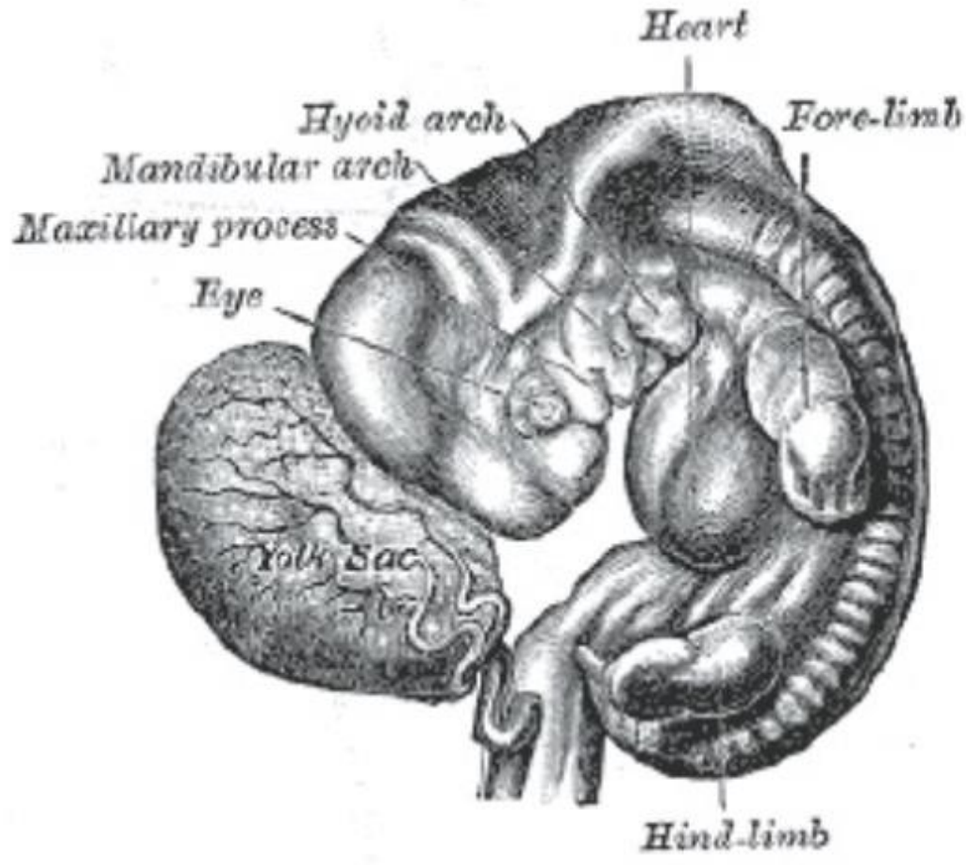
In the name of God

Presenting by F.Malek
Assistant professor of Hematology and Oncology

- On average, bone marrow constitutes 4% of the total body mass of humans
- In adults weighing 65 kg (143 lbs), bone marrow accounts for approximately 2.6 kg (5.7 lbs)
- The hematopoeitic compartment of bone marrow produces approximately 500 billion blood cells per day

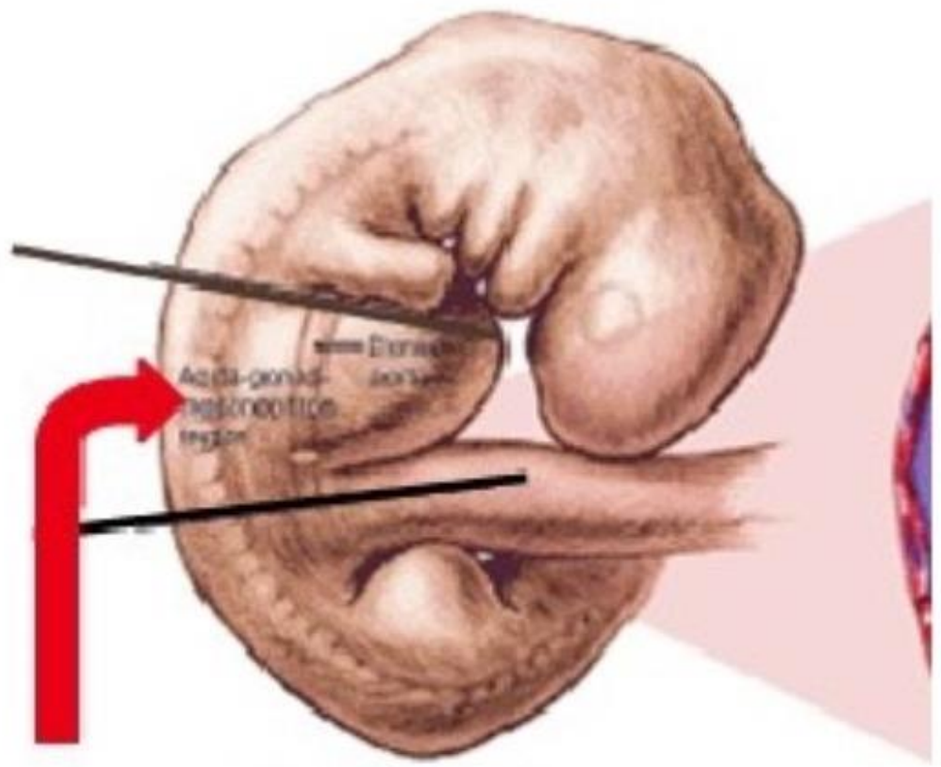
Primitive Hematopoiesis

- Derived from the extra- embryonic **YOLK SAC**;
- Consists mainly of nucleated erythroid cells that carry oxygen to the developing embryonic tissues... an early circulatory system.
- Probably starts ~ 4 weeks in humans



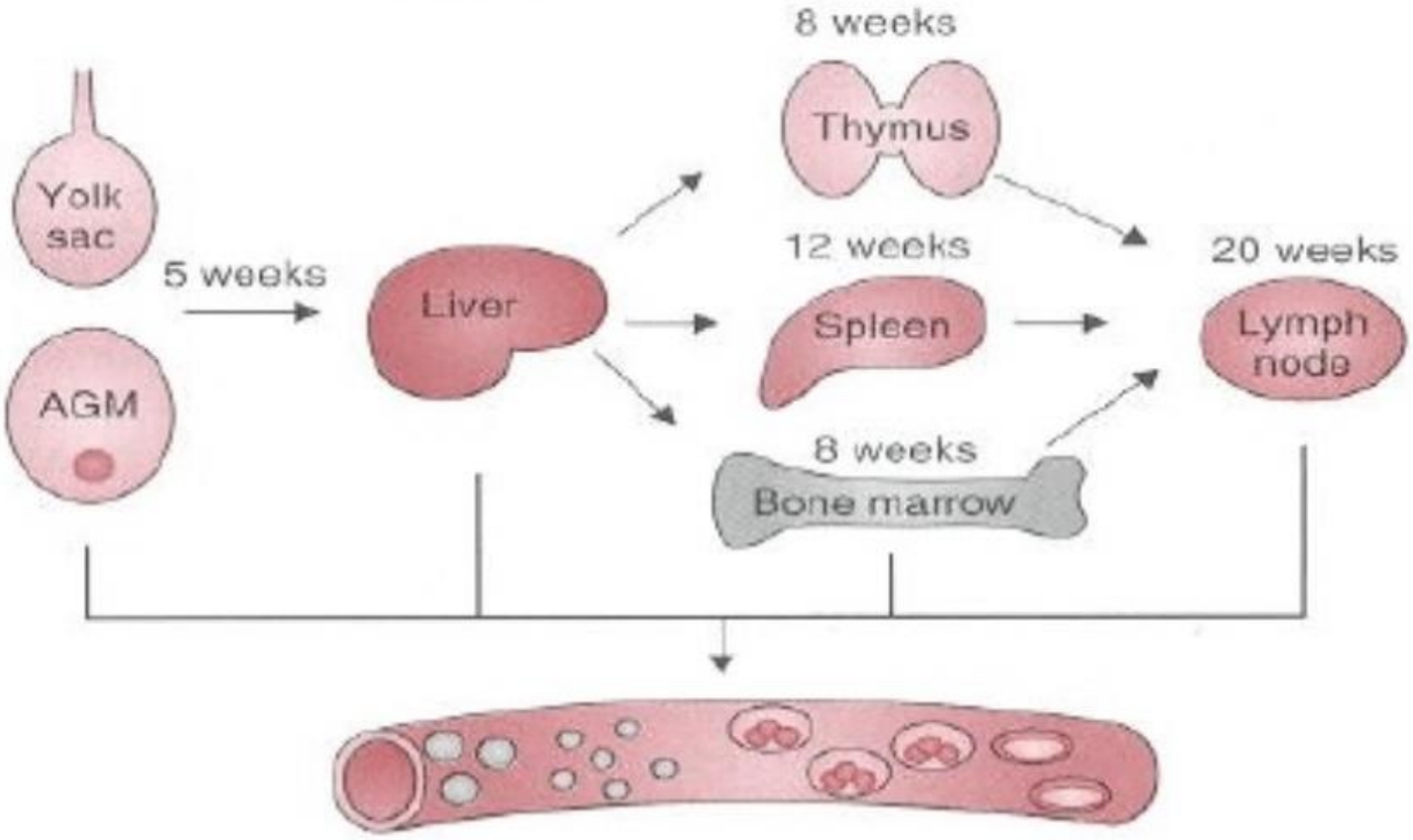
Definitive Hematopoietic System

- As the embryo's size increases, primitive system superceded by definitive hematopoietic system, which originates in the embryo itself and continues throughout adult life.
- **1st definitive multipotent hematopoietic stem cells are generated within the embryonic AGM region** of the para-aortic splanchnopleuric mesoderm (day 30-37 in human)



AGM region – aorta-gonad-mesonephros

Subsequent sites of Hematopoiesis in fetal life

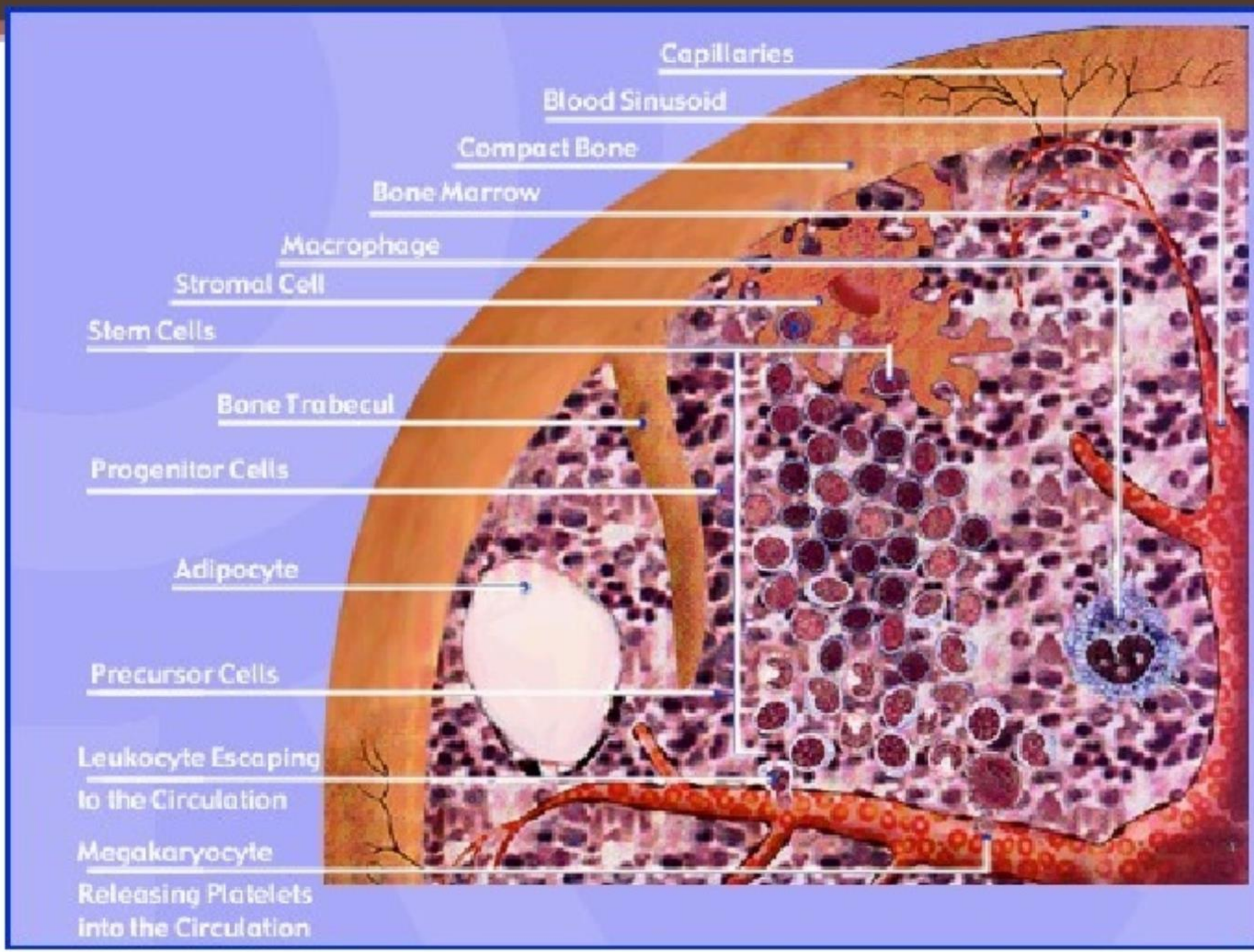


After birth, BONE MARROW becomes main hematopoietic organ

- Throughout fetal life, the liver is the chief organ for production of myeloid and erythroid cells

Bone Marrow

- Bone marrow is specially designed to support the proliferation, differentiation, and maintenance of hematopoietic cells
- The **stroma** of the bone marrow is all tissue not directly involved in the primary function of hematopoiesis.
- Cells that constitute the bone marrow stroma are:
  - fibroblasts (reticular connective tissue)
  - macrophages
  - adipocytes
  - osteoblasts
  - osteoclasts
  - endothelial cells, which form the sinusoids.


Capillaries
Blood Sinusoid
Compact Bone
Bone Marrow
Macrophage
Stromal Cell
Stem Cells
Bone Trabecul
Progenitor Cells
Adipocyte
Precursor Cells
Leukocyte Escaping
to the Circulation
Megakaryocyte
Releasing Platelets
into the Circulation

BONE MARROW EXAMINATION

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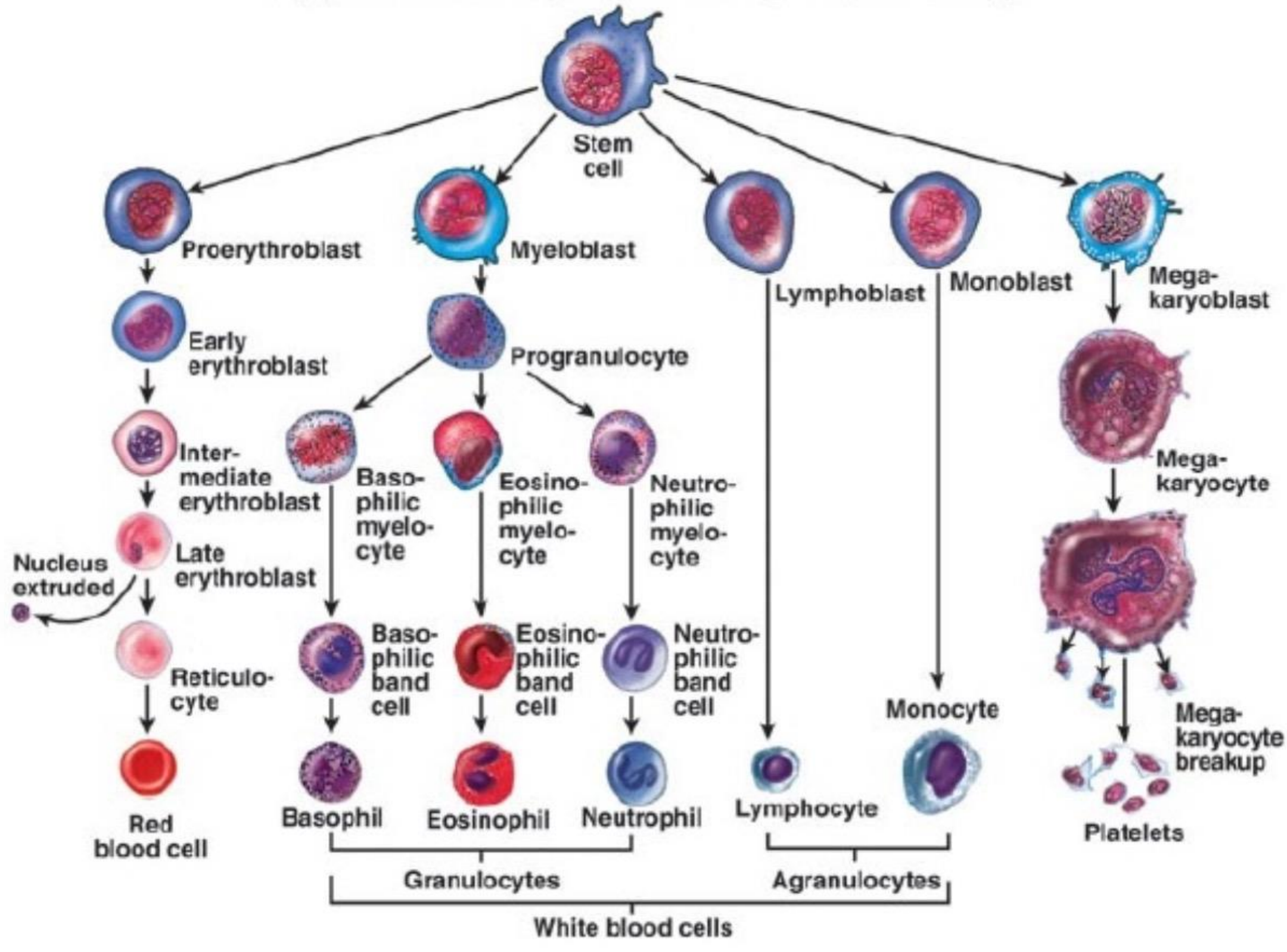


Erythroid series

*Proerythroblast

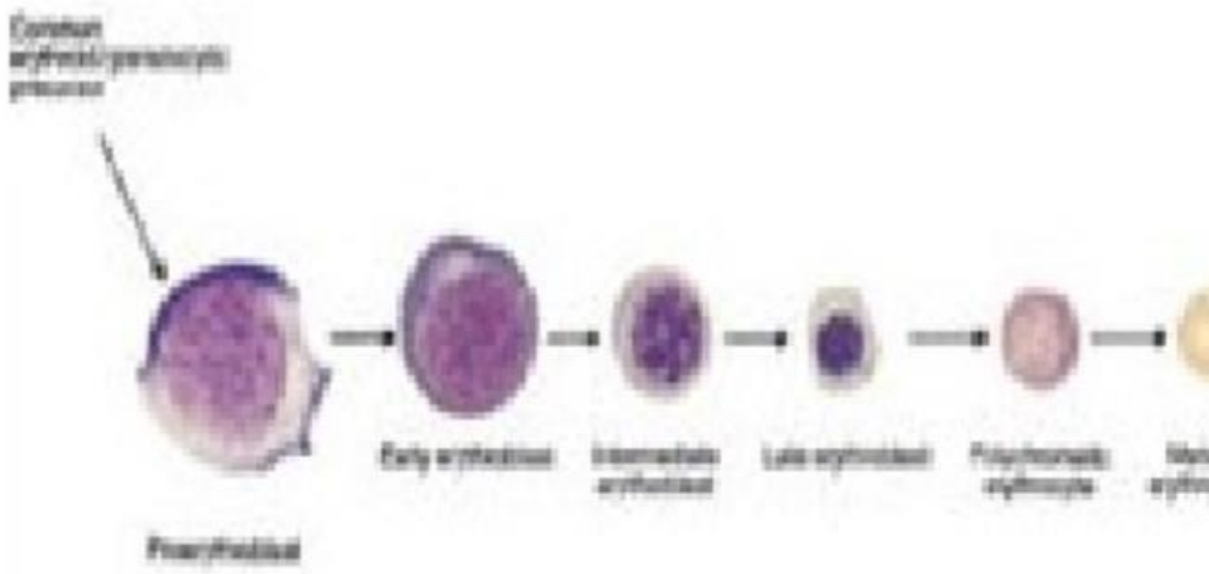
**Early erythroblast (Basophillic)

***Intermediate erythroblast (Polychromatic)

****Late erythroblast (Orthochromatic)

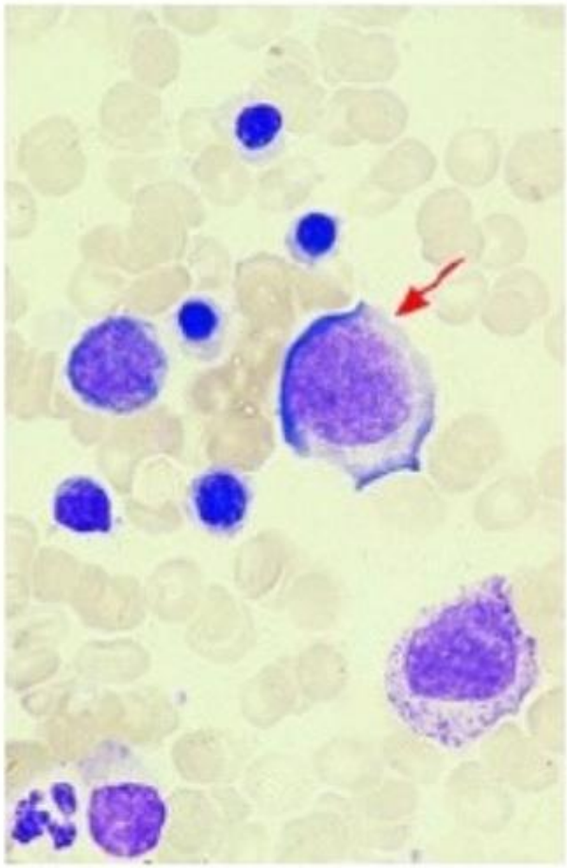
Erythroid precursors

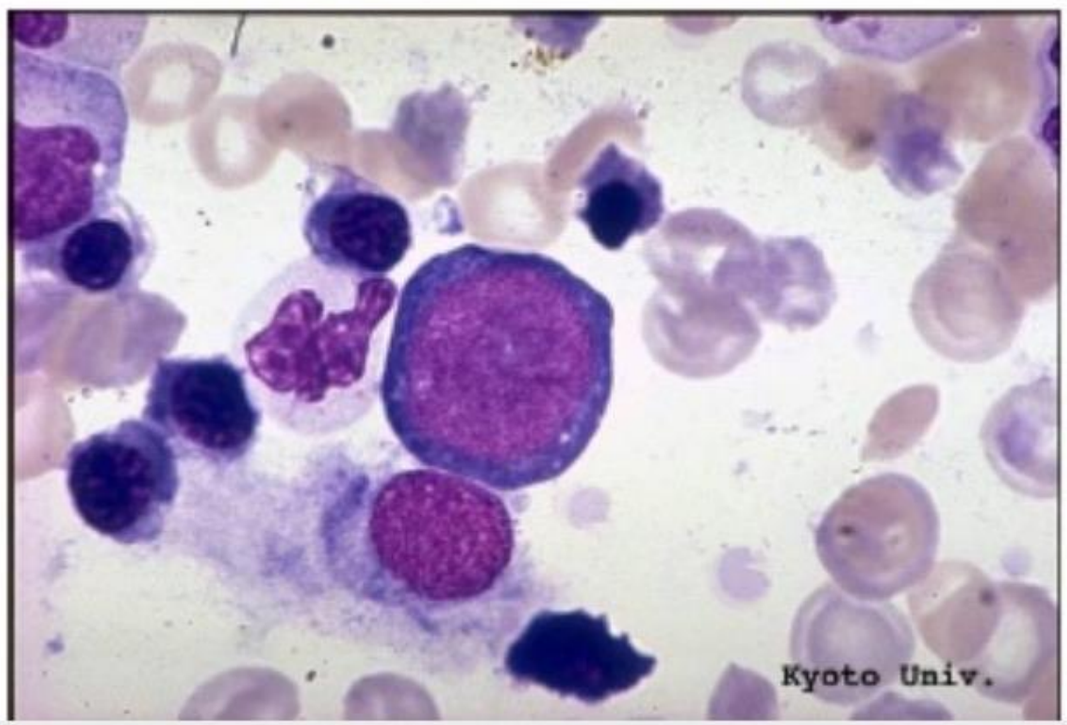
- Normal red cells are produced in the bone marrow from erythroid precursors or erythroblasts.
- The earliest morphologically recognisable red cell precursor is derived from an erythroid progenitor cell which in turn is derived from a multipotent haemopoietic progenitor cell.



proerythroblast

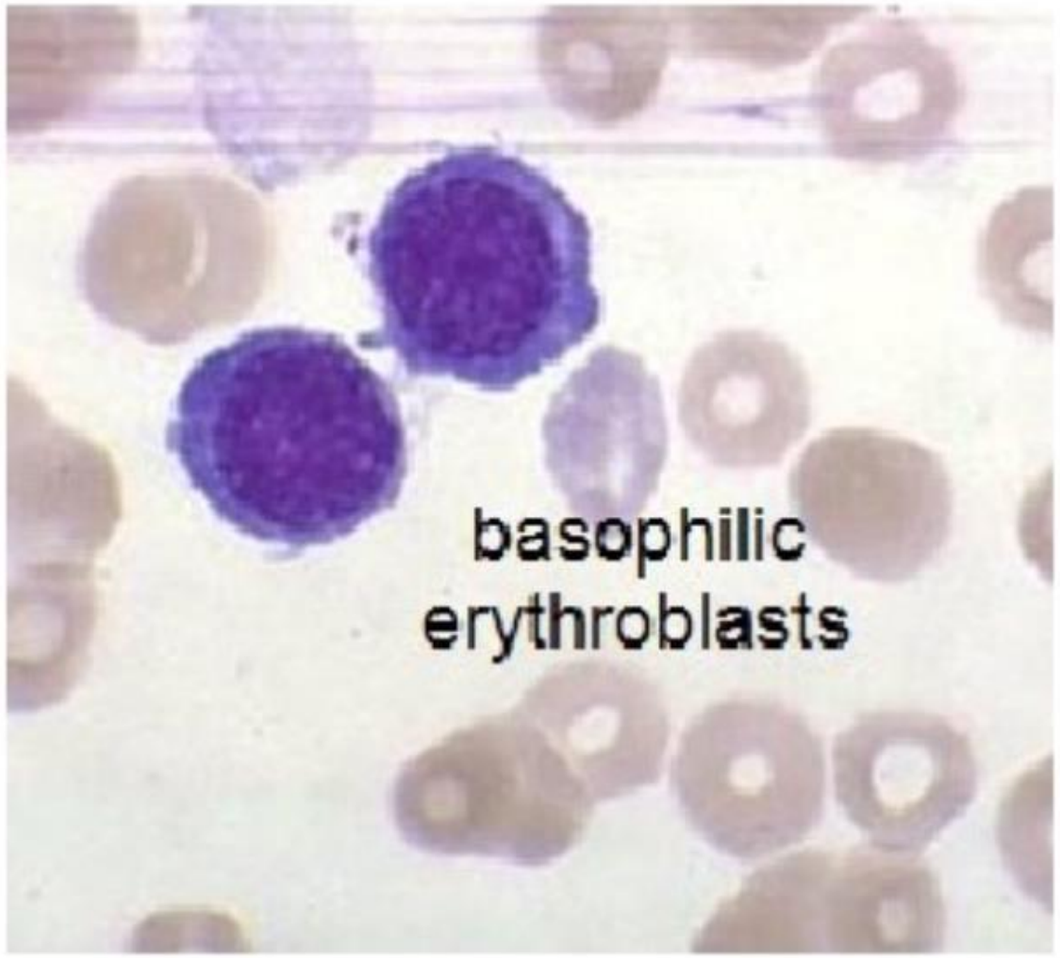
- Normal proerythroblast [dark red arrow] in the bone marrow. This is a large cell with a round nucleus and a finely stippled chromatin pattern. Nucleoli are sometimes apparent.
- The cytoplasm is moderately to strongly basophilic.





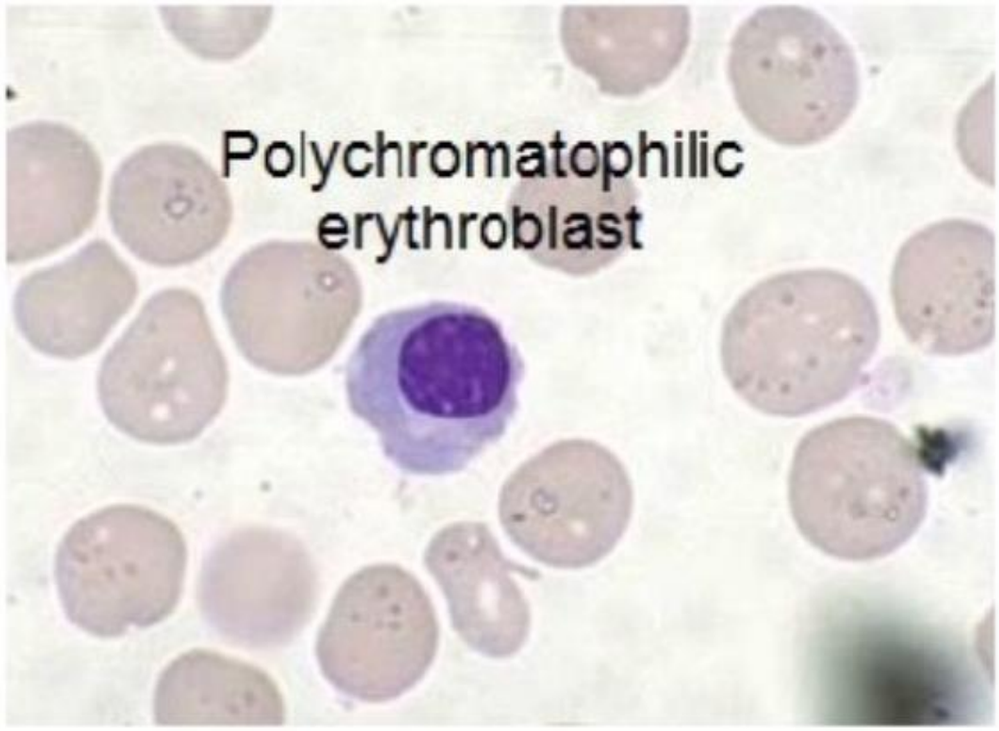
Basophilic erythroblast

- Spherical nucleus, nucleoli not visible, basophilic cytoplasm



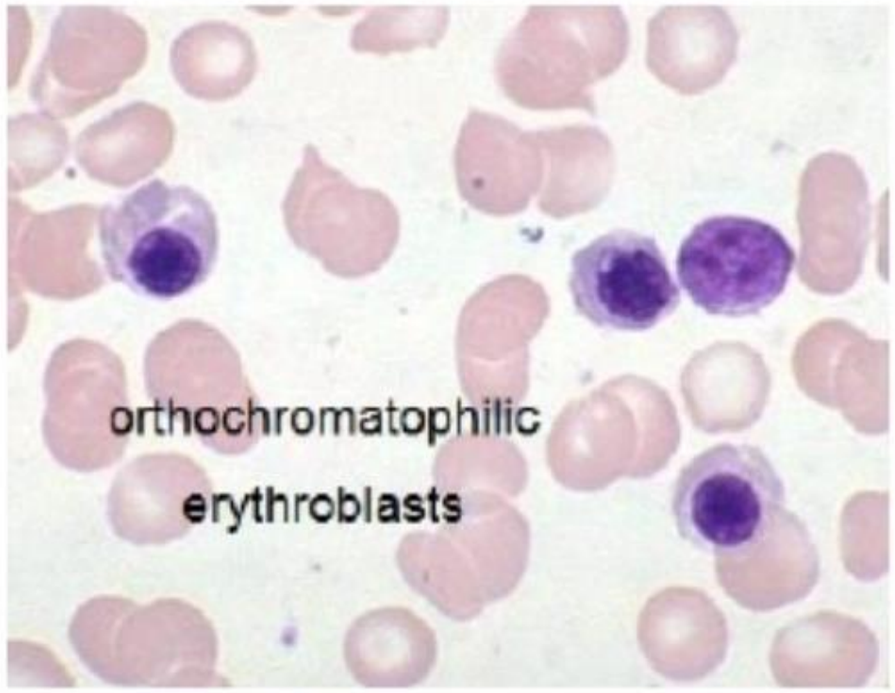
Polychromatophilic erythroblast

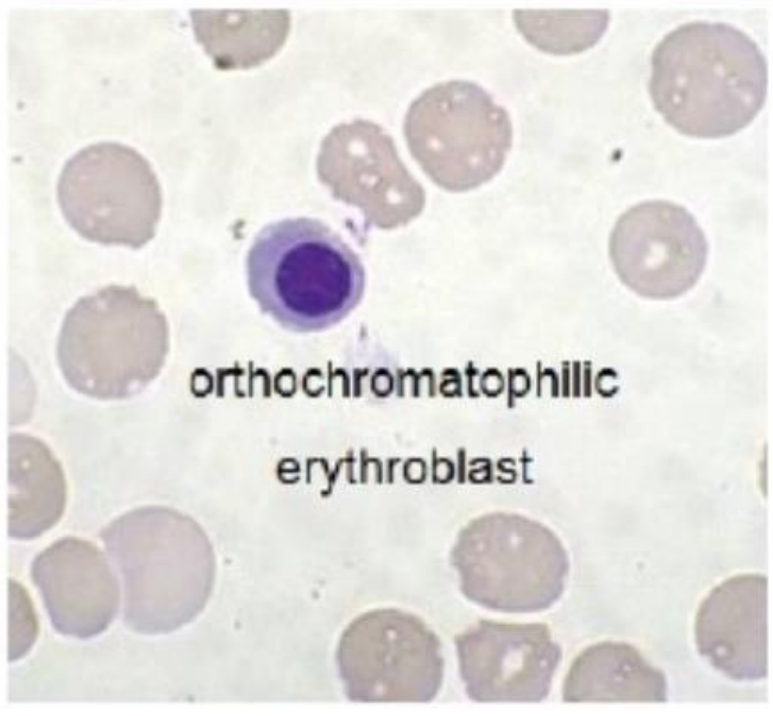
- smaller nucleus –condensed chromatin, baso- and eosinophilia in the cytoplasm



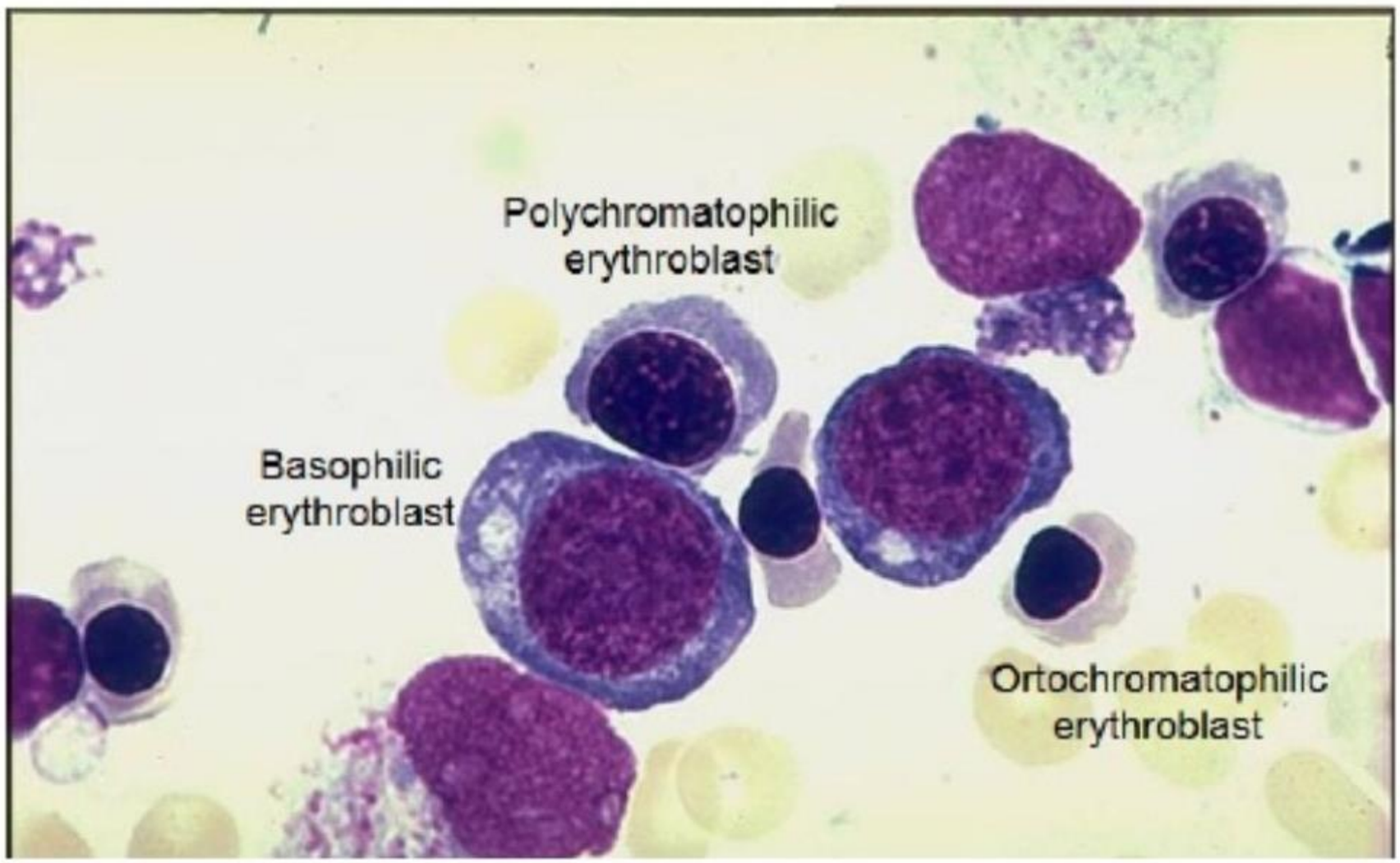
Orthochromatophilic erythroblast

- small nucleus with highly condensed chromatin, nucleus extruded, eosinophilic cytoplasm

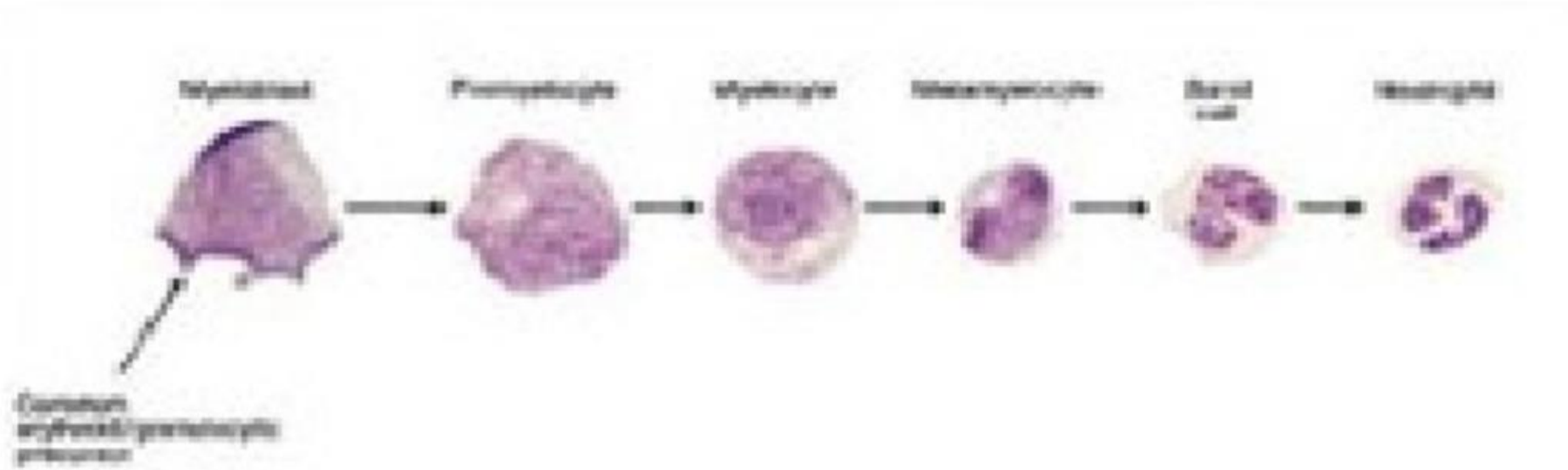




Normal erythroblasts in the BM



Myeloid precursors

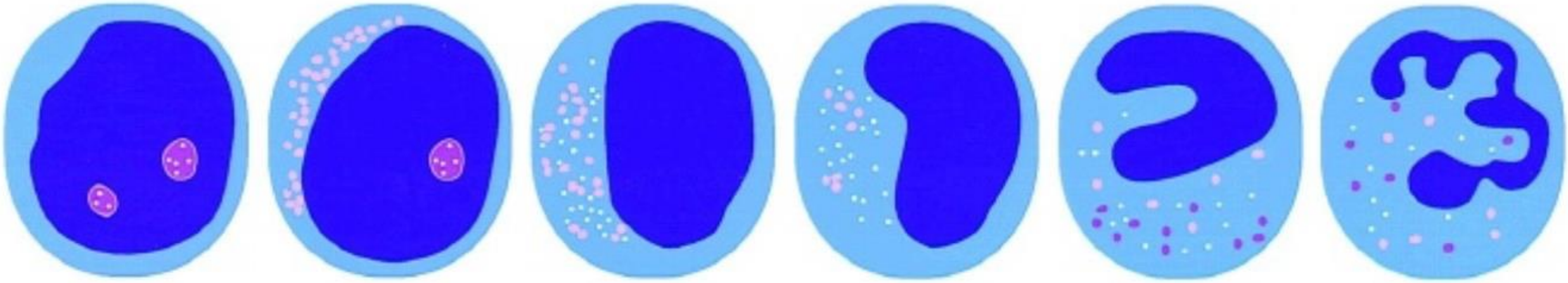


- Myeloblast→ promyelocyte→ myelocyte → metamyelocyte → band form → mature neutrophil.

G-CSF

↓

C/EBPε^{32}



Myeloblast | Promyelocyte | Myelocyte | Metamyelocyte | Band | Segmented

1°granules (azurophilic) — MPO, Collagenase, β-glucocuronidase; Defensins

2°granules (specific)

3°granules (gelatinase)

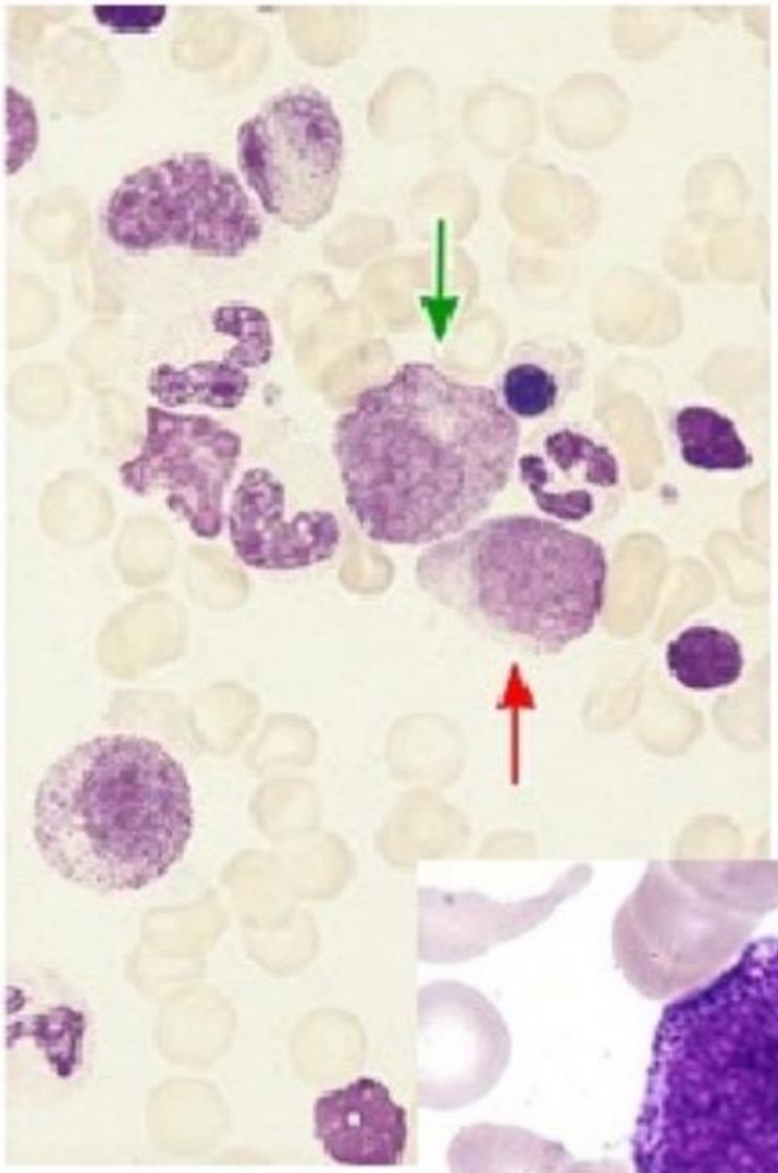
credit-Dr. Lekstrom Hines, JEM 1999

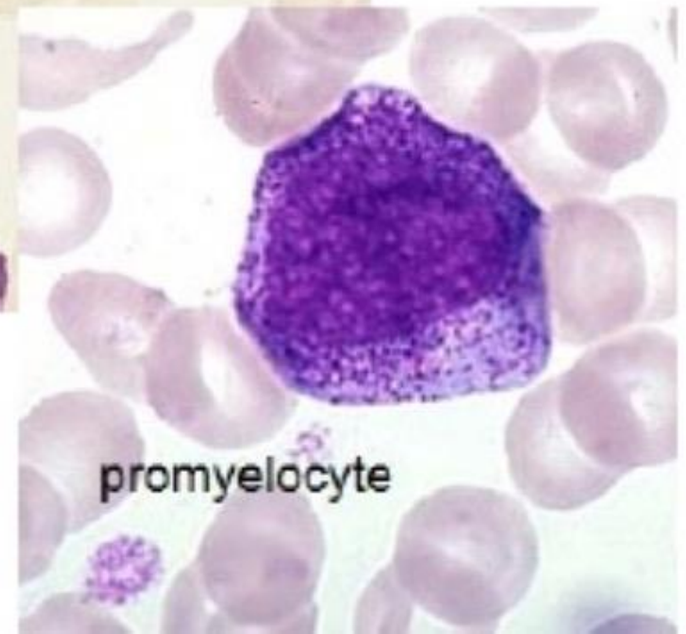
Myeloid precursors

- Normal myeloblasts have no granules but abnormal myeloblasts may have a few granules.
- Myeloblasts undergo one cell division and mature into promyelocytes.
- Promyelocytes have primary or azurophilic granules. They have a Golgi zone a pale area adjacent to the nucleus that is the site of granule production. The chromatin pattern of a promyelocyte shows some condensation or clumping, in contrast to the diffuse chromatin pattern of a myeloblast, but nucleoli are still visible.

Normal granulocyte precursors in the bone marrow

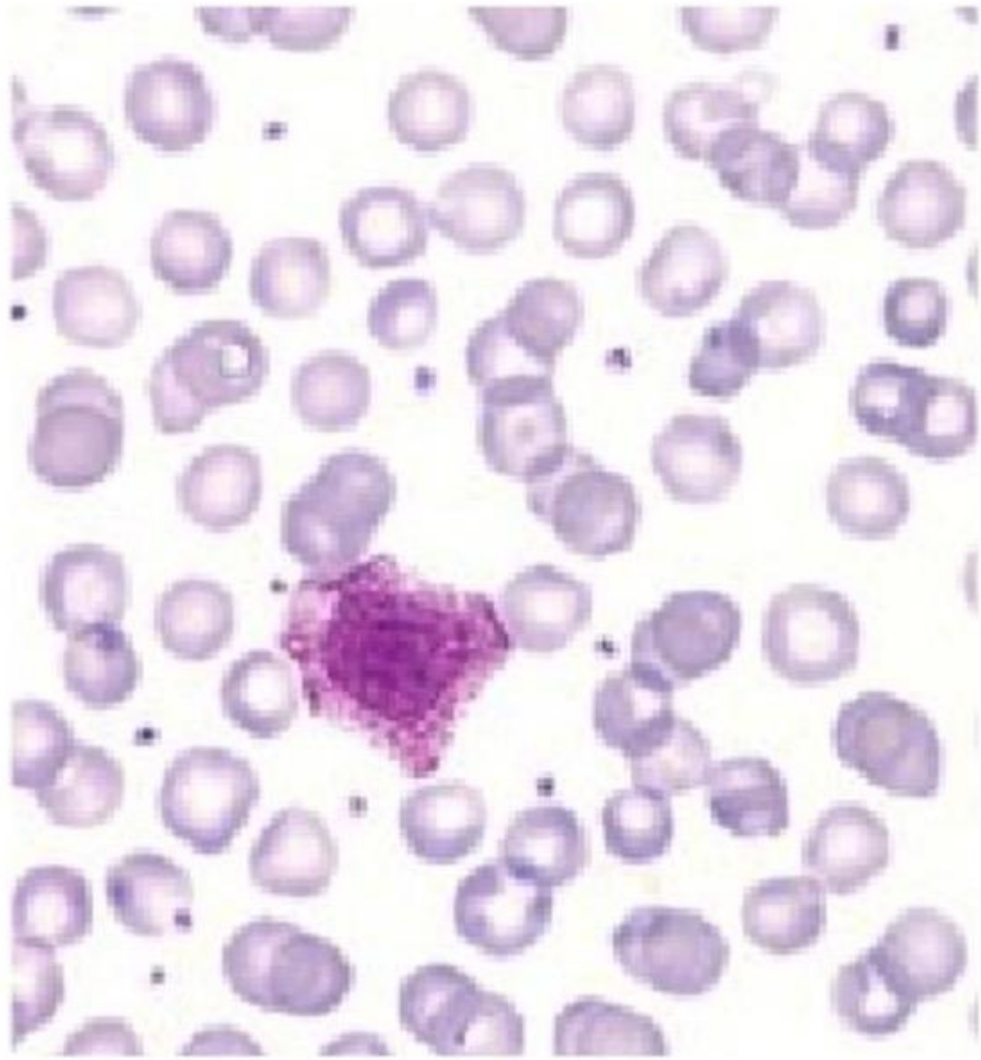
- Note the myeloblast [dark red arrow] with a high nucleocytoplasmic ratio, diffuse chromatin pattern and nucleolus.
- There is a promyelocyte [green arrow] which is larger and has a lower nucleocytoplasmic ratio and abundant azurophilic granules.





Myelocytes

- Myelocytes are smaller than promyelocytes and have specific granules that indicate whether they are of neutrophil, eosinophil or basophil lineage.
- The nucleolus is no longer visible.



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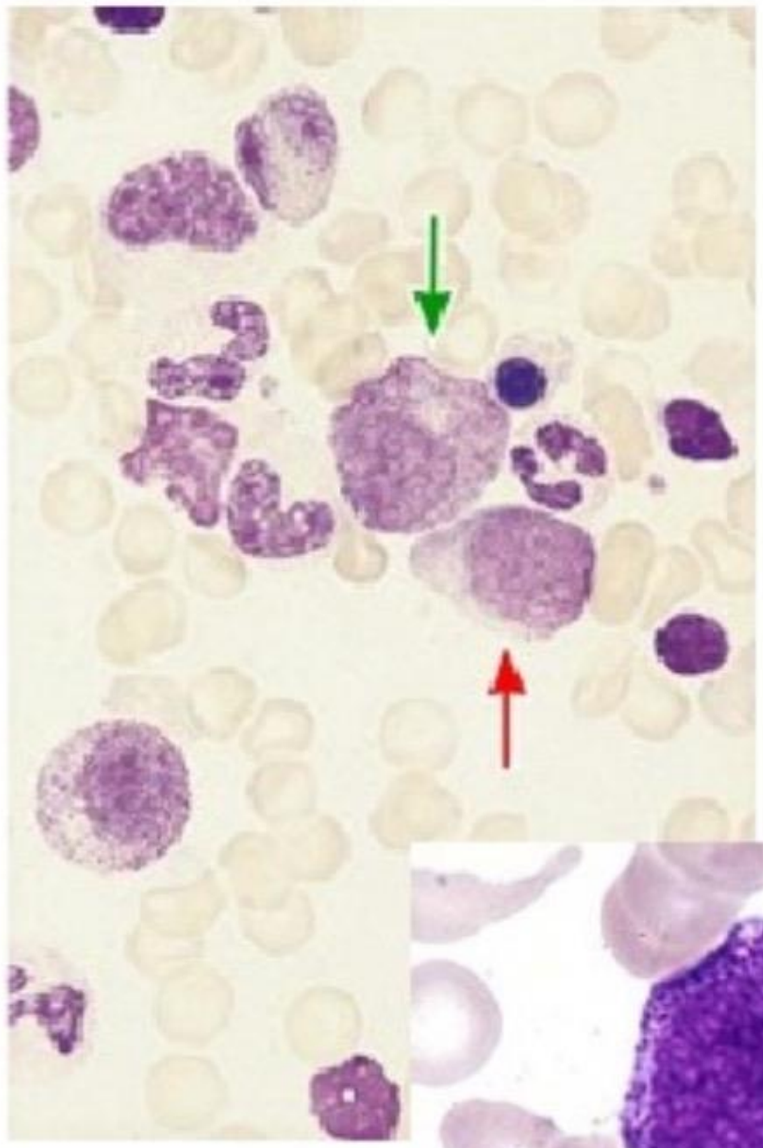
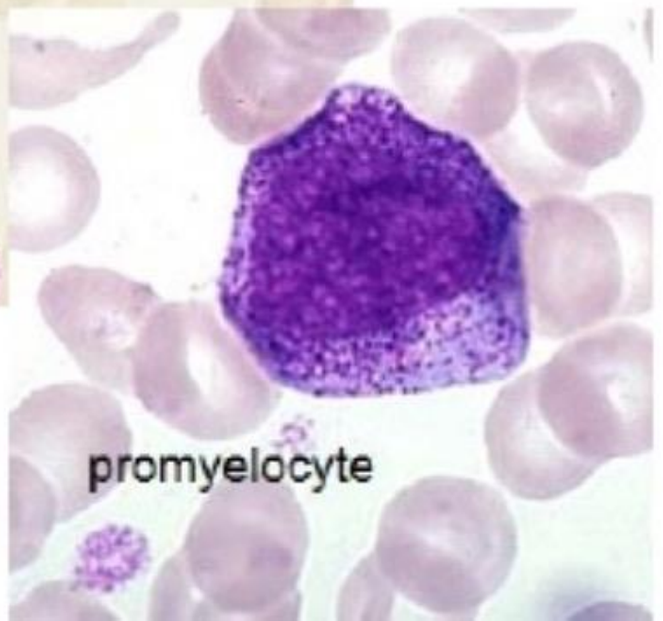
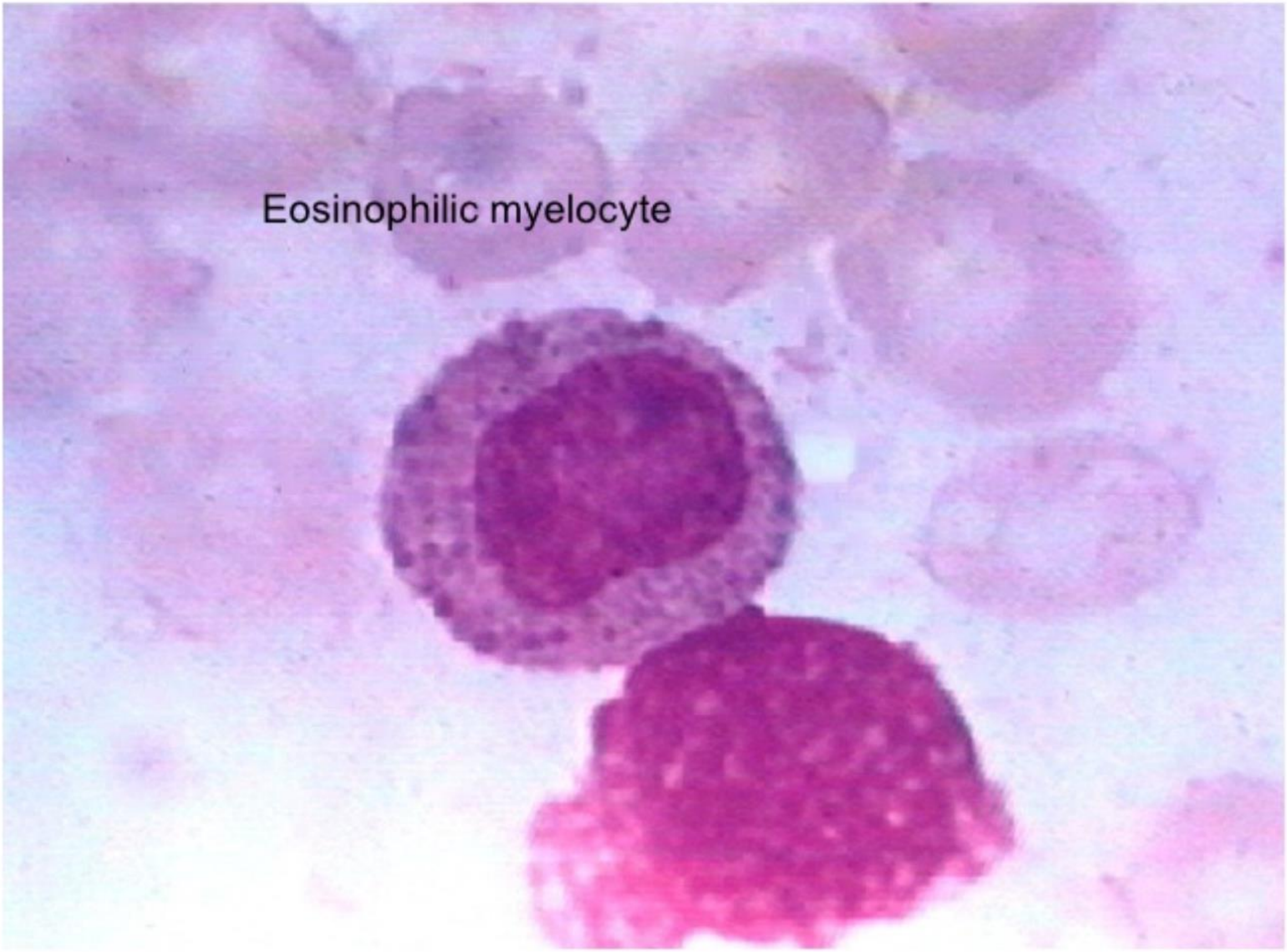
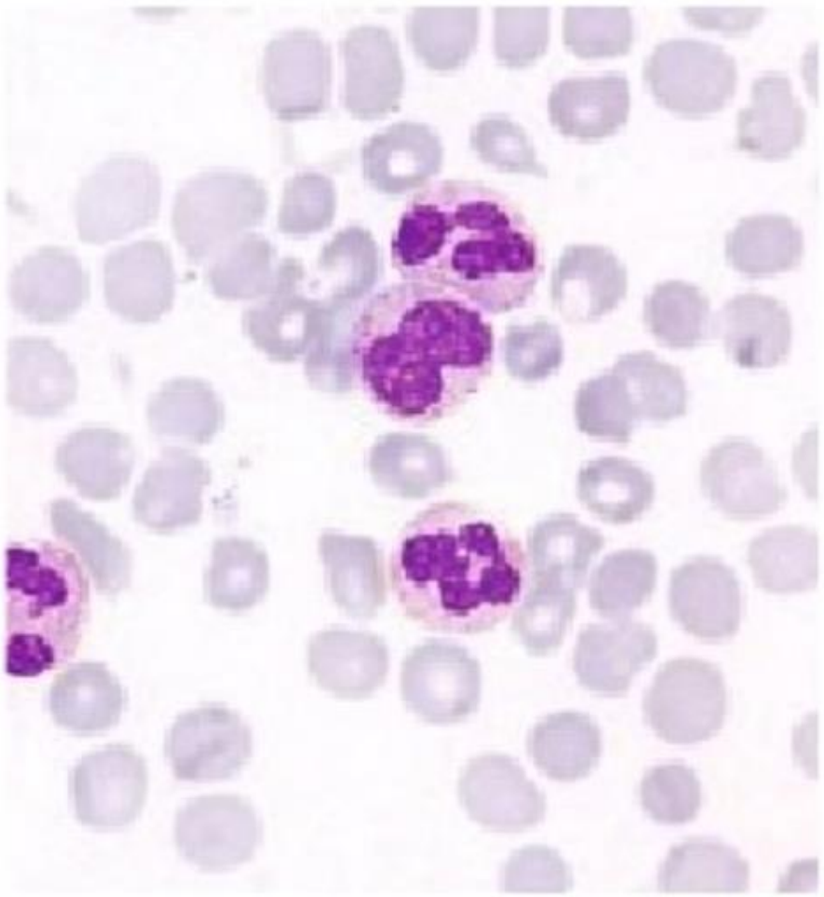


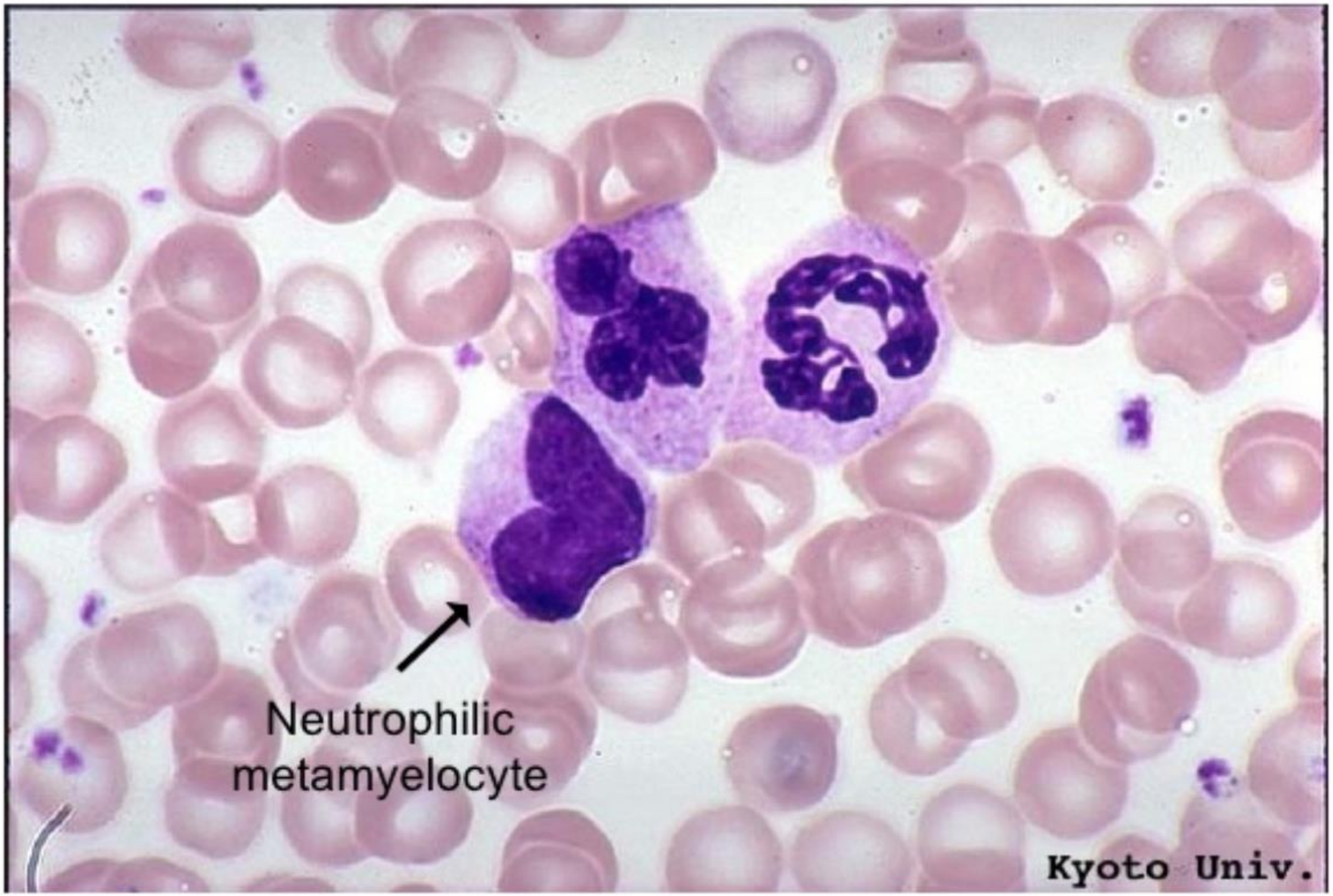


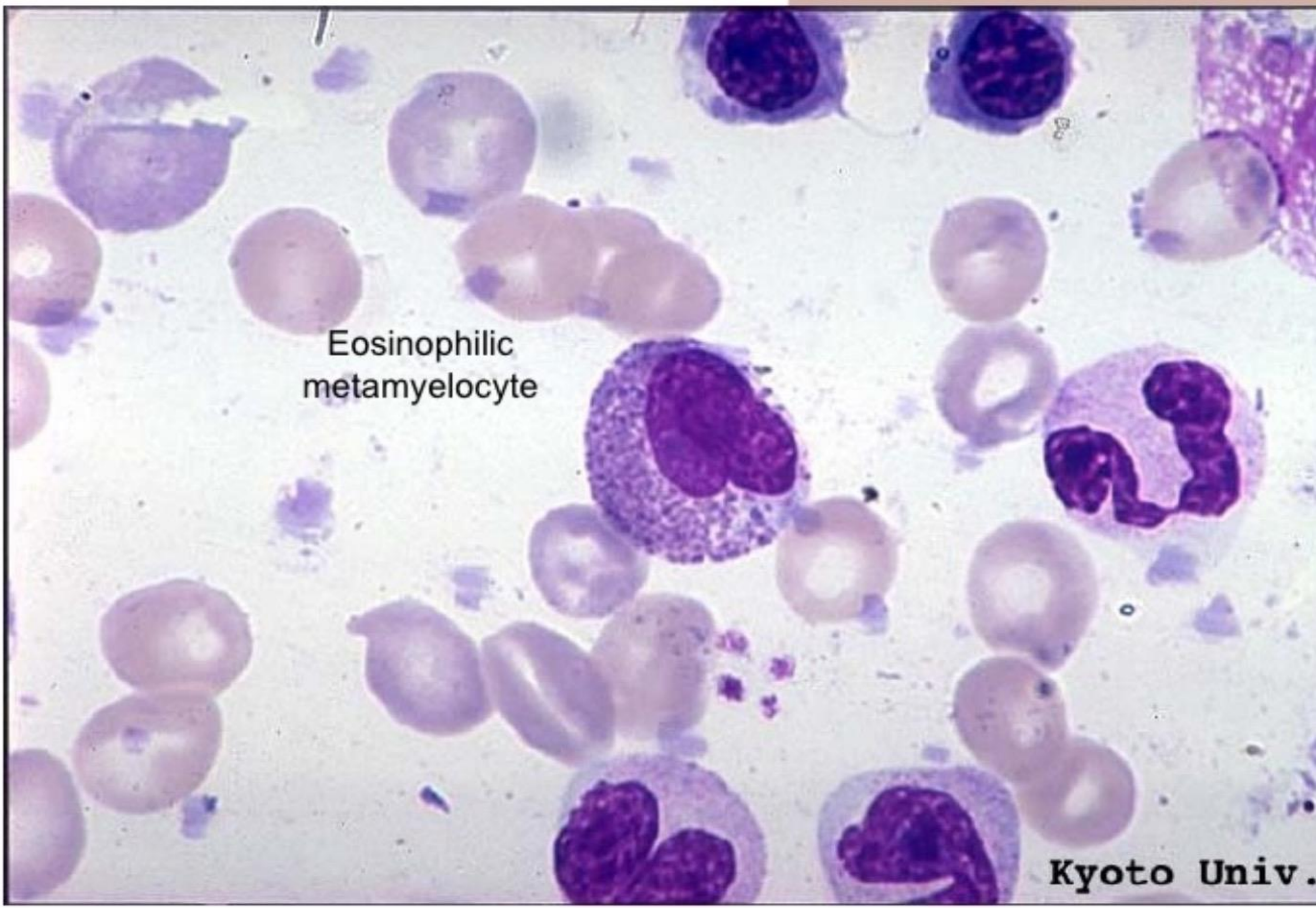

Eosinophilic myelocyte

A neutrophil metamyelocyte

- The metamyelocyte differs from a myelocyte in having some indentation of the nucleus
- It differs from a band form in not having any part of its nucleus with two parallel edges

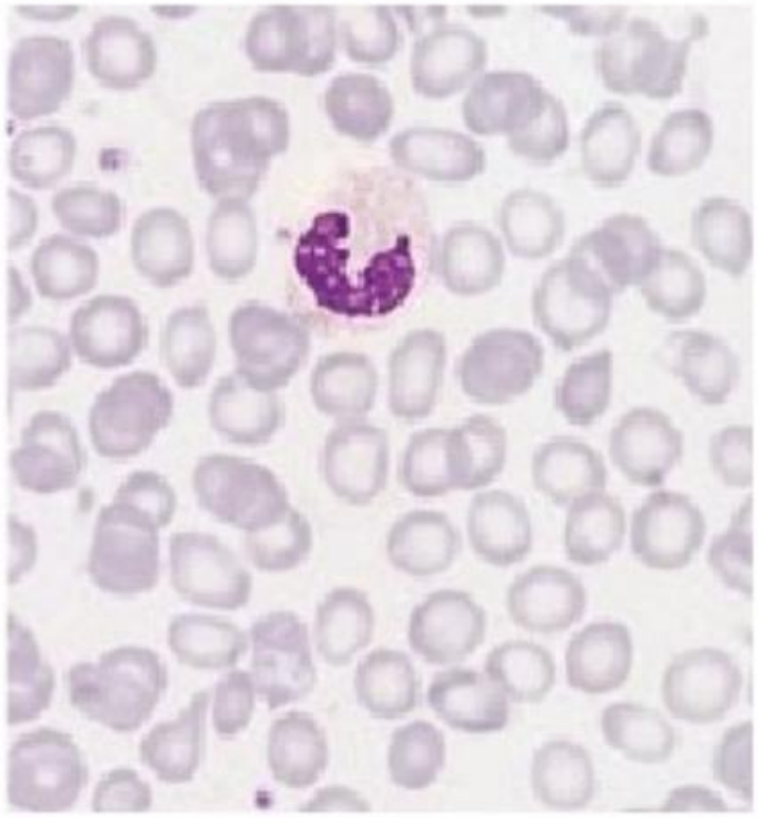



Neutrophilic
metamyelocyte
Kyoto Univ.

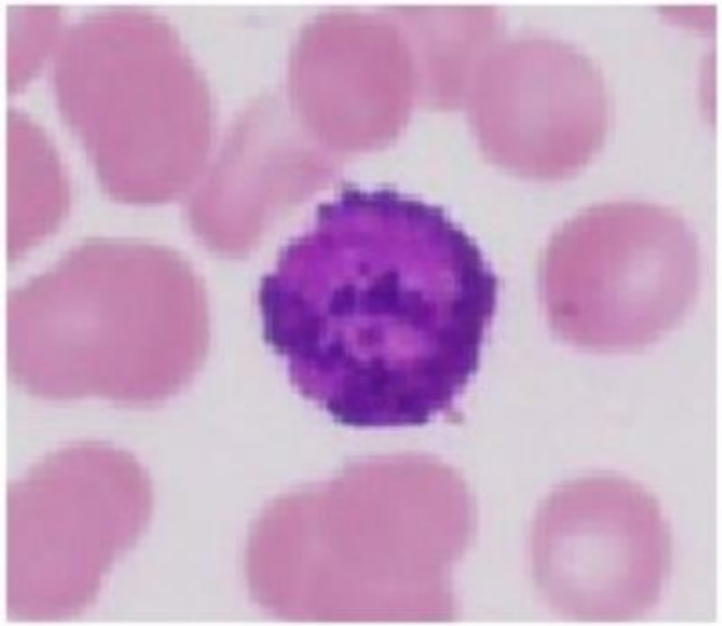

Eosinophilic
metamyelocyte
Kyoto Univ.

Band or juvenile Neutrophils

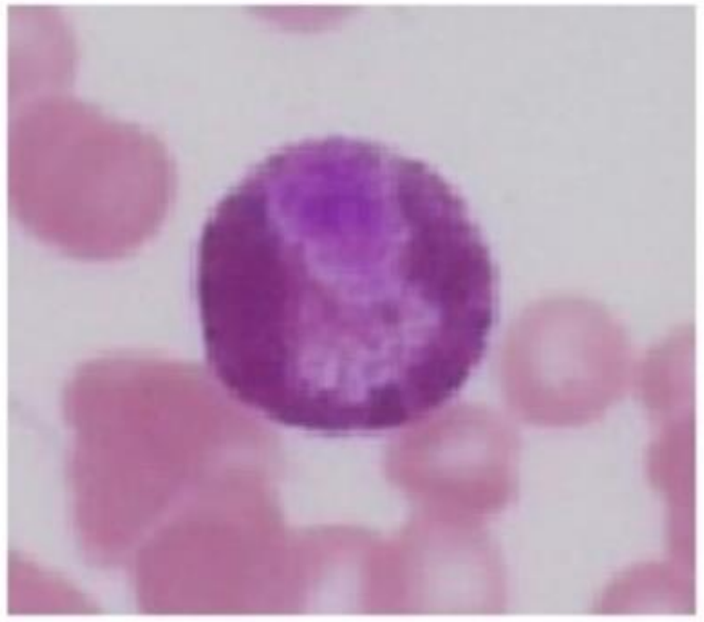
- There are smaller numbers of cells of neutrophil lineage with non-segmented nuclei. They are referred to as neutrophil band cells or band forms. They are less mature than segmented neutrophils.



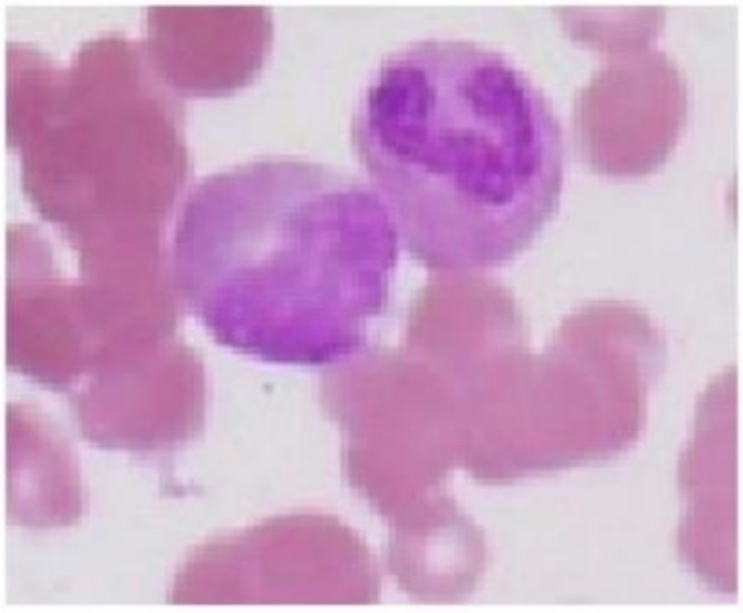
Compare the different cell types:



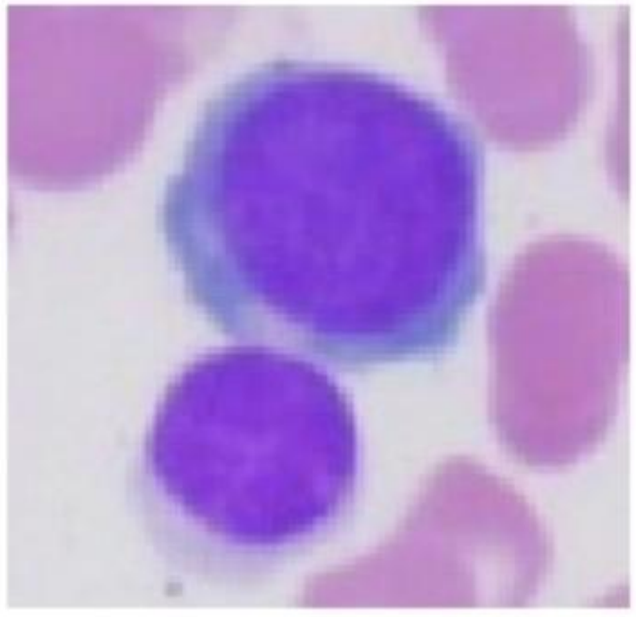
basophilic (myeloid)



eosinophilic (myeloid)



neutrophilic (myeloid)



erythroid

Megakaryocytic series

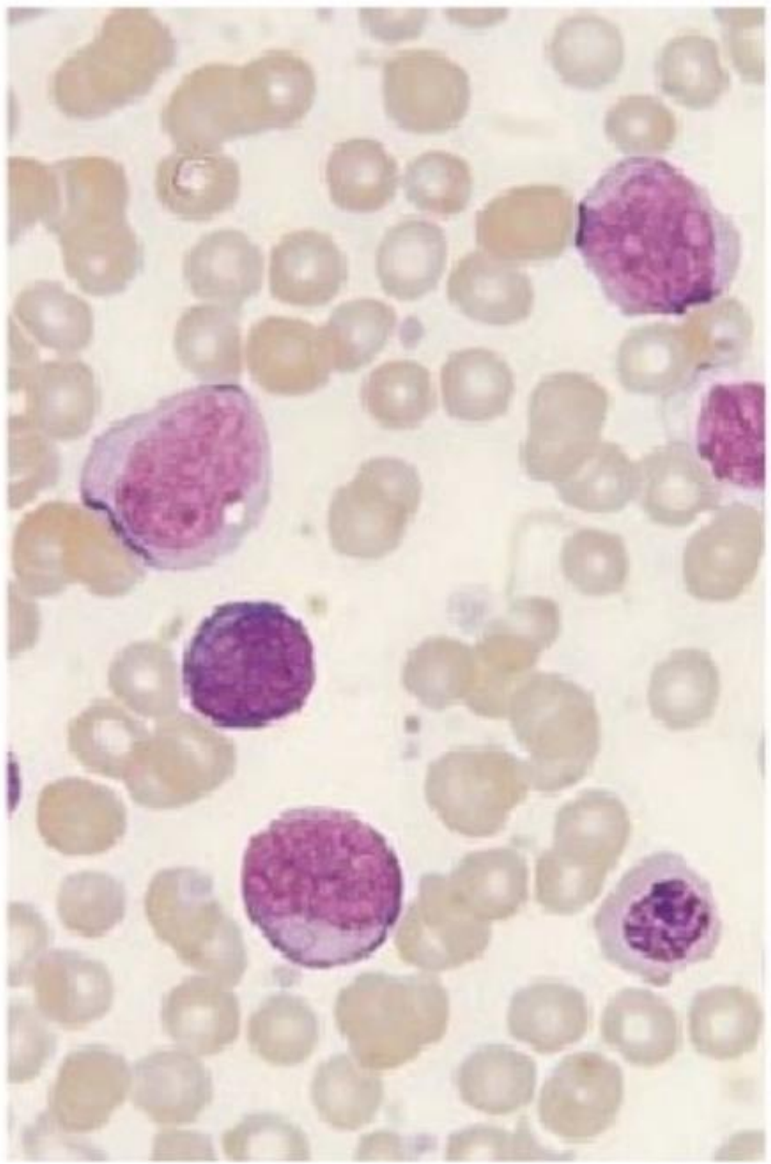
*Megakaryoblasts

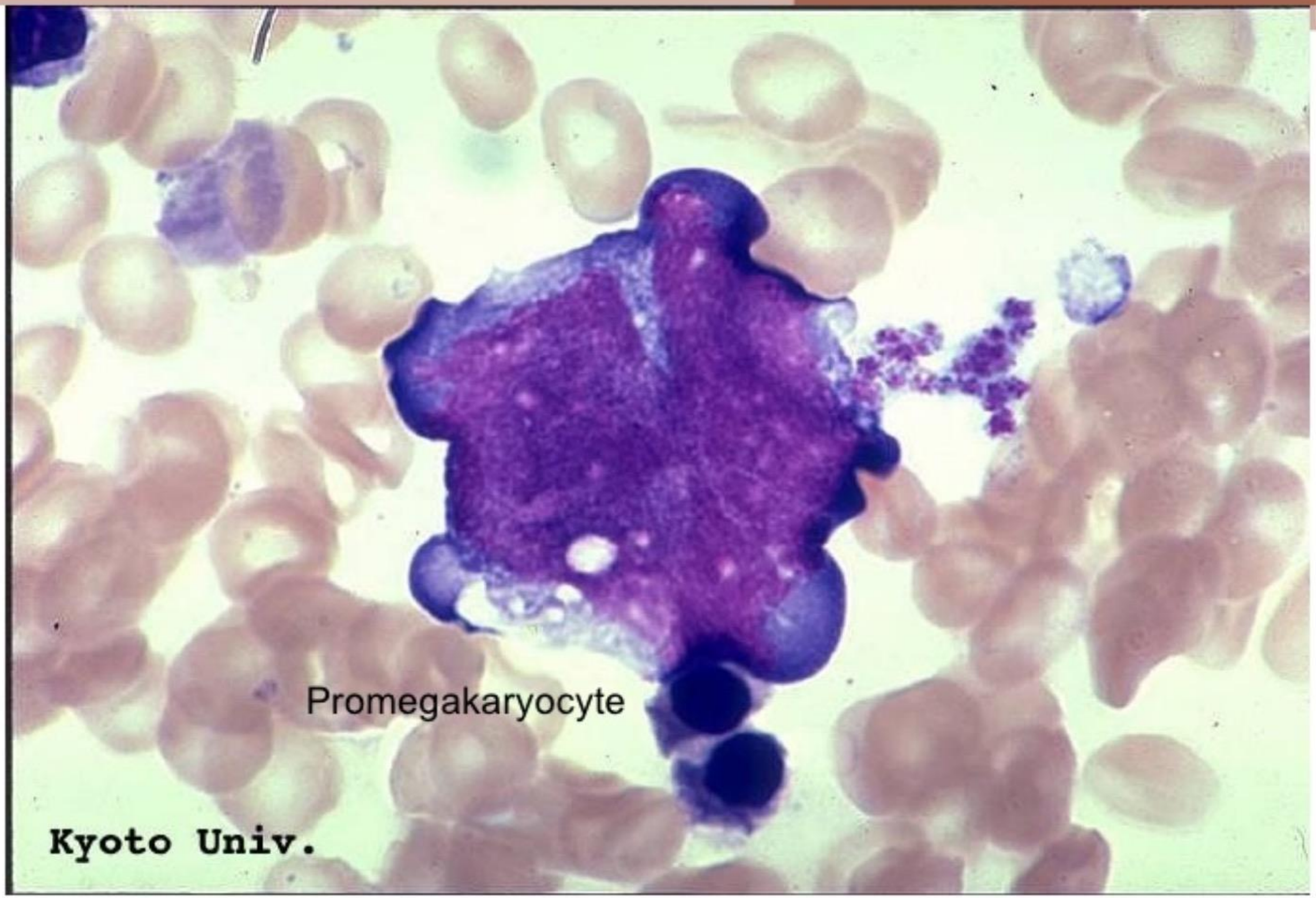
** Promegakaryocytes

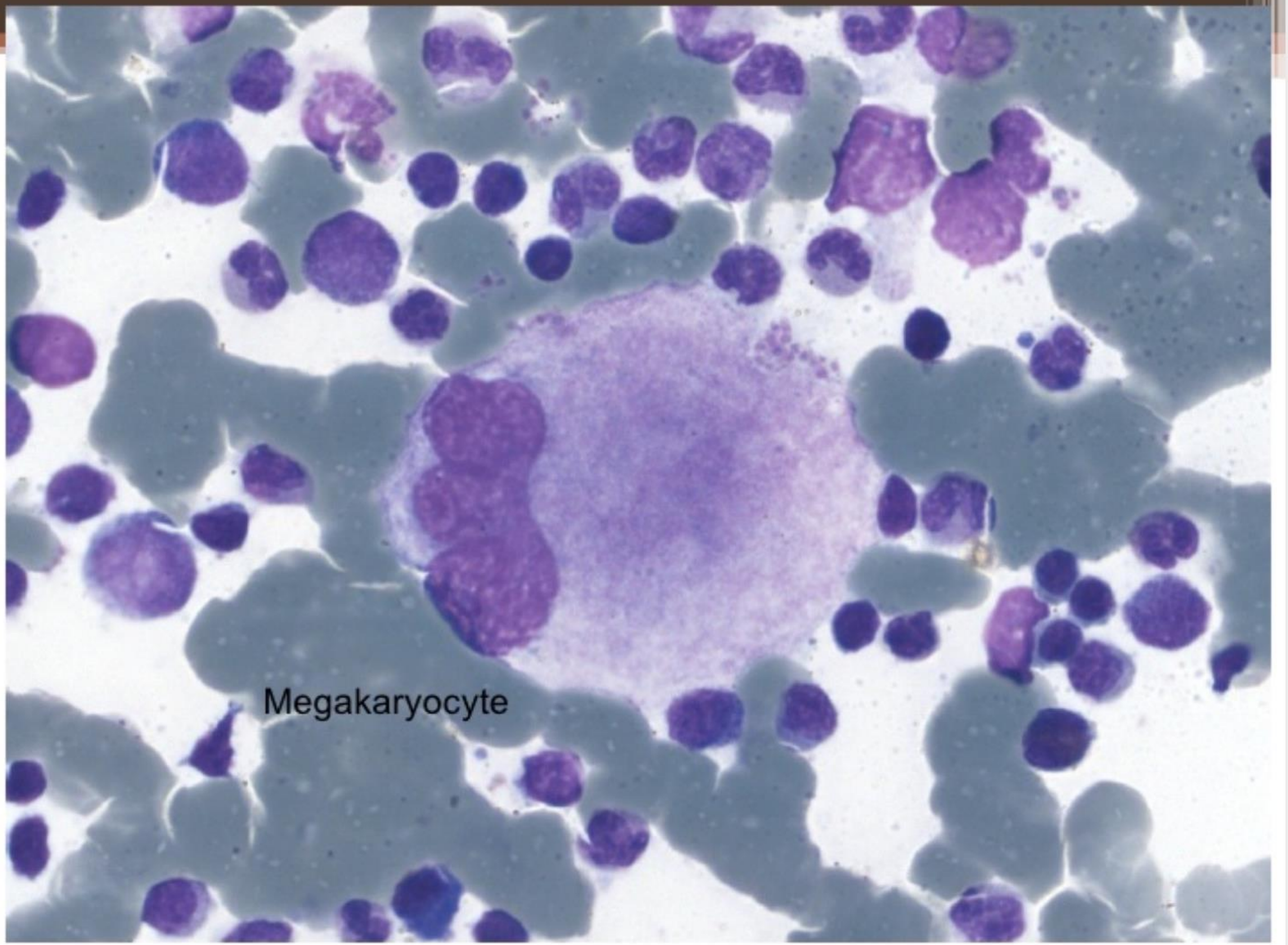
***Megakaryocytes

Megakaryoblasts

- Megakaryoblasts are the precursors of the megakarycytes.
- They may show cytoplasmic blebbing.




Promegakaryocyte
Kyoto Univ.

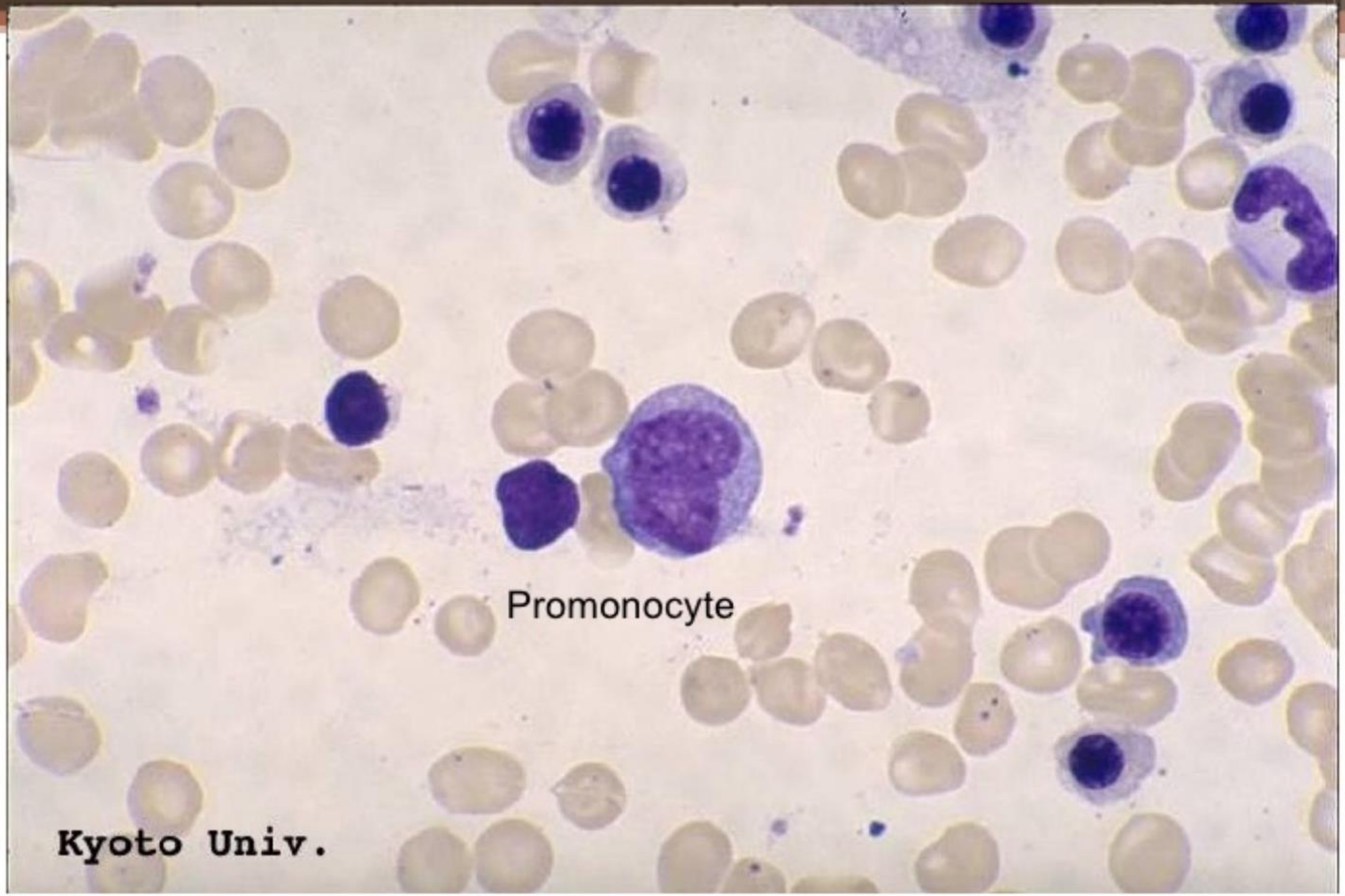

Megakaryocyte

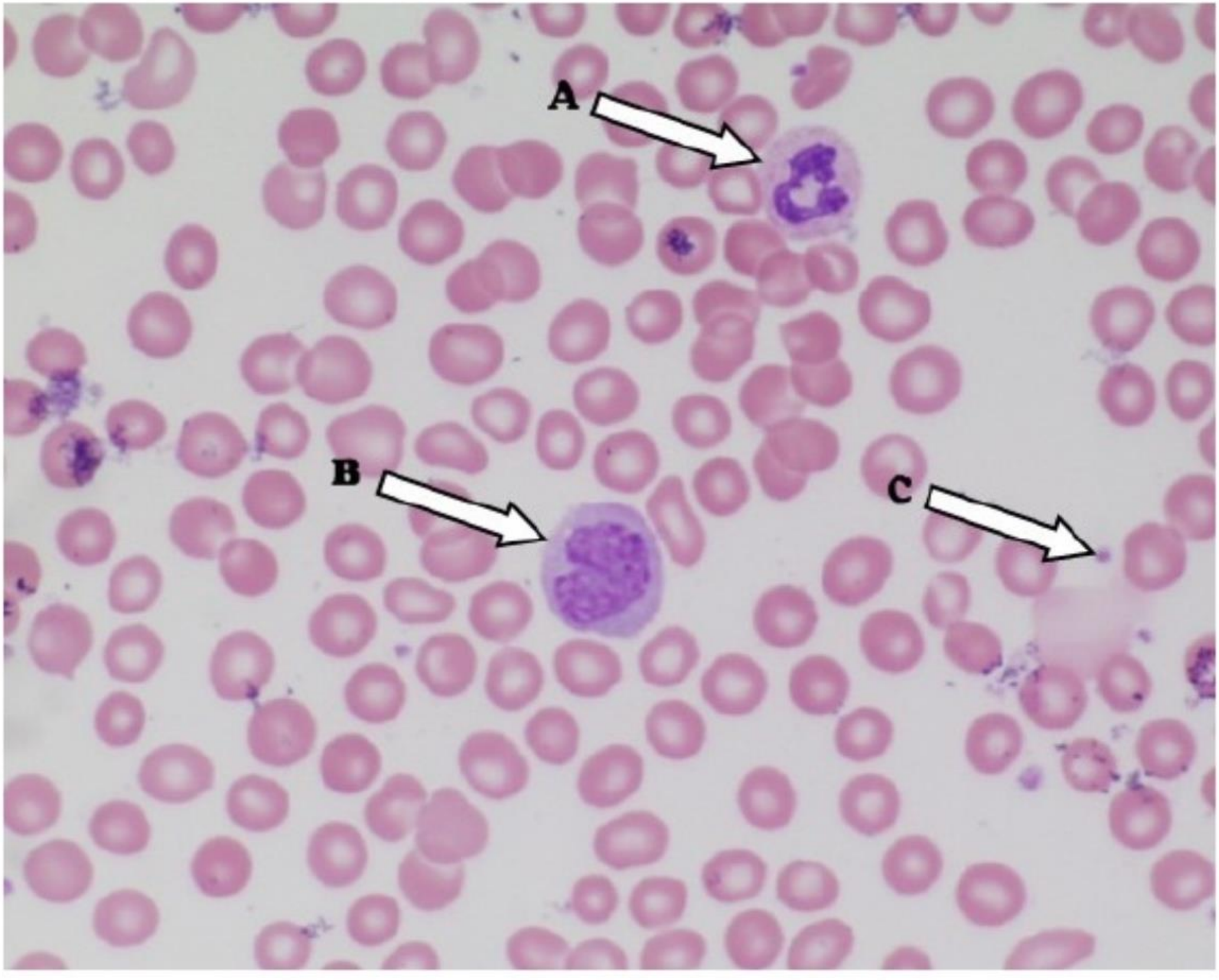
Monocytopoiesis

- Monoblast

- Promonocyte

- Monocyte


Promonocyte
Kyoto Univ.


A
B
C

Systemic scheme for Examining aspirated BM films

- Low power (x10)
  - Determine cellularity
  - Identify megakaryocytes
  - Look for clumps of abnormal cells
  - Identify macrophages

Systemic scheme for Examining aspirated BM films

- Higher power (x40, x100)
  - Identify all stages of maturation of myeloid and erythroid cells.
  - Determine the M:E ratio
  - Perform a differential count
  - Look for areas of BM necrosis.
  - Assess the iron content.

Assessment of BM cellularity

- Cellularity cannot be assessed without knowing the age of a patient.
- A young child on average has about 80% of the intertrabecular space occupied by haemopoietic cells whereas in a 75-year-old the average has fallen to around 30%.
- **100 – Age of Patient**

M:E ratio

- The M:E ratio is the ratio of all granulocytic plus monocytic cells (Myeloid) to all erythroblasts (Erythroid).
- For all bone marrow aspirates examined, the report should specify the M:E ratio and the percentage of lymphocytes and plasma cells.
- A differential count of at least 200-300 cells should be performed.
- If there is any borderline abnormality, e.g. in the number of blasts, lymphocytes or plasma cells, a 500 cell differential count should be performed.

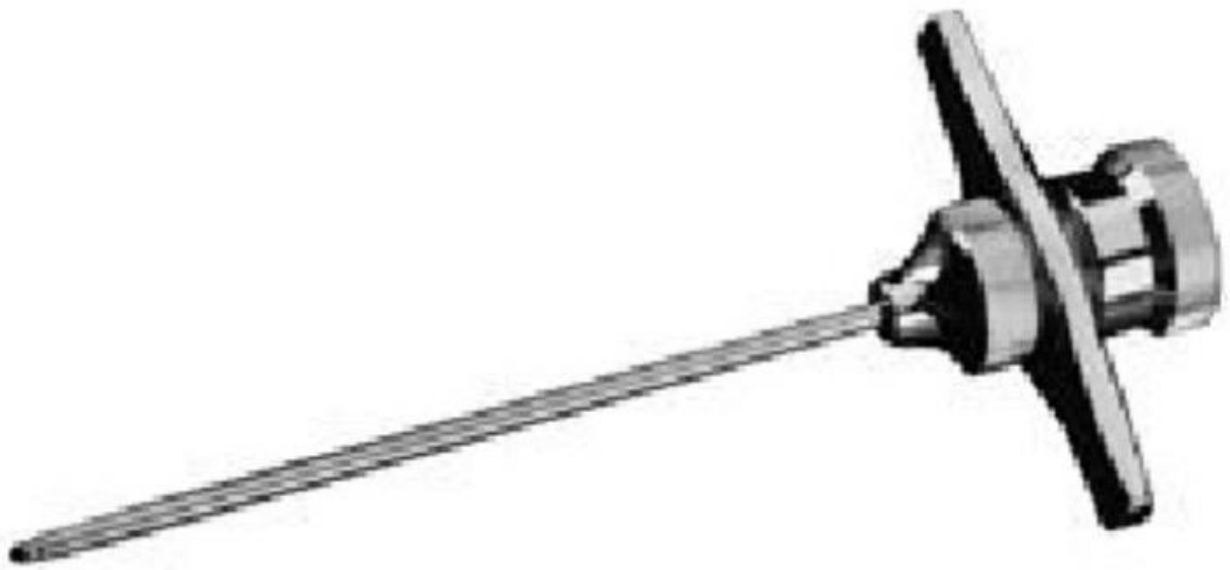
- Only after the bone marrow has been carefully assessed on low and medium power the X100 oil should be used to assess cellular detail

Reporting results

- List the various descriptive comments regarding all the cell lines present in the BM
- Mention the striking abnormalities separately.
- Write your impression and any recommendations to the clinician.

Trephine biopsy

- Jamshidi Type Bone Marrow Biopsy Needle,
  - 8swg (4.0mm) x 100mm
  - 11swg (3.0mm) x 100mm
  - 13swg (2.3mm) x 90mm
  - 14swg (2.0mm) x 90mm (In child)



Comparison

	Aspiration	Biopsy
Advantages	• Fast • Gives relative quantity of different cell types • Gives material to further study, e.g. molecular genetics and flow cytometry	• Gives cell and stroma constitution • Represents all cells • Explains cause of "dry tap" (aspiration gives no blood cells)
Drawbacks	Doesn't represent all cells	Slow processing

Adequacy of biopsy

- should contain at least five to six intertrabecular spaces and, after processing,
- should be at least 2–3 cm in length
- Others have considered that 1.5–2 cm is an acceptable length

Contraindications

- The only absolute reason to avoid is the presence of a severe bleeding disorder

Complications

- While mild soreness lasting 12-24 hours is common after a bone marrow examination, serious complications are extremely rare.

Thank You..