Pediatric Congenital Hematologic
Disorders Research Center
مرکزتحقیقات بیماری های خونی مادرزادی کودکان

Examination of Bone Marrow Aspirates

Aspiration of the BM

- Satisfactory samples can usually be aspirated from the
  - Sternum
  - Anterior or posterior iliac spines
- Aspiration from only one site can give rise to misleading information; this is particularly true in aplastic anaemia as the marrow may be affected partially.

- There is little advantage in aspirating more than 0.3 ml of marrow fluid from a single site for morphological examination as this increases peripheral blood dilution.

Bone marrow aspirate

- A bone marrow film should first be examined macroscopically to make sure that particles or fragments are present.
- Bone marrow aspirates which lack particles may be diluted with peripheral blood and may therefore be unrepresentative. An ideal bone marrow film with particles is shown.

- Even films without fragments are worth examining as useful information may be gained.
- However, assessment of cellularity and megakaryocyte numbers is unreliable and dilution with peripheral blood may lead to lymphocytes and neutrophils being over-represented in the differential count.

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Erythroid series Myeloid series

Megakaryocytic series

Cells of the bone marrow

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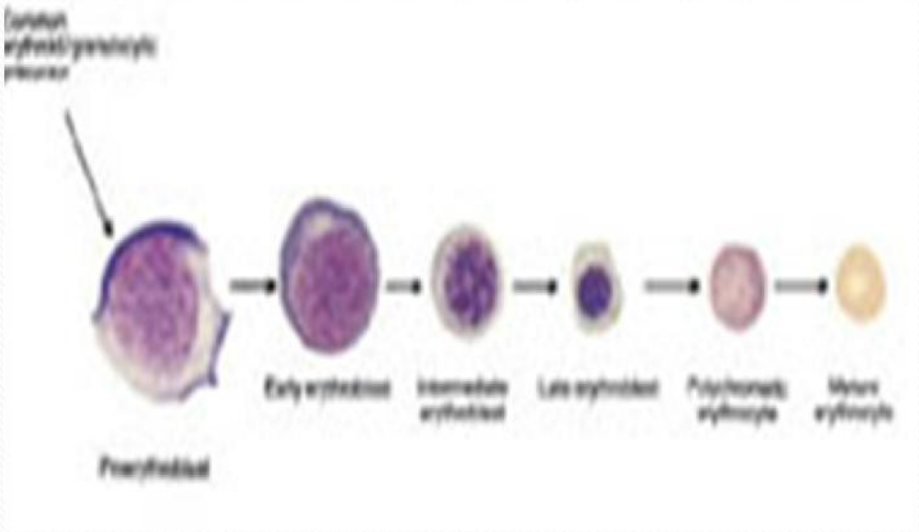
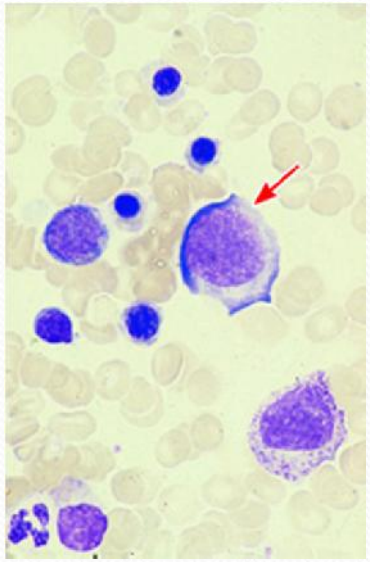
Erythroid series

Erythroid precursors



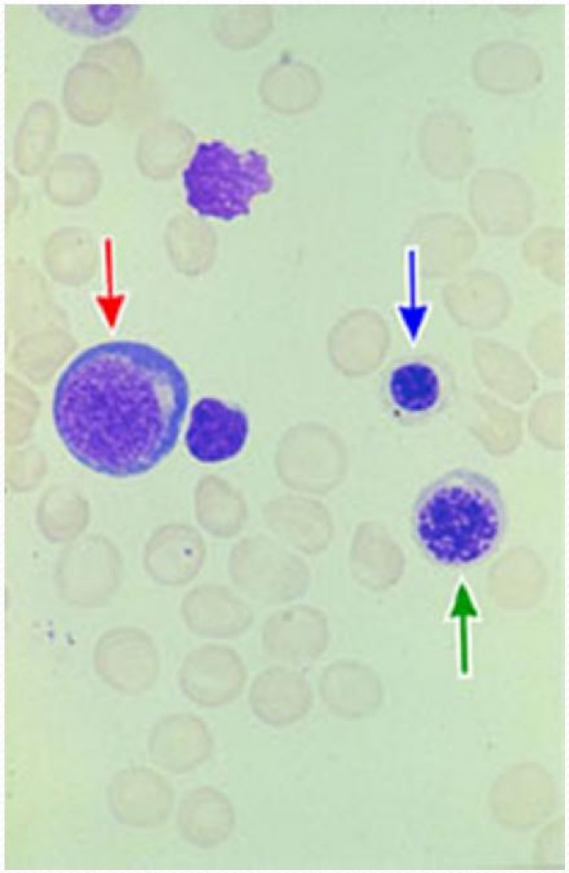
- Normal red cells are produced in the bone marrow from erythroid precursors or erythroblasts.
- The earliest morphologically recognisable red cell precursor is derived from an erythroid progenitor cell which in turn is derived from a multipotent haemopoietic progenitor cell.

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.
- There may be a paler staining area of cytoplasm surrounding the nucleus.

Normal erythroblasts in the BM



- . The early erythroblast [red arrow] is similar to a proerythroblast but is smaller and no longer has visible nucleoli.
- The intermediate erythroblast [green arrow] and the late erythroblast [blue arrow] show a progressive reduction in cell size, reduction in cytoplasmic basophilia and increase in chromatin clumping.
- The cytoplasm of the late erythroblast may have a pink tinge attributable to haemoglobin.

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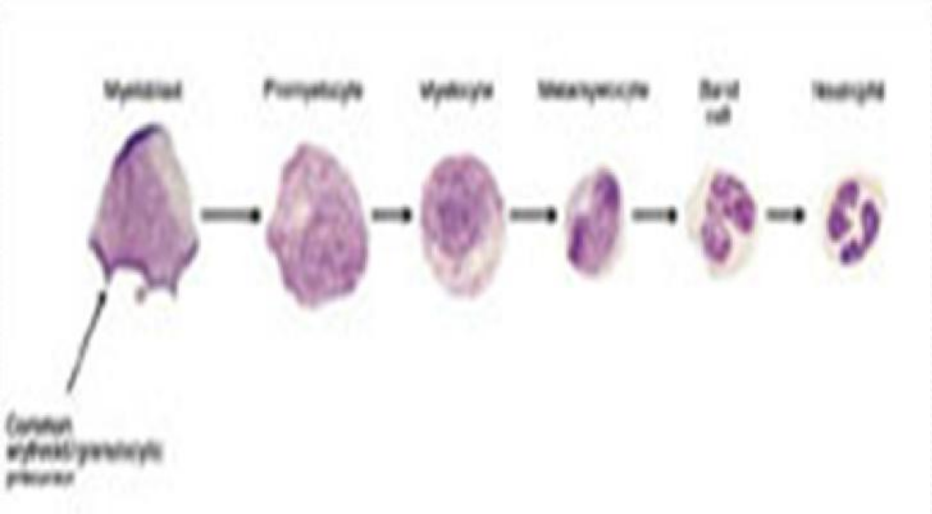
Myeloid series

Myeloid Precursors

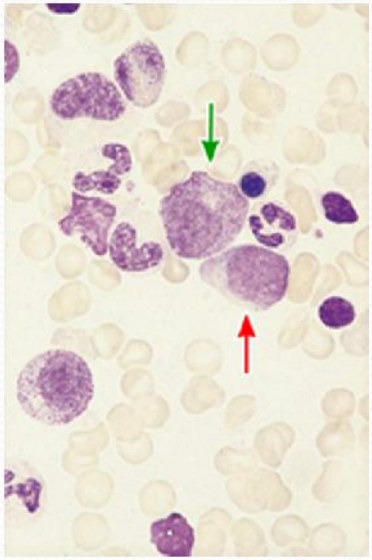


- Myeloblast→ promyelocyte→ myelocyte → metamyelocyte → band form → mature neutrophil.

Myeloid precursors

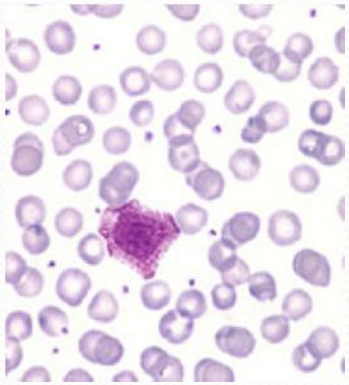
- Normal myeloblasts have no granules but abnormal myeloblasts may have a few granules.
- Myeloblasts undergo one cell division and mature into promyelocytes.
- Promyelocytes have primary or azurophilic granules. They have a Golgi zone a pale area adjacent to the nucleus that is the site of granule production. 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



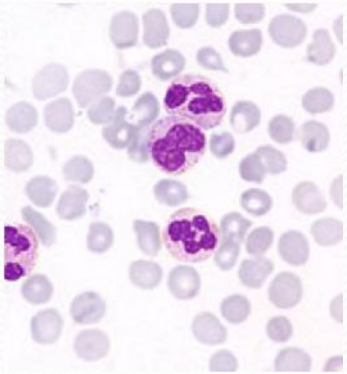
- . Note the myeloblast [dark red arrow] with a high nucleocytoplasmic ratio, diffuse chromatin pattern and nucleolus.
- There is a promyelocyte [green arrow] which is larger and has a lower nucleocytoplasmic ratio and abundant azurophilic granules.

Myelocytes



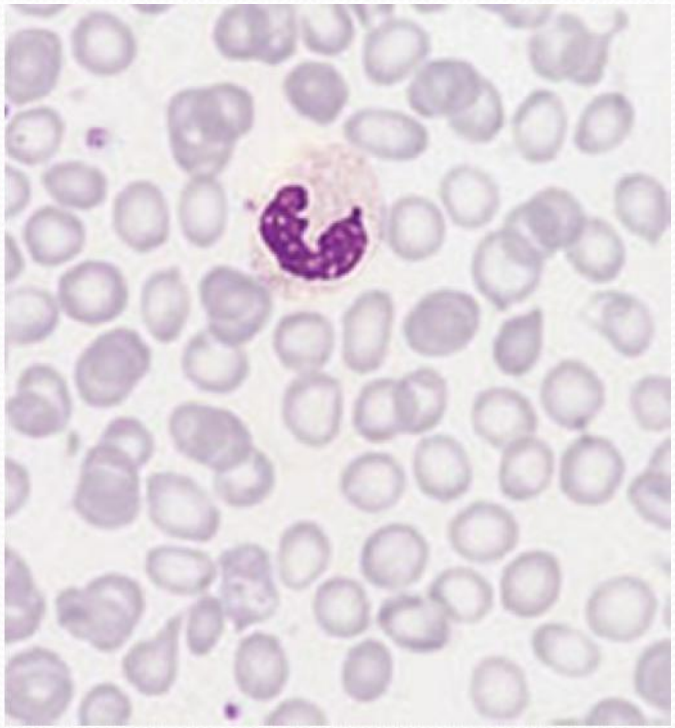
- Myelocytes are smaller than promyelocytes and have specific granules that indicate whether they are of neutrophil, eosinophil or basophil lineage.
- The nucleolus is no longer visible.

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.

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.

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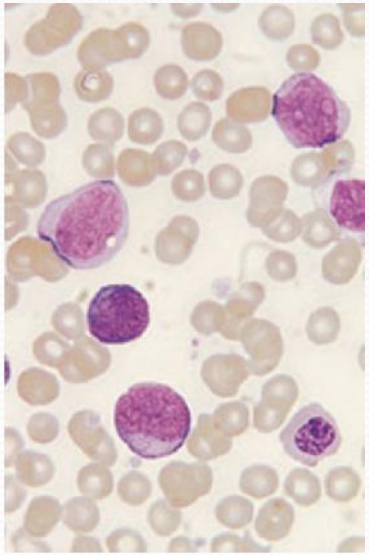
Megakaryocytic series

*Megakaryoblasts

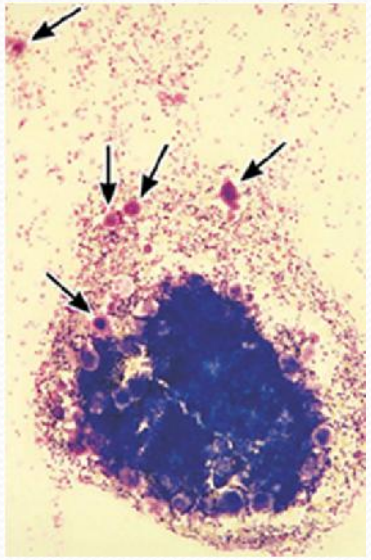
**Megakaryocytes

Megakaryoblasts



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

Megakaryocytes in the BM



- . Most megakaryocytes [arrows] are large cells which can be identified with low power. Their numbers are very variable in normal bone marrow films, being partly related to the number of fragments present.
- This image shows increased megakaryocyte numbers.

Points to be considered in BMA reporting

- 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 should the X100 oil be used to assess cellular detail.

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%.

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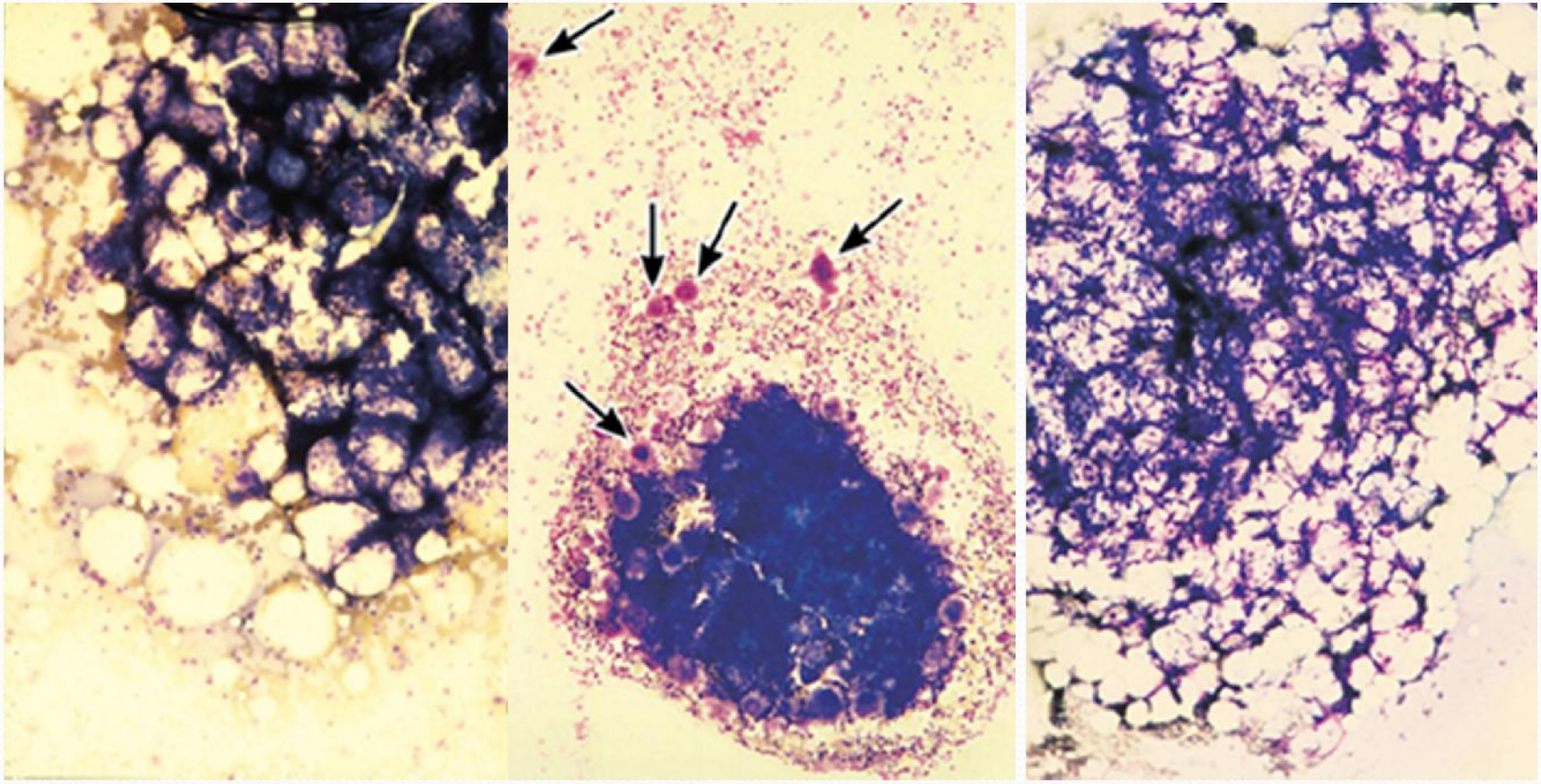
Comparing Normo, Hyper, & Hypocellular Marrows



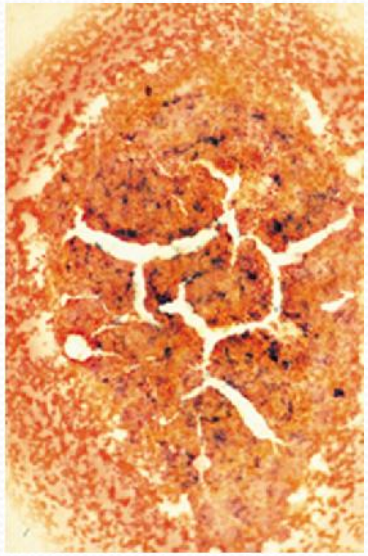
Systemic Scheme For Examining Aspirated BM Films

- 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.

BM iron stores



- Once all normal and abnormal bone marrow cells have been assessed on a routine stain an iron stain should be examined, using a medium power objective (X 40 or X 50). Storage iron, which stains blue, should be assessed in bone marrow fragments. This image shows normal bone marrow iron.

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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.

Normal ranges of bone marrow cells

- M:E ratio 2.4 (1.3-4.6)
- myeloblasts 1.4 (0-3)
- Promyelocytes 7.8 (3.2-12.4)
- Myelocytes 7.6 (1.9-13.3)
- Metamyelocytes 4.1 (2.3-5.9)
- Neutrophils plus band cells 34.2 (23.4-45)
- Eosinophils 2.2 (0.3-4.2)
- Basophils 0.1 (0-0.4)
- Monocytes 1.3 (0-2.6)
- Erythroblasts 25.9 (13.6-38.2)
- Lymphocytes 13.1 (6-20)
- Plasma cells 0.6 (0-1.2)