BHS
Belgian Hematology Society

BLOOD AND BONE MARROW MORPHOLOGY

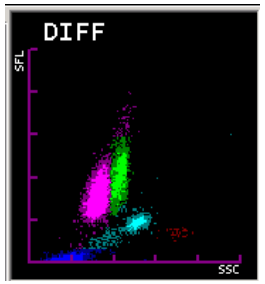
LABORATORY HEMATOLOGY
BHS COURSES
OCTOBER 14, 2023

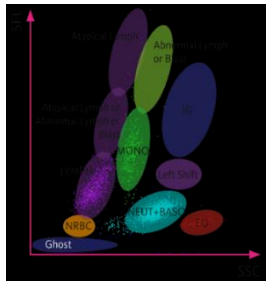
LHUB-ULB

Laurence Rozen

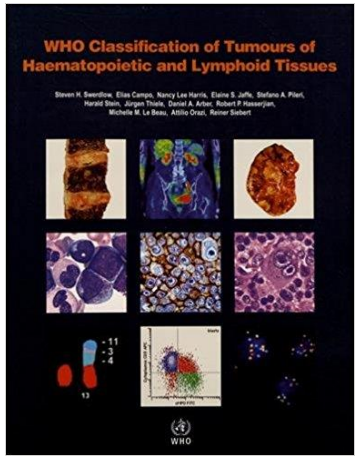
CHU | UVC BRUGMANN

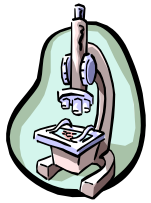
Diagnostic of malignant hemopathy (leukemia, lymphoma…)

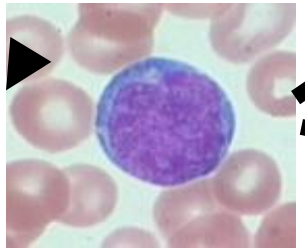




Flow cytometry







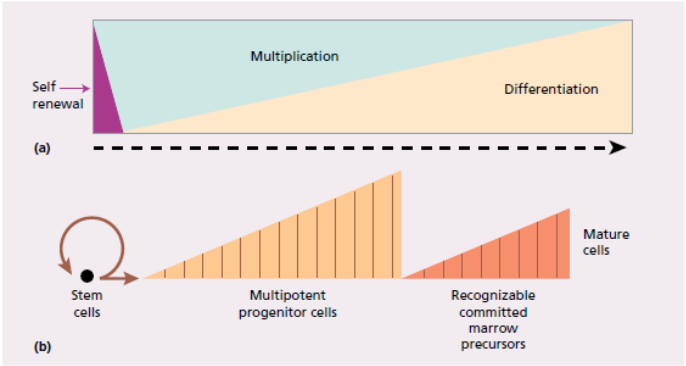
- Karyotype
- Molecular Biology (NGS)

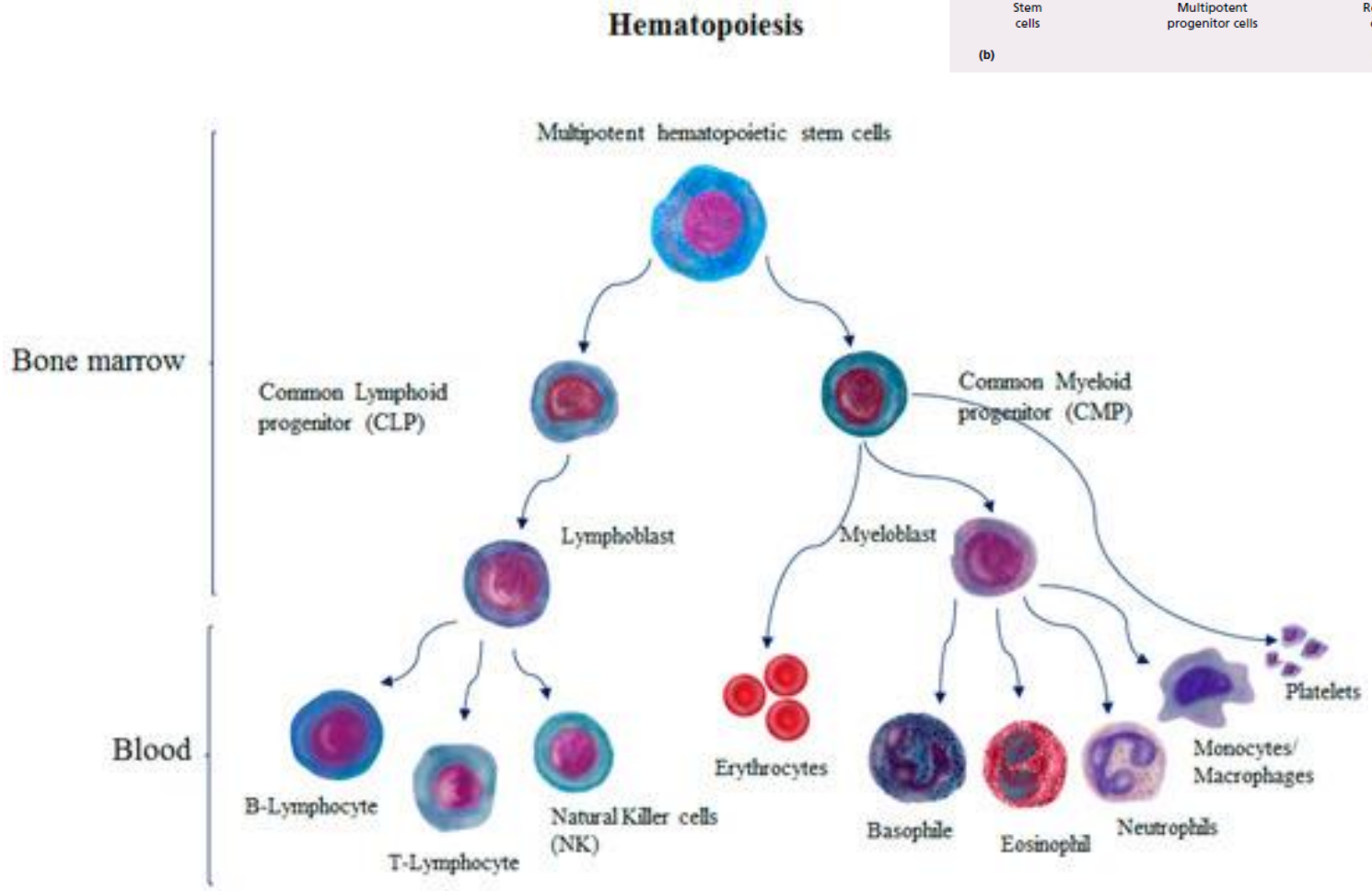
WHO 2022 Revised

Roles of morphology

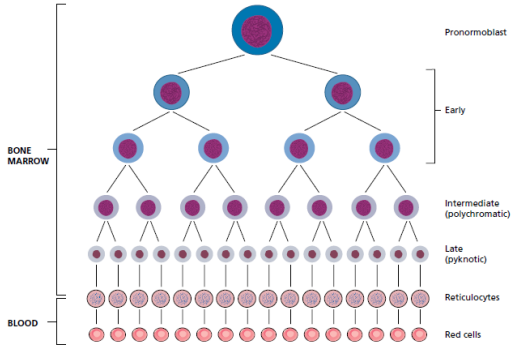
- Morphology offers primary diagnostic “screening”.
- Morphology allows provisional sub-classification.
- Morphology guides further investigation.
- Sometimes morphology is associated with recurrent genetic abnormalities.
- Morphology is also a part of the follow-up.

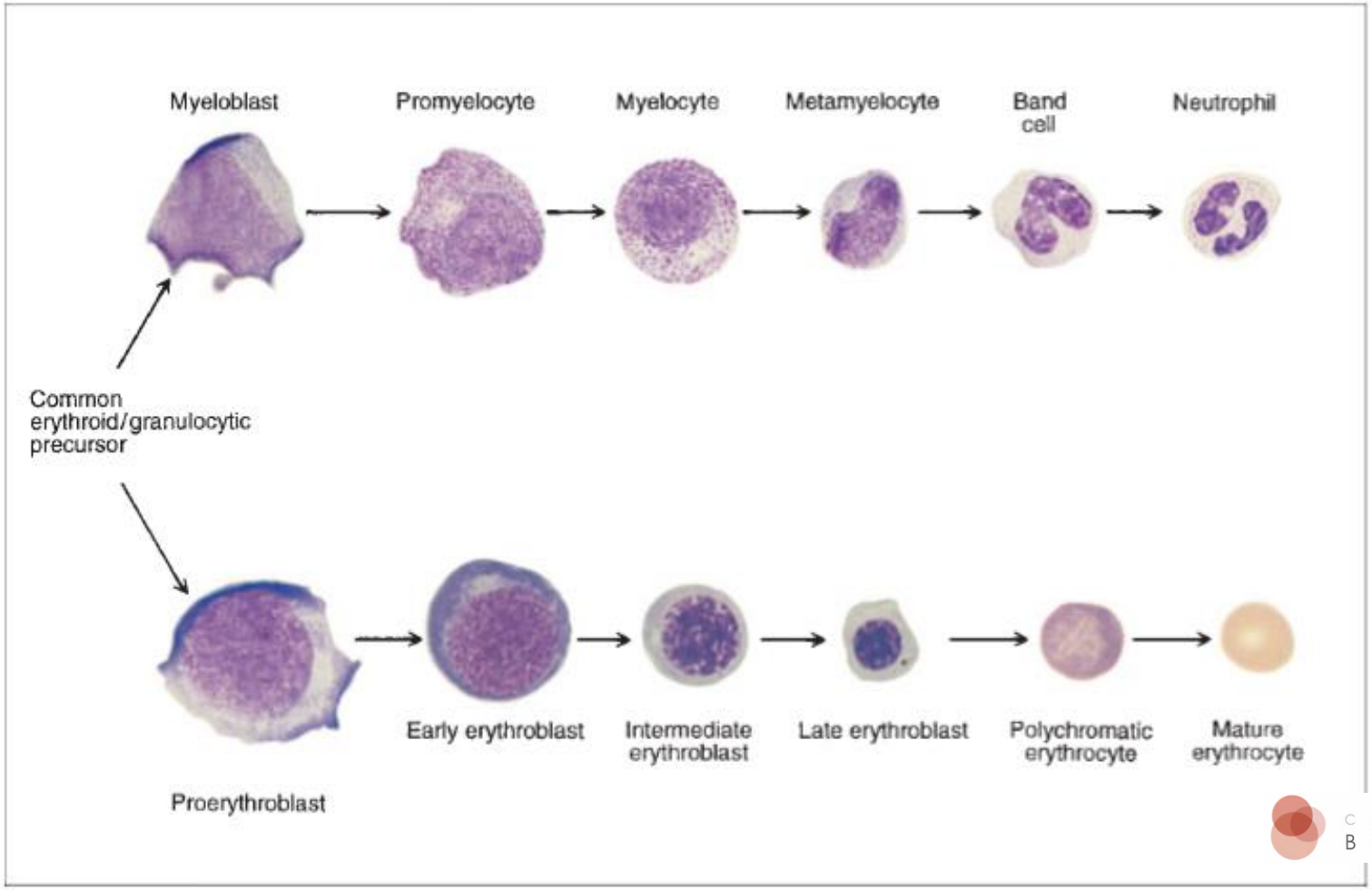
Hematopoiesis





Hematopoiesis

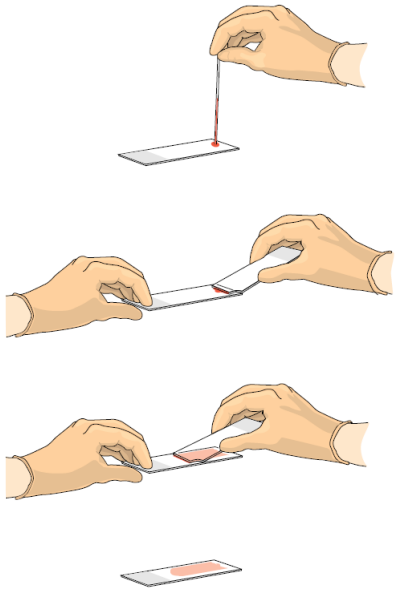


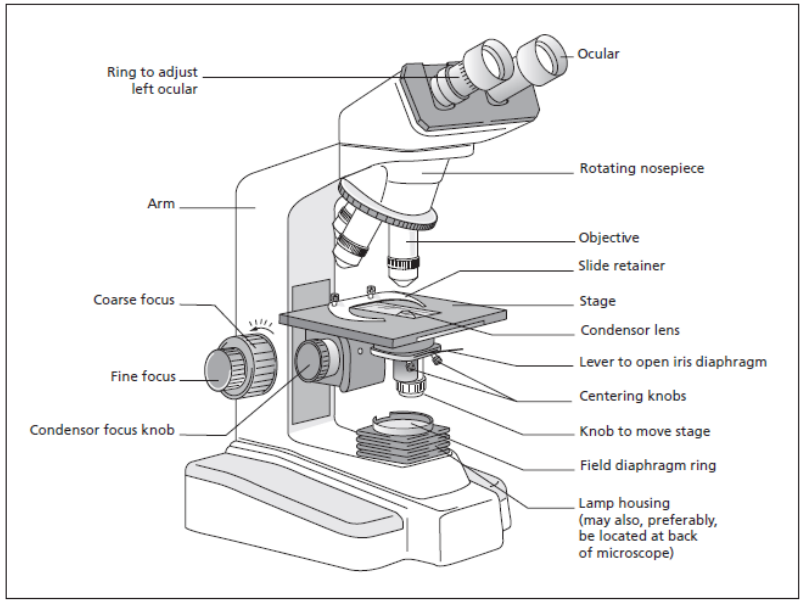


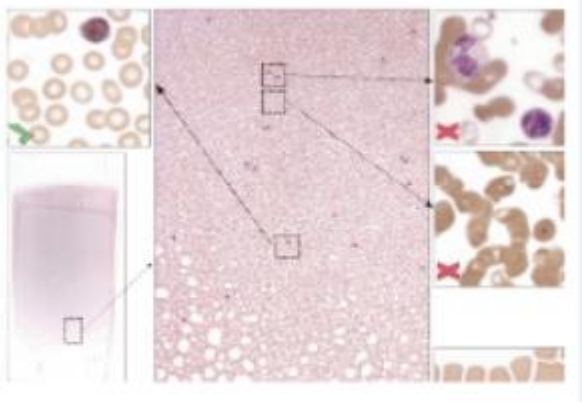
Indication of blood morphology

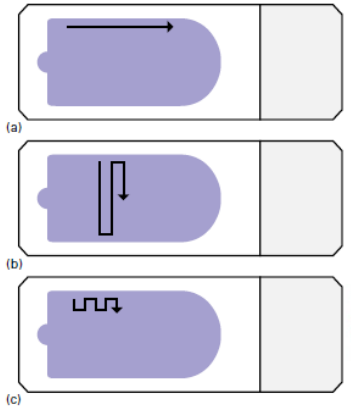
- Blood morphology may be request by the **clinician** on account of a clinical suspicion.
- The **laboratory** may initiate peripheral blood film based on
  - abnormal findings from an automated count
  - or patients clinical information whose diagnosis may be supported by a peripheral blood film.
- Common indication:
  - Cytopenia(s) : Anemia, leucopenia, thrombocytopenia
  - Unexplained leukocytosis, lymphocytosis, monocytosis
  - Unexplained hemolysis, jaundice
  - Features of hemolytic anemia
  - Suspected chronic or acute myeloproliferative disease

Blood microscopic review









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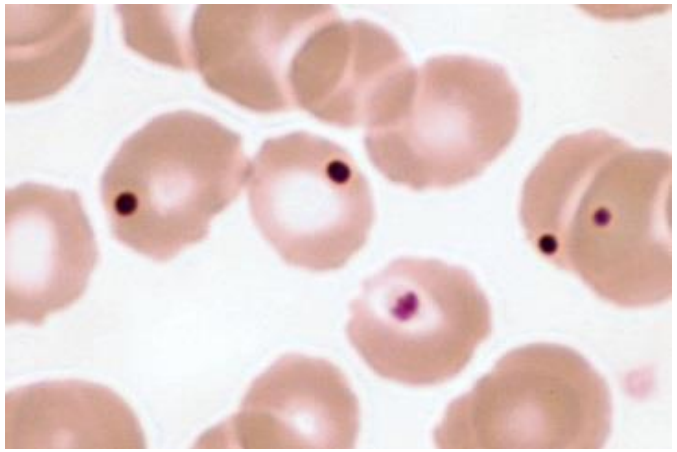
RBC morphology anomality

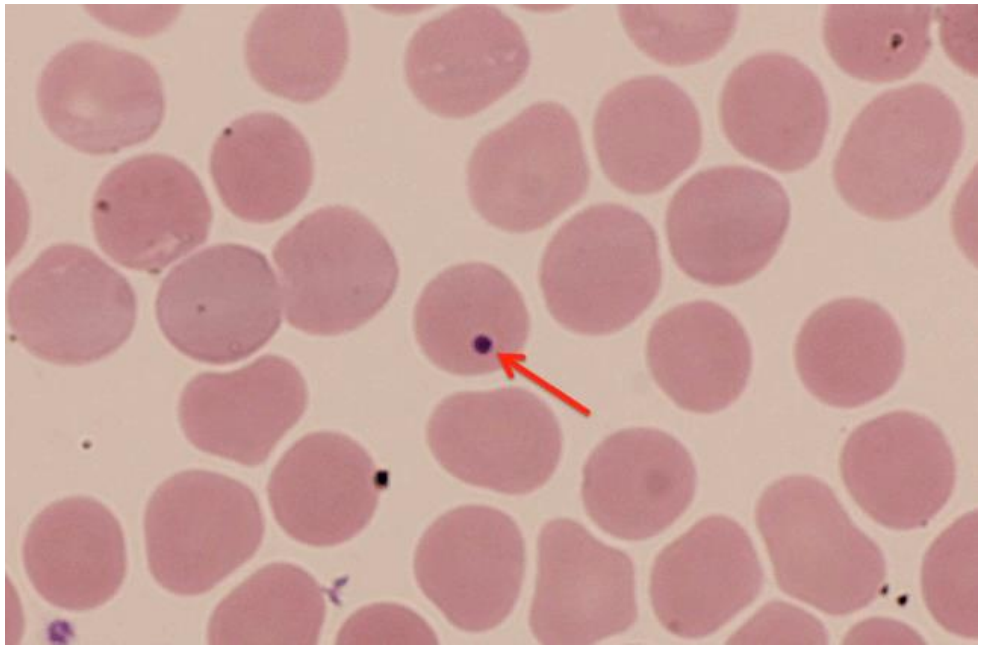
	Red cell abnormality	Causes
	Normal	
	Macrocyte	Liver disease, alcoholism. Oval in megaloblastic anaemia
	Target cell	Iron deficiency, liver disease, haemoglobinopathies, post-splenectomy
	Stomatocyte	Liver disease, alcoholism
	Pencil cell	Iron deficiency
	Echinocyte	Liver disease, post-splenectomy. storage artefact
	Acanthocyte	Liver disease, abetalipo-proteinaemia, renal failure

	Red cell abnormality	Causes
	Microspherocyte	Hereditary spherocytosis, autoimmune haemolytic anaemia, septicaemia
	Fragments	DIC, microangiopathy, HUS, TTP, burns, cardiac valves
	Elliptocyte	Hereditary elliptocytosis
	Tear drop poikilocyte	Myelofibrosis, extramedullary haemopoiesis
	Basket cell	Oxidant damage– e.g. G6PD deficiency, unstable haemoglobin
	Sickle cell	Sickle cell anaemia
	Microcyte	Iron deficiency, haemoglobinopathy

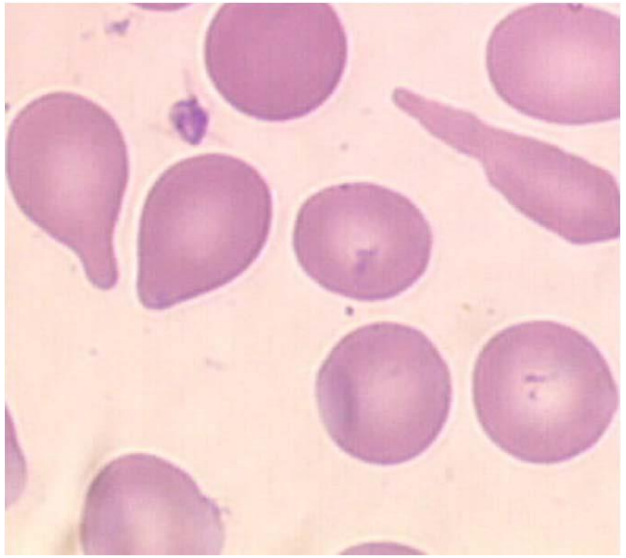
LHUB-ULB

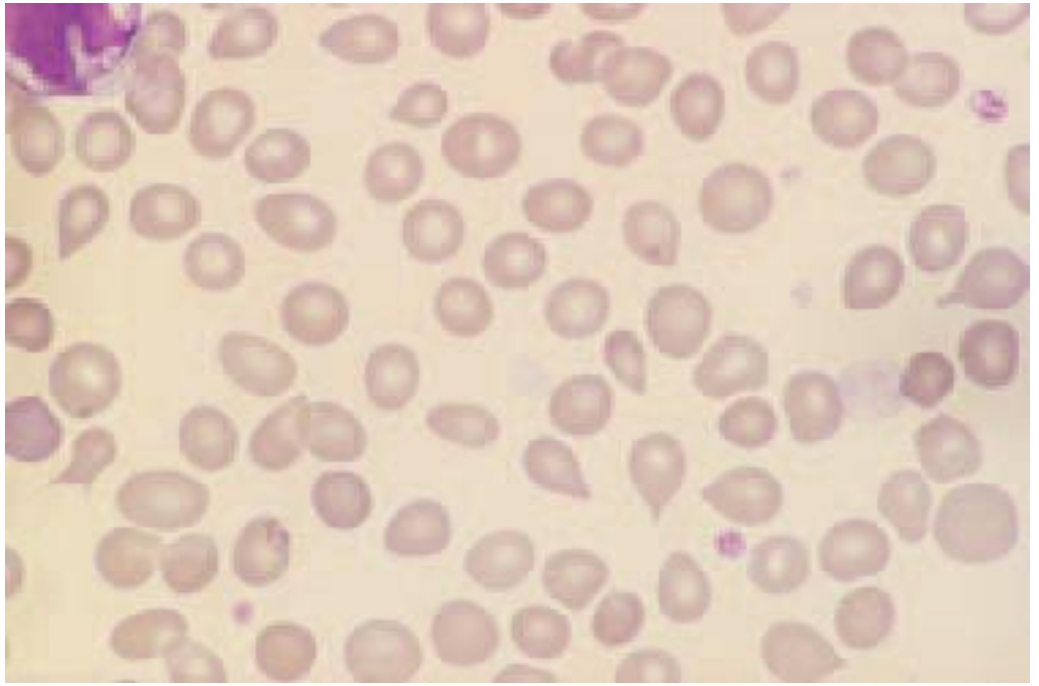
Howell-Jolly bodies



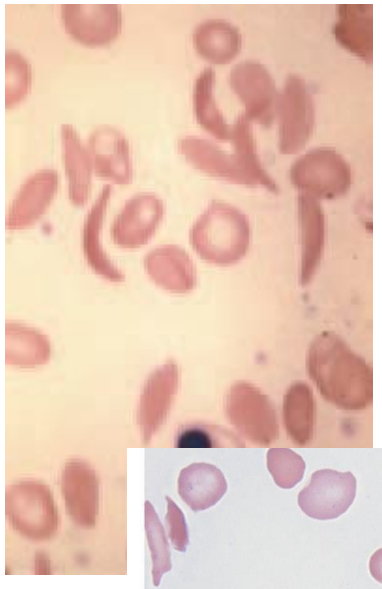


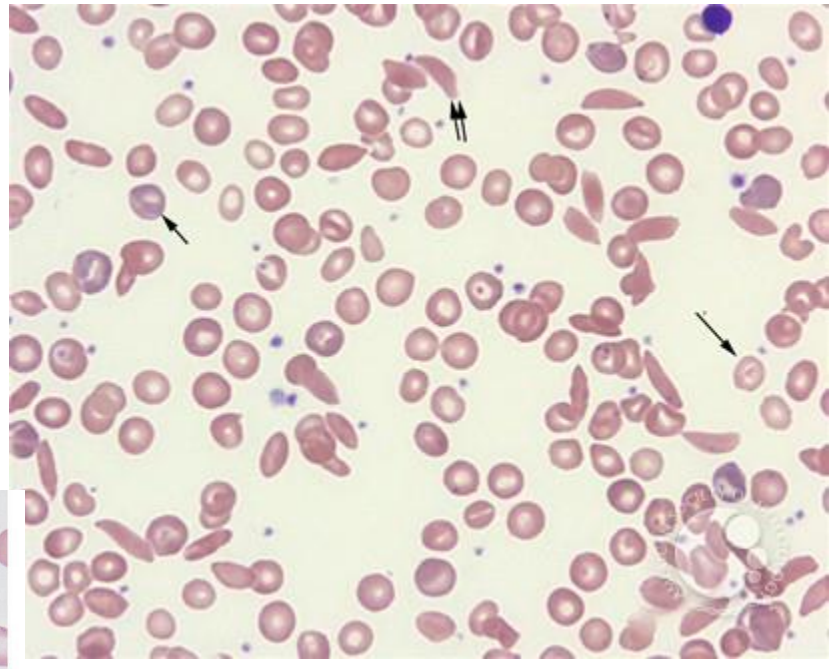
Dacryocytes or teardrop cells

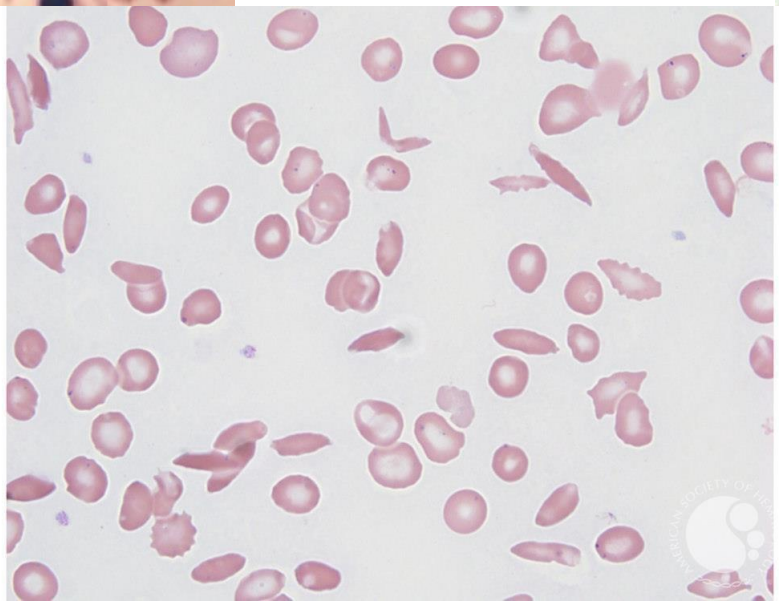




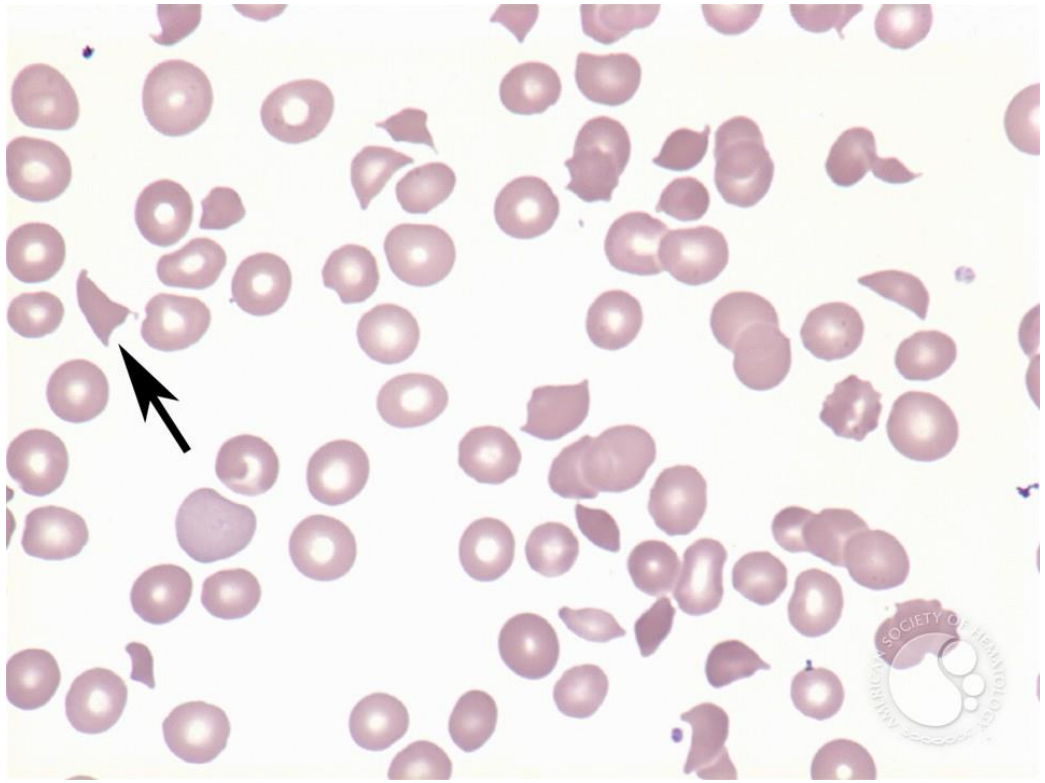
Sickle cells







Schizocytes



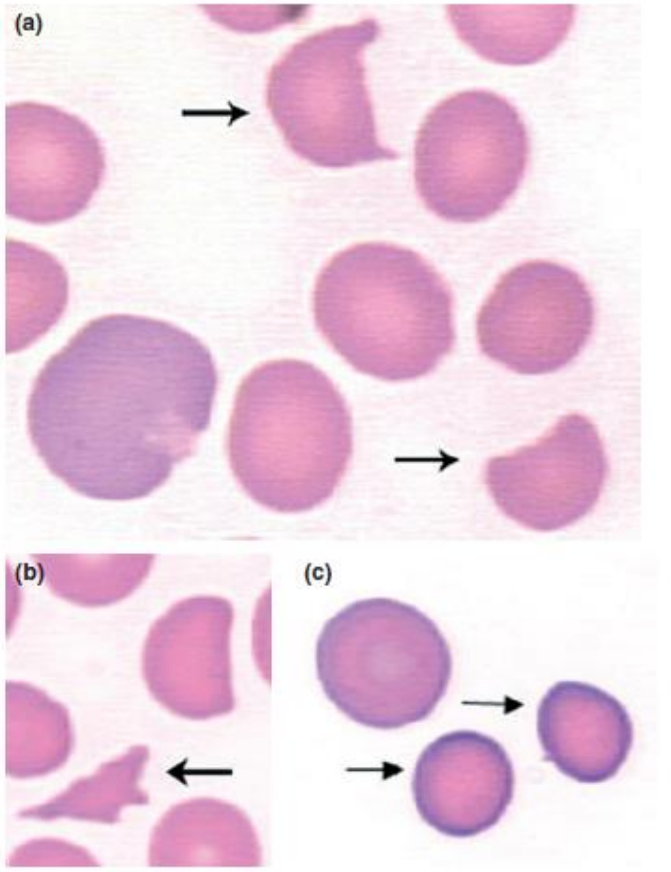
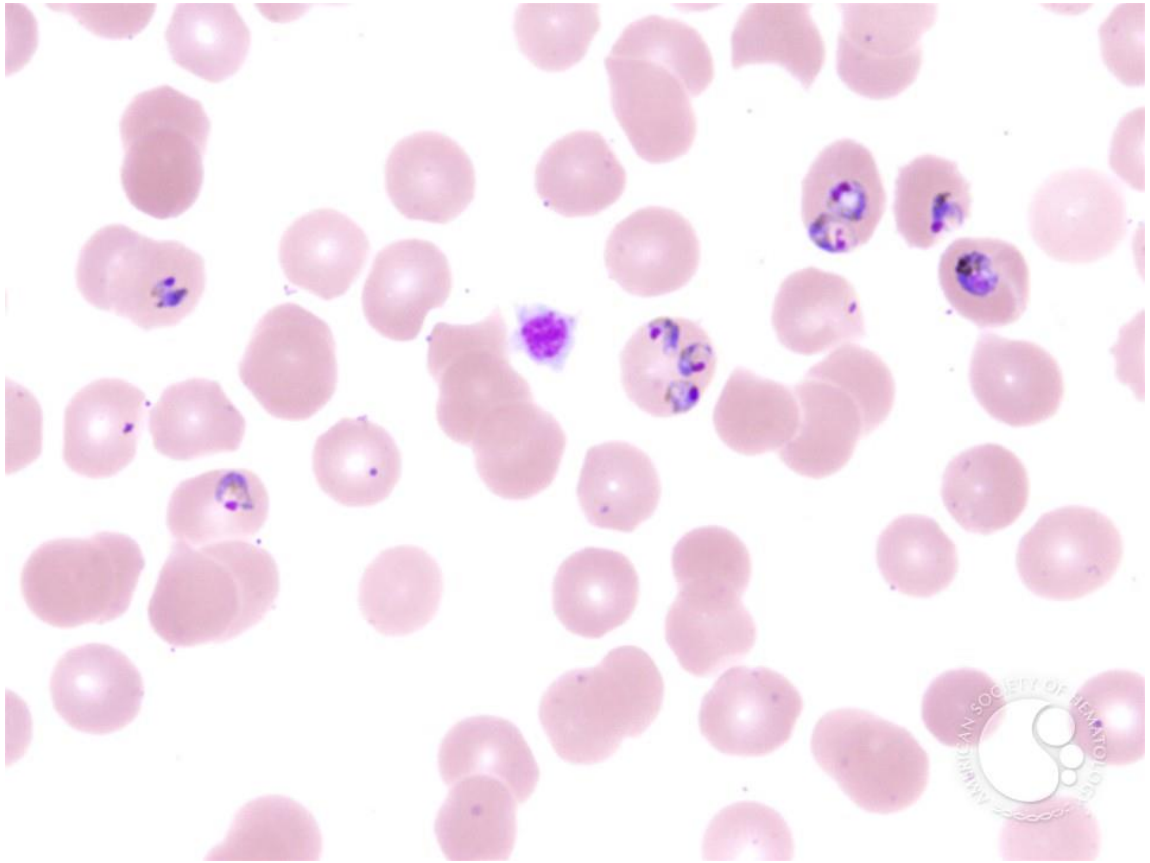


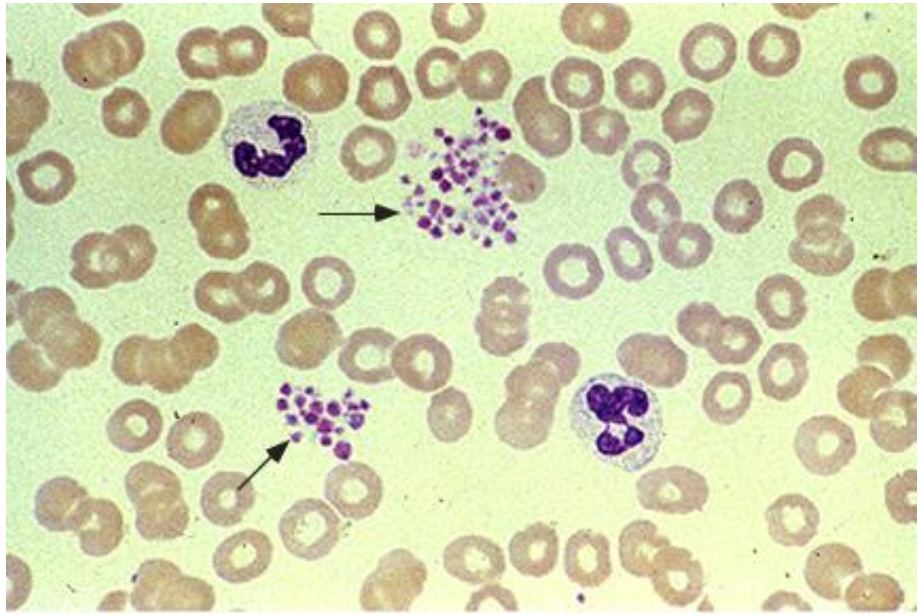
Figure 1. Typical shapes for specific identification of schistocytes. (a) keratocyte (upper arrow) and helmet cell (lower arrow), close to a polychromatophilic erythrocyte in the left lower corner; (b) a triangle schistocyte (arrow) with a helmet cell on the upper right; (c) two microspherocytes (arrows); they are derived, in a context of thrombotic microangiopathic anemia, from schistocytes.

Parasites

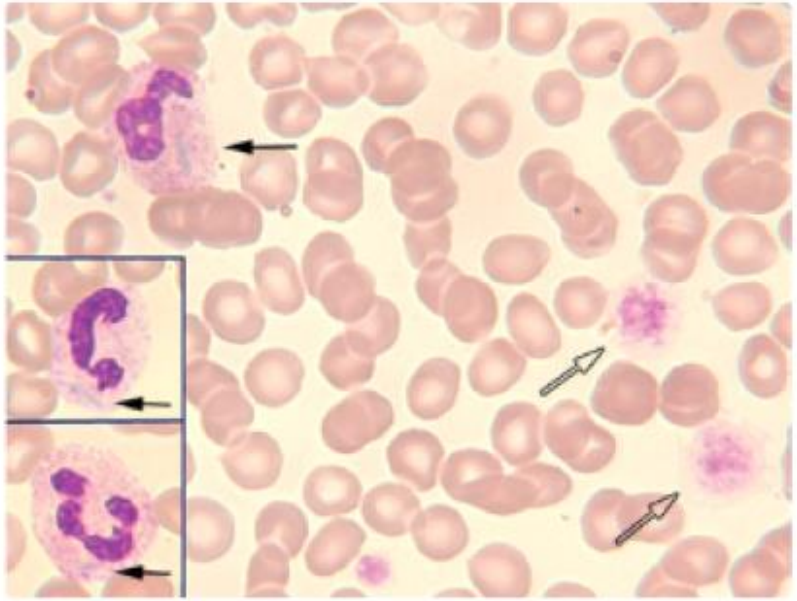


Platelet morphology

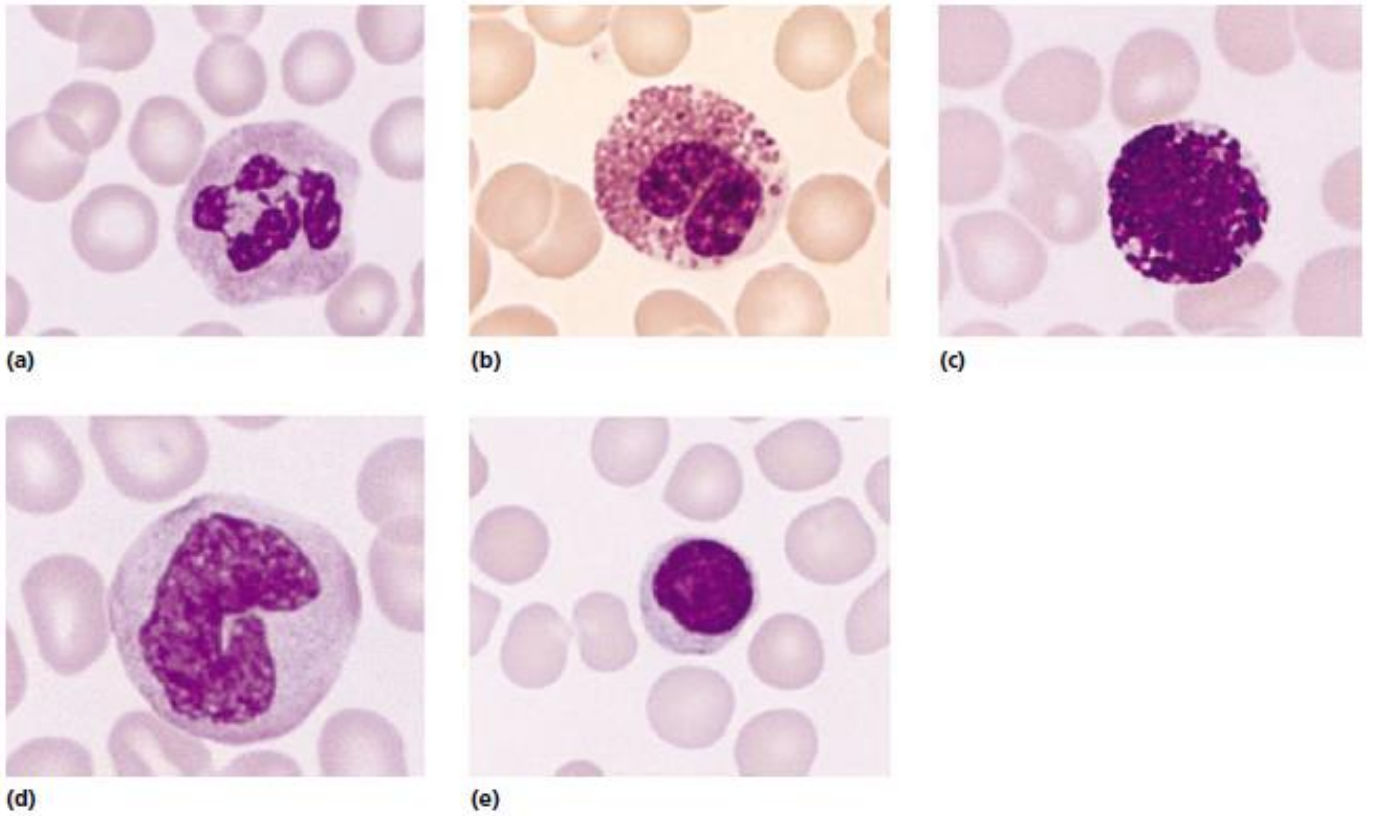
EDTA-dependent Pseudothrombocytopenia



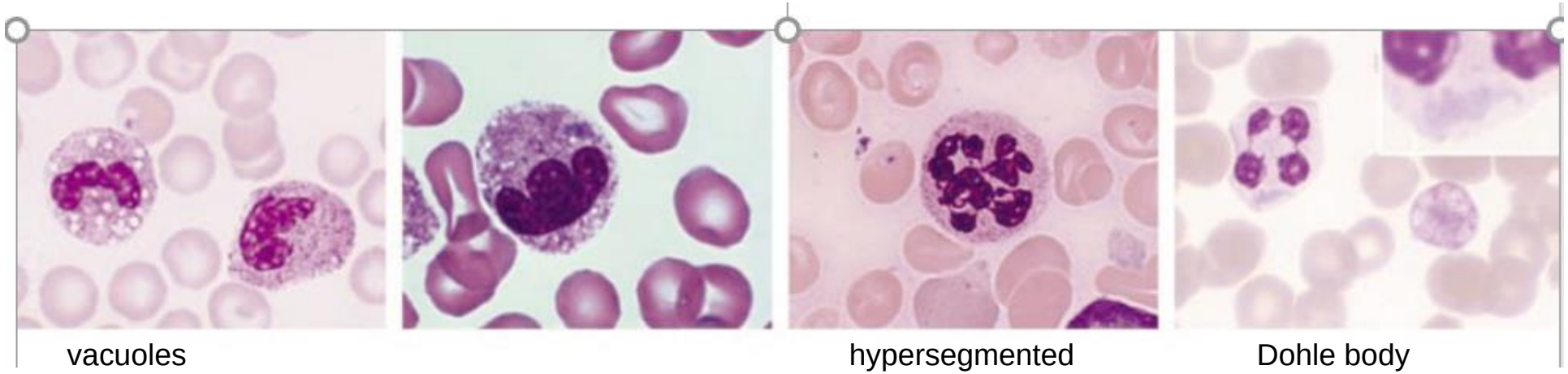
May-Hegglin



Normal white blood cells



Abnormal neutrophils



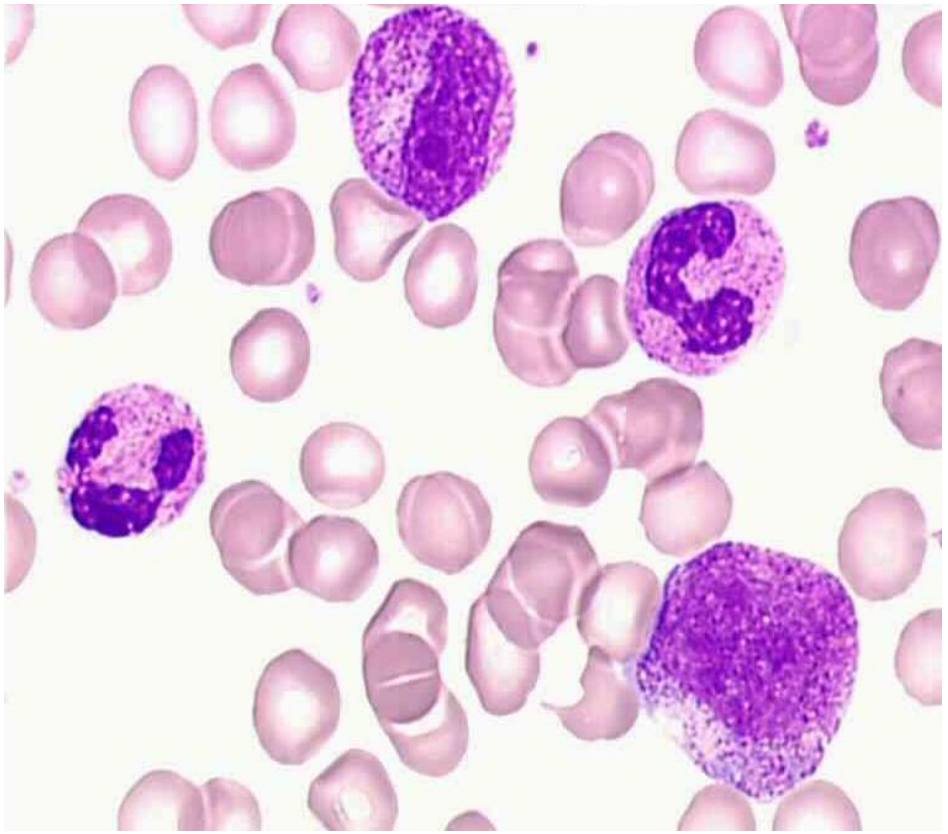
vacuoles

hypersegmented

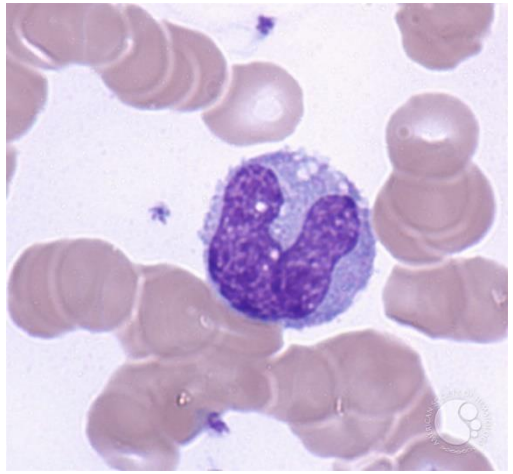
Dohle body

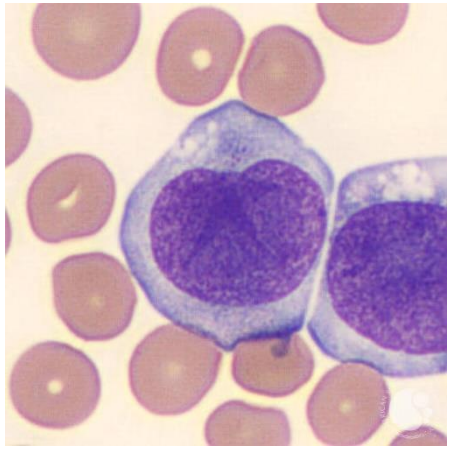
MYELEMIA

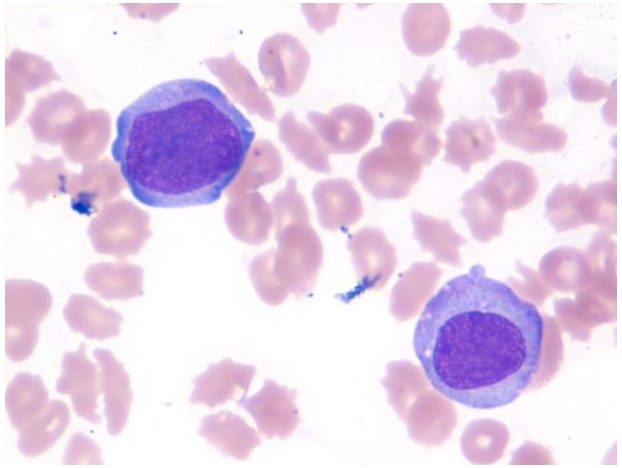
Circulating immature granulocyte



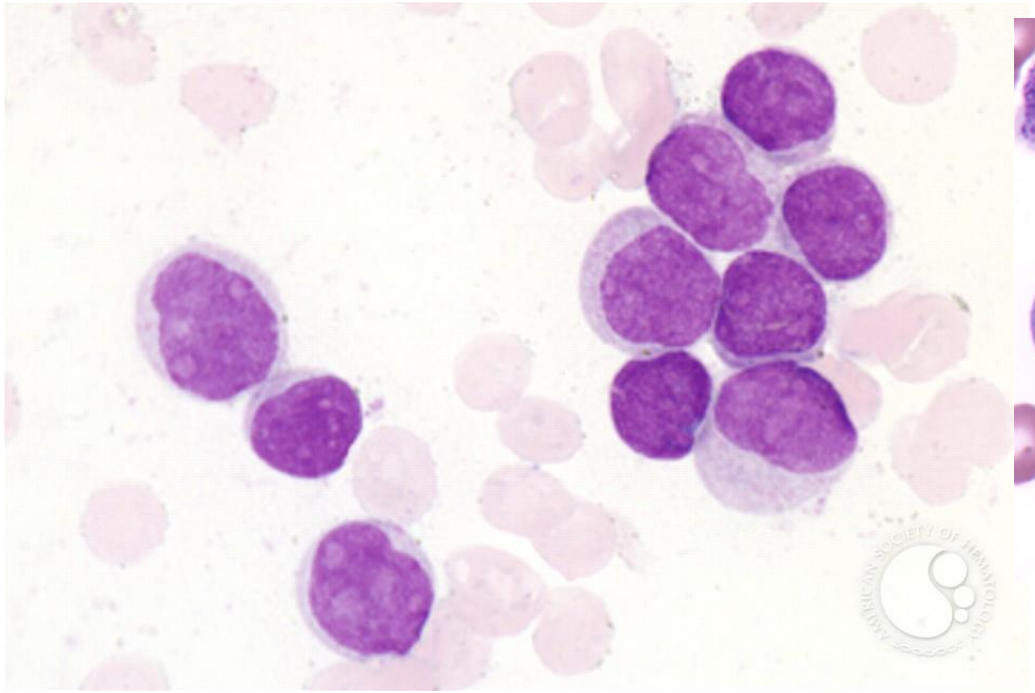
Monocytes – promonocytes - monoblasts

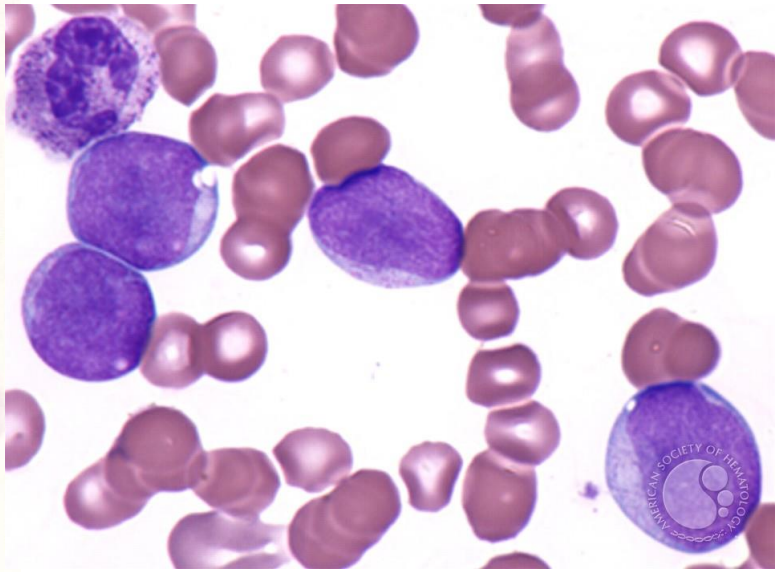




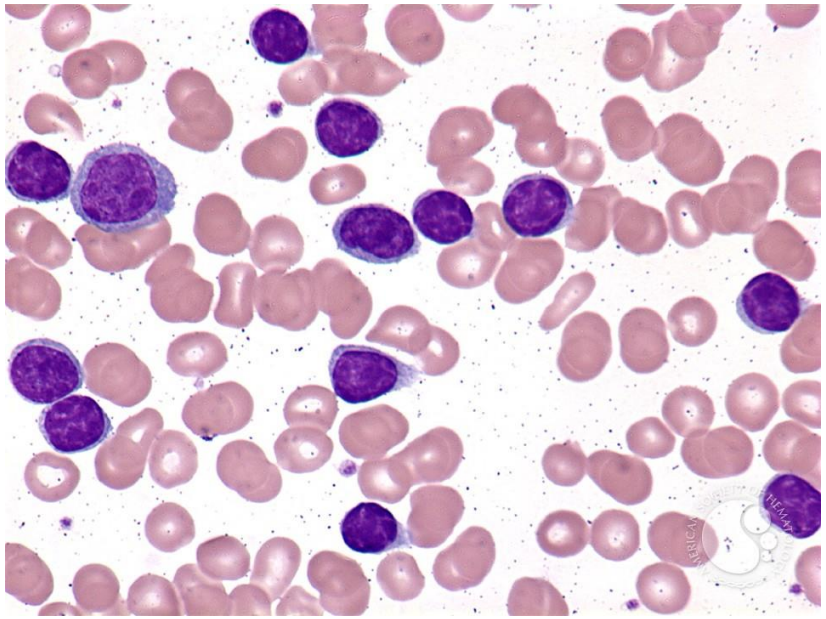


Blasts

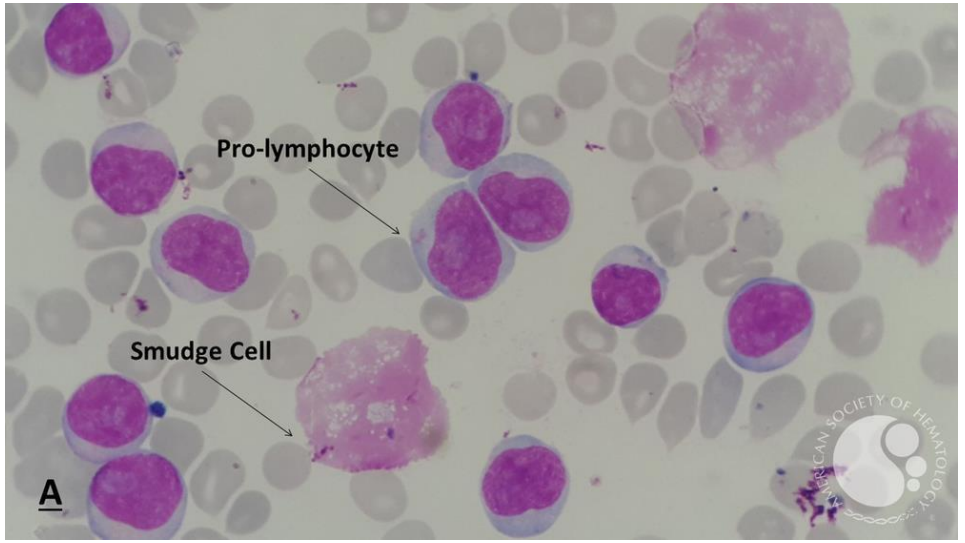


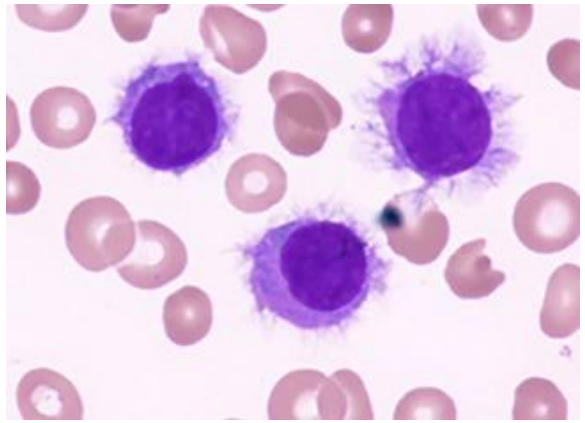


Lymphoma cells



CLL





HCL

Bone marrow aspiration - Indication

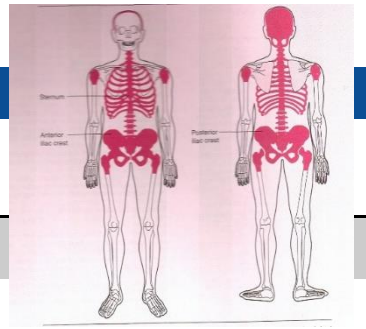
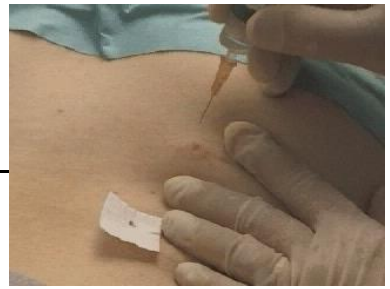


Table 1. Indications for bone marrow examination

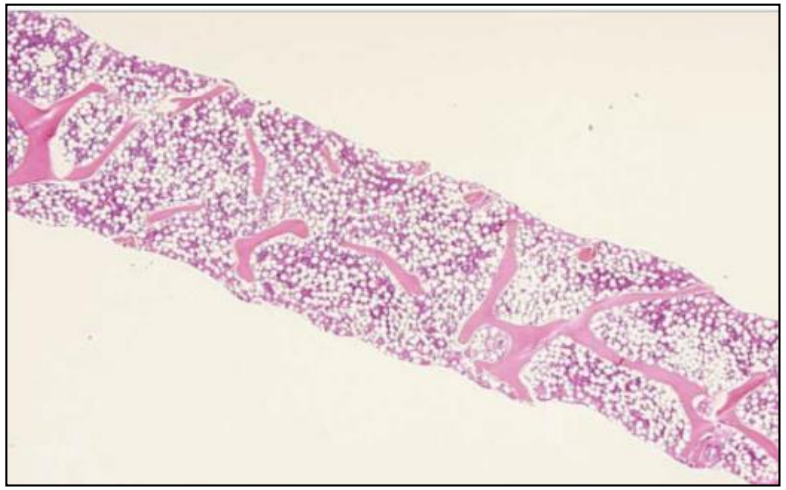
Investigation of unexplained anaemia, abnormal red cell indices, cytopenias or cytoses
Investigation of abnormal peripheral blood smear morphology suggestive of bone marrow pathology
Diagnosis, staging and follow-up of malignant haematological disorders (e.g. acute and chronic leukaemias, myelodysplastic syndromes, chronic myeloproliferative disorders, lymphomas, plasma cell myeloma, amyloidosis, mastocytosis)
Investigation of suspected bone marrow metastases
Unexplained focal bony lesions on radiological imaging
Unexplained organomegaly or presence of mass lesions inaccessible for biopsy
Microbiological culture for investigations of pyrexia of unknown origin or specific infections, e.g. military tuberculosis, leishmaniasis, malaria
Evaluation of iron stores
Investigation of lipid/glycogen storage disorders
Exclusion of haematological disease in potential allogeneic stem cell transplant donors

 Int. Jnl. Lab. Hem. 2008, **30**, 349–364



Bone marrow trephine biopsy - Indication

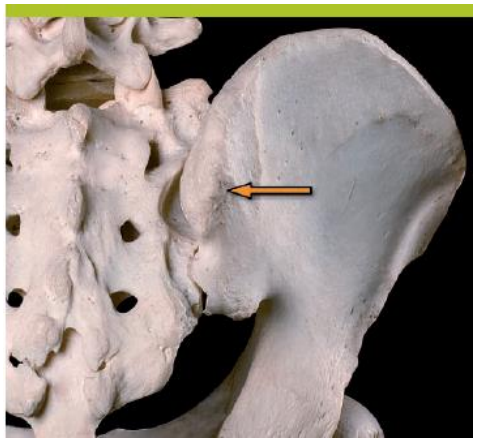
- Lymphoma extension assement.
- Suspicion of myelofibrosis.
- Suspicion of aplasia.
- Diagnosis/follow-up of multiple myeloma.
- Search for neoplastic cells in solid tumor.

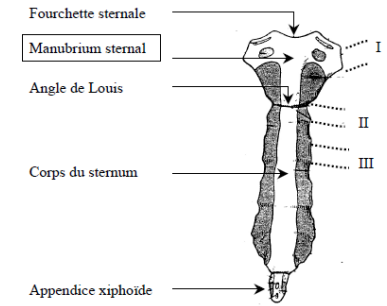


Punction site

It depends on the age of the patients and their medical history:

- Iliac crest (posterior superior or anterior iliac spine): for bone biopsy, in paediatrics, in the event of sternotomy or irradiation on the thorax in adults
- Manubrium sternal for adults (not in multiple myeloma)
- Tibia: for baby (low weight)

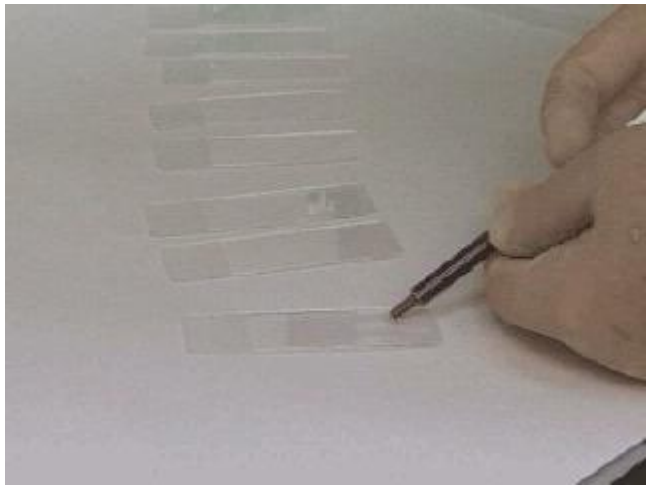


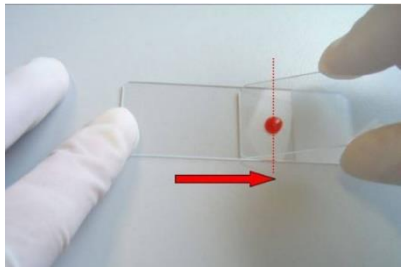


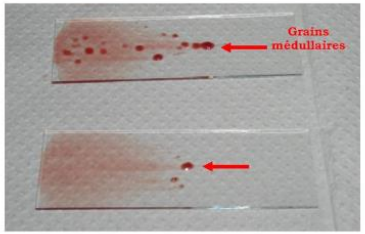
Face antérieure du sternum.
En chiffres romains sont signalées les 3 premières échancrures costales. La zone de ponction de moelle se situe dans le manubrium sternal.

Bone marrow punction – smear spreading

On the first drop







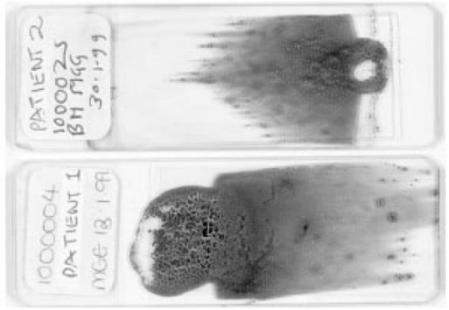


Figure 1 Stained bone marrow films showing a film of appropriate length, spread towards the frosted end where the label is applied (patient 1), and a film that is too long and has been spread, incorrectly, away from the frosted end where the label is applied (patient 2).

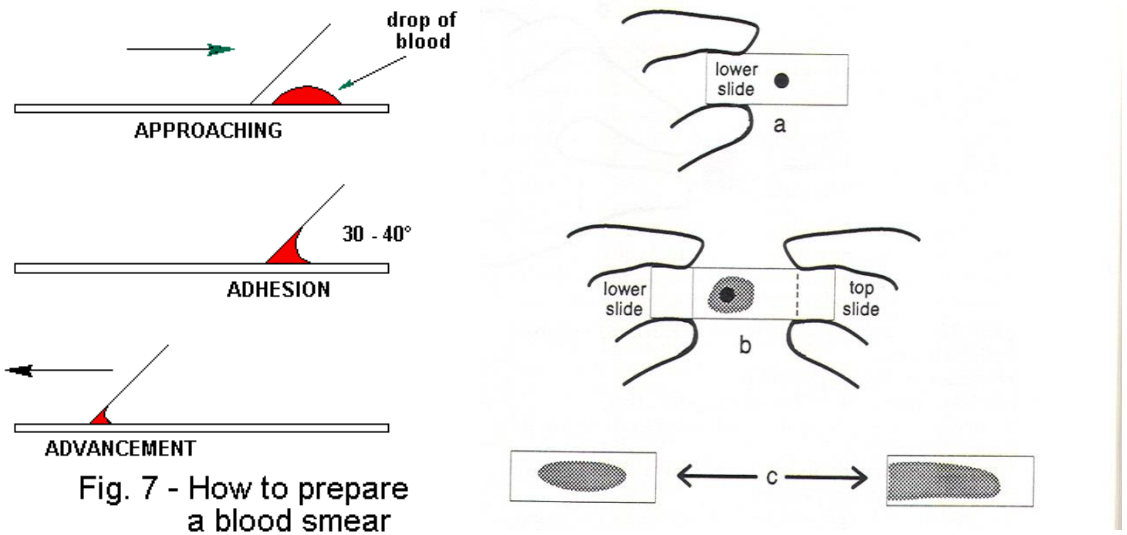
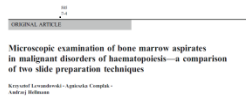


Fig. 7 - How to prepare a blood smear

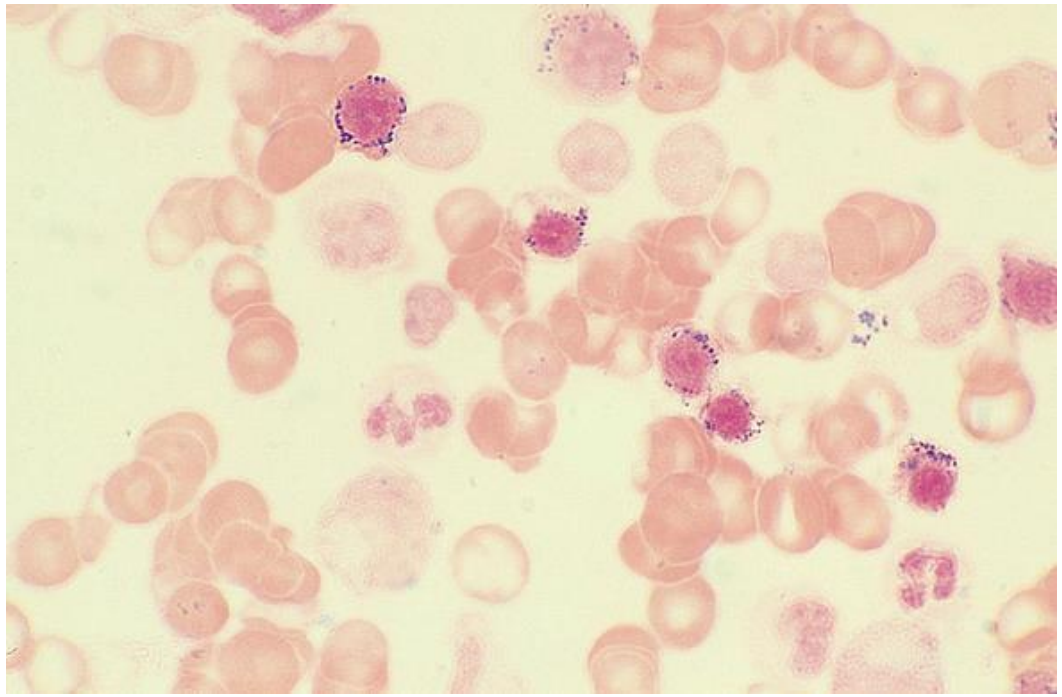
Microscopic examination of bone marrow aspirate in healthy adults – comparison of two techniques of slide preparation



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

Staining of the smears

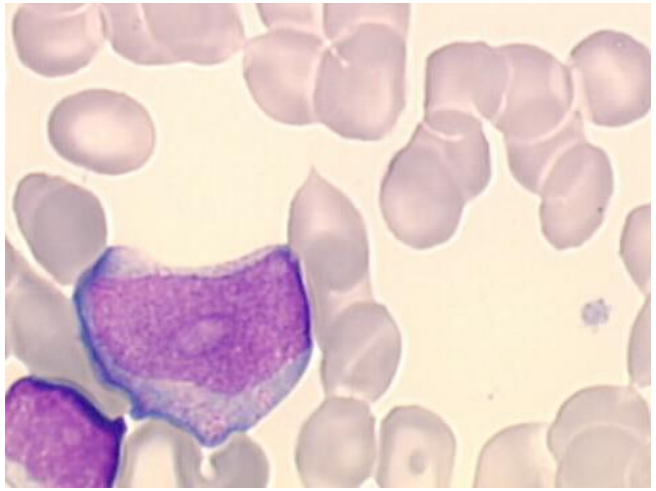
- Bone marrow smears are fixed and colored with May-Grunwald Giemsa.
- If MDS is suspected, a bone marrow smear is also colored by Perls Prussian blue.

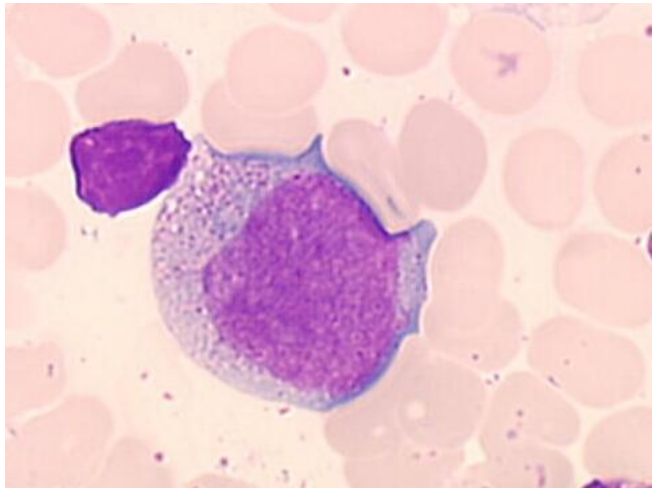


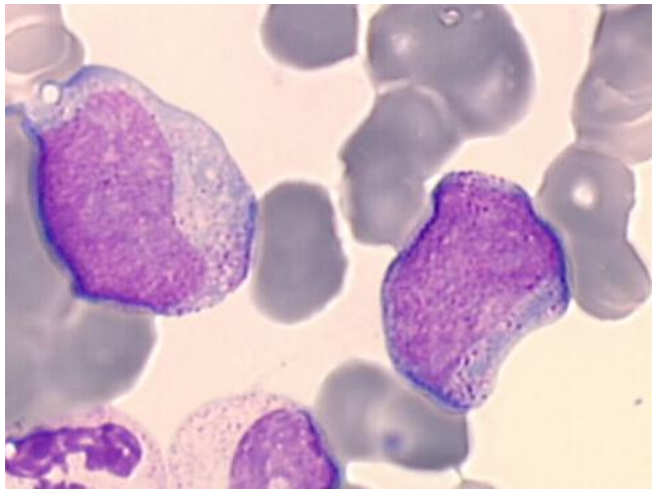
Microscopy

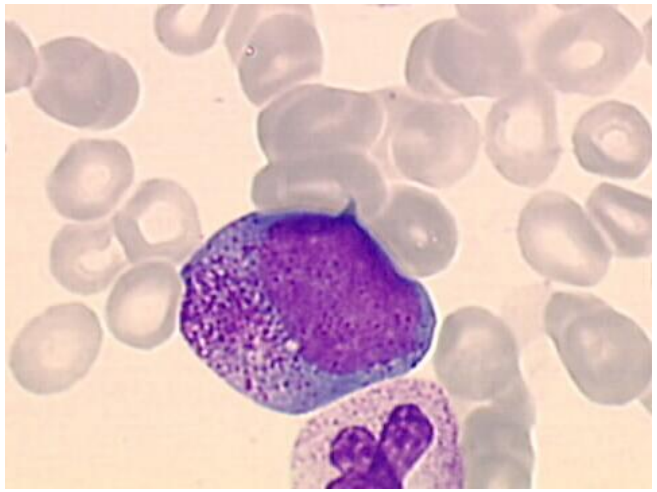
- Observation at low magnification 100x:
  - Determination of cellularity
  - Search for megakaryocytes
  - Search for suspicious cell clusters, neoplastic cells
- Observation at highest magnification (500x and 1000x) in a well-spread area:
  - Detailed observation of morphology
  - More specific research: parasites, inclusions, cellular details
- Performing the count:
  - Counting nucleated elements
  - Ideally: 2 x 250 cells

Myeloid precursor

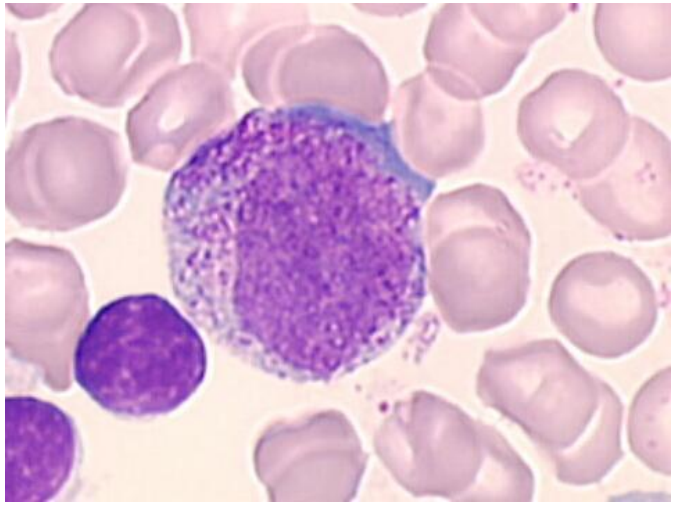


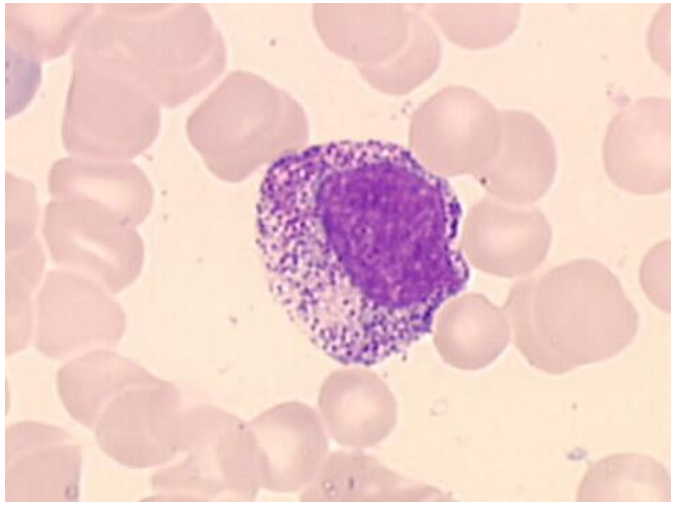


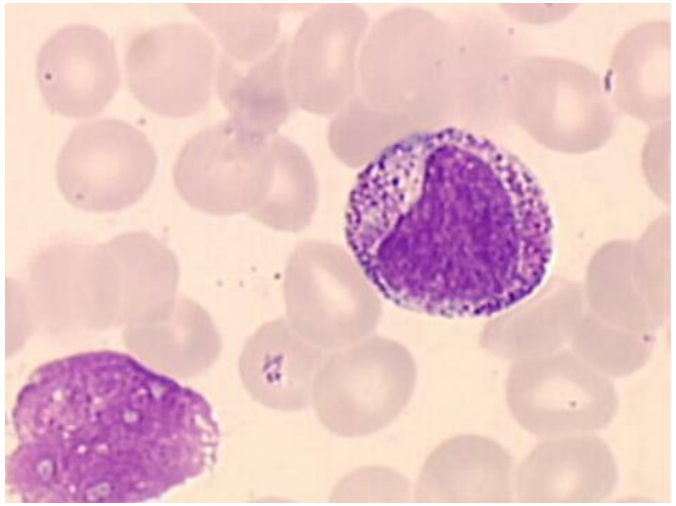


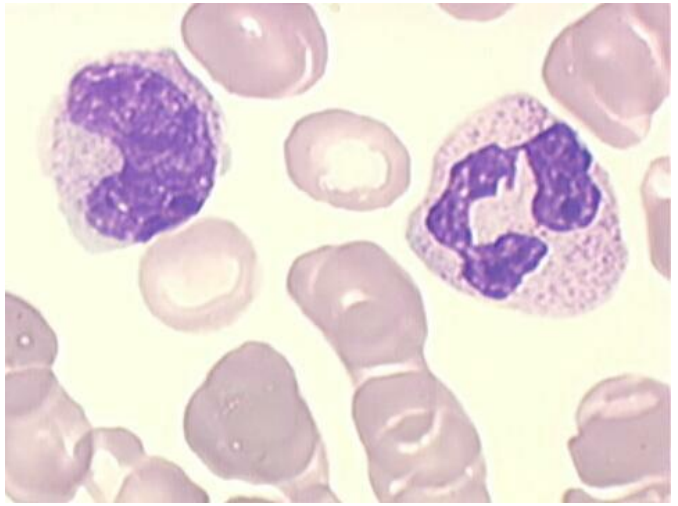


Granulopoiesis

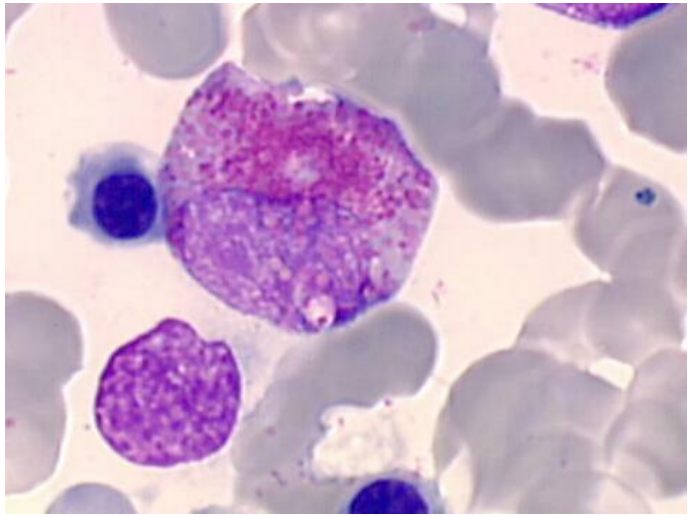


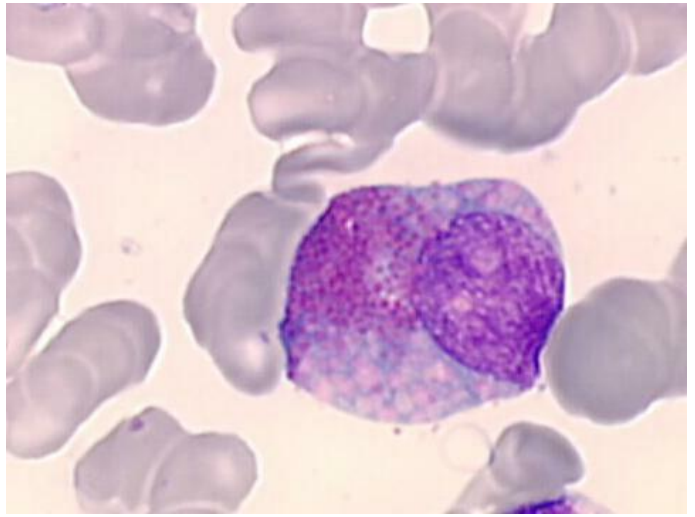


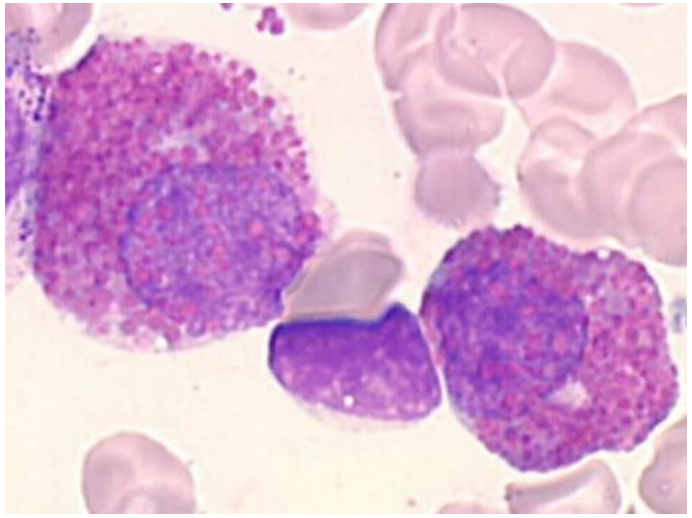


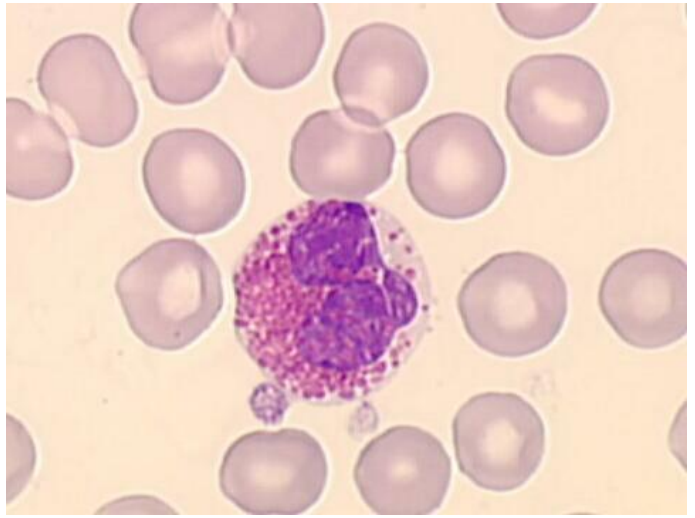


Eosinophilia lineage

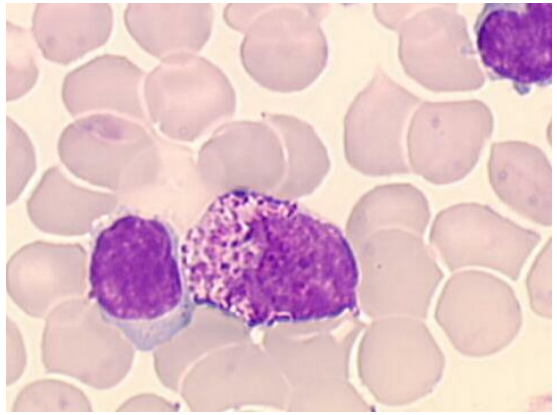


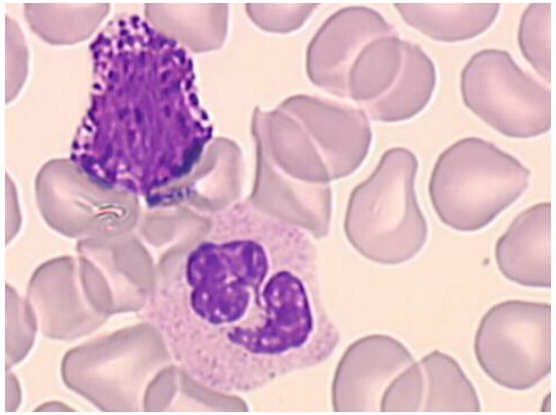


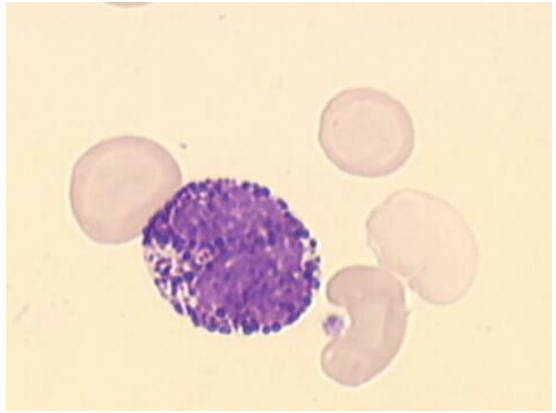


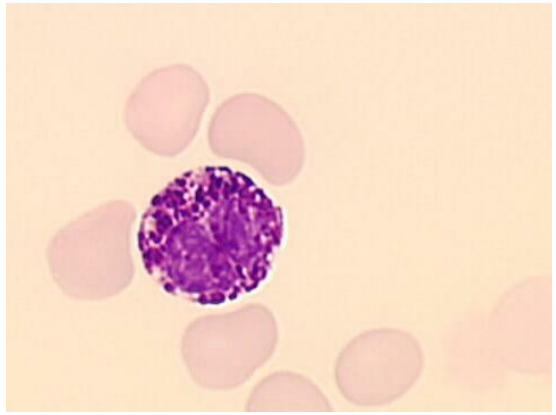


Basophilia lineage

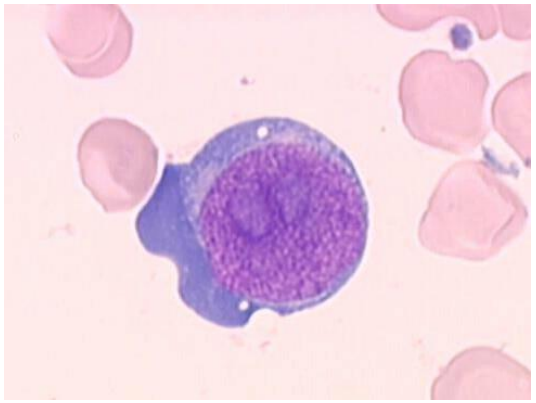
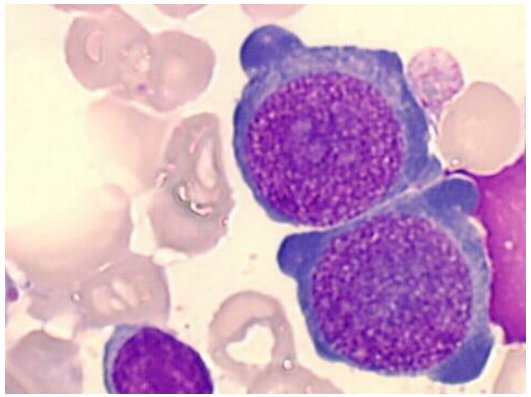
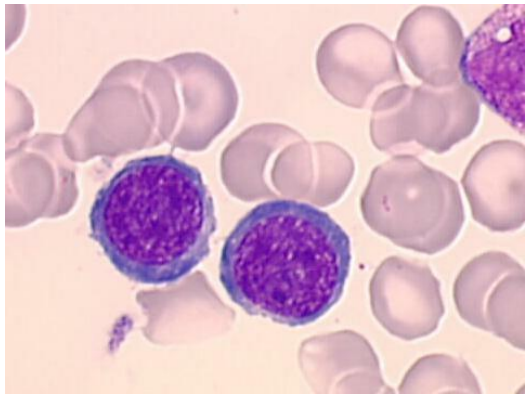
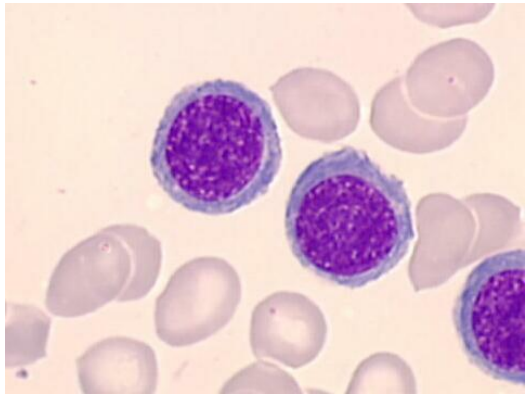
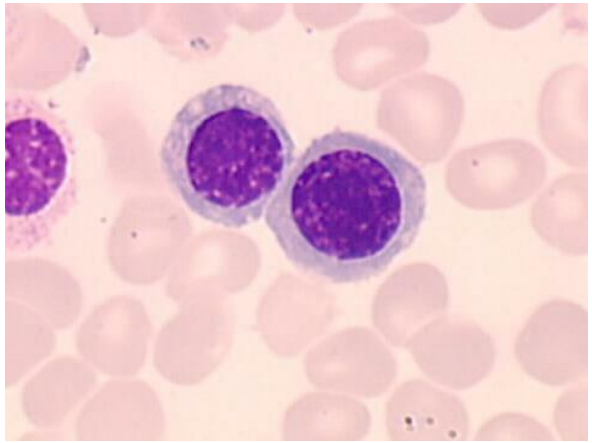
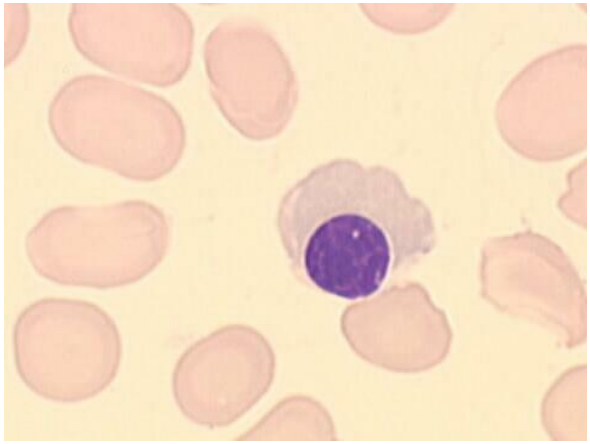




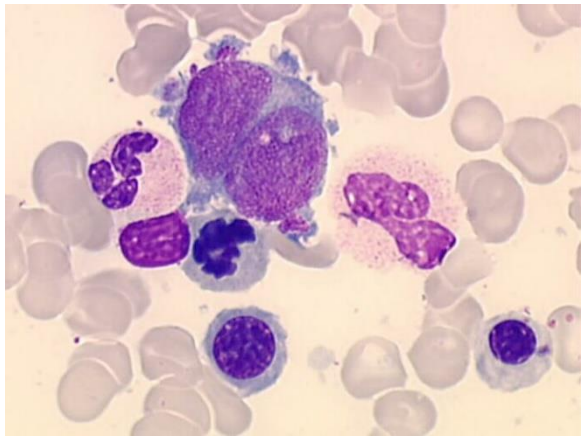


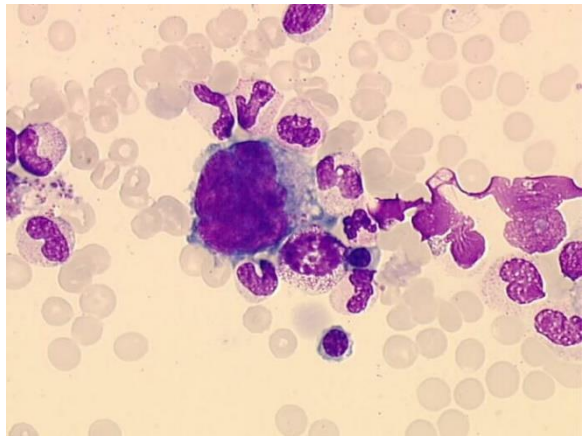


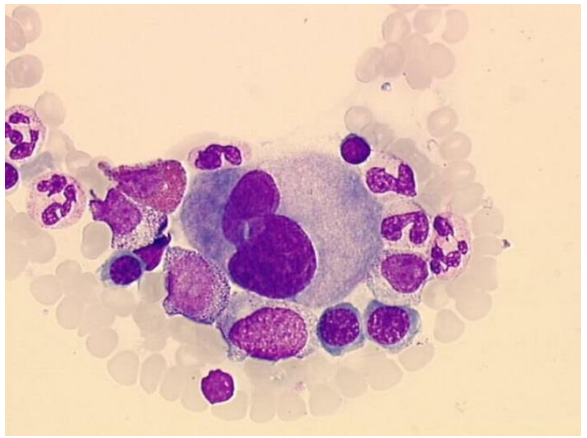
Erythropoiesis

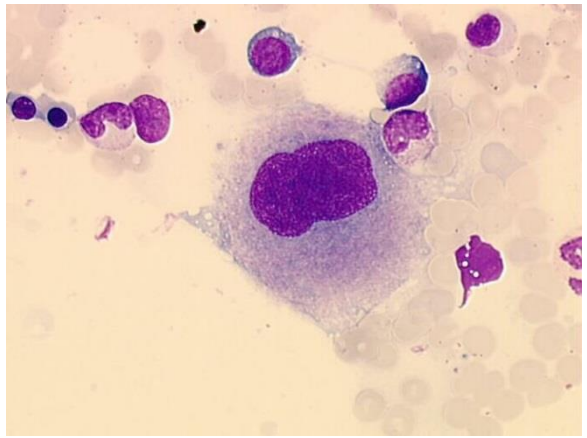







Megacaryopoiesis

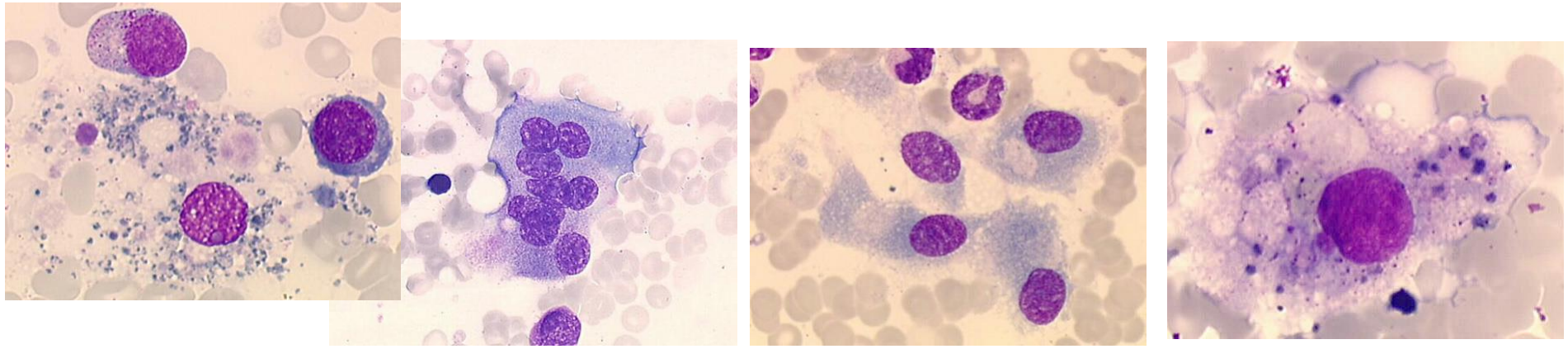


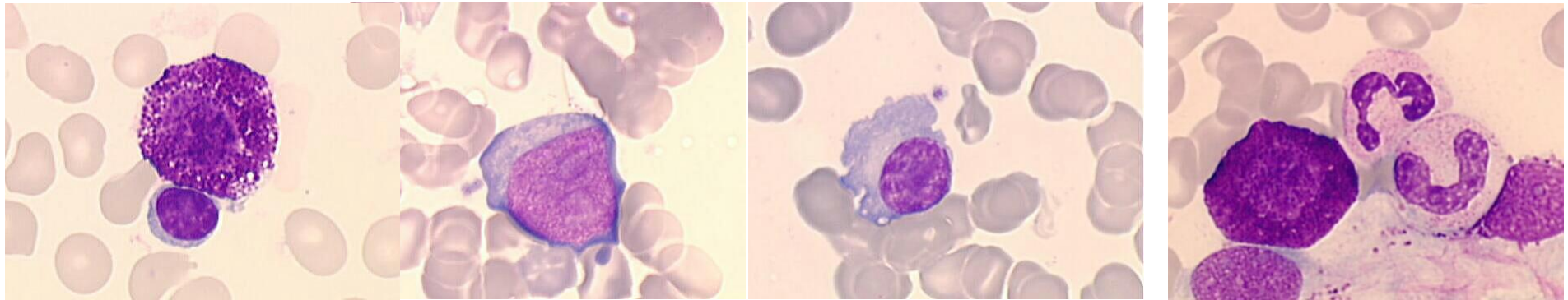




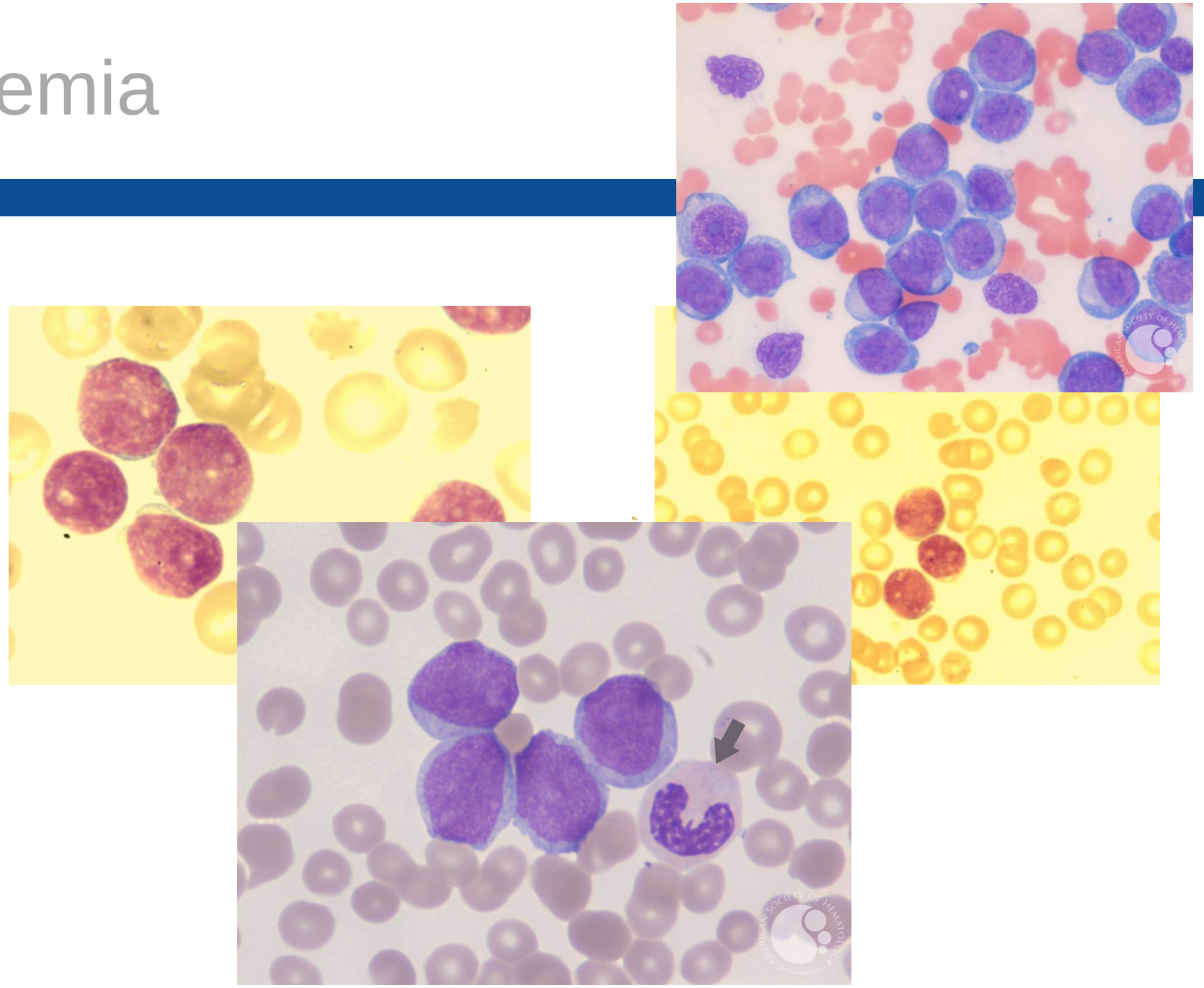


Rare cells



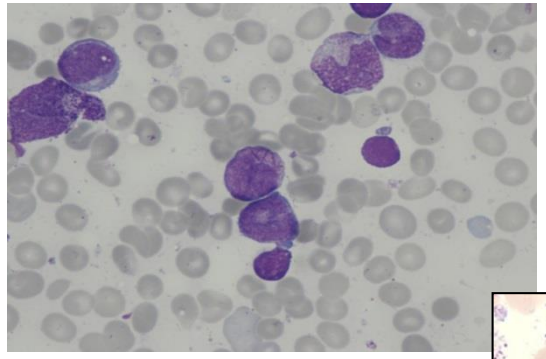


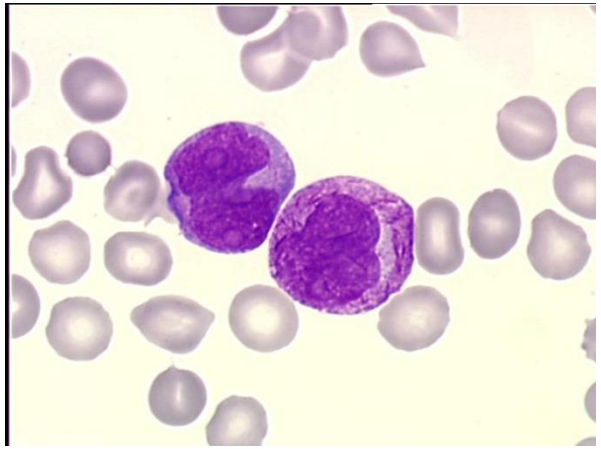
Leukemia

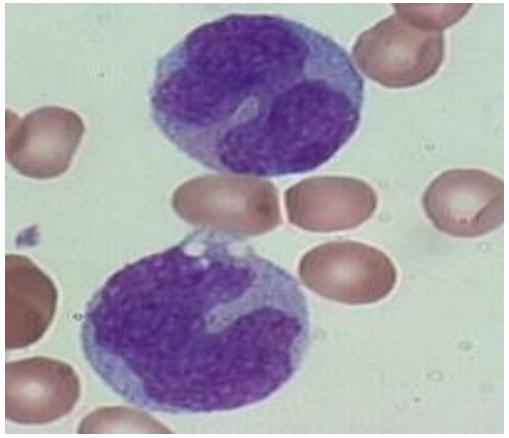


Acute Promyelocytic Leukemia (APL) – Emergency in hematology

Maturation: In the classic M3 the majority of the proliferating cells are abnormal promyelocytes with numerous primary type granules. Auer rods are frequent and often multiple.





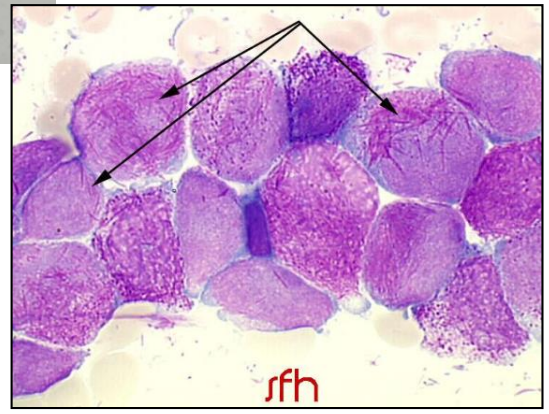


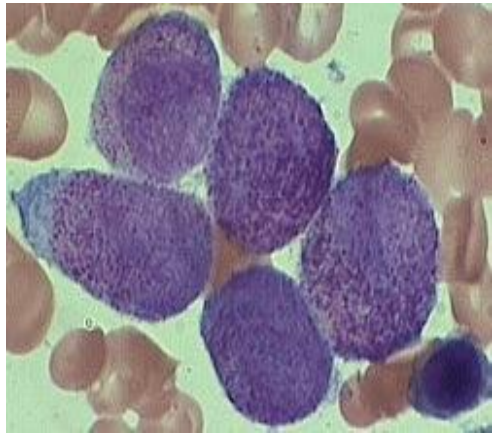
M3v AML

!! DIC

t(15;17)

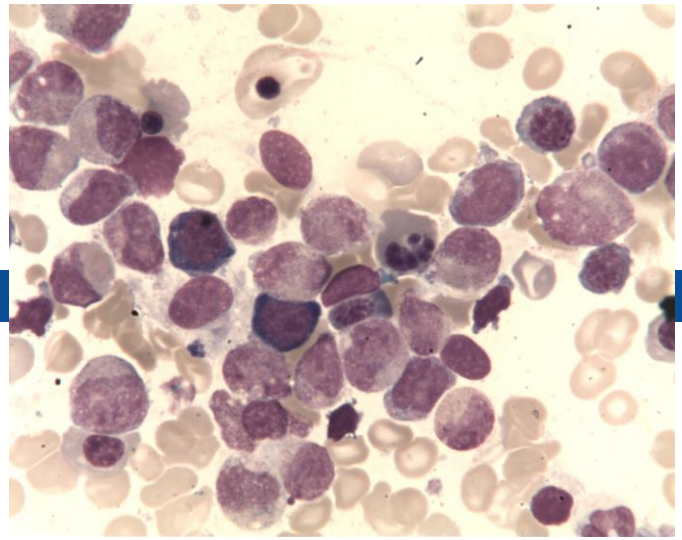
CD34-, HLA-DR -

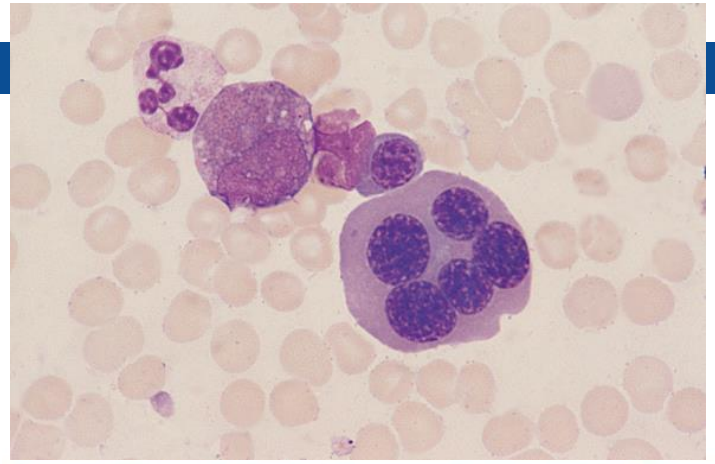


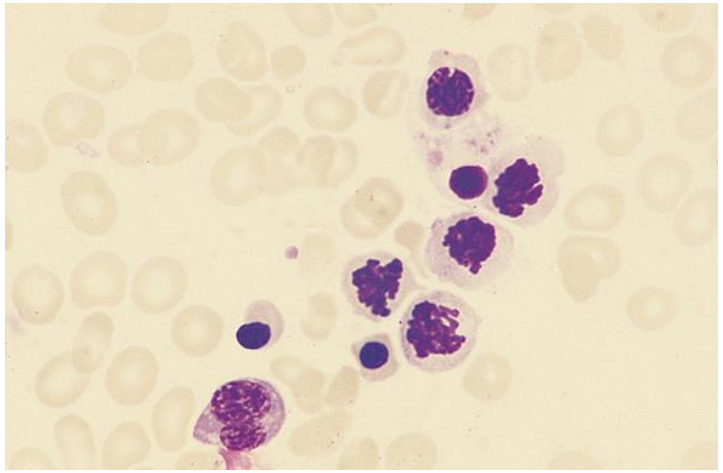


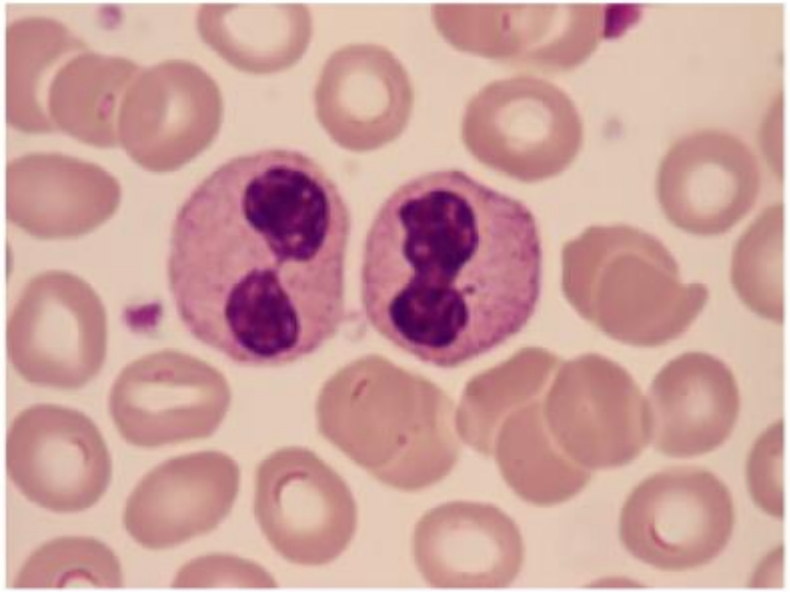
LHUB-ULB

Myelodysplasia

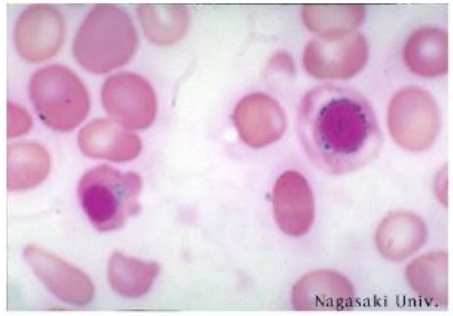


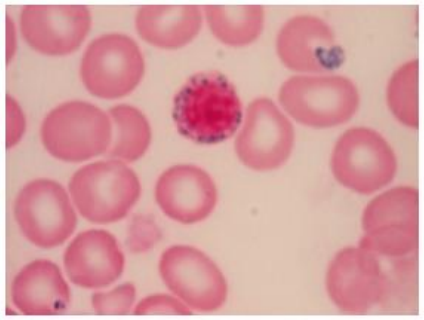




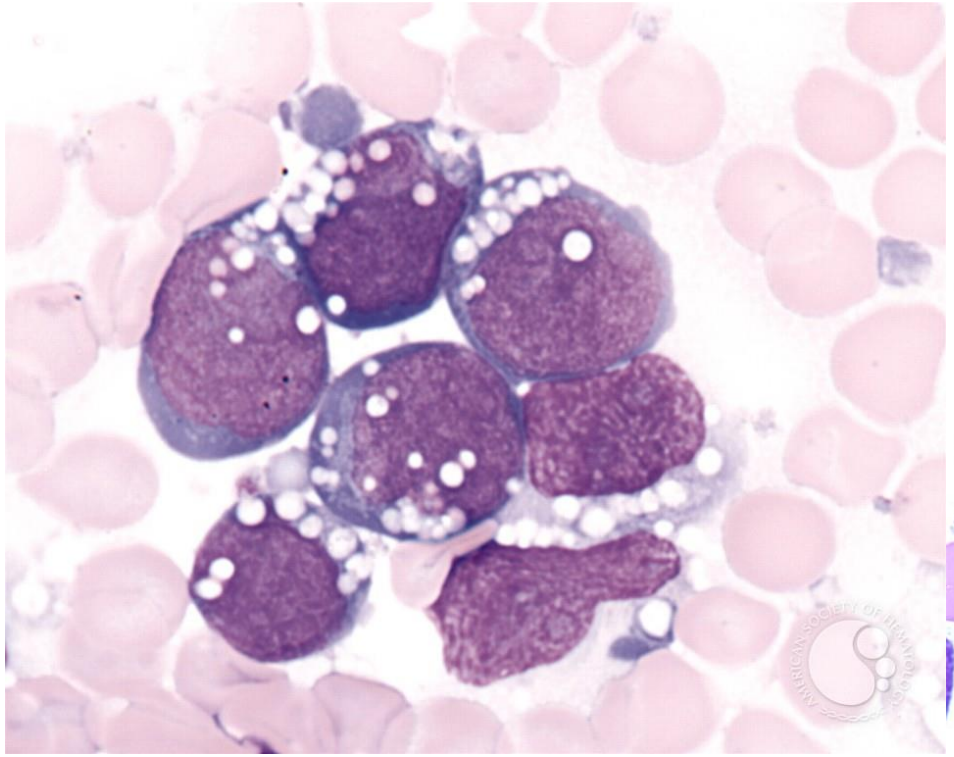


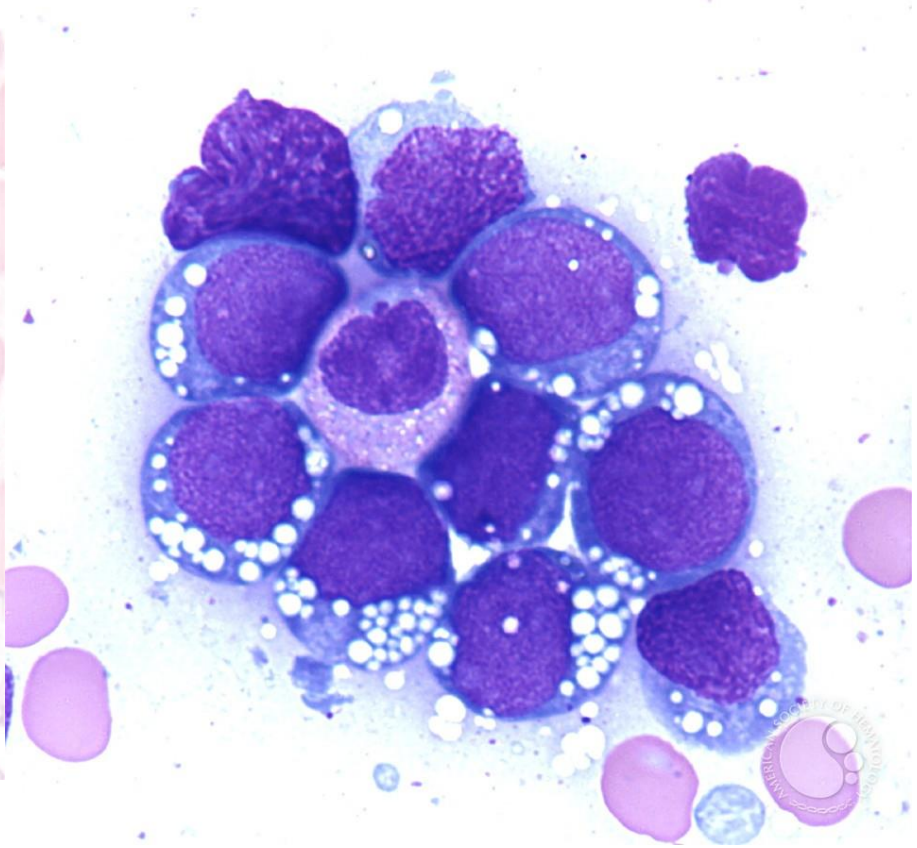
Bi-lobed PMN



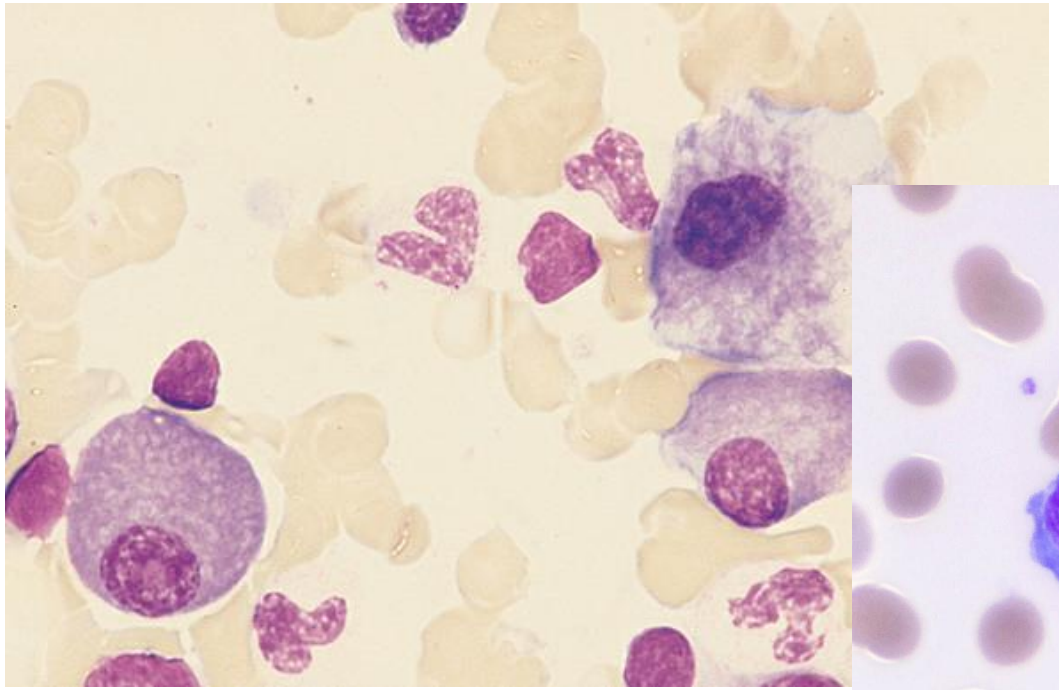


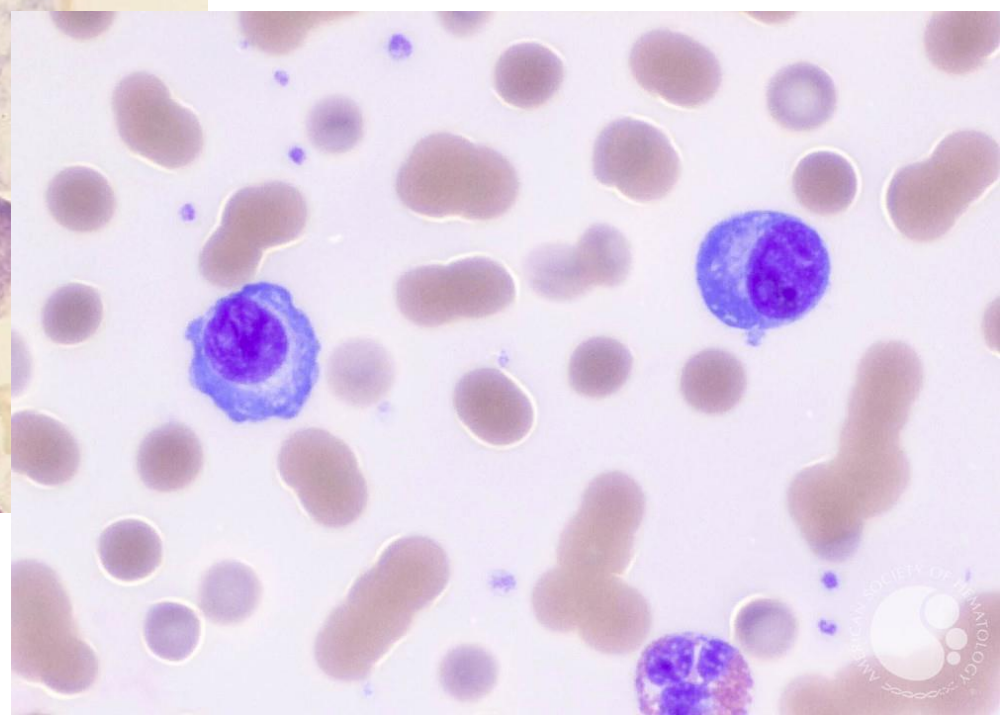
Burkitt lymphoma



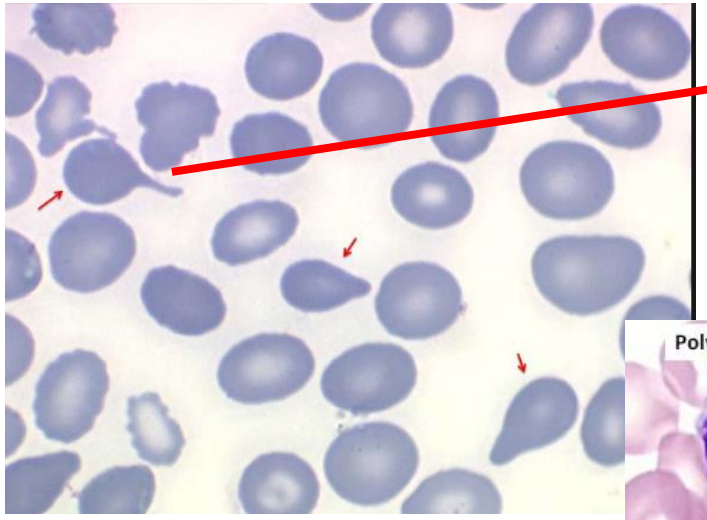


Multiple myeloma



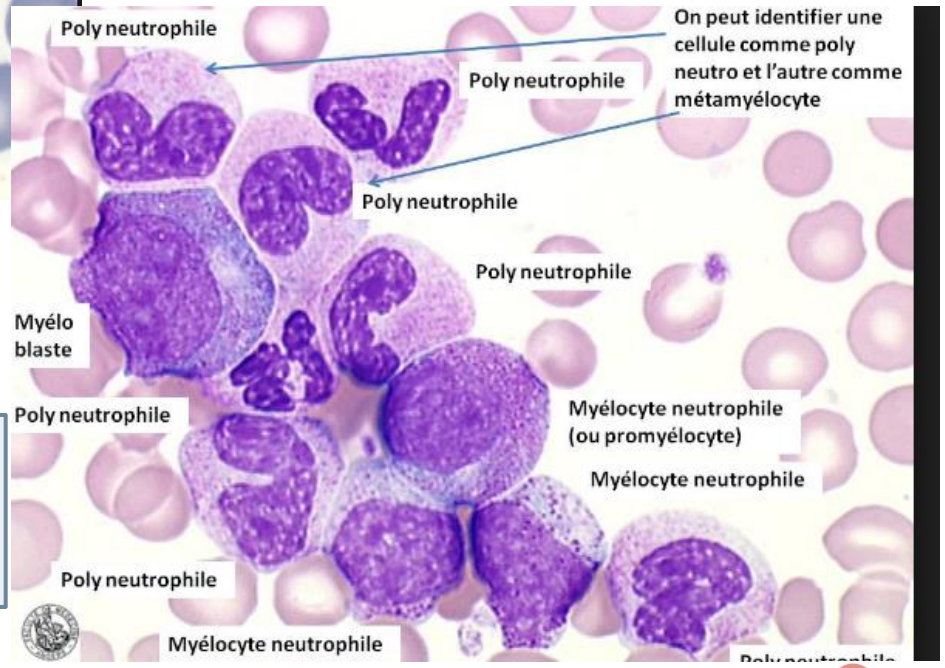


Myeloproliferative neoplasm



Tear drop cells

CML
T(9;22)



Hemophagocytic Lymphohistiocytosis

Table 1. Diagnostic criteria for HLH used in the HLH-2004 trial[3,4]

The diagnosis of HLH may be established by:

A. A molecular diagnosis consistent with HLH (Table 2)

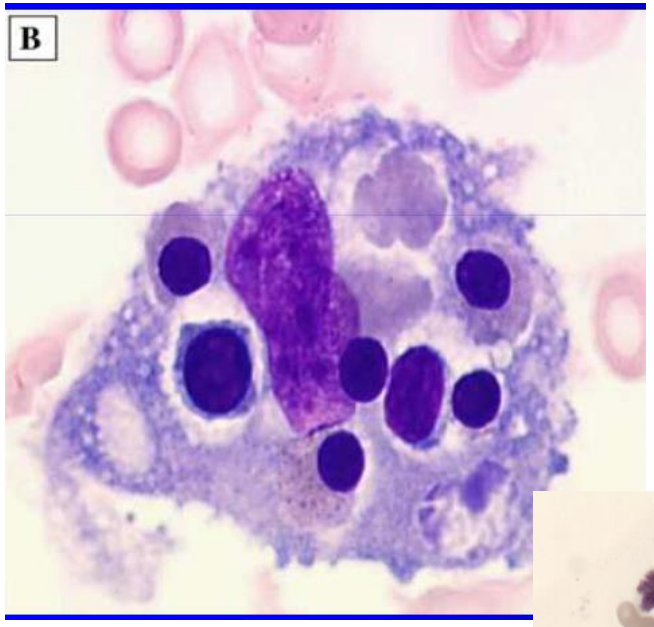
-OR-

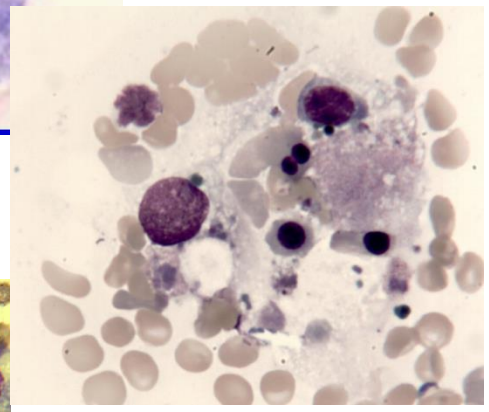
B. Five of 8 criteria listed below are fulfilled:

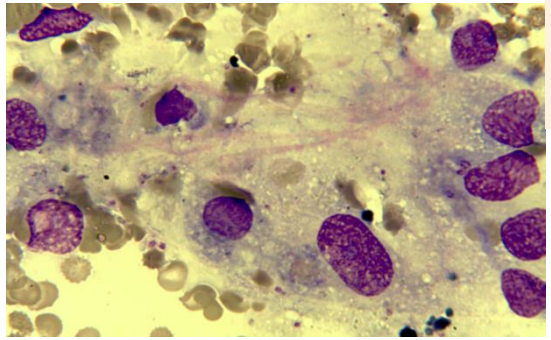
1. Fever [mtequ]38.3°C
2. Splenomegaly
3. Cytopenias (affecting at least 2 of 3 lineages in the peripheral blood):
   Hemoglobin <9 g/dL (in infants <4 wk: hemoglobin <10 g/dL)
   Platelets $<100 \times 10^3$/mL
   Neutrophils $<1 \times 10^3$/mL
4. Hypertriglyceridemia (fasting, >265 mg/dL) and/or hypofibrinogenemia (<150 mg/dL)
5. Hemophagocytosis in bone marrow or spleen or lymph nodes or liver
6. Low or absent NK-cell activity
7. Ferritin >500 ng/mL*
8. Elevated Soluble CD25 (soluble IL-2 receptor alpha)†

* Whereas the HLH-2004 protocol uses ferritin >500 ng/mL, we generally view ferritin >2000 ng/mL as concerning for HLH, and ferritin >10 000 as highly suspicious in pediatric patients.[46,47]

† Elevations above age-adjusted, laboratory-specific normal levels (defined as >2 SD from the mean) appear more meaningful than the original designation of ">2400 U/mL," because of variations between laboratories.



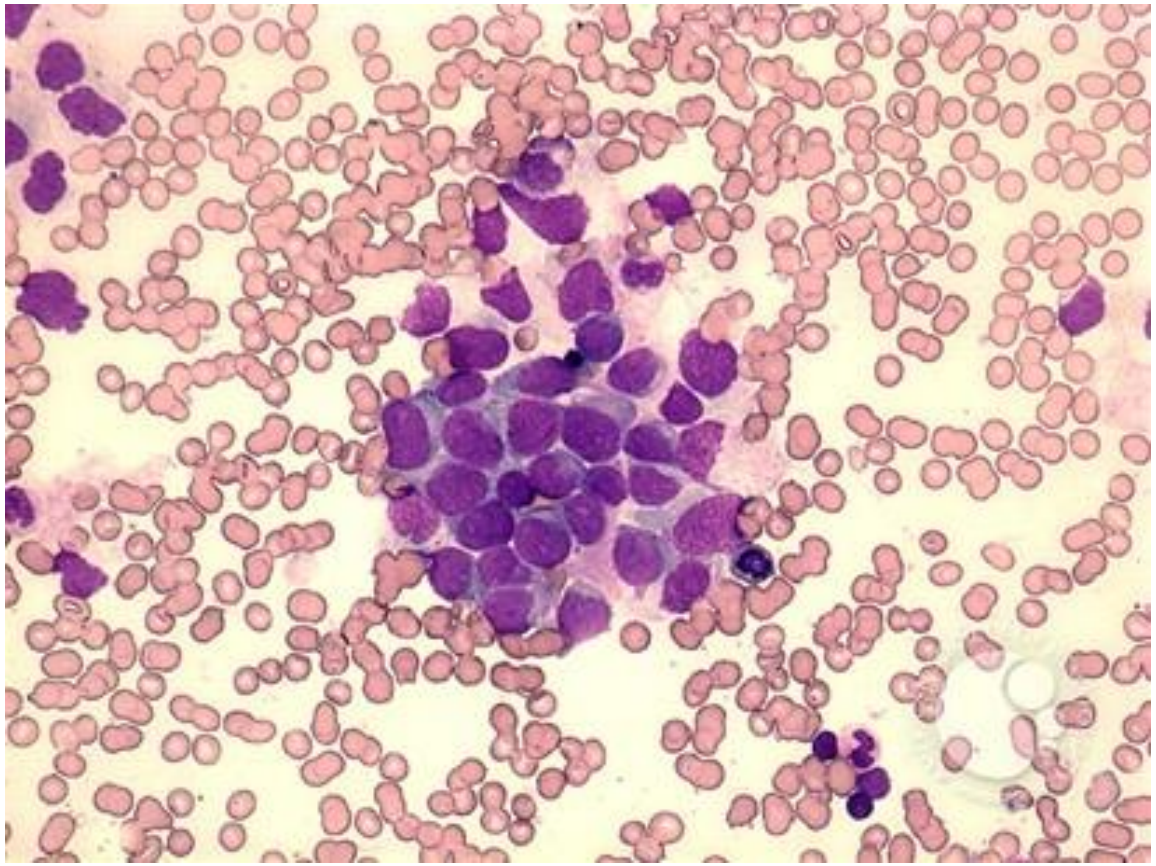




LHUB-ULB

CHU | UVC BRUGMANN

Infiltration by solid tumor



Conclusion

- Blood and bone marrow morphology are important key in the diagnosis of hemopathies, cytopenia, abnormalities of the hemogram.
- Hemopathies are diagnosed thanks to multidisciplinary approach and the pooling of the results of the hematology analysis including microscopy, immunophenotyping, cytogenetics, molecular biology and pathology.

THANK YOU

References

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