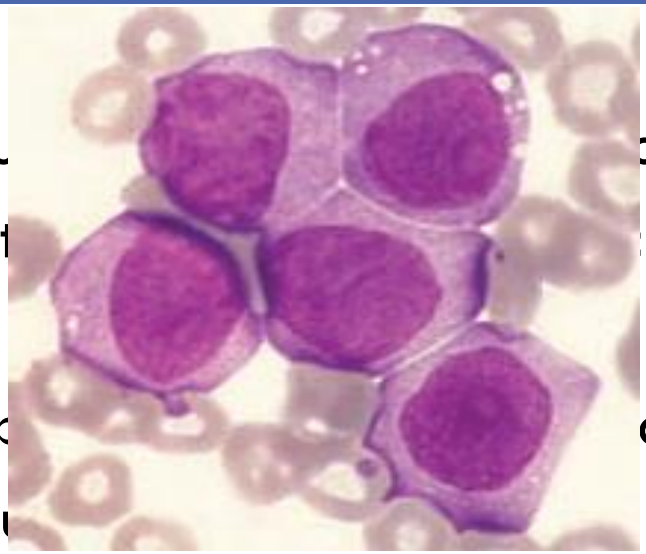
- ***ROUTINE STAIN VS MYELOPEROXIDASE***

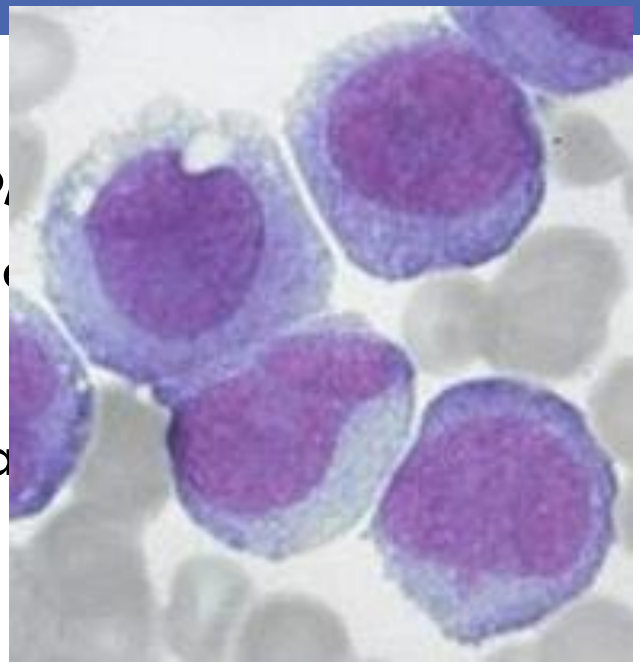
AML-M3v



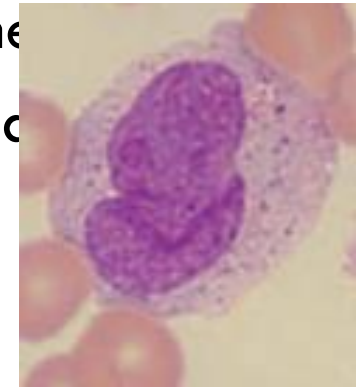
Morphologic Features of Blasts and Other Immature Cells (Blast Equivalents)

- **Monoblast**
- Moderate to low nu
  finely dispersed wit
  folded
- Abundant, slightly b
  and occasional vacu



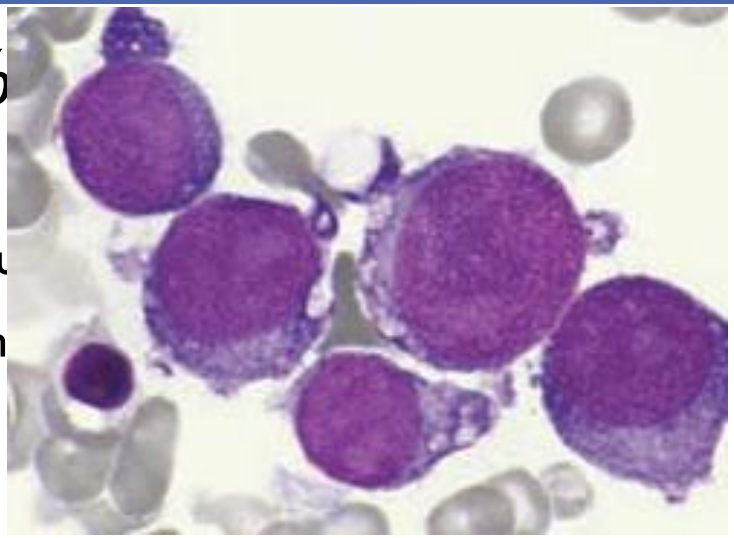


- **Promonocyte (Blast equivalent)**
- Slightly condensed nuclear chromatin; variably promine
- Abundant finely granular blue/gray cytoplasm that ma
  vacuolated
- Very monocytic appearance with nuclear immaturity

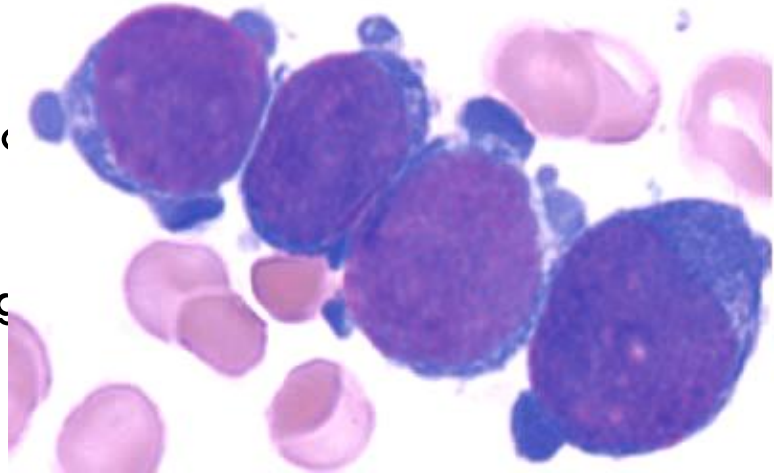


Morphologic Features of Blasts and Other Immature Cells (Blast Equivalents)

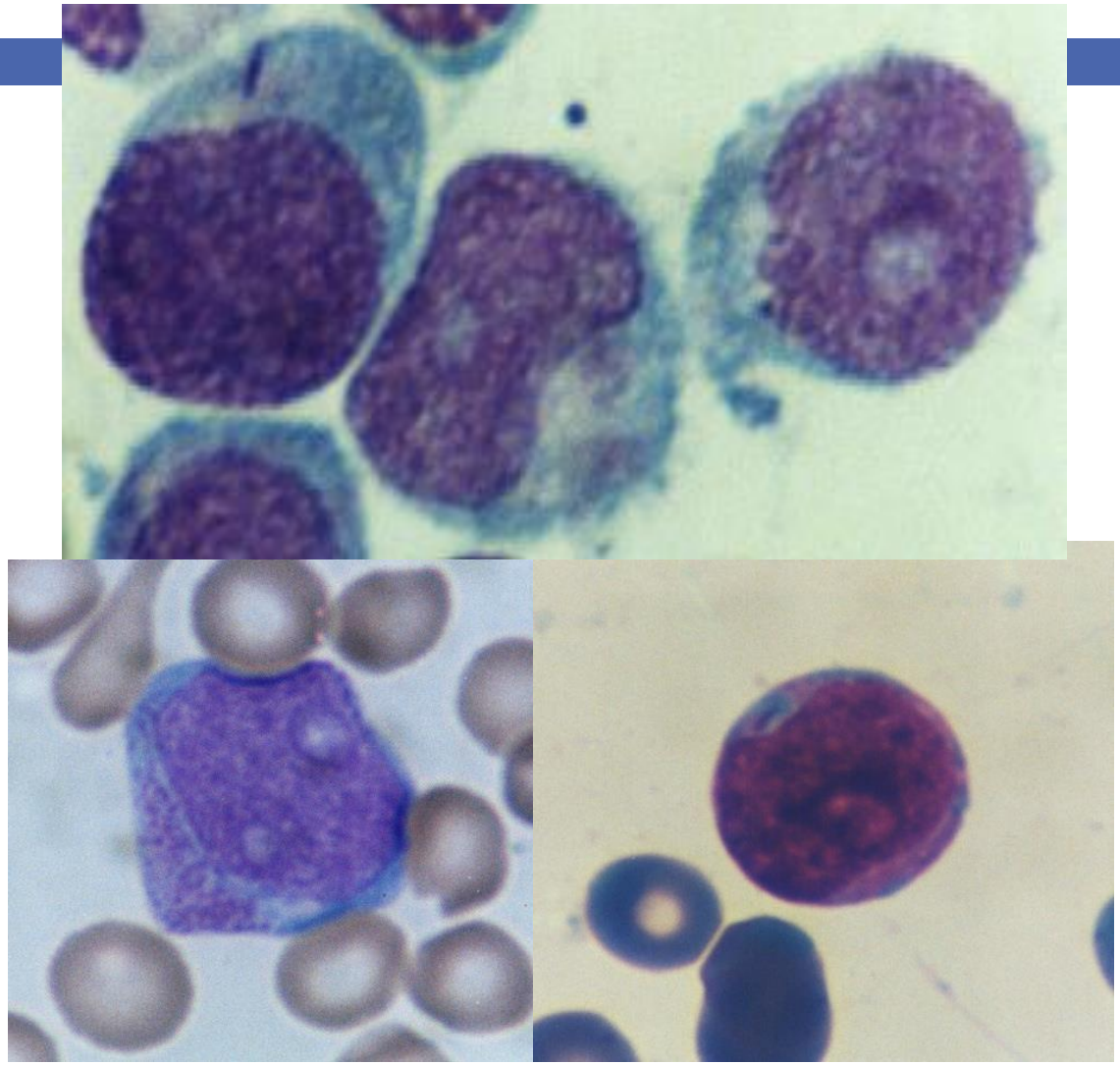
- **Erythroblast** *(not included in blast %*
- Relatively high nuclear/cytoplasmic ratio
- Nucleus round with slightly condensed chromatin; nu
- Moderate amounts of deeply basophilic cytoplasm



- **Megakaryoblast**
- Highly variable morphologic features
- Often not recognizable without special studies
- May be lymphoid-appearing with high nuclear t
- Nuclear chromatin fine to variably condensed
- Cytoplasm may be scant to moderate; usually ag
- Blasts may form cohesive clumps



CYTOPLASMIC GRANULES



- MYELOBLAST TYPE 1 TO 3

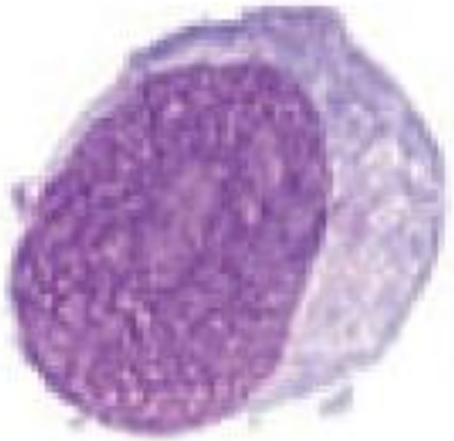
Definition of myeloblasts

After reviewing all the available bone marrow smears, the IWGM group recommended that :

- Myeloblasts in MDS should be classified as **agranular** or **granular.**
- The agranular blasts correspond to the type I blasts of the FAB classification.
- Granular blasts are cells that have the nuclear features of blast cells but also have cytoplasmic granules.

Blasts

Agranular

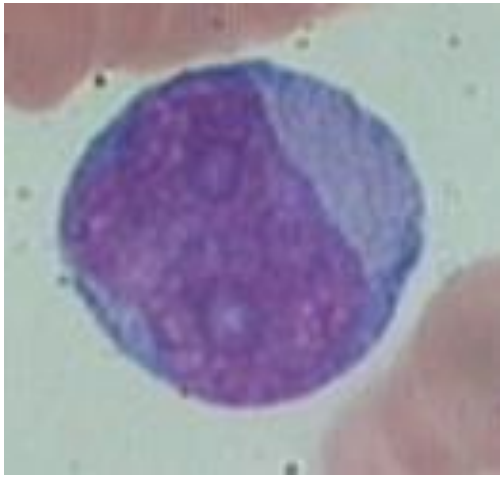


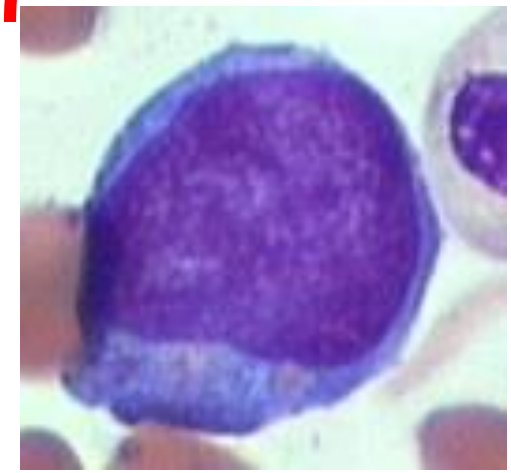
- Basophilic cytoplasm
- Fine chromatin
- Nucleoli

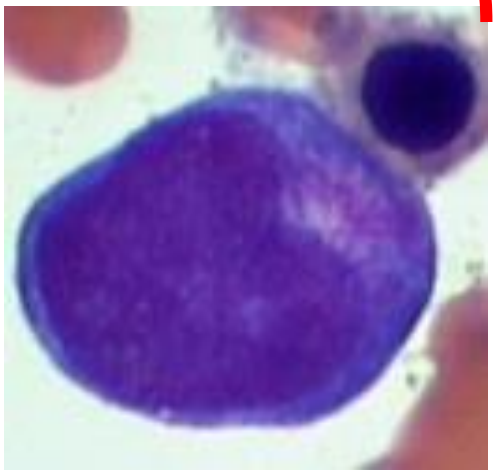
Granular

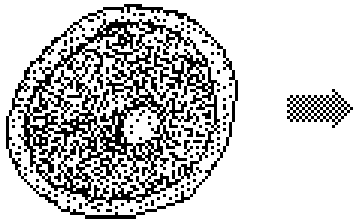
- Azurophilic granulation
- Absence of Golgi zone

Agranular Blast vs granular Blsats

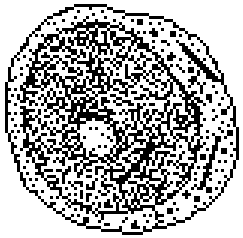








Myeloblast Type I



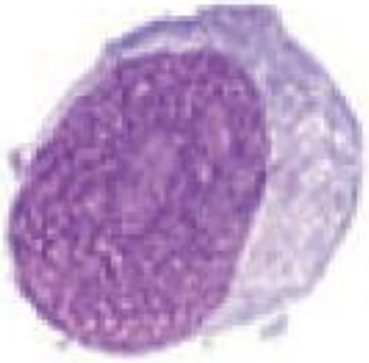
Myeloblast Type II

Myeloblast Type III

Blasts, promyelocytes, abnormal promyelocytes.

Blasts

Agranular

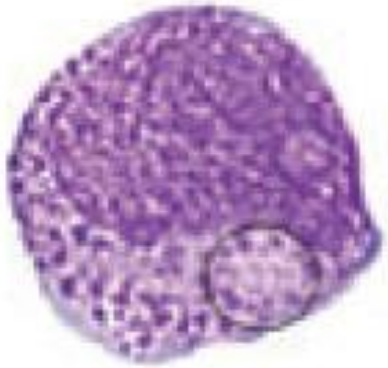


- Basophilic cytoplasm
- Fine chromatin
- Nucleoli

Granular

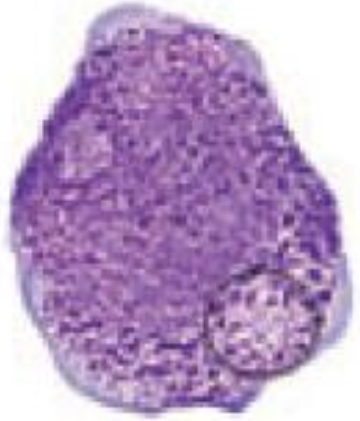
- Azurophilic granulation
- Absence of Golgi zone

Promyelocyte



- Azurophilic granulation+
- **Clearly visible Golgi zone**

Abnormal promyelocyte



Azurophilic granulation +++

Significant changes in the diagnosis and classification of precursor B- and T- cell neoplasms:

- Although the distinction between lymphoblastic leukemia and lymphoblastic lymphoma is obvious when the patient ***has a mass comprised of B or T lymphoblasts and no blasts in the blood or marrow***,
- It is more arbitrary when there is a mass and limited marrow involvement.
- When a **mass** is present and **20% or more** of the nucleated cells in the bone marrow are lymphoblasts, a diagnosis of lymphoblastic leukemia is preferred over lymphoblastic lymphoma.

Significant changes in the diagnosis and classification of precursor B- and T- cell neoplasms:

- Because ALL rarely presents with low BM blast counts, the diagnosis of ALL should be deferred if there are **<20% blasts** in the BM until there is definitive evidence to confirm the diagnosis.
- However, in the unusual case that a patient presents with **<20%** lymphoblasts in the BM and no evidence of an extramedullary mass, but demonstrates one of the known **recurring cytogenetic abnormalities** associated with ALL ,the patient may be considered to have lymphoblastic leukemia.

Significant changes in the diagnosis and classification of precursor B- and T- cell neoplasms:

- However, the finding of <20% unequivocal lymphoblasts in the BM should also prompt a search for lymphoblastic lymphoma in an **extramedullary location**.

Highlights in routine lab.

- Use ***good quality smear*** (prep., distribution, fix, stain)
- Review of smear ***by experts*** (low & high power)
- Recognition of leukemic blasts
- ***Observation of blast is not equal to Acute leukemia***
- Reporting presence, percentage & morphological features
- Notice to other finding in CBC, clinical presentation, previous lab result
- Consultation with ordering physician (recommendations) & critical value report

Critical Values

Test Report Name	Age	Critical Low	Critical High	Units
HEMATOLOGY				
Hemoglobin	**0-7weeks**	**≤ 6.0**	**≥ 24.0**	**g/dL**
Hemoglobin	**>7weeks**	**≤ 6.0**	**≥ 20.0**	**g/dL**
Hematocrit		**<21**	**>65**	**%**
Leukocytes		**≤ 2.0**	**≥ 25.0**	**x10(9)/L**
Neutrophilic Segs (%)		**≤ 10**	**= 100**	**%**
Neutrophilic Segs		**≤ 0.5**	**-**	**x10(9)/L**
Neutrophils		**≤ 0.5**	**-**	**x10(9)/L**
Platelets, Blood		**≤ 40**	**≥ 1000**	**10(9)/L**
Blast			**Any**	

Thank you, any question?