Interpretation of bone marrow biopsy

DR.

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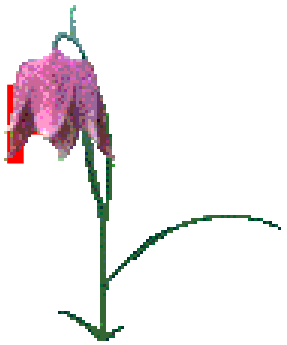


Objective

- Component of bone marrow biopsy
- Assessment of bone marrow biopsies
- Feature of dysplasia in each lineage

Bone marrow is the site of origin ,maturation and development of all hematopoietic elements that are then released into peripheral blood

Structure of bone marrow :

A) cellular elements

Haematopoetic stem cells , Progenitors , precursors .

B) Stroma - unique microenvironment of the marrow

Marrow architecture

- The BMTB enables the assessment of bone marrow architecture, the distribution of cellular elements and the bone and stromal cells.
- The outermost elements of the biopsy are composed of collagenous periosteal connective tissue, followed by a zone of cartilage or cortical bone (depending on the age of the patient).
- After this the bone breaks up into a meshwork of trabeculae, between which are the intertrabecular spaces. Hemopoietic cells are present within these intertrabecular spaces and are supported by fat cells, stromal cells, histiocytes extracellular matrix and blood vessels .
- The hemopoietic cells are located within the intertrabecular spaces.
- The intertrabecular areas can be divided into three zones which contain different hemopoietic cell types

Indications for B M Examination

1- neoplasms hematopoietic and non hematopoietic

2- staging

3-Further assessment of peripheral blood abnormality

4-Fever of unknown etiology

5- Unexplained splenomegaly

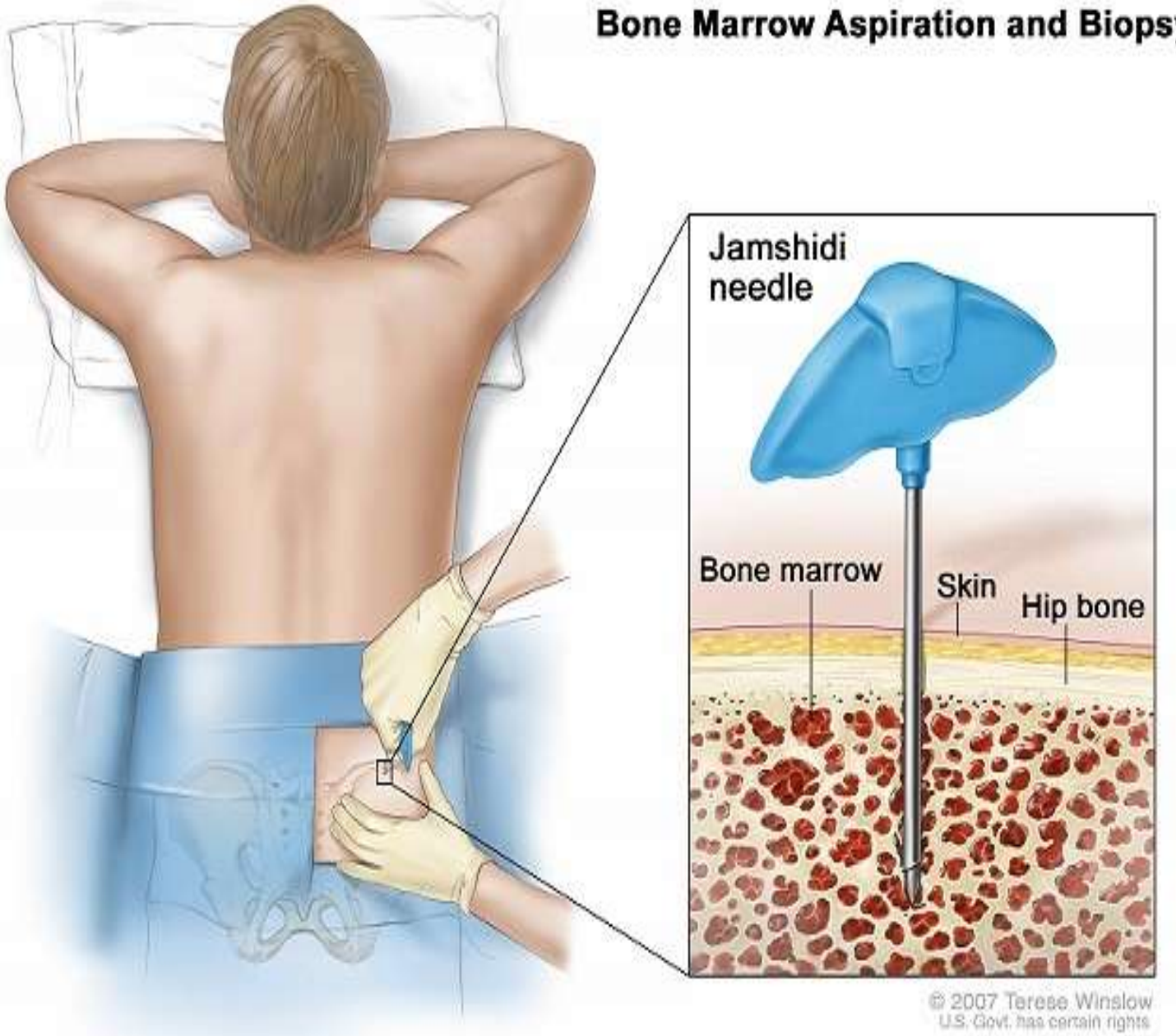
6- monitoring : response to treatment ,residual disease ,recurrent

Sits of bone marrow biopsy :

- **Crest of the posterior superior iliac spine – Preferred site**
- Anterior superior iliac spine – Rarely performed
- Anterior tibial plateau (Tibial tuberosity) – Very young children

Sternum – Rarely performed ,need experienced operator

Bone Marrow Aspiration and Biopsy



Contraindications

- Hemophilia (factor transfusion before the procedure)
- Sternal in children ,myeloma and carcinoma patients

Processing of bone marrow biopsy:

1. FIXATION

   10% buffered formalin

   Zenkers fluid

2. DECALCIFICATION

   Nitric acid 2-3 hours

3.EMBEDDING

   Paraffin

4. STAINING

   H & E , Reticulin , trichrome .

BM Biopsy evaluation :

Adequacy of biopsy :

Length 1.6 cm (1.5 –2.5cm)

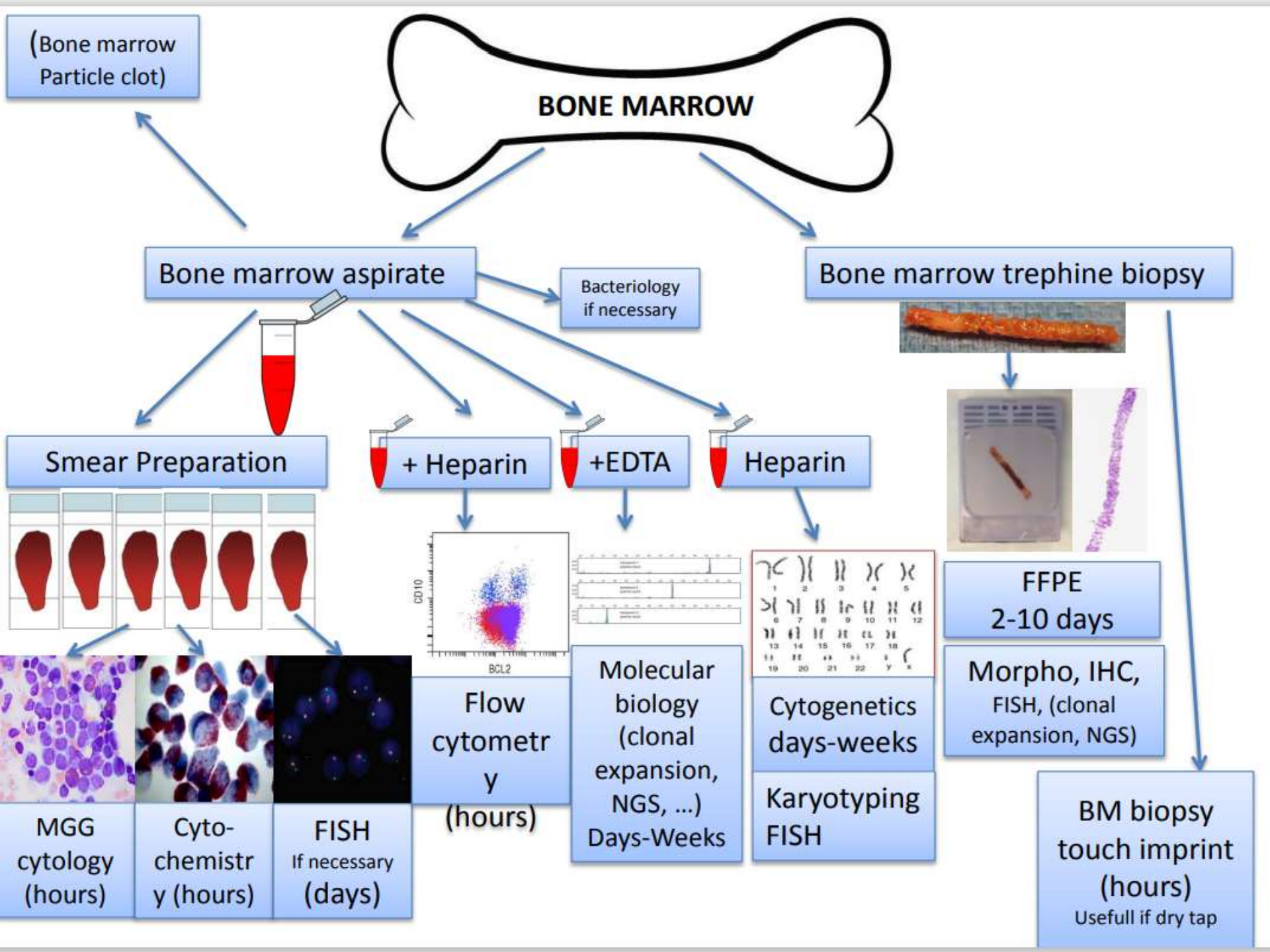
25% shrinkage during processing

5-6 trabecular spaces

Good quality staining (free of crush artifact or interstitial hemorrhage or fragmentation)

Evaluation of bone marrow

- History
- CBC
- Peripheral blood smear
- **Core biopsy**
- Clot section
- Aspirate smear
- Touch imprint
- IHC (Immunohistochemistry)
- Special stains
- Flow cytometry
- Cytogenetic \FISH
- Molecular


(Bone marrow Particle clot)
BONE MARROW
Bone marrow aspirate
Bacteriology if necessary
Bone marrow trephine biopsy
Smear Preparation
+ Heparin
+EDTA
Heparin
CD10
BCL2
FFPE 2-10 days
Morpho, IHC, FISH, (clonal expansion, NGS)
MGG cytology (hours)
Cyto-chemistry (hours)
FISH If necessary (days)
Flow cytometry (hours)
Molecular biology (clonal expansion, NGS, …) Days-Weeks
Cytogenetics days-weeks
Karyotyping FISH
BM biopsy touch imprint (hours) Usefull if dry tap

Touch imprints of the core biopsy

Benefit of biopsy

- Better estimation of **cellularity**
- Shows architecture
- Distribution of cells
- Stromal cells
- Pattern of involvement

BM ASPIRATE

- Quick results
- Fine cytological detail
- Enumeration of marrow cellular elements
- Wider cytochemical stains can be used
- Ideal for flow cytometry, cytogenetics/molecular studies

BM BIOPSY

- Complete assessement of cellularity and architecture
- More sensitive for focal lesions
- Allows grading of fibrosis
- Use of IHC
- Useful for assessment of AA, metastasis, some infections

BM biopsy examination include:

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

Marrow cellularity

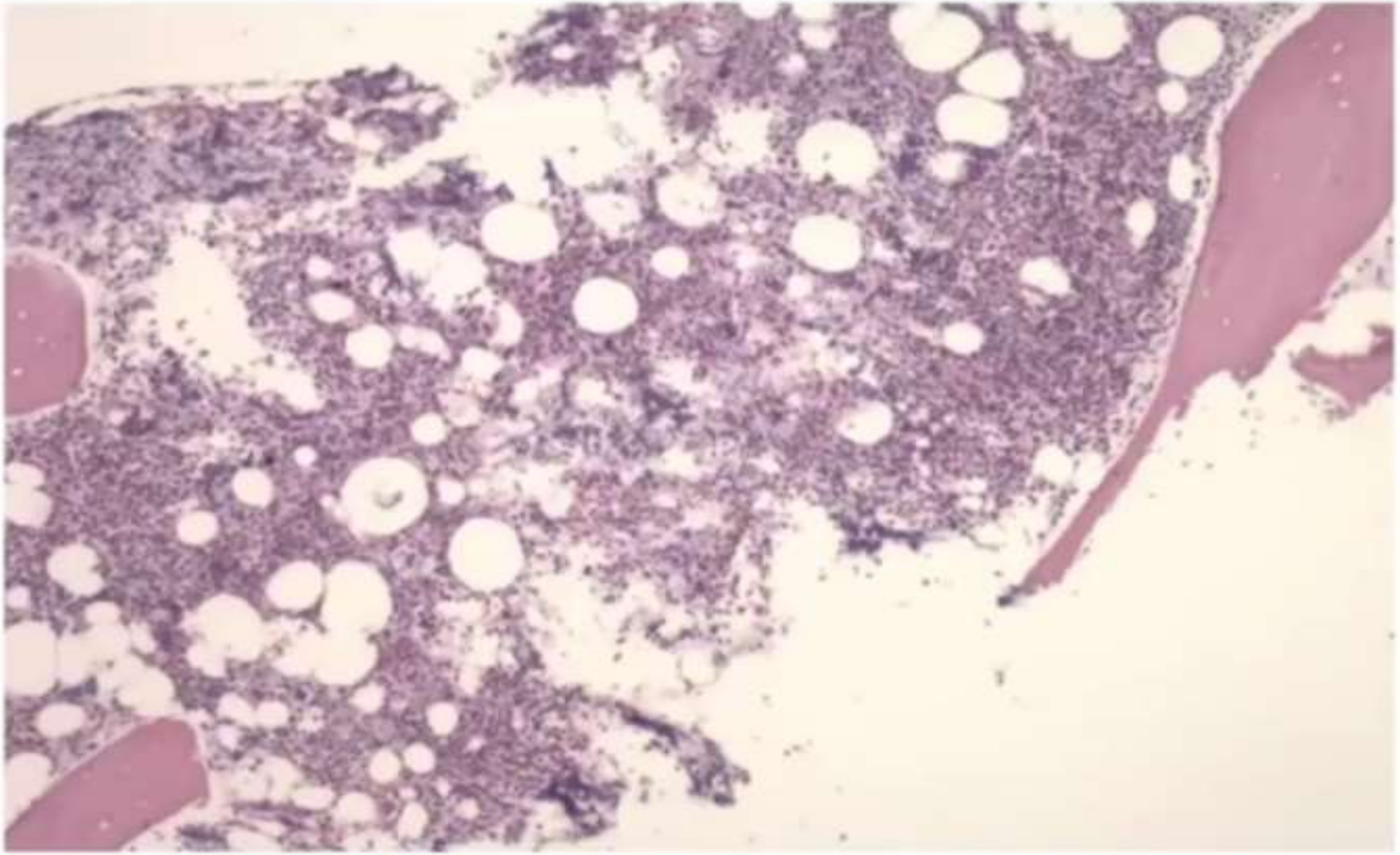
- The BMTB is particularly useful for the assessment of marrow cellularity.
- **This is the relative amount of BM cells to adipocytes, which is assessed subjectively and should be interpreted in the context of the age of the patient.**
- The terms normocellular (normal for age), hypercellular (increased cellularity for age) and hypocellular (reduced cellularity for age) are used.
- Cellularity reduces with increasing age

- In practice, the formula (cellularity = 100 – patient age) can be applied for adults; however, it does not correlate with cellularity at the extremes of the age range.
- The intertrabecular spaces adjacent to the marrow cortex tend to be hypocellular and should not be assessed when determining overall BM cellularity.

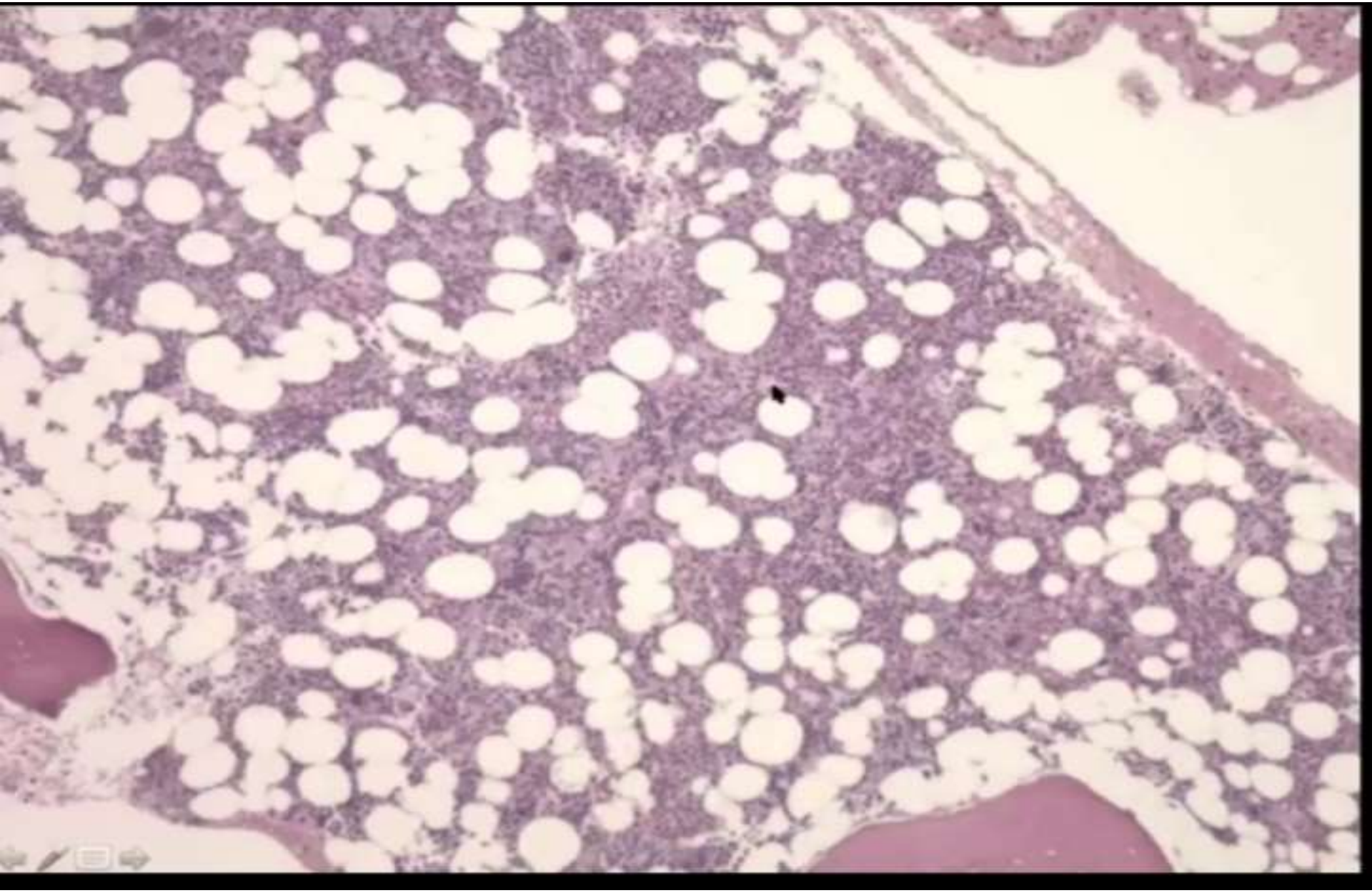
  – In adults - sub cortical marrow is hypoplastic

Table 3.2 Cellularity ranges for various age groups

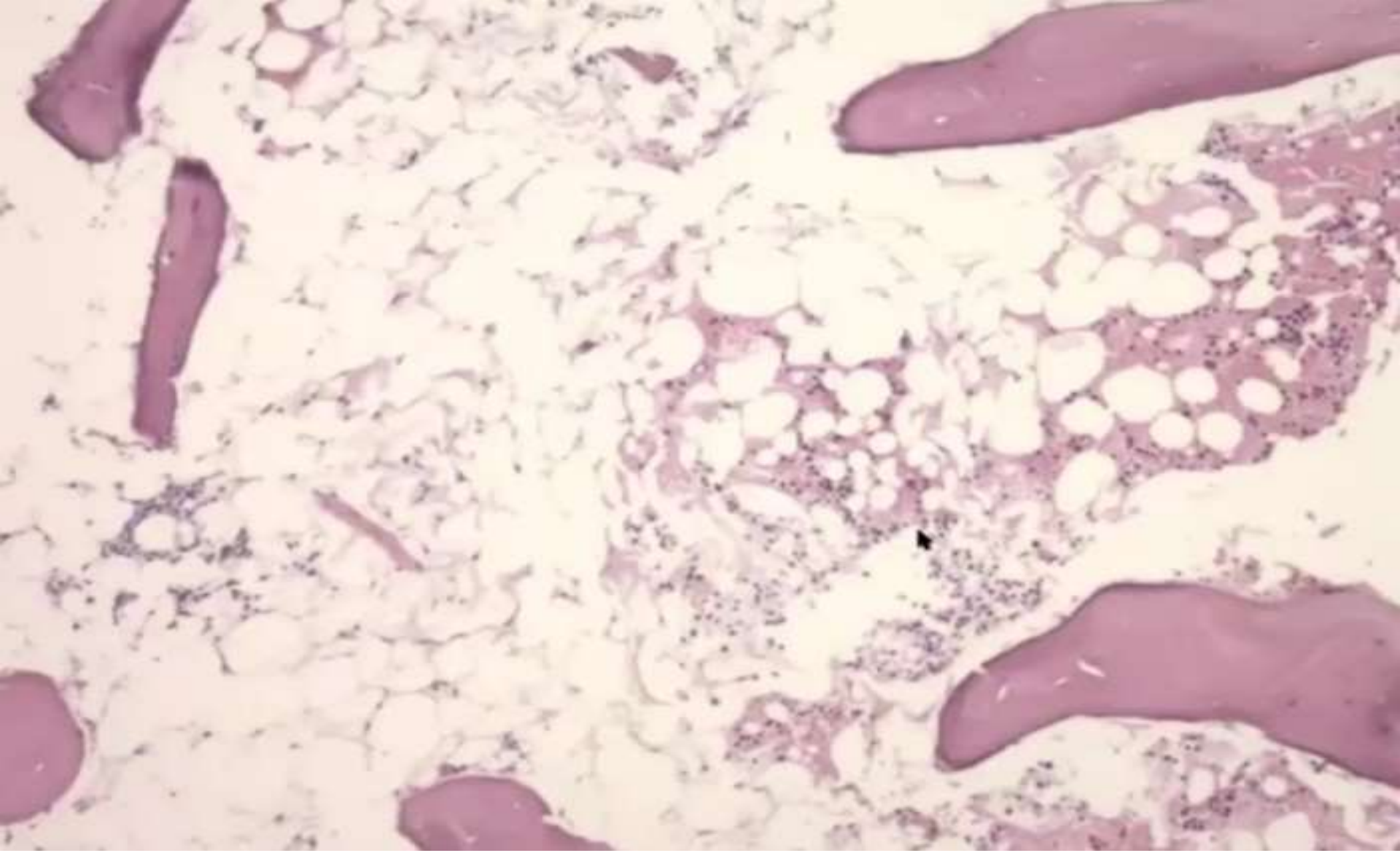
Age	Cellularity
Newborn to 3 months	80–100%
Childhood	60–80%
20–40 years	60–70%
40–70 years	40–50%
>70 years	30–40%



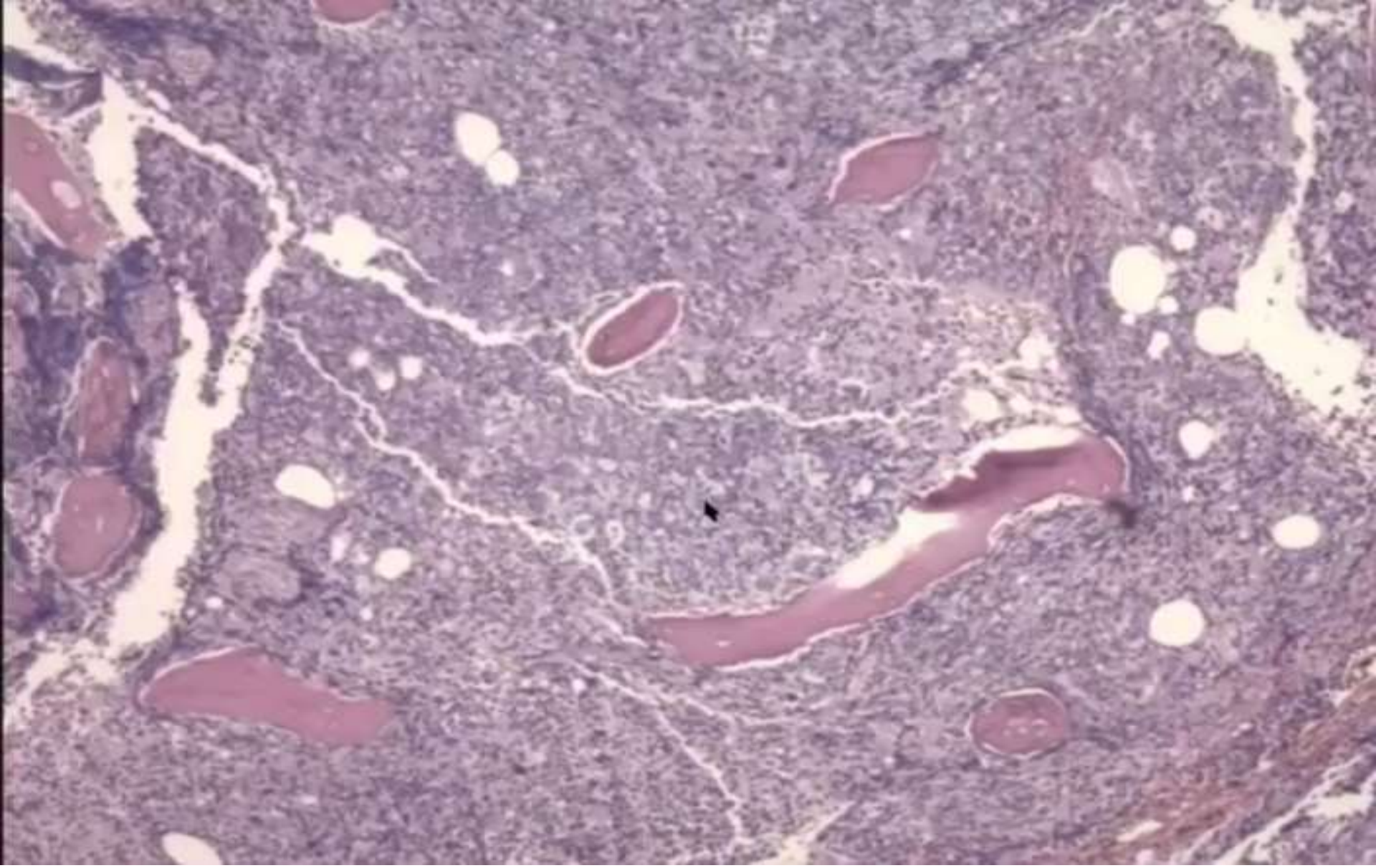
Marrow cellularity is about 60%



Marrow cellularity is about 50%



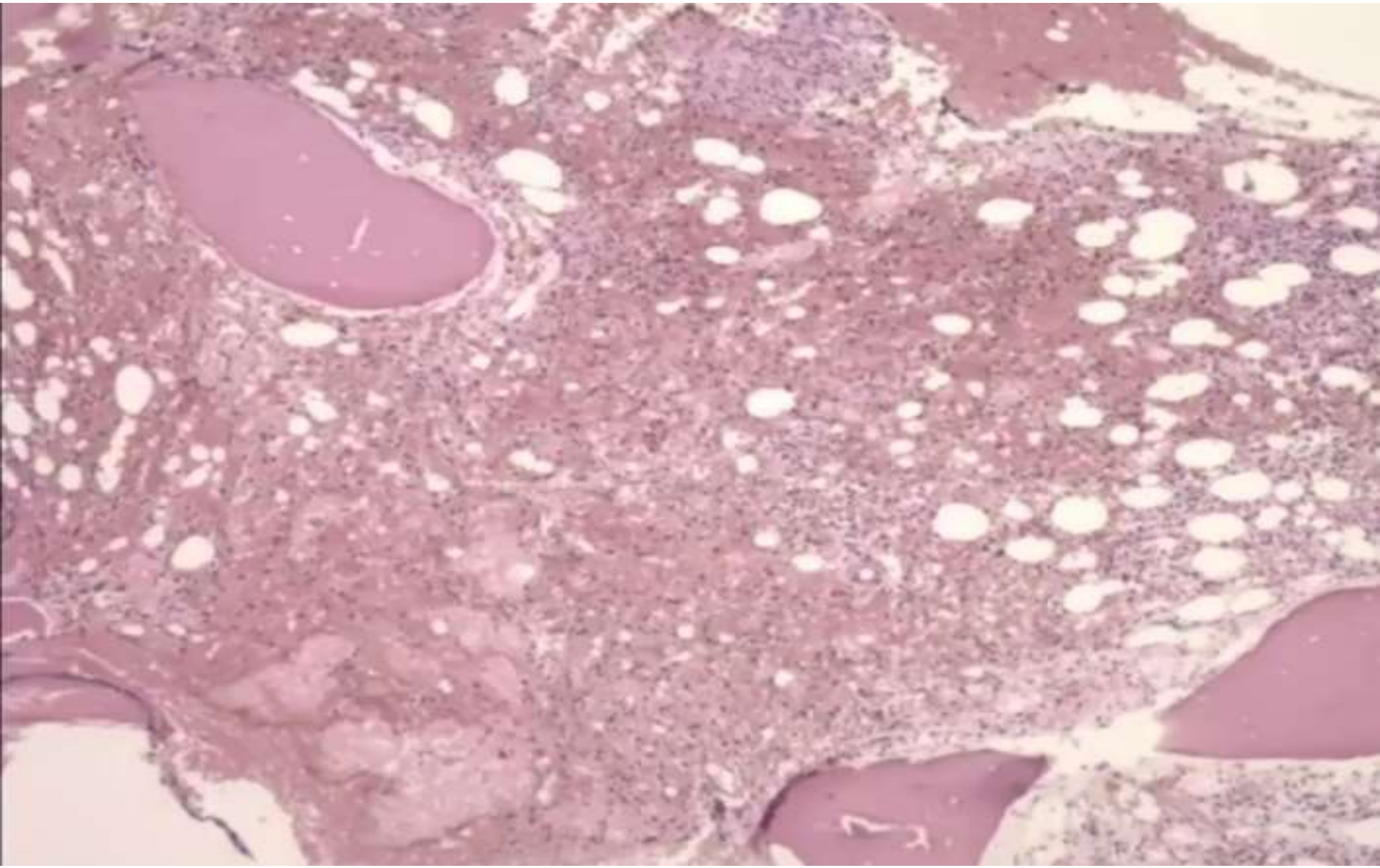
Marrow is hypo cellular or even acellular



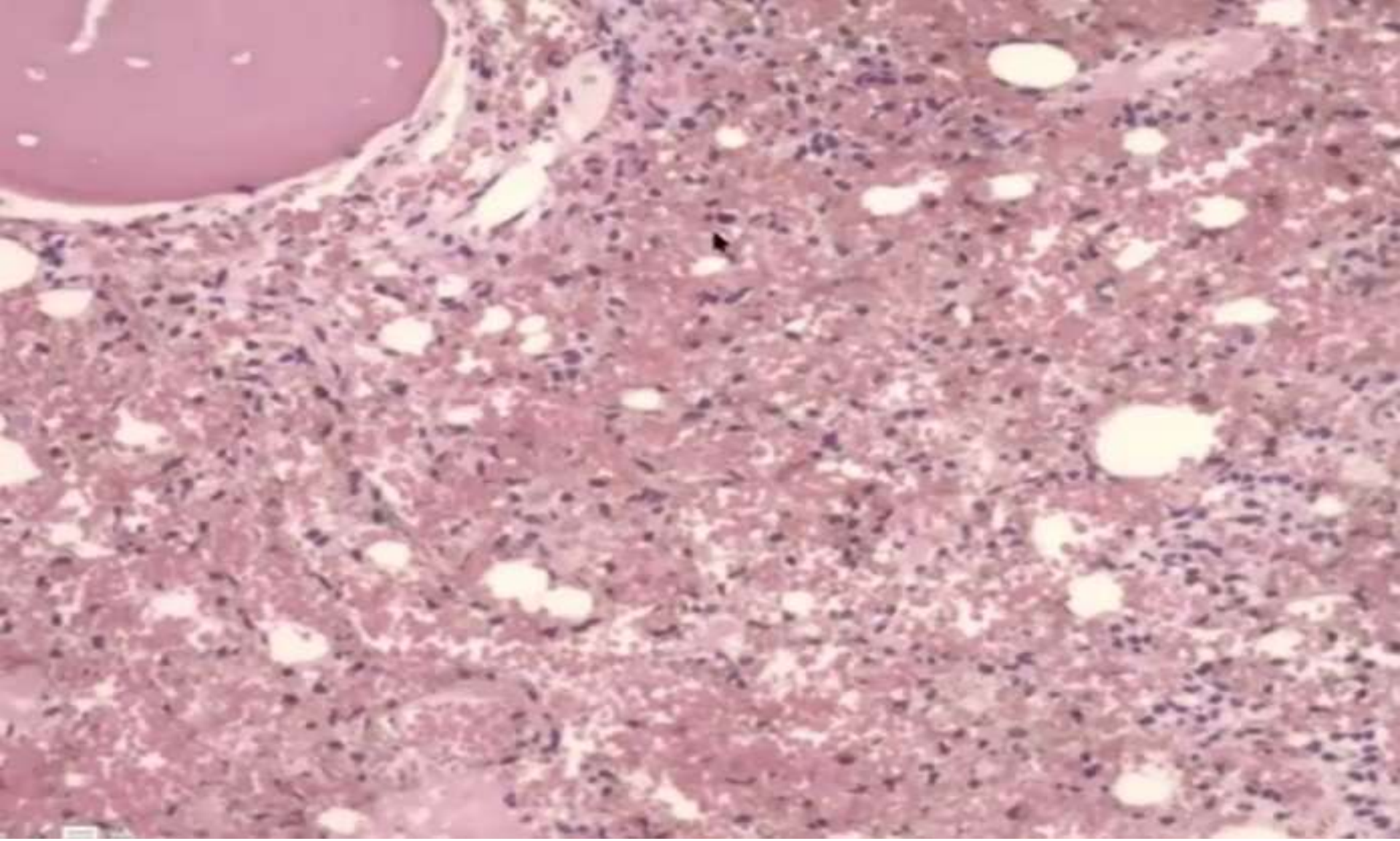
Marrow cellularity is 90-100 %

Examples on problematic B M biopsy:

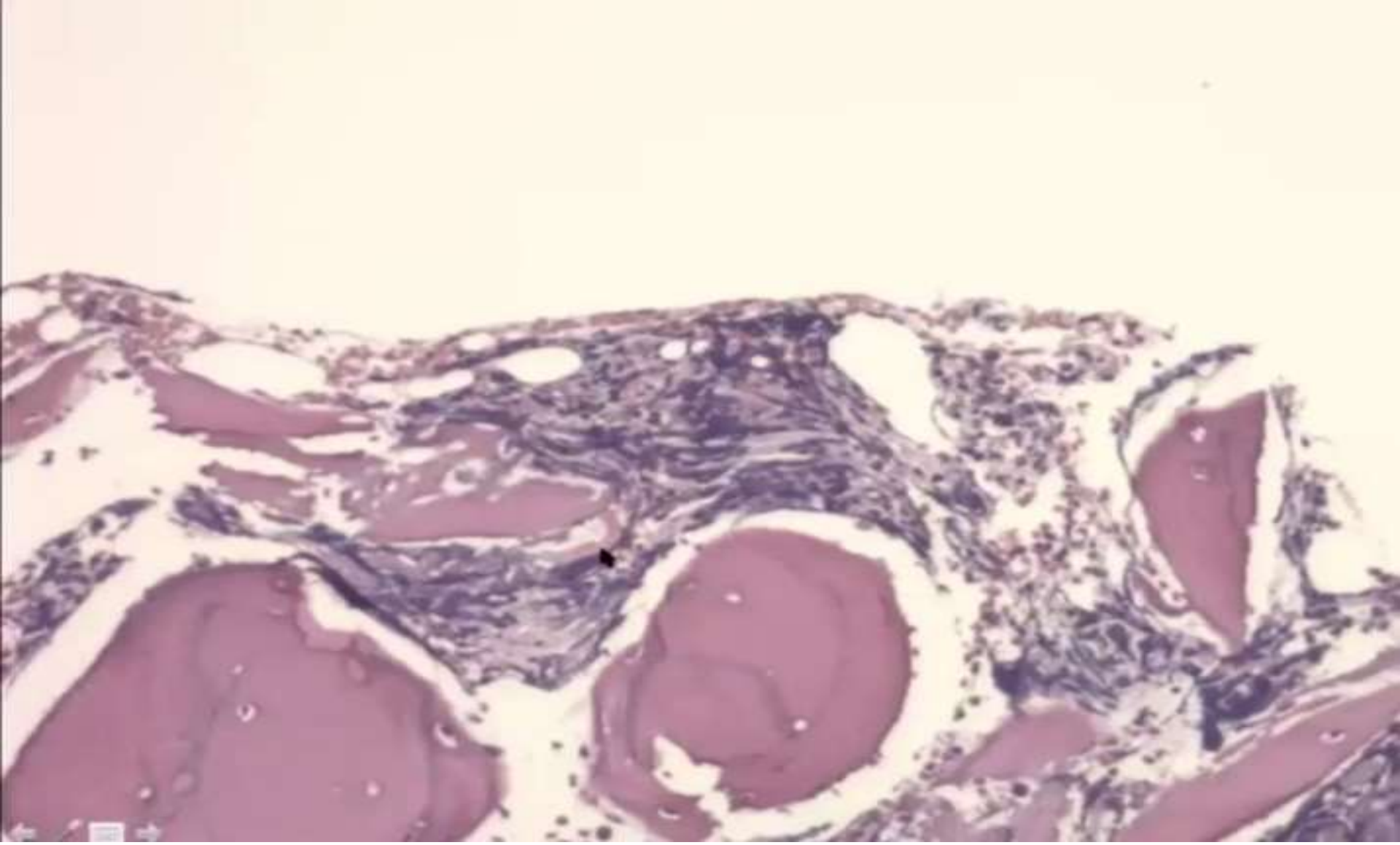
- Suboptimal
- Crush artifact
- Aspiration artefact
- Subcortical (normally hypocellular)



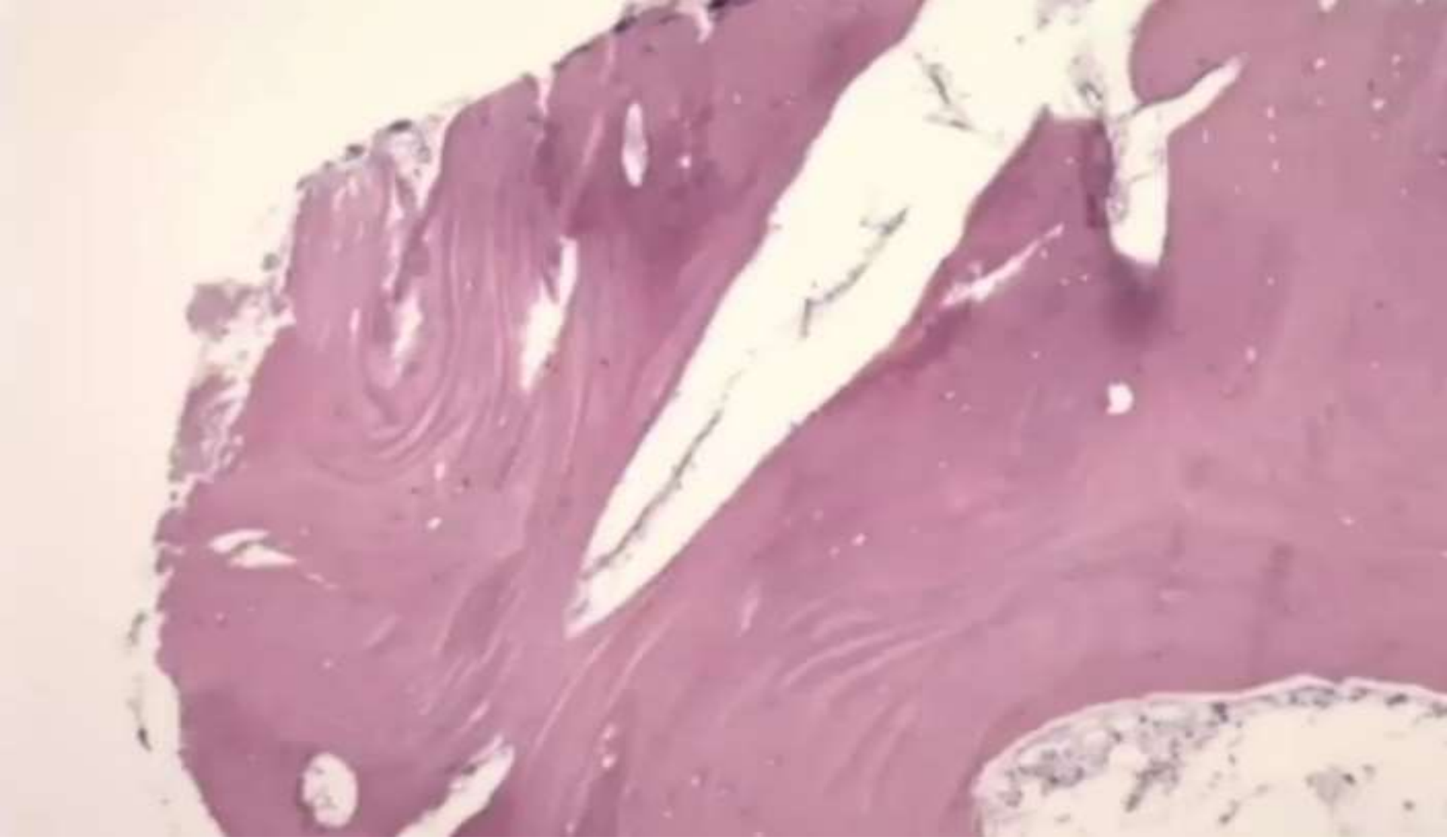
Cellularity ??



This is the same previous slide at high power showing mature RBCS which is not normal component of BM this is aspiration artifact due to aspiration of peripheral blood



Crush artifact : the cells are crushed so looking with high N\C ratio



Suboptimal biopsy

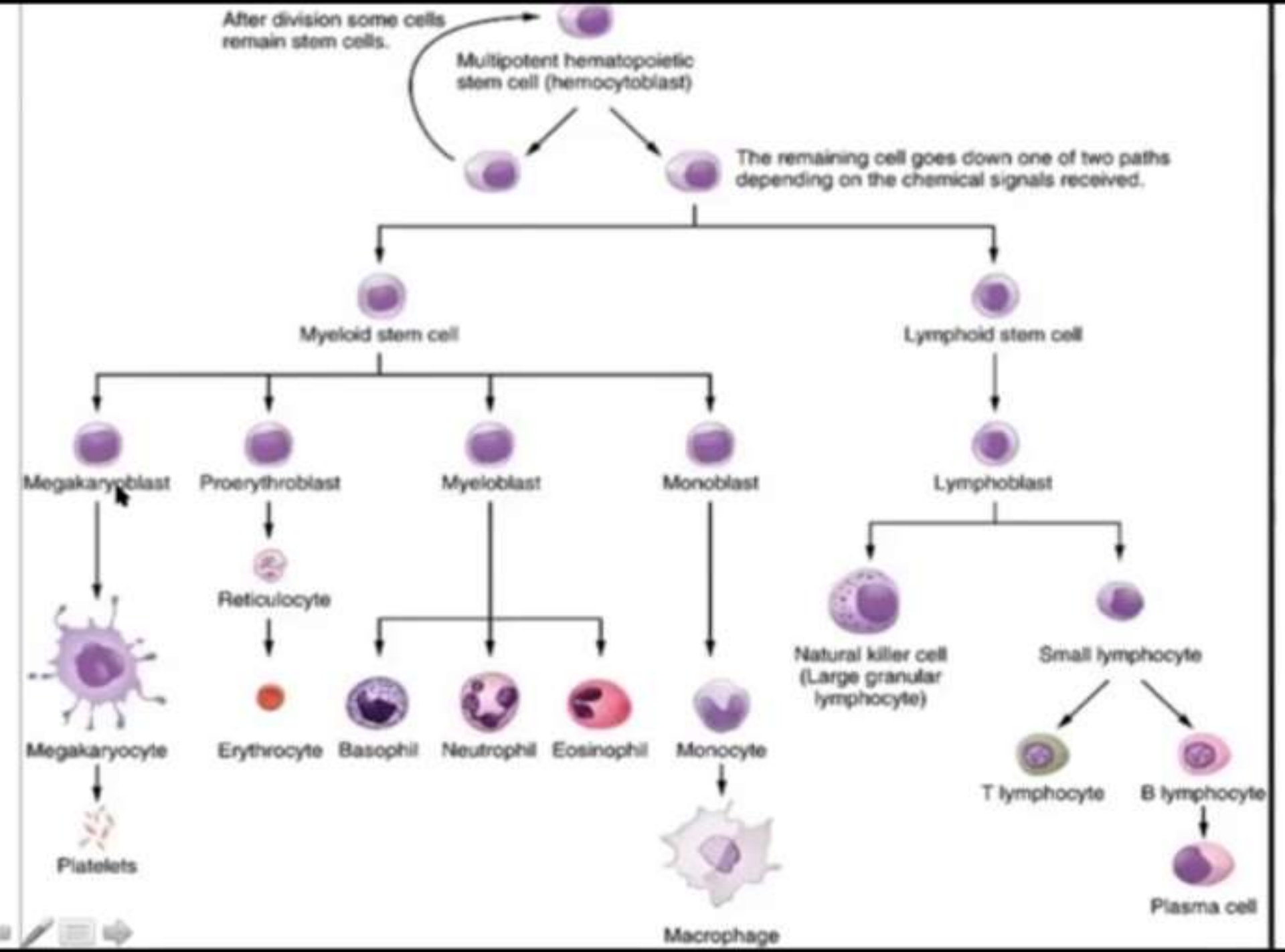
there is no hematopoietic elements, only bone

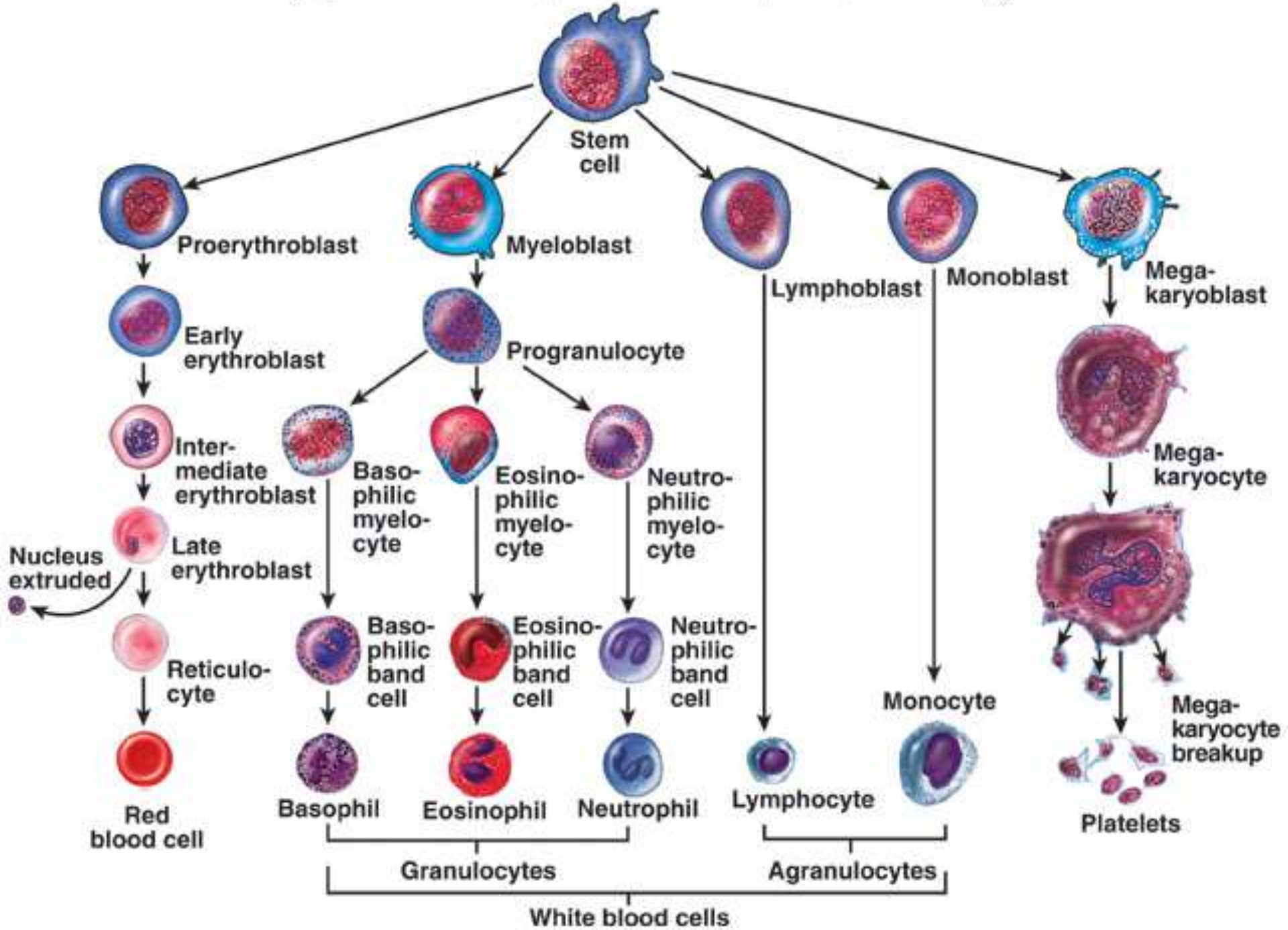
Hematopoiesis

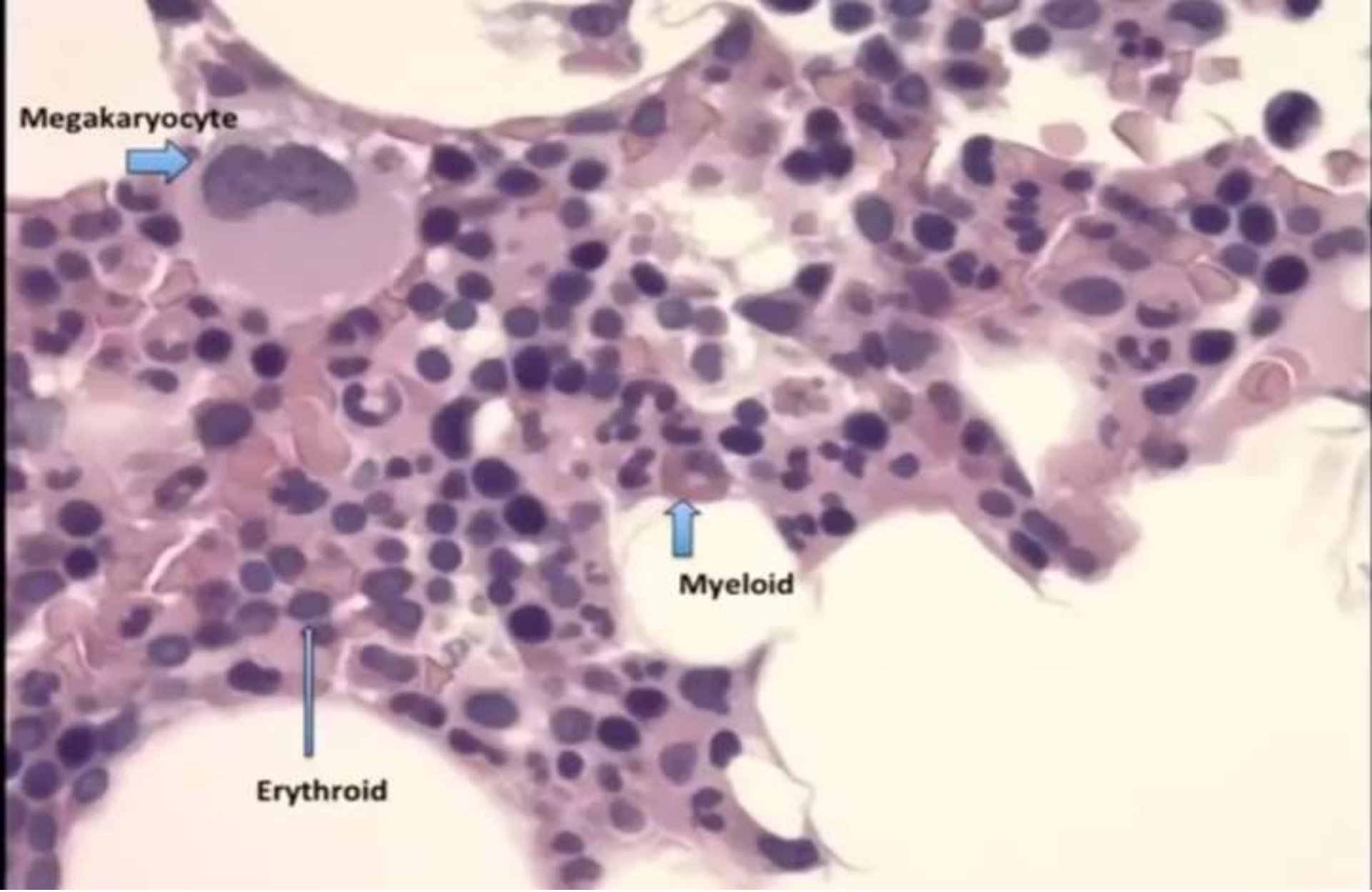
- Three main linage :

granulocytic (myeloid),Erythroid and megakaryocytic

- Genes involved: GATA1, GATA2,CBFB, RUNX1,RUNX2

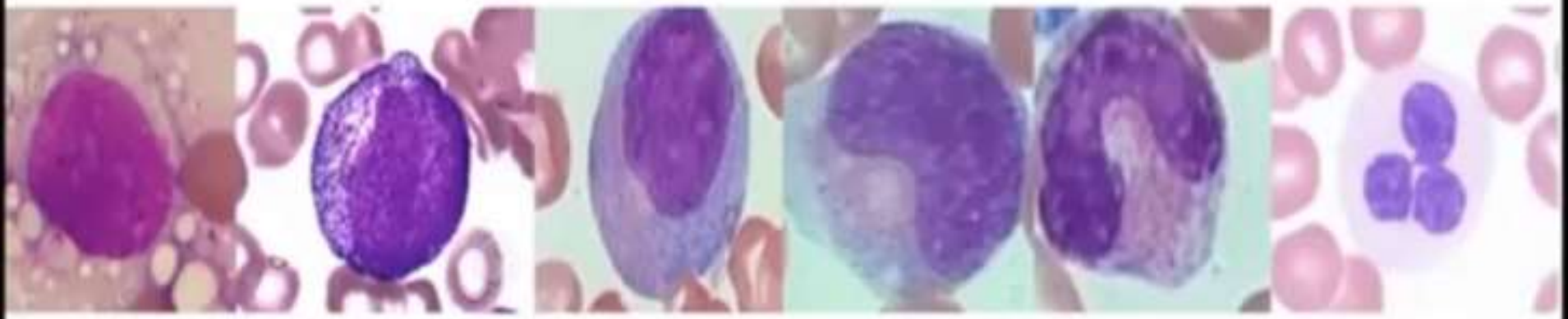

After division some cells remain stem cells.
Multipotent hematopoietic stem cell (hemocytoblast)
The remaining cell goes down one of two paths depending on the chemical signals received.
Myeloid stem cell
Lymphoid stem cell
Megakaryoblast
Proerythroblast
Myeloblast
Monoblast
Lymphoblast
Reticulocyte
Natural killer cell (Large granular lymphocyte)
Small lymphocyte
Megakaryocyte
Erythrocyte
Basophil
Neutrophil
Eosinophil
Monocyte
T lymphocyte
B lymphocyte
Platelets
Plasma cell
Macrophage





Normal marrow components

Myeloid lineage



Myeloblast Promyelocyte Myelocyte Metamyelocyte Band Neutrophil

Myelocyte is last to proliferate

Myeloid linage:

- Immature form located next to the bone
- More mature form located more central
- G-CSF binds to receptors on these cells and stimulates them
- **Markers for amyloid:**

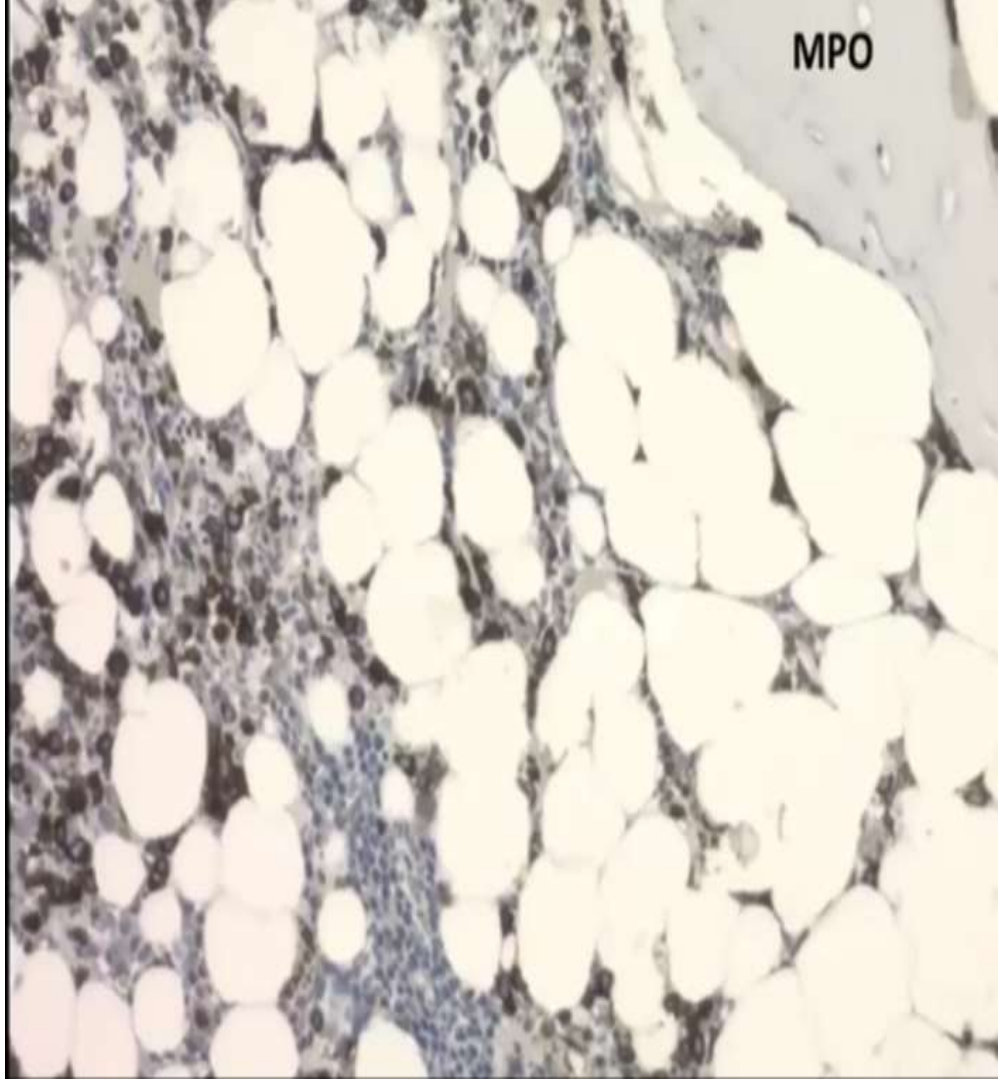
Myeloperoxidase (MPO)

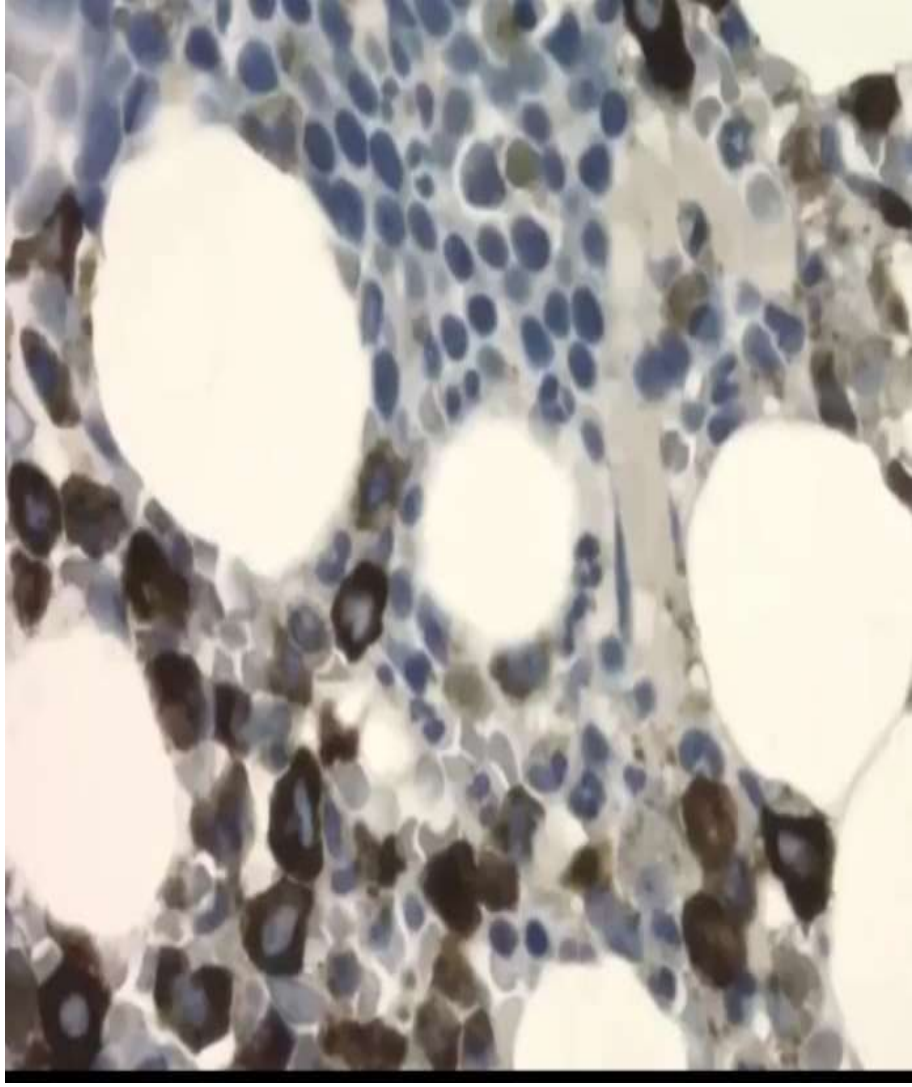
CD13

CD33

CD34

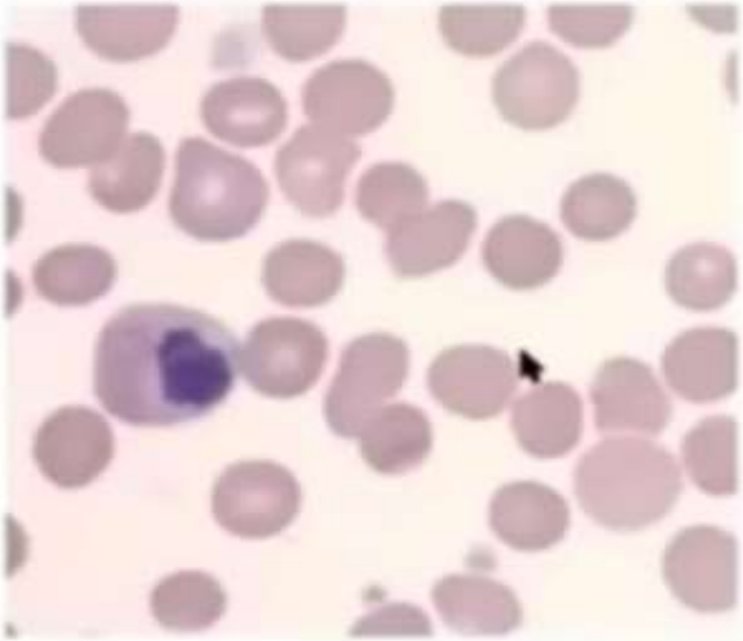
CD117

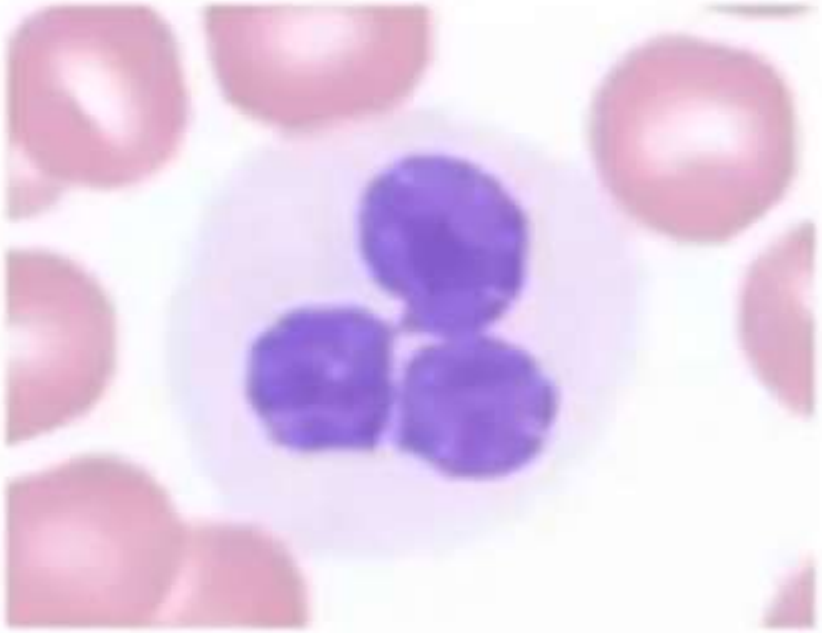




Myeloperoxidase (MPO) it stains immature cells more strongly than mature cells

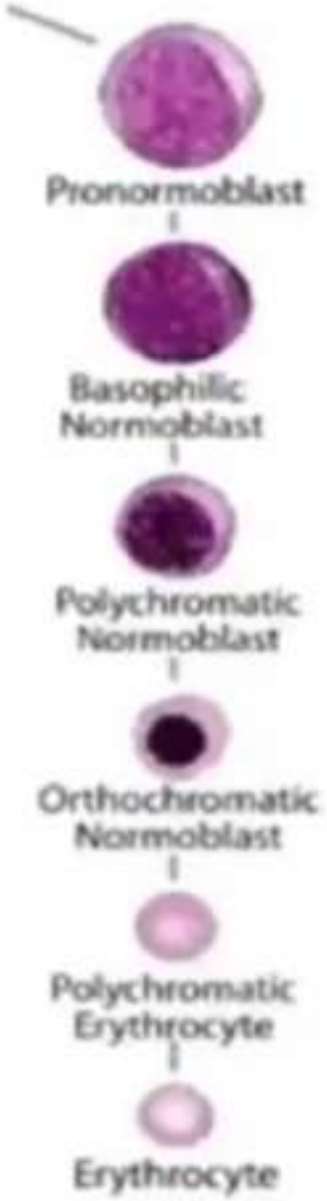
Signs of dysplasia



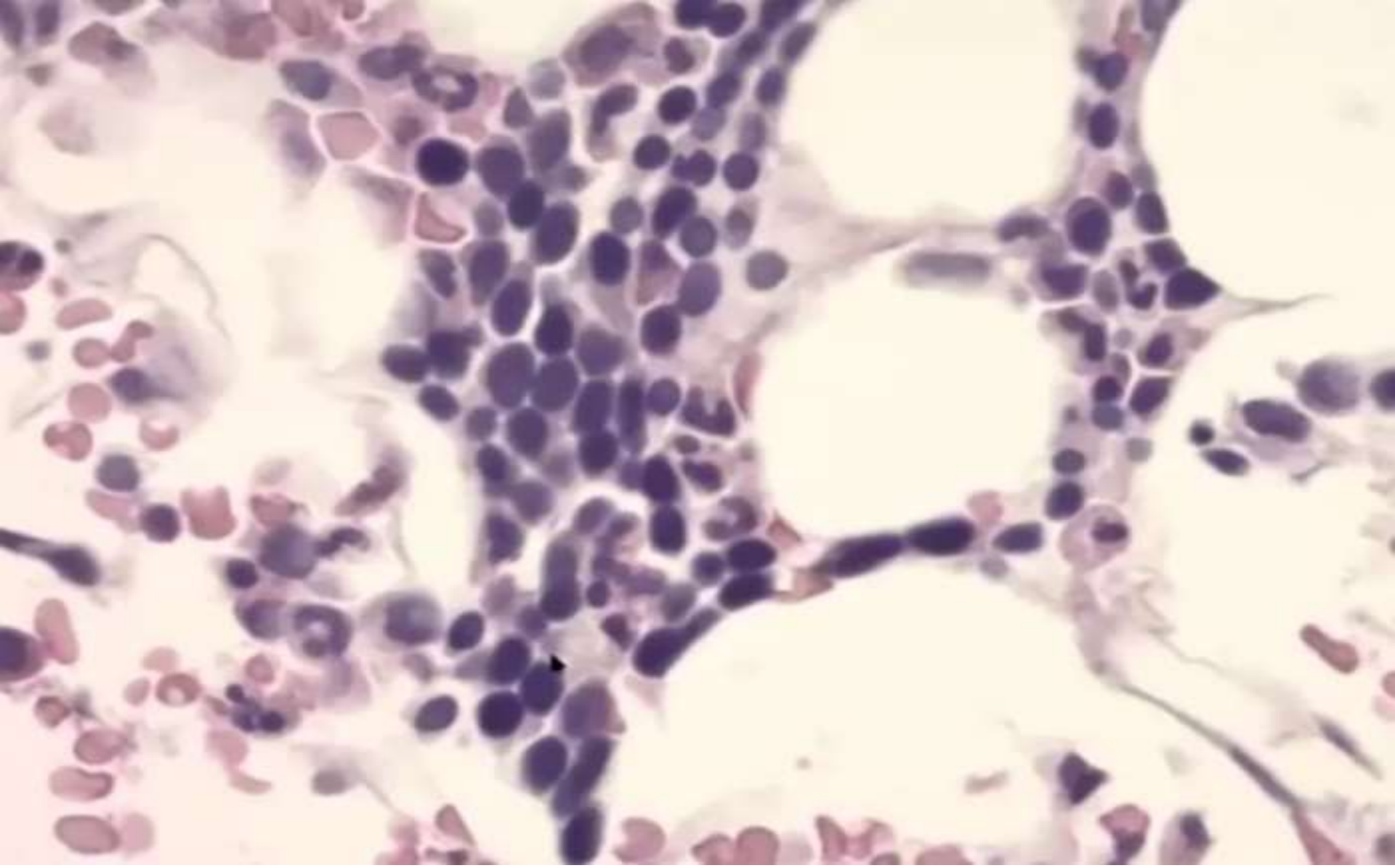


Signs of dysplasia in myeloid :Hypo granular cytoplasm hypo segmented nuclei

Erythroid lineage



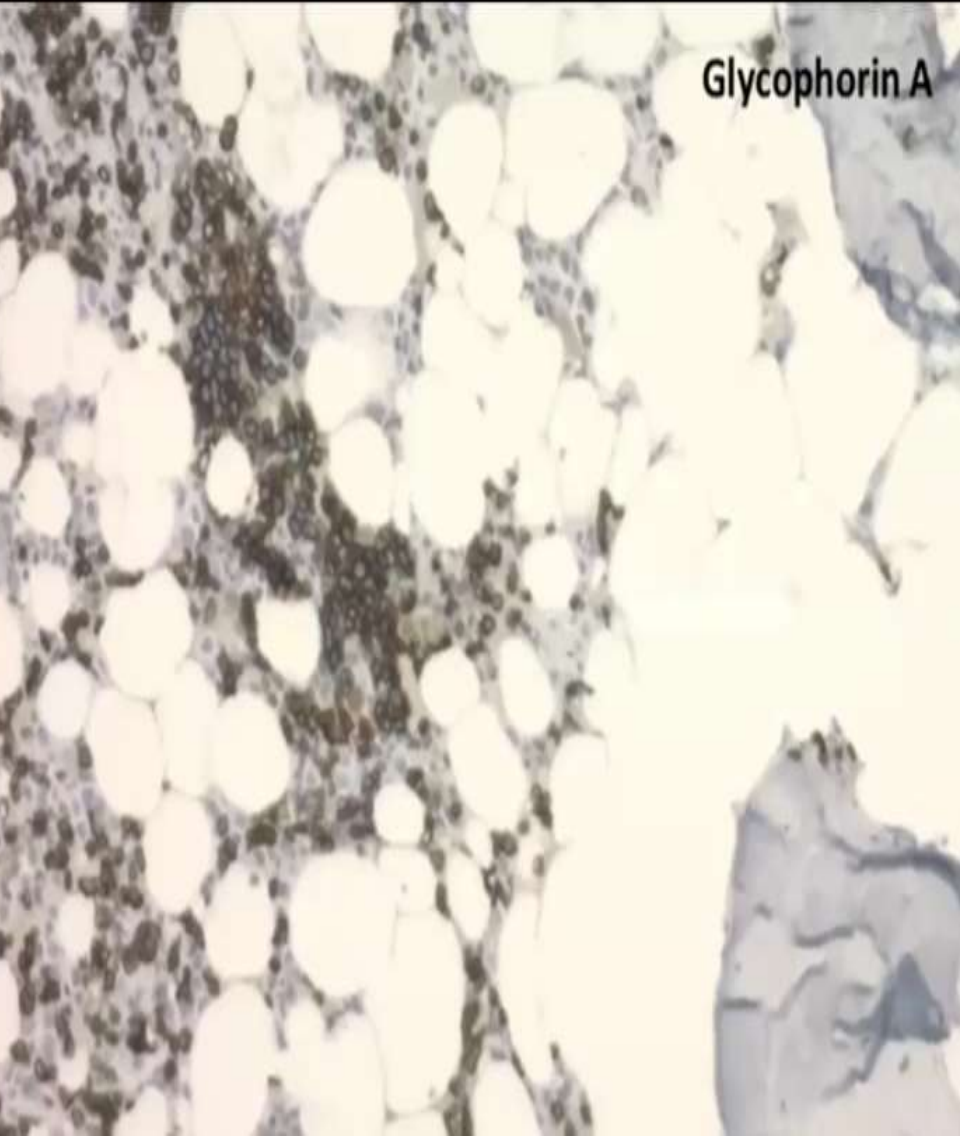
Early polychromatophilic normoblast is last to proliferate



B. M. biopsy showing group of erythroid cells

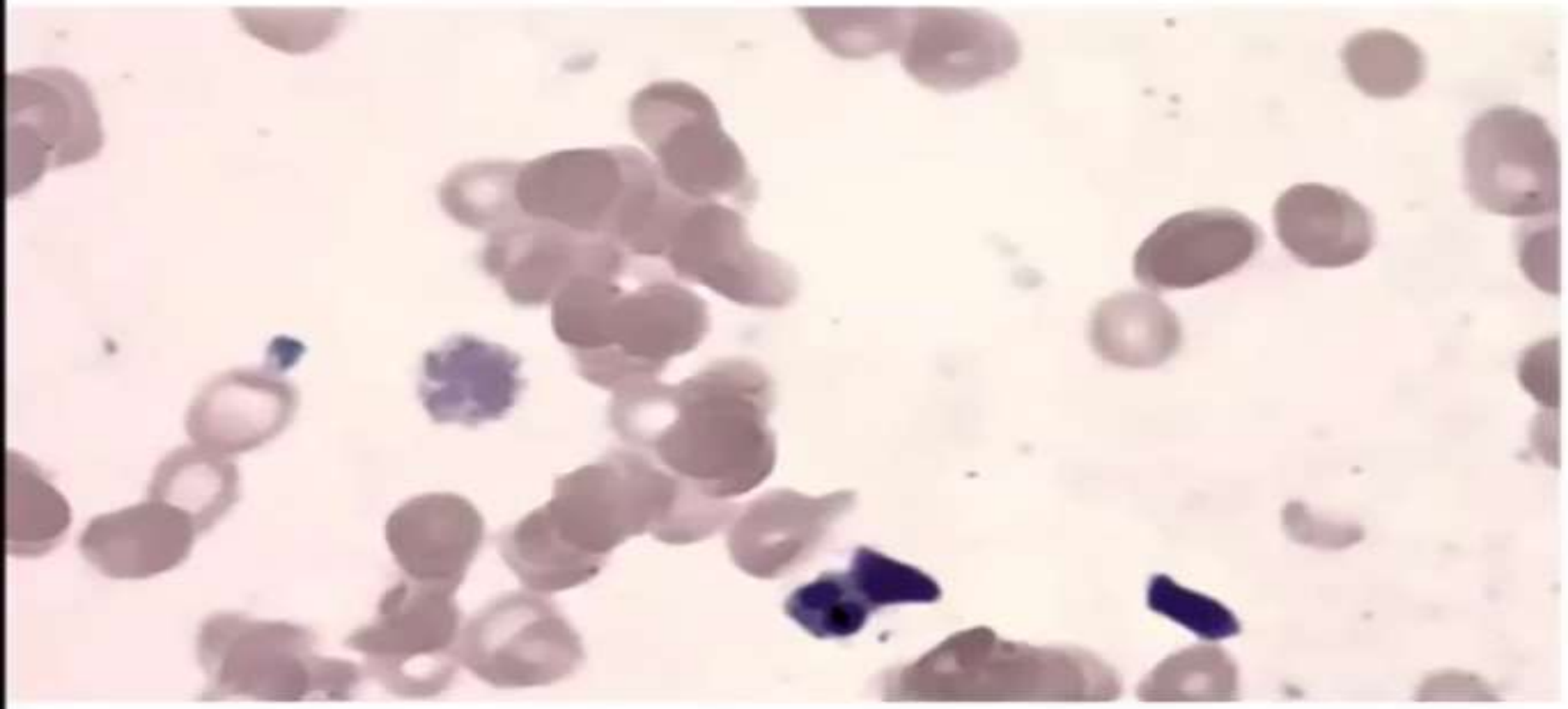
Markers for erythroid linage

- Glycophorin A
- E- cadherin
- Hemoglobin A



Glycophorin A staining erythroid precursors

Signs of dysplasia



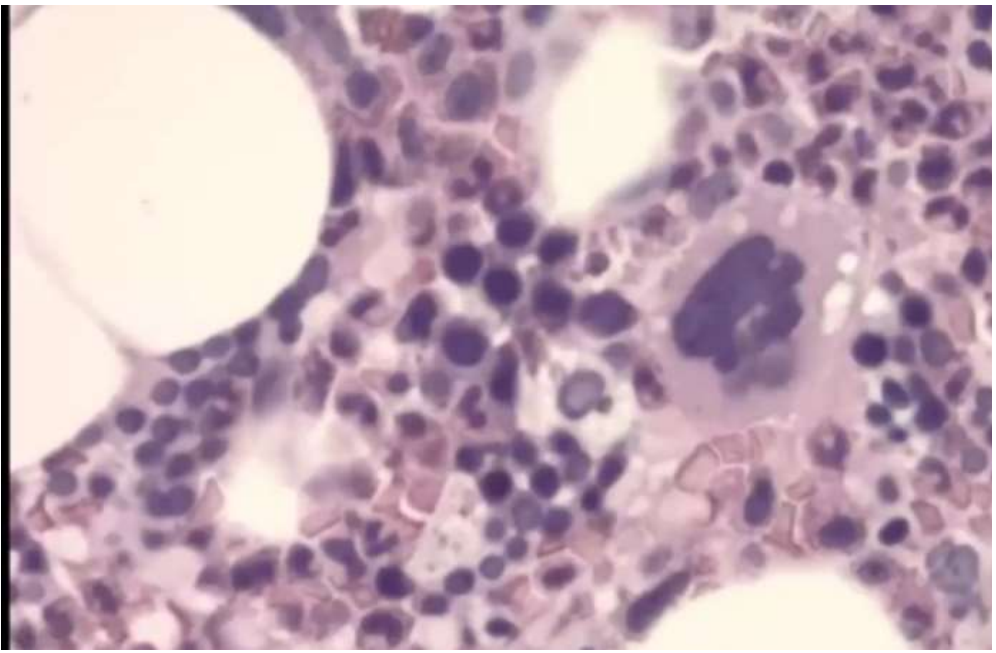
Signs of dysplasia in erythroid : bi nucleation, nuclear irregularities

M:E ratio

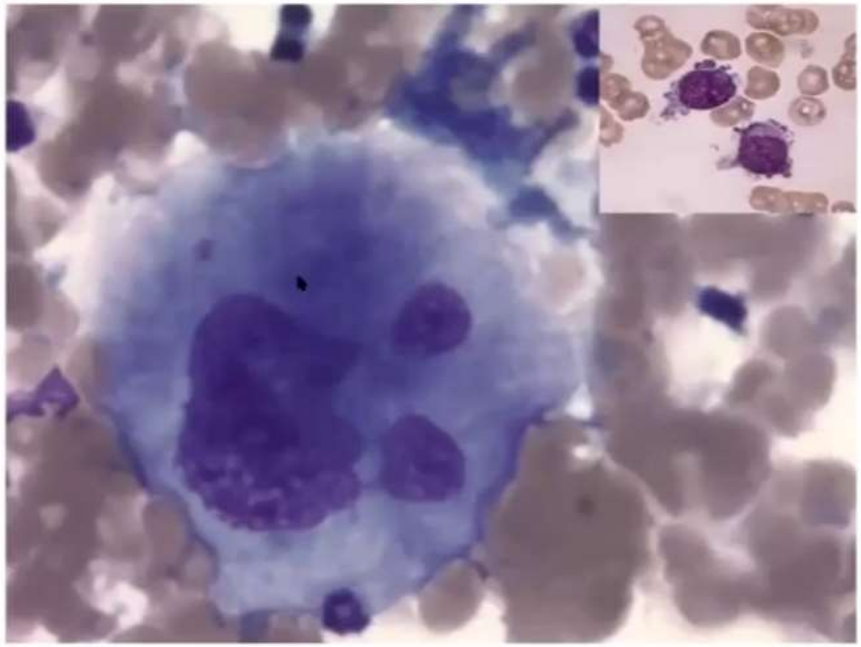
- The M:E ratio is the ratio of all granulocytic plus monocytic cells (Myeloid) to all erythroblasts (Erythroid).
- **Normally M\E = 2-3\1**
- For all bone marrow aspirates examined, the report should specify the M:E ratio and the percentage of lymphocytes and plasma cells.
- A differential count of 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.

Megakaryocyte :

largest cell in BM biopsy ,usually located next to the capillary ,lobulated nuclease and abundant pink cytoplasm, if cytoplasm is blue mean immature or degenerated megakaryocyte



Megakaryocyte

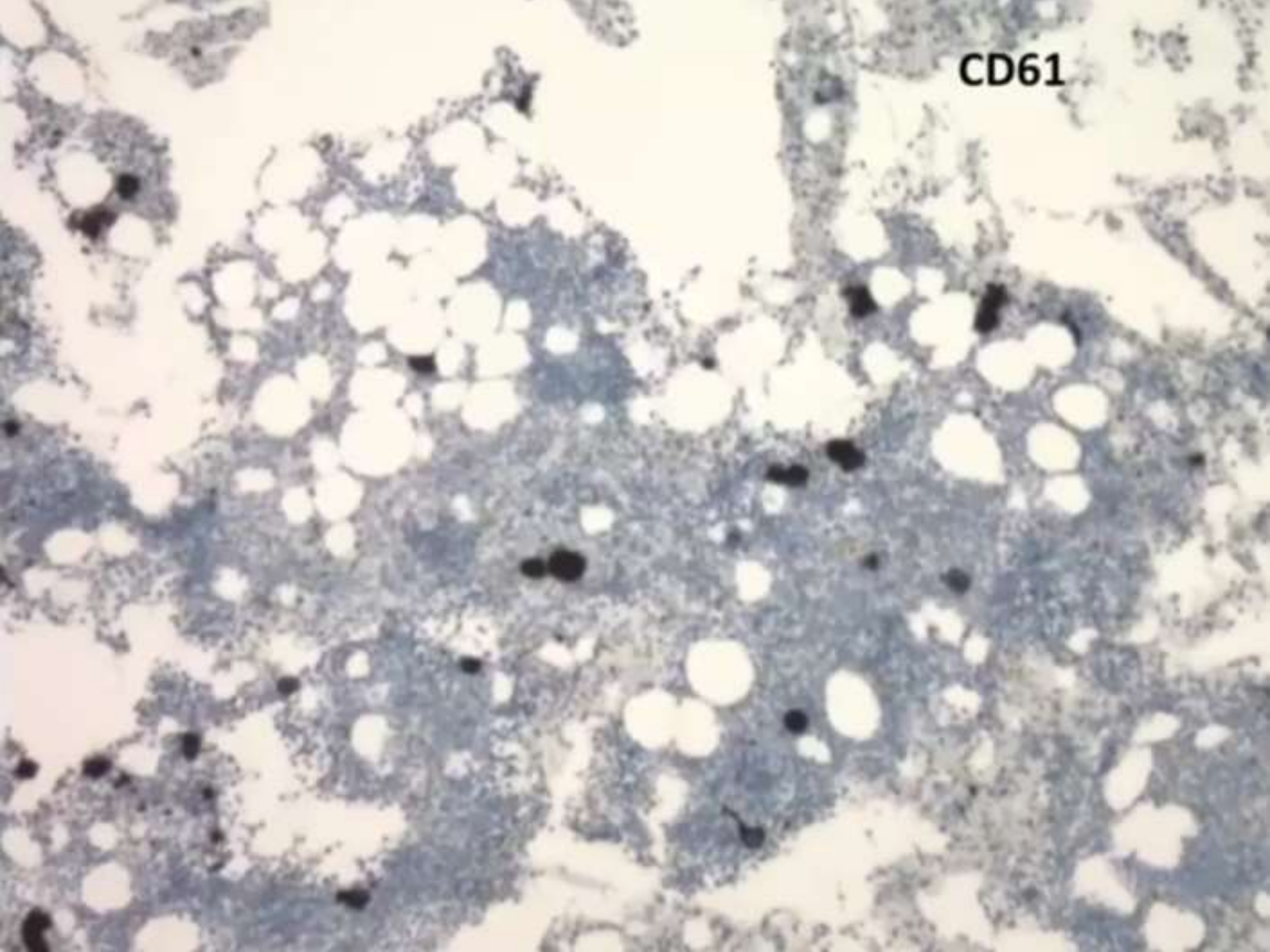


Megakaryoblast showing cytoplasmic blebs

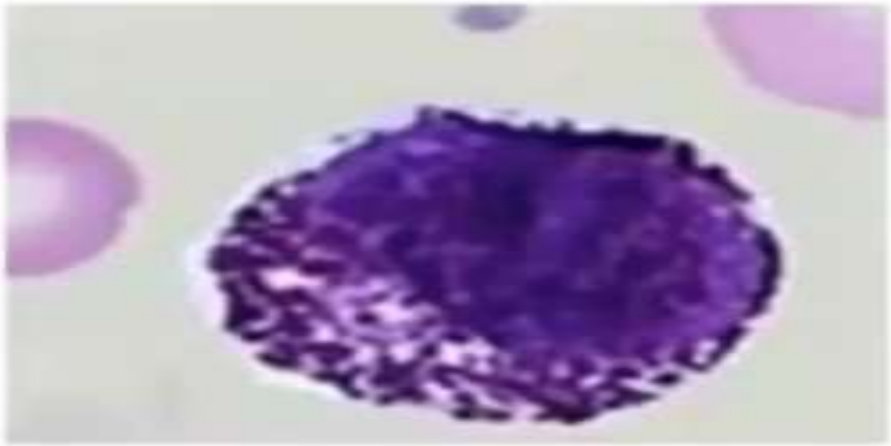
Signs of dysplasia in megakaryocytes : hypo lobulation of nuclei , clustering

Markers of megakaryocytes:

- CD61
- CD42b
- CD41
- CD31
- PAS(non specific)


CD61

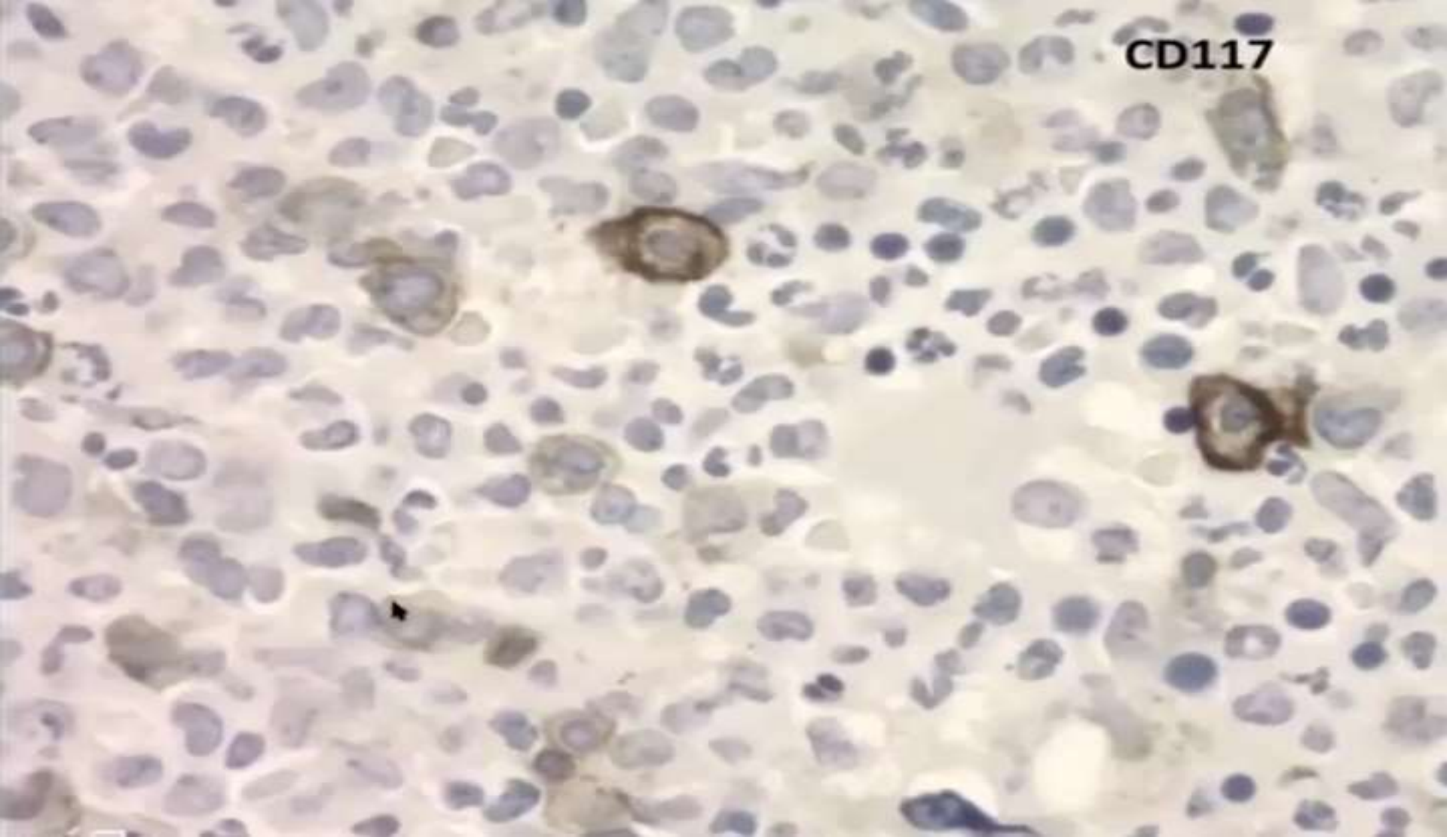
Mast cells



Markers of mast cells

CD117

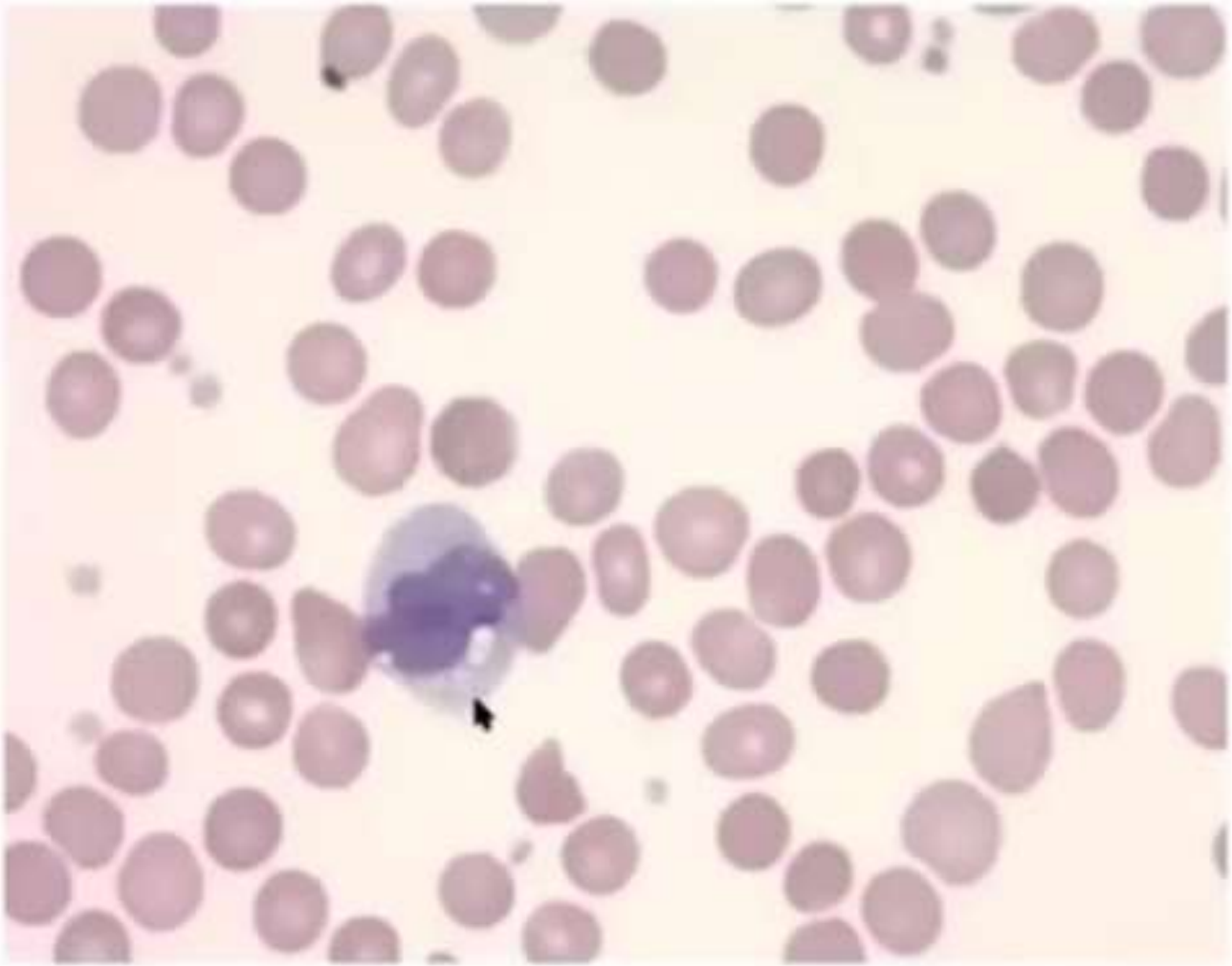
Tryptase



CD117 stain also myeloid

Monocytes

Monoblasts → promonocyte → monocyte

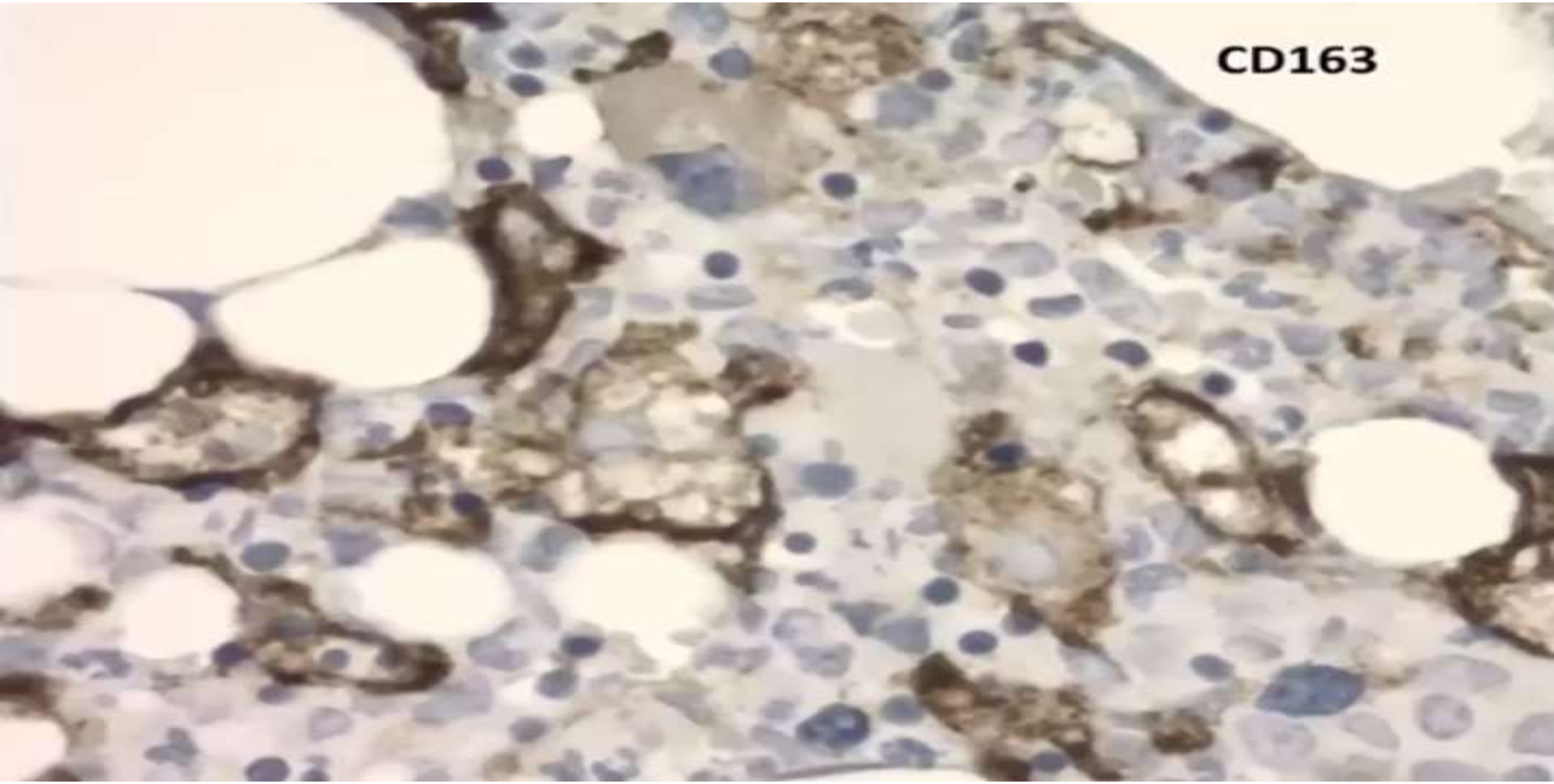


Markers for monocytes

- CD14
- CD13
- CD33
- CD4
- HLADR

Macrophages

- Increased in BM biopsy after chemotherapy.
- Markers : CD68, CD163.



Lymphoid elements

- T>B
- Lymphoid aggregate: normal increased with age.

Common HL lymphoma involving bone marrow

Incidence of bone marrow involvement 2to 30 %.

1- Follicular lymphoma: paratrabicular.

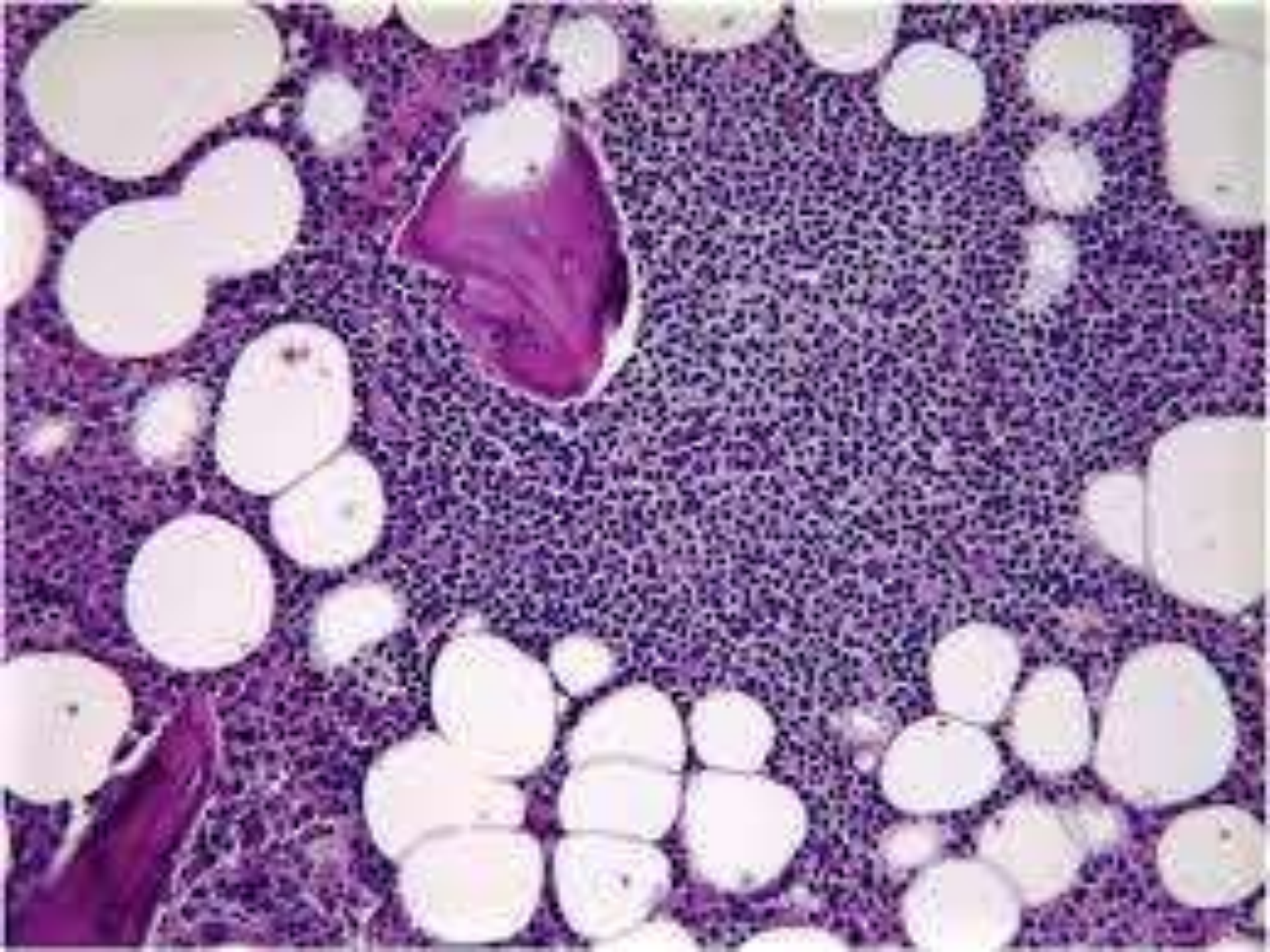
2- DLBCL: diffuse.

3- Mantle cell lymphoma: nodular.

4- Lymphoplastic lymphoma :intestinal.

CRITERIA FOR BM INVOLVEMENT

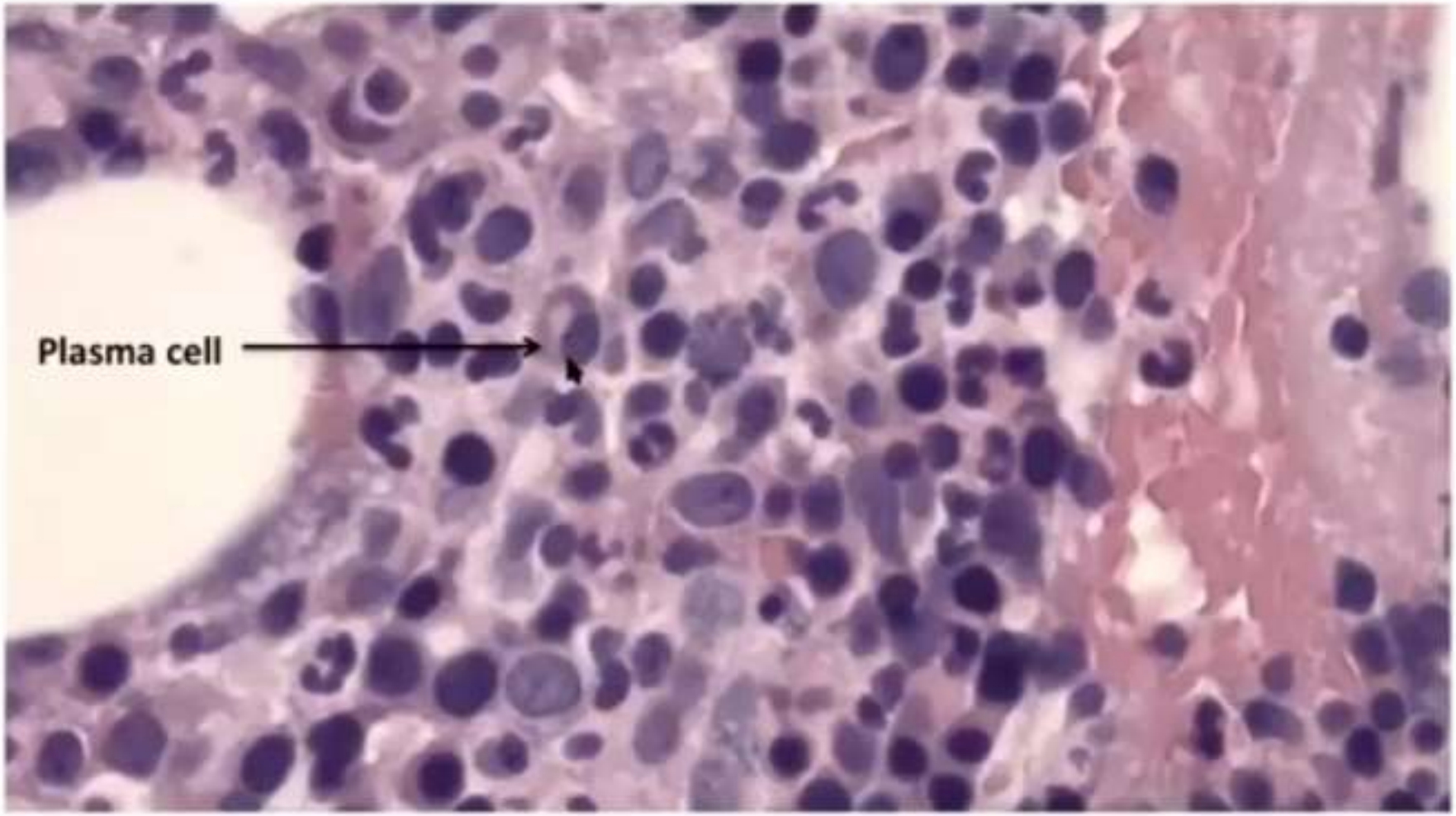
CERTAIN	TYPICAL RS CELLS OR MONONUCLEAR VARIANTS IN CHARACTERISTIC CELLULAR ENVIRONMENT
SUGGESTIVE	ATYPICAL HISTIOCYTE OR CHARACTERISTIC CELLULAR BACK GROUND
SUSPICIOUS	FIBROSIS / NECROSIS ALONE .

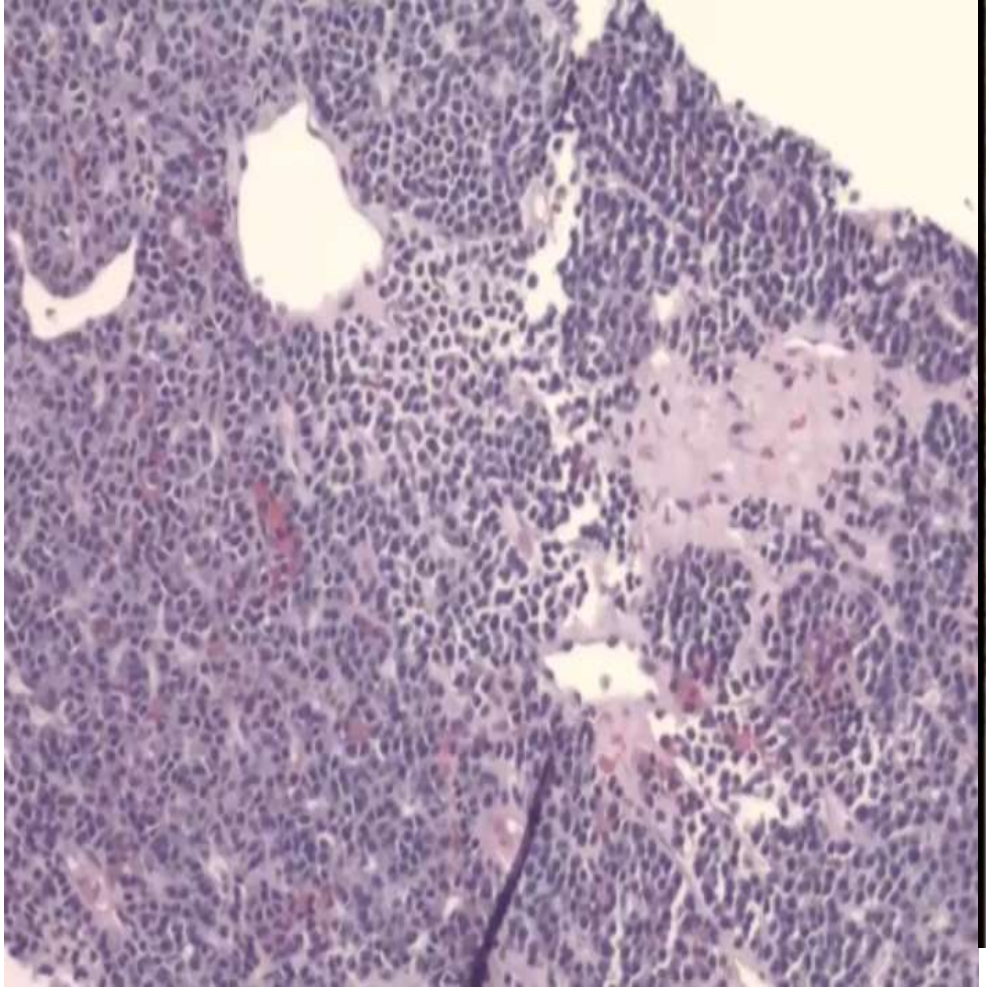


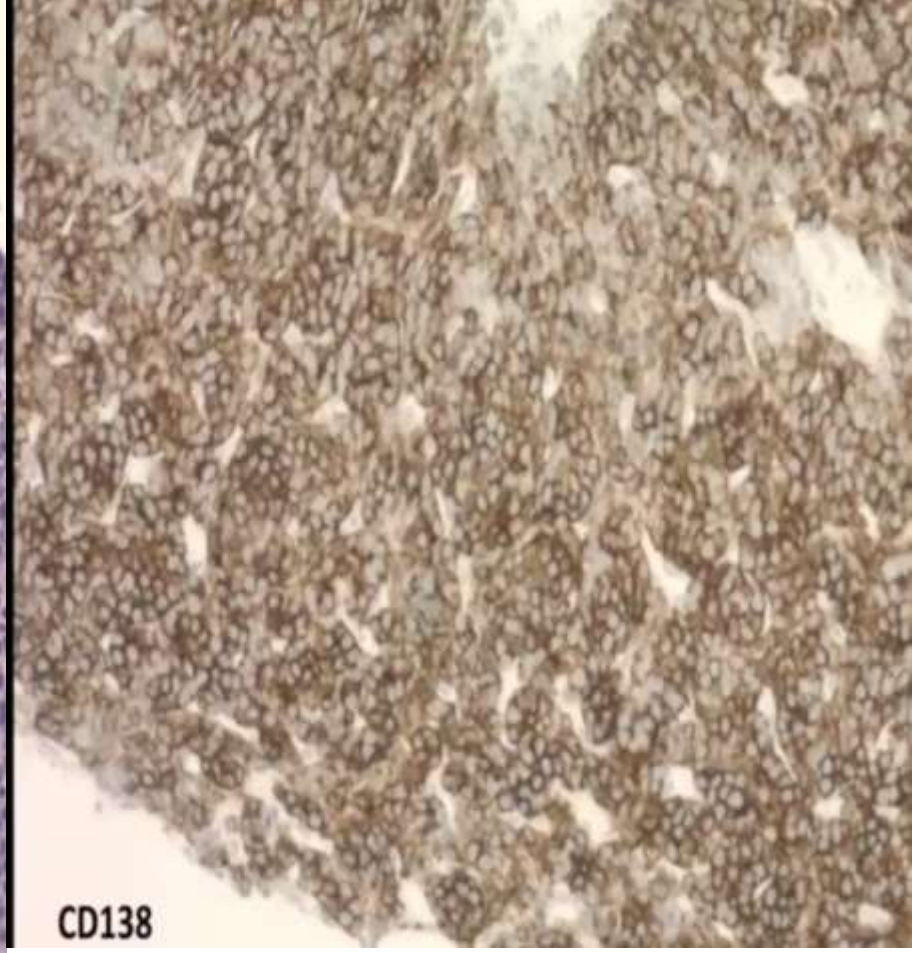
BONE MARROW IN NHL

- Affect staging
- Marrow involvement is more frequent in low grade NHL
- Less common in high grade NHL – poor prognosis .
- Predictor of high risk for CNS involvement

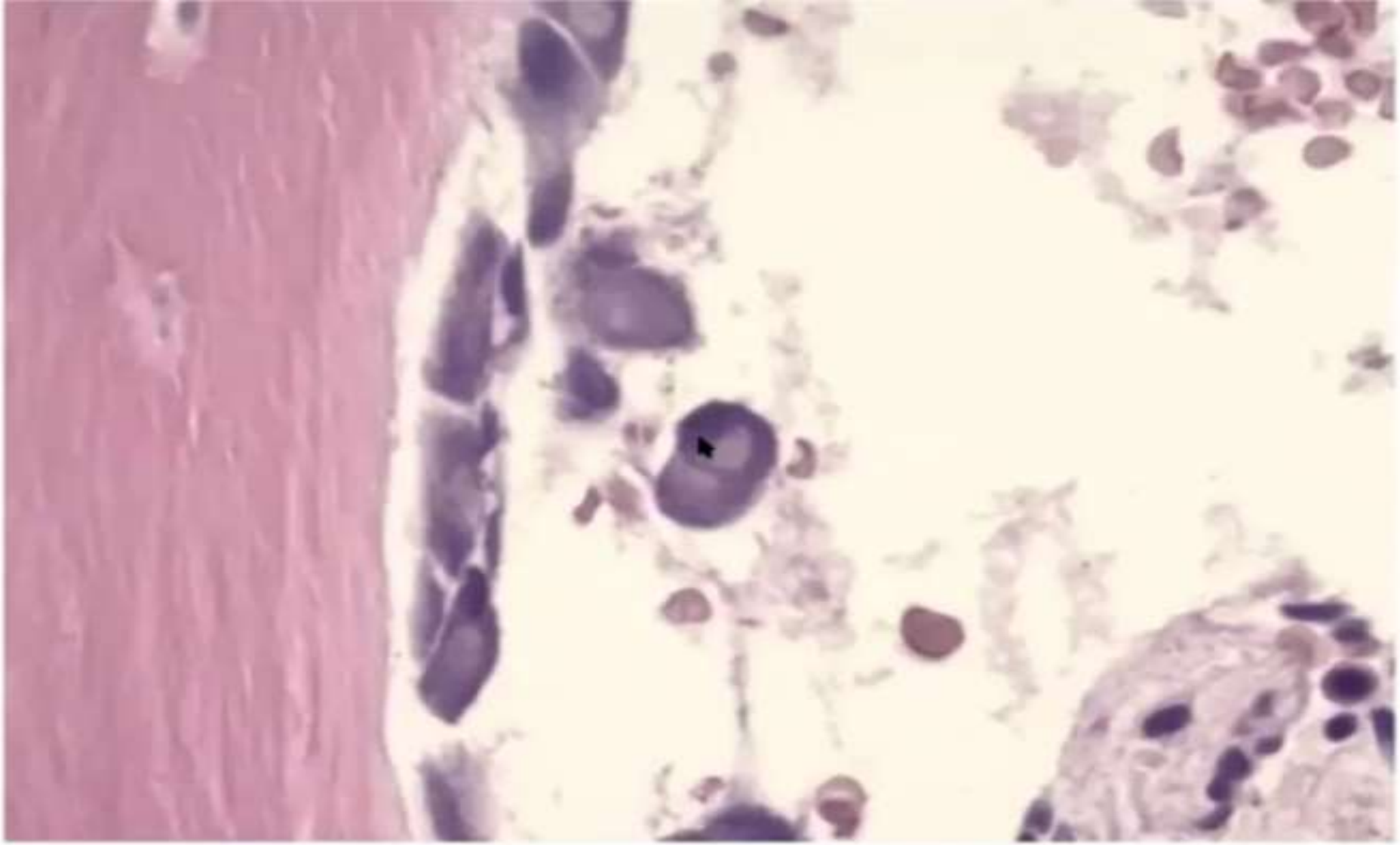
Plasma cells







In plasma cell myeloma plasma cell become larger with nucleolus



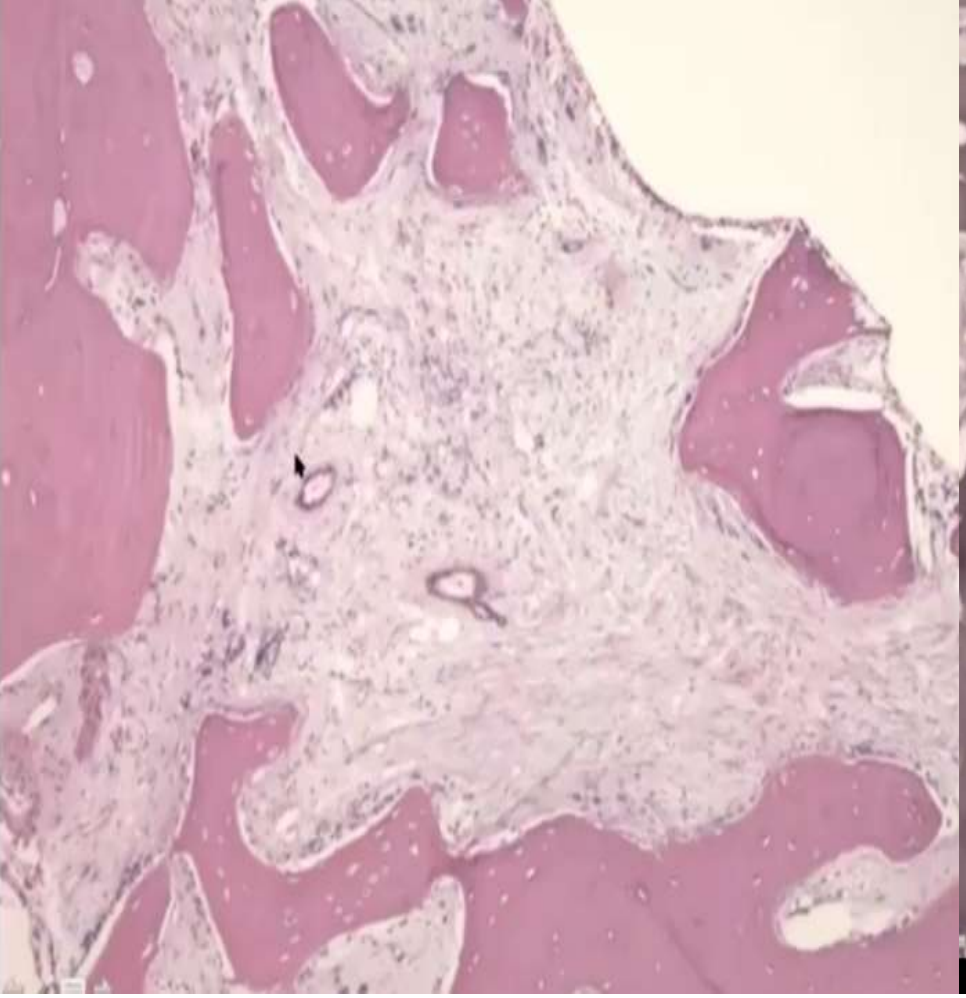
Osteoblast in BM biopsy mistaken with plasma cell

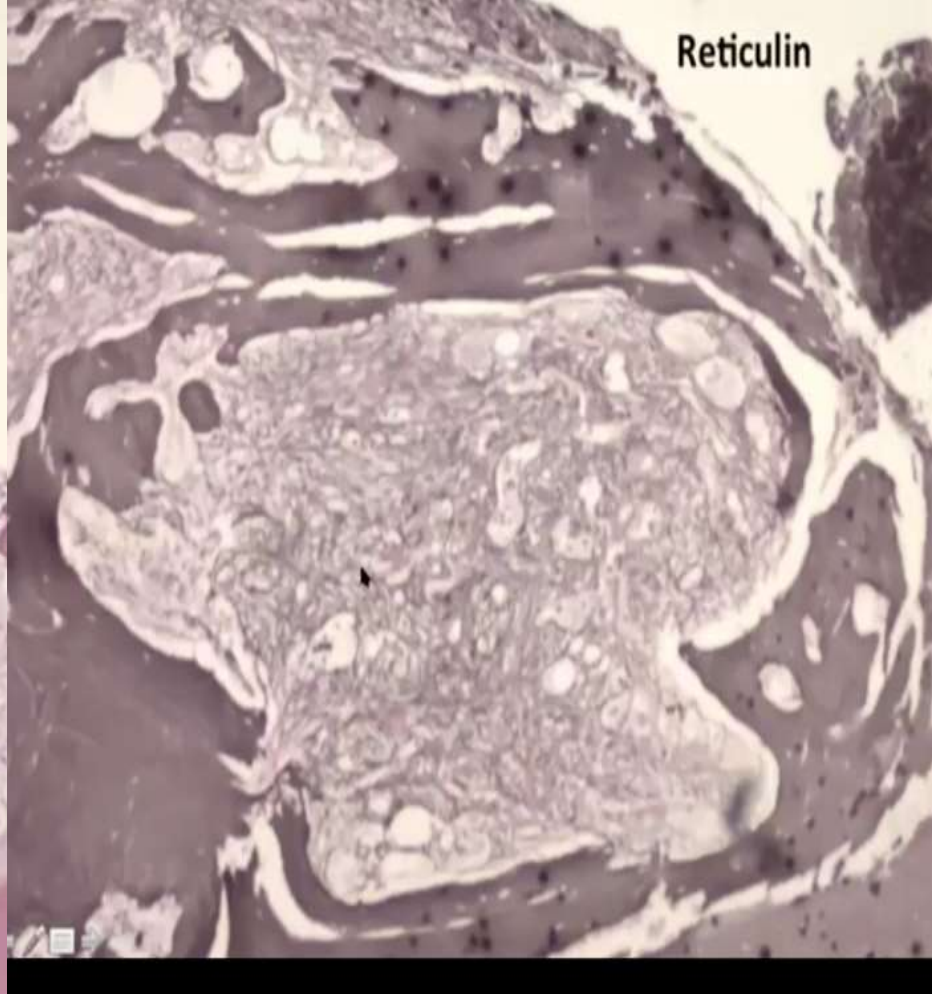
Fibrosis of BM

Bone marrow fibrosis – indicates increase in reticulin or collagen

CAUSES

- idiopathic / primary myelofibrosis
- CML , MDS with fibrosis , Hodgkin deposit in marrow , Hairy cell leukemia , metastatic deposit in marrow
- Reticulin stain ,trichrome stain for collagen (+ve in MF3)
- MF0 (normal),MF1,MF2,MF3





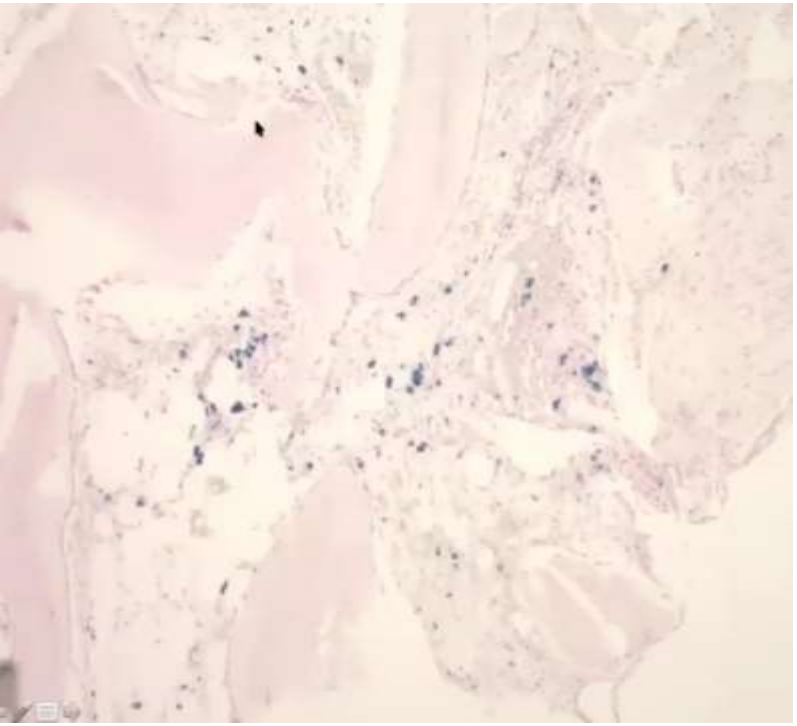
Sever BM fibrosis (MF3) stain with reticulin stain

FIBROSIS GRADING – MODIFIED BAUERMEISTER

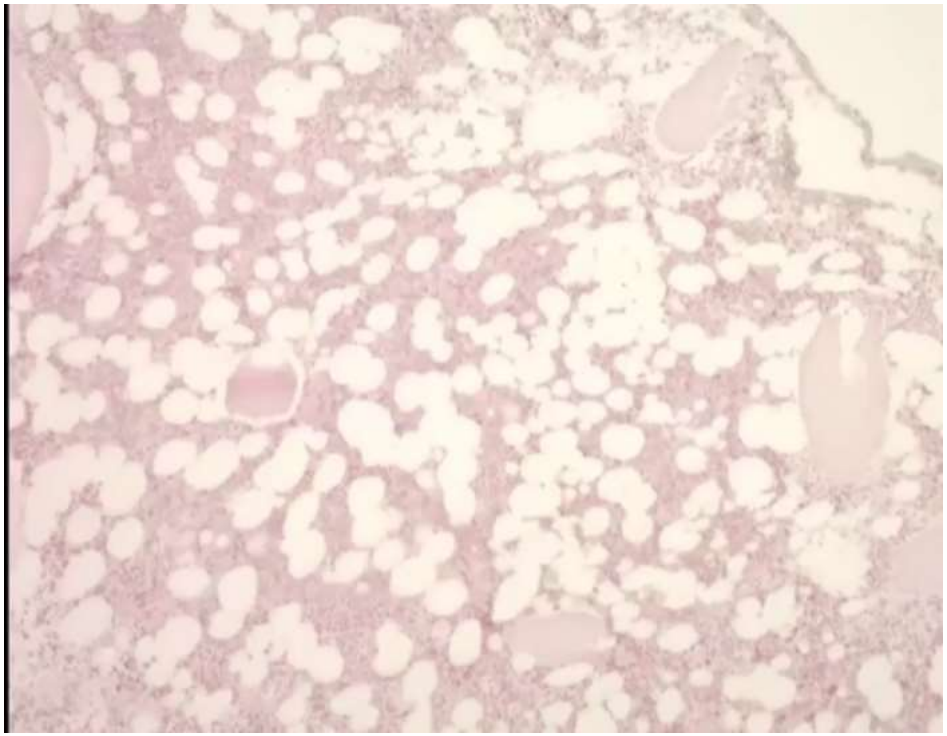
GRADE 0	No reticulin fibres demonstrable
GRADE 1	Occasional fine individual fibres or foci of fine fibre network
GRADE 2	Fine fibre network throughout most of the marrow section , no coarse fibres
GRADE 3	Diffuse fibre network with scattered thick coarse fibres but no more collagen
GRADE 4	Diffuse , often coarse fibre network with areas of collagenisation

Iron stain

- Adequate , increased .decreased.
- Sideroblast which its presence may indicate MDS.
- Ring sideroblast.

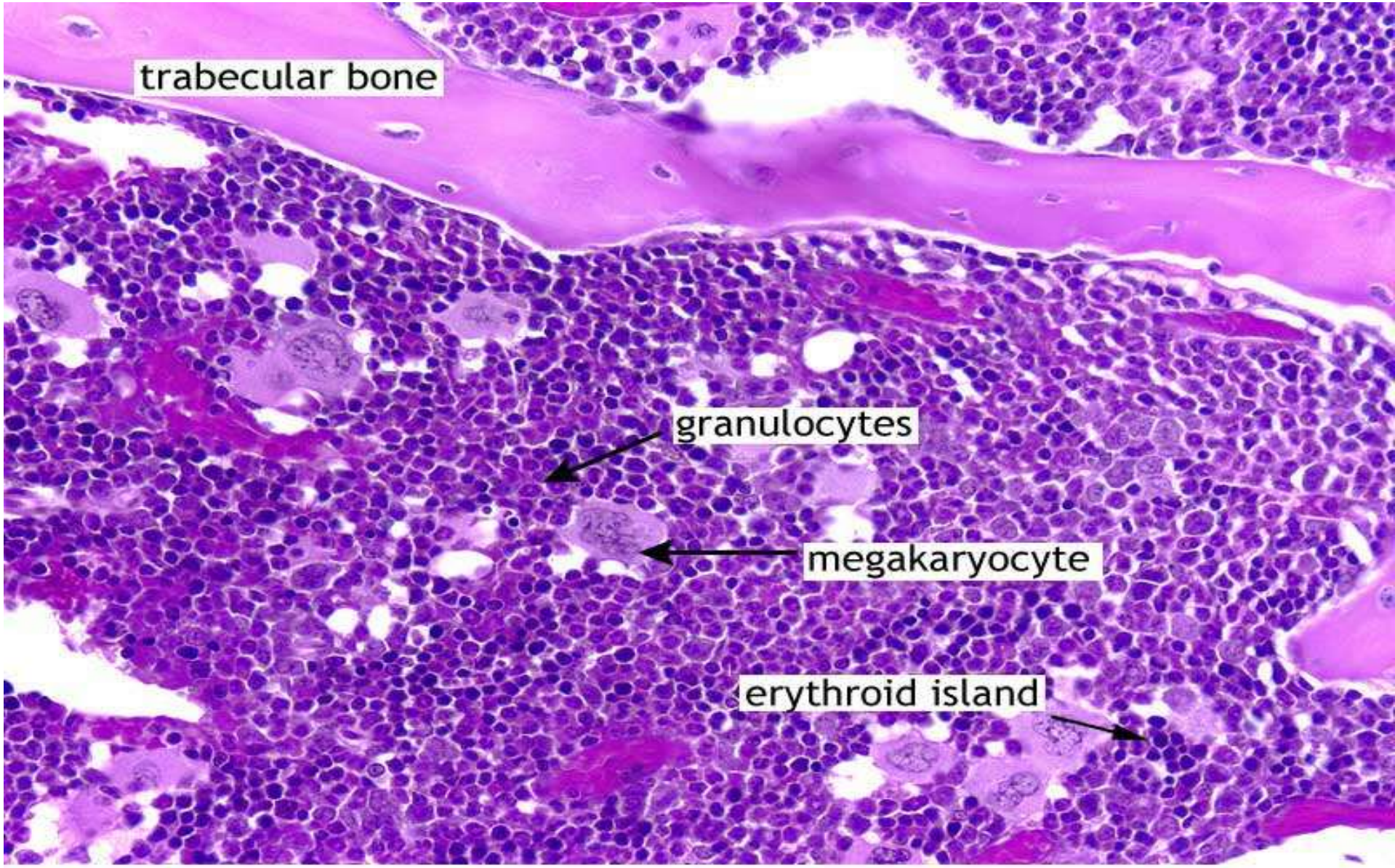


Increased iron

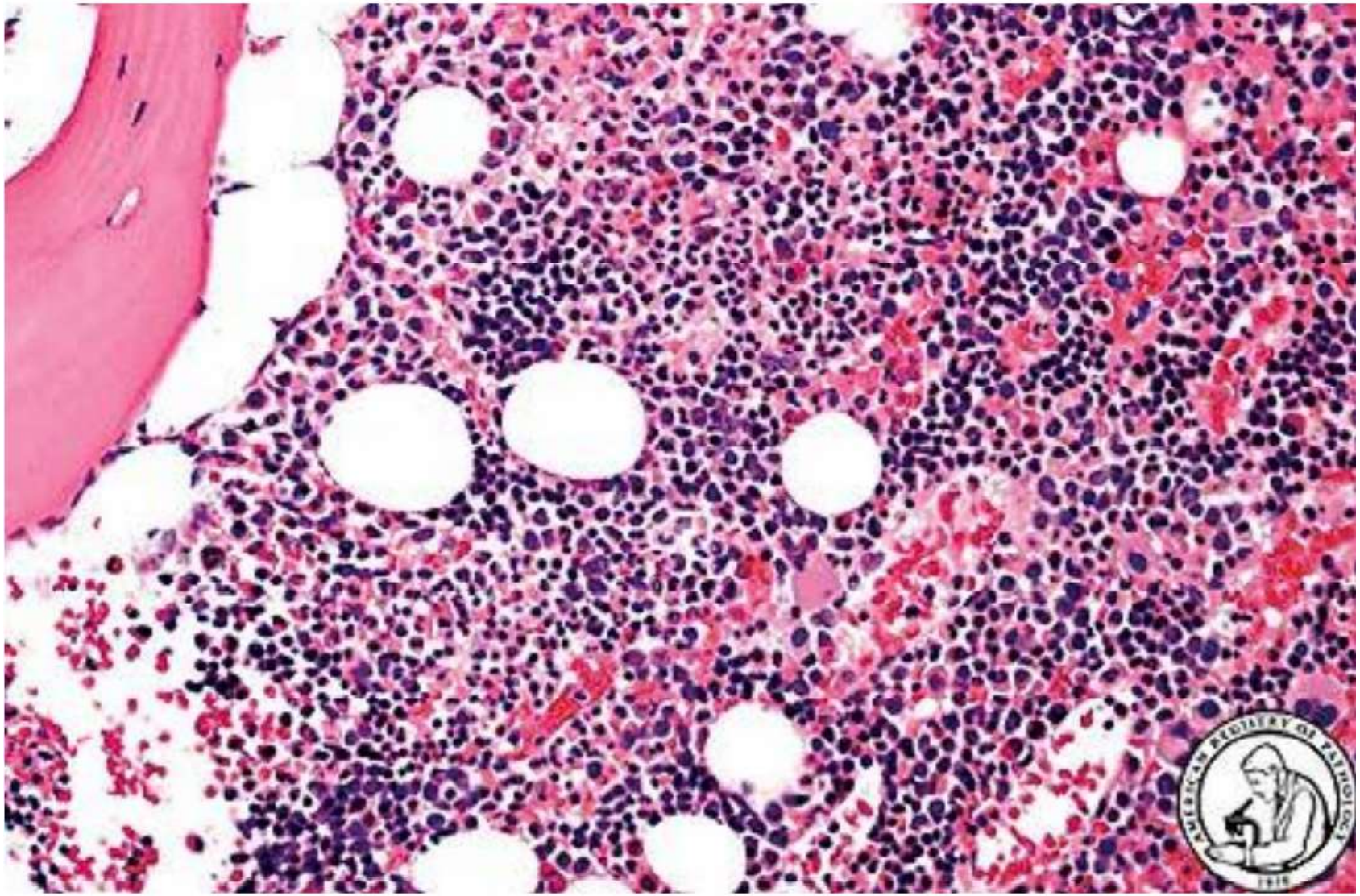


decreased iron

Myeloid VS Erythroid



Erythroid hyperplasia

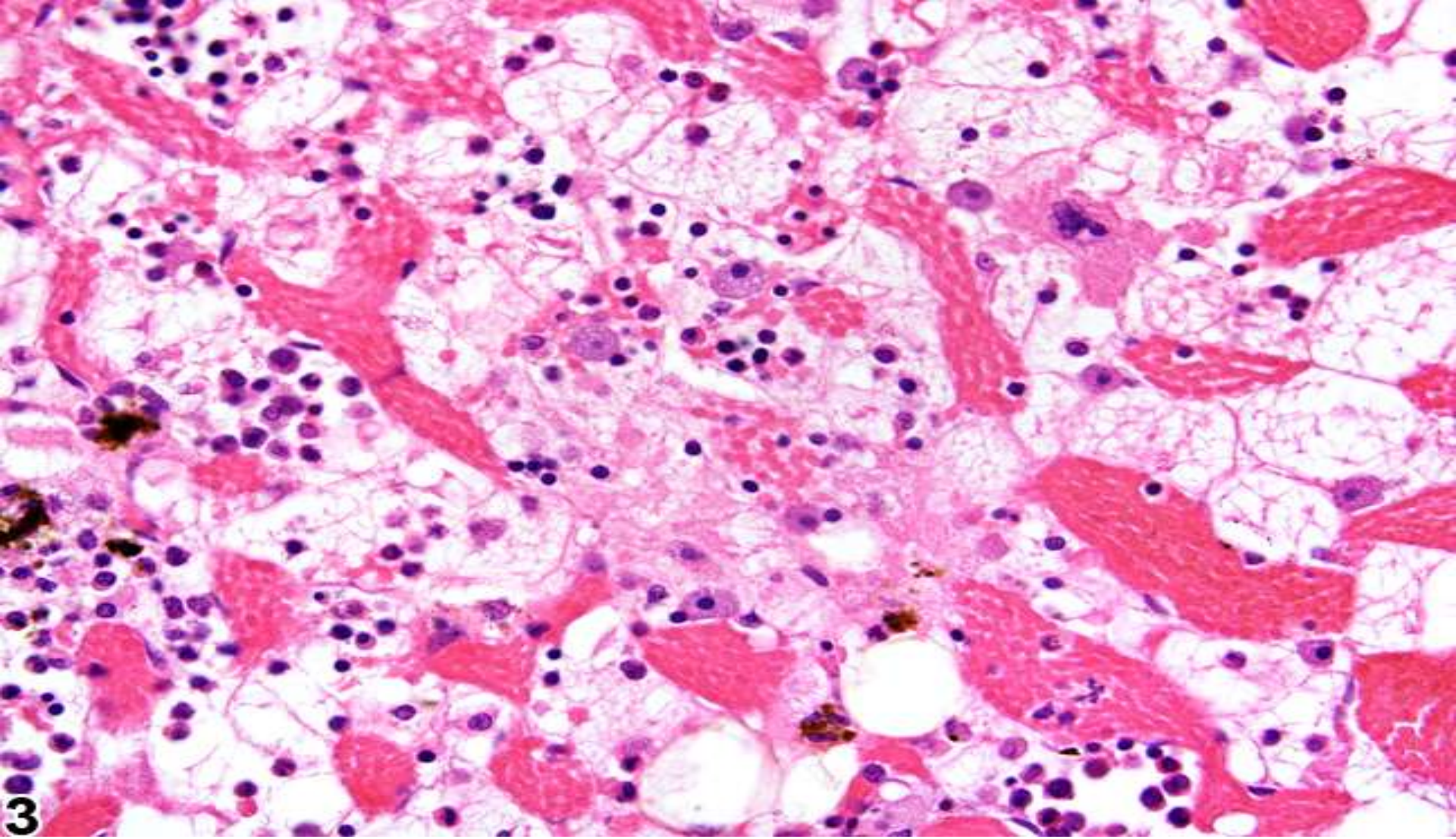


GELATINOUS TRANSFORMATION OF BONE MARROW

- Also known as **STARVATION MARROW**
- Characterized by focal or diffuse extracellular deposition of gelatinous material in between fat cells and hypocellular marrow
- **CAUSES**

  post chemotherapy , malnutrition , anorexia nervosa , HIV , chronic tuberculosis , chronic liver disease .

  acid mucopolysaccharides in the gelatinous material stain with ALCIAN BLUE



Gelatinous transformation of bone marrow .

The marrow is characterized by severe hypo cellularity, atrophied fat cells, and the presence of eosinophilic granular ground substance.

BONE MARROW NECROSIS

- Necrosis of hematopoietic cells or necrosis of neoplastic cells that have replaced normal marrow elements .

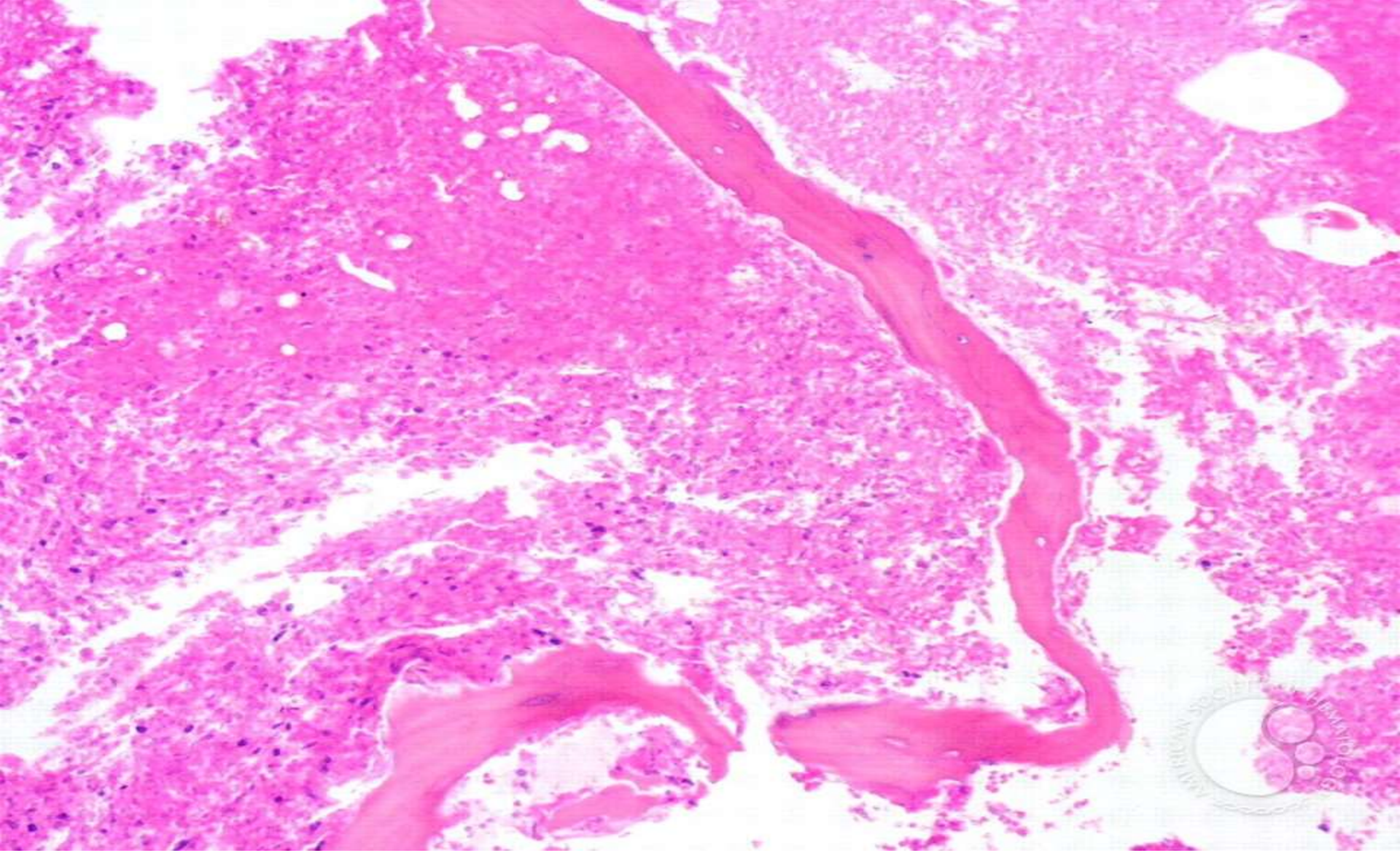
- may be associated with osteonecrosis - absence of osteoblasts lining the trabeculae & osteocytes in the lacunae

- Necrotic areas – anucleate pink ghost cells

- Degree of necrosis variable – focal , moderate or extensive

CAUSES

- acute leukemia (pre / post chemotherapy)
- sickle cell anemia
- CML , NHL , HODGKINS DISEASE
- metastatic deposits



Bone marrow necrosis

APLASTIC ANEMIA

- Progressive pancytopenia , reticulocytopenia

- Bone marrow biopsy < 25 % of normal cellularity of that age.

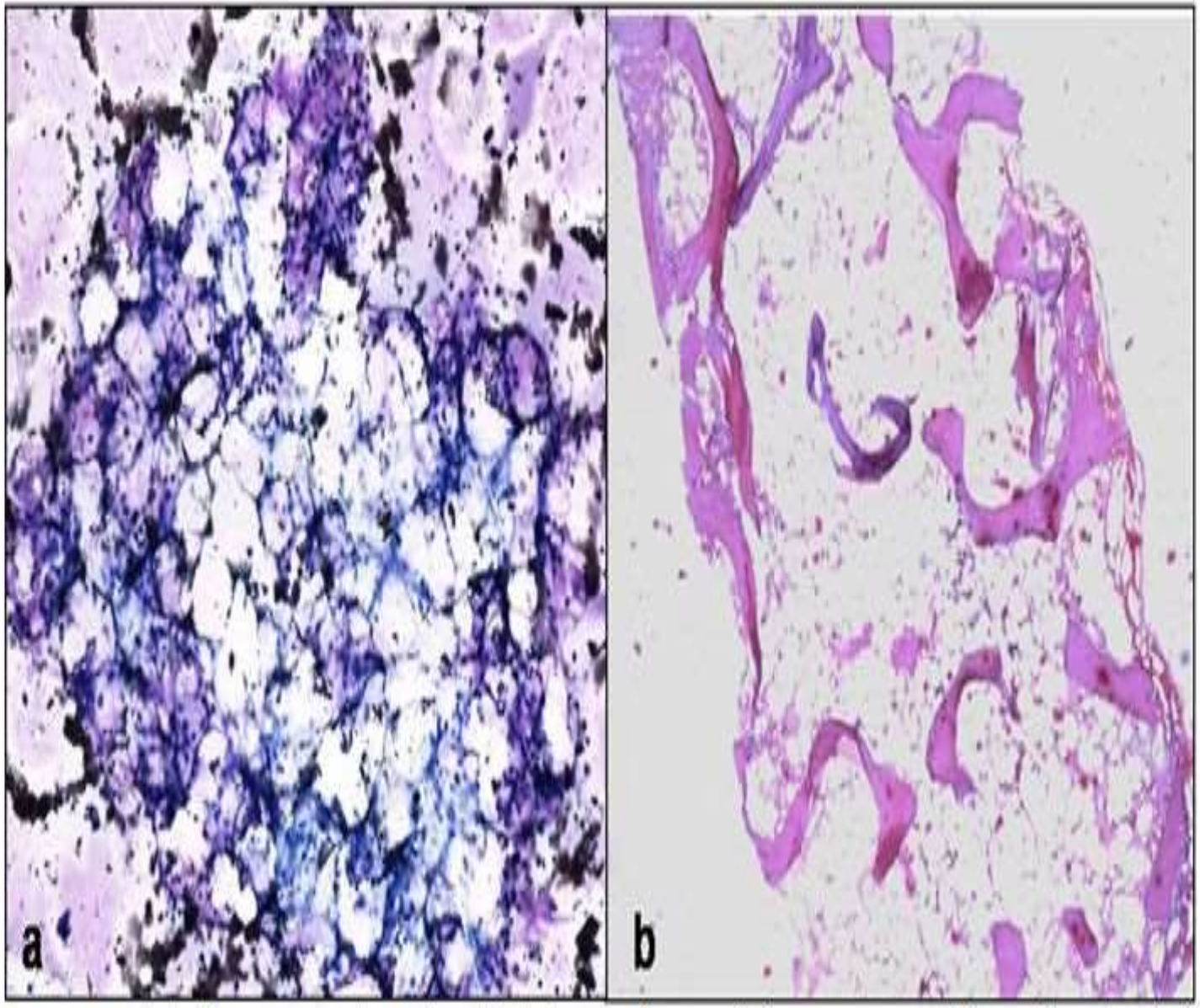
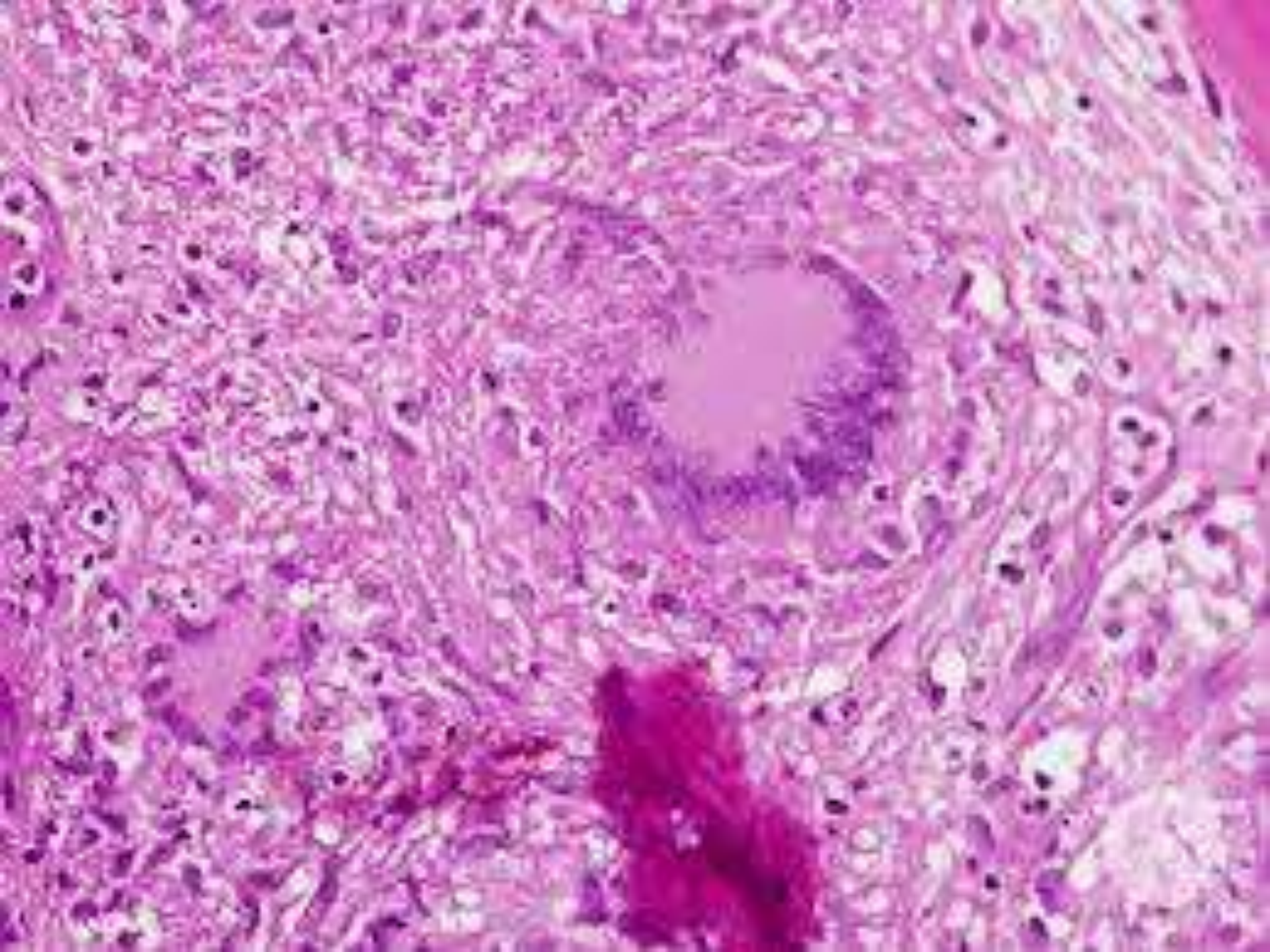


Figure 7. a) Bone marrow aspirate from a pancytopenic patient showing hypocellular marrow particles with entangled lymphocytes and plasma cells and occasional erythroid precursors (Wright stain x 400) b) Biopsy shows markedly hypocellular marrow with increased fat spaces, confirming the diagnosis of aplastic anemia (H&E x 100)

GRANULOMA

- Bacterial - TB , leprosy , syphillis , brucellosis ,
- Fungal – cryptococcosis , histoplasmosis
- Sarcoidosis



METASTASIS

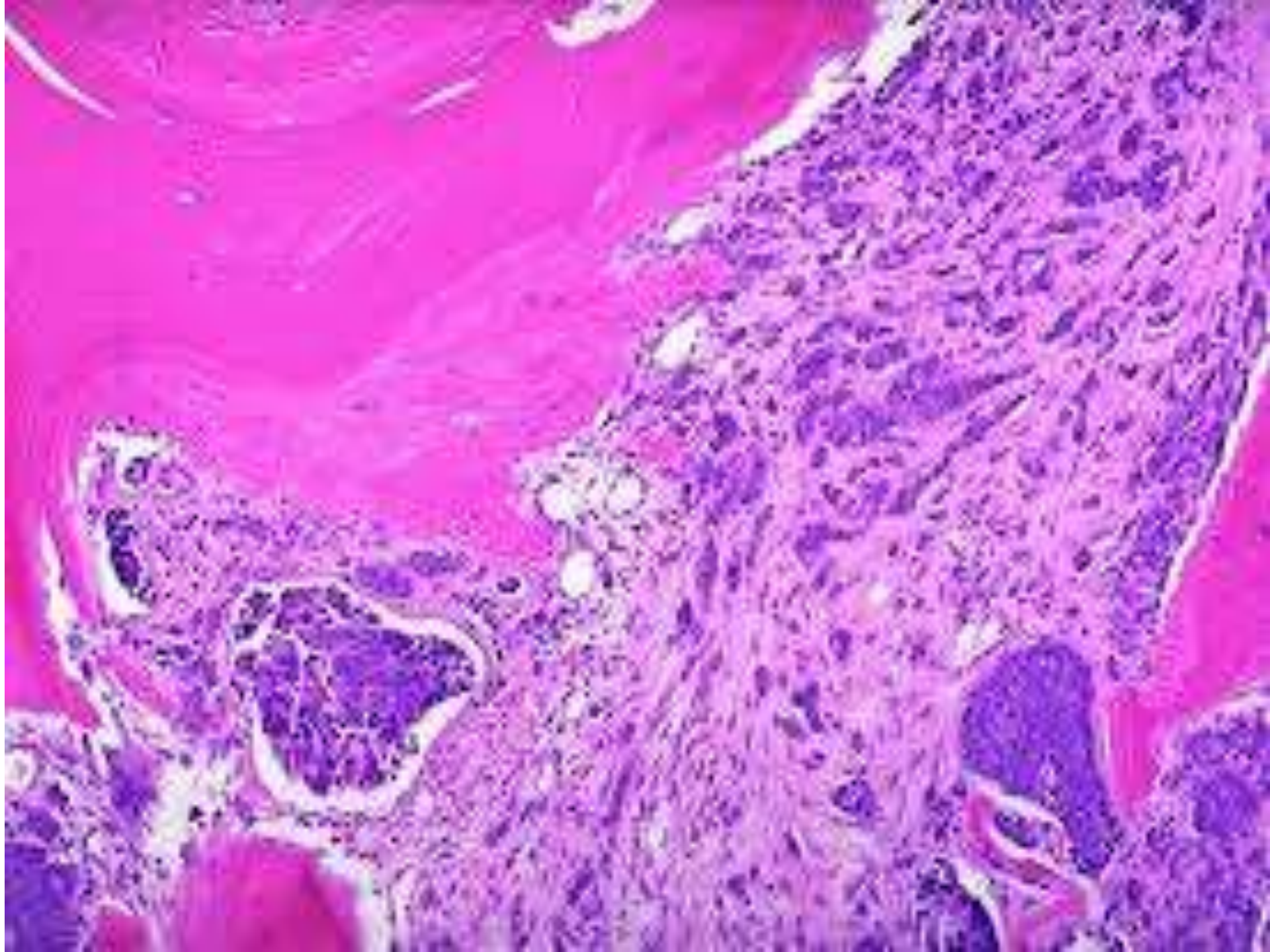
- Carcinoma / lymphoma
- For staging of malignancy
- Evaulate occult malignancy

Adults

ca breast, thyroid , prostate , stomach , kidney , lung .

Children

neuroblastoma , RMS, retinoblastoma , PNET , Ewings sarcoma



THANK YOU