Bone marrow basics
- Hematopoiesis
- Bone marrow structure
- Obtaining bone marrow
- Interpreting bone marrow
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### Architecture of bone marrow

#### Vascular space
- Central artery -> carry food $O_2$ to hematopoietic tissue
- Central vein -> carry waste product, hematopoietic cell to circulation

#### Cellular compartment
- Hematopoietic tissue
- Reticular cells: reticular fiber for hematopoietic cell attachment
- Adipose cell (fat cells)
Architecture of bone marrow

Compact Bone & Spongy (Cancellous Bone)

- Lacunae containing osteocytes
- Lamellae
- Canaliculi
- Osteon
- Haversian canal
- Volkmann’s canal

Bone Marrow

- Volume: 30-50 mL/Kg of body weight
- Composition (Red : Yellow)
  - Hemopoietic compartment (Hemopoietic cell)
  - Non-hemopoietic compartment
    - stromal cell
    - vascular nerves
    - reticulum
    - fat cell
- Adult (Cell : Fat cell) ~ 1:1
Bone marrow

Stromal
- Indirectly involved in hematopoiesis.
- Provide the hematopoietic environment
  - by parenchymal cell,
    - fibroblast (reticular connective tissue)
    - macrophage
    - adipocyte
    - osteoblast
    - blood vessels (sinusoid)

Bone marrow

Marrow barrier
- Inhibit the immature cell leaving from the bone marrow.
- Mature cell have the membrane protein for endothelial cell attachment.

Bone marrow

Stem cell
- Multipotent stem cell.

Type of the stem cell
- Hematopoietic stem cell
- Mesenchymal stem cell
- Endothelial stem cell
1. Red marrow
   : cellularity (hematopoietic cell/fat cell) = 1:1
   : found in area which active marrow,
   : particularly in children marrow

2. Yellow marrow
   : cellularity < 1:1
   : found in area which decrease (non-active) marrow,
   : particularly in older marrow
Different patterns of Hematopoiesis

A Normal
B Thal
C AA
D MF

Normal Bone Marrow
Bone Marrow Activity

Bone marrow examination

Refers to pathologic analysis from the BM

BM biopsy and aspiration

For diagnosis

- Leukemia
- MM
- Anemia
- Pancytopenia
Objective of Bone Marrow Examination

- **To evaluate hematopoiesis**
- To diagnose malignancy of primary and metastatic origin

- **To determine the cause of infection**
- To evaluate the progression of some hematologic diseases

**To response of the marrow to treatment follow up**

To primary diagnosis of systemic diseases

Miscellaneous

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To evaluate hematopoiesis

1. Primary diagnosis of hematolympoid malignancies
   - Acute leukemias
     - Chronic myeloproliferative disorder
     - Chronic lymphoproliferative disorder
     - Myelodysplastic syndromes
     - Hodgkin and non-Hodgkin lymphomas
     - Multiple myeloma

2. Staging of lymphoid malignancies and solid tumors

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To determine the cause of infection

- Mycobacterium and fungal infections
- Granulomas
- Unknown infection agents using culture and special stains
- Hemophagocytic syndrome
**Objective of Bone Marrow Examination**

- Follow up to treatment
- Post chemotherapy and radiation
- Post bone marrow transplant

**Indication for Bone Marrow Studies**

1. Unexplained anemia/erythocytosis
2. Unexplained leukocytosis/leukocytopenia
3. Appearance of immature or abnormal cell in circulation
4. Unexplained thrombocytosis/thrombocytopenia
5. Leukemia
6. Infiltration disease; Cancer metastasis, TB
7. Fever unknown origin (FUO)

**Contraindication for Bone Marrow Studies**

Coagulopathy
- Factor activity > 50%

Thrombocytopenia

Skin infection or recent radiotherapy at sampling sites

Bone marrow disorders
- Osteomyelitis
- Osteogenesis imperfecta

**Obtaining Bone Marrow**

- Sternum
- Tibia
- Posterior iliac crest
- Anterior iliac crest
Equipments

Preparation Marrow film

1. Two cover slips
2. Smearing
3. Squashing
Types of marrow specimen

Aspirated sample: the best for
- cytologic morphology
- cytochemical staining

1. Aspirate
   - Cytogenetic
   - Immunologic marker
   - Microbiology
   - Microscopic marrow examination
   - Iron storages
   - Cytochemical stain

2. Biopsy sample: the best for
   - evaluation of marrow cellularity
   - evaluation marrow involvement by the proliferative cells
   - evaluation marrow structure

Bone Marrow Specimens
Bone Marrow Specimens

2. Trephine biopsy
   - Imprint (touch prep)
   - microscopic marrow examination
   - Histochemical & Immunologic stain
Bone Marrow Examination

1. Cellularity
2. Differential cell count
3. Myeloid:Erythroid ratio (M:E ratio)
4. Iron accumulation

Bone Marrow Cellularity

Marrow Hypoplasia

Marrow Hyperplasia

Bone Marrow Examination

1. Cellularity
   Cellularity is a hematopoietic cell/fat cell ratio
   : Normal cellularity = 1
   : Cellularity > 1 refer as “Marrow Hyperplasia”
   : Cellularity < 1 refer as “Marrow Hypoplasia”
   The mostly evaluated from biopsy specimen

2. Differential cell count
   - 500-1000 cells had been counted
Normal Bone Marrow

Granulocytic series (65%) (M)
Erythrocytic series (20%) (E)
Lymphocyte (10%)
Others (5%)

Myeloid : Erythroid = 2:1-4:1

M:E Ratio

- Megakaryocyte
- R.E. Cell (Histiocyte)
- Monocyte, Plasma cell
- Mitotic cell
  - Ostioblast, Ostiocyte
  - Tissue eosinophil, Mast cell
  - Recticulin fiber, Fat cells
3. Myeloid/Erythroid ratio (M/E ratio)
   - Normal M:E ratio responsible by age
   - At birth = 3-4.5
   - Up to 1 month 3-4.5
   - Children = 1.5-4
   - Adult = 2-4

4. Iron Stores
   - Storage iron is “hemosiderin”
   - It contained by nucleated erythroid cell
   - Unstained -golden-yellow granules
   - Wright’s stain- brownish-blue granules
   - Prussian blue is the mostly stain for marrow iron storage
   - Iron stores is benefit for evaluation of anemia
Abnormal results

Anemia
- Iron deficiency anemia
- Sideroblastic anemia
- Megaloblastic anemia
- Aplastic anemia

Leukemia

Multiple Myeloma
Hodgkin's disease
Lymphoma

Abnormal results

Metastatic Bone Cancer
Macroglobulinemia
Agammaglobulinemia
Myelofibrosis
Collagen disease
Infection

Aplastic anemia

Leukemia
Tuberculosis infection

**Bone Marrow Iron Stores**

**Grading Iron Stores**

- 0 - No stainable iron
- 1+ - Small intracellular iron stores using oil objective
- 2+ - Small, sparse intracellular iron particles at low power
- 3+ - Numerous small intracellular iron particles
- 4+ - Larger particles with a tendency to aggregate into clumps
- 5+ - Dense, large clumps
- 6+ - Very large clumps and extracellular iron

- Perl's Prussian blue
- Best performed on bone marrow aspirate smears
- Intracellular stores should be evaluated, extracellular stores can be confused with artifact
- Most intracellular iron is in macrophages, a small amount in erythroblasts (sideroblasts)
- Normally 20-50% of erythroblasts are sideroblasts
- Ringed sideroblasts are atypical, with iron in mitochondria forming a ring around nucleus

**Perl's Prussian Blue**
Sideroblastic anemia

Bone Marrow Reticulin

Grading Reticulin Content

- 0: No reticulin fibers
- 1+: Occasional fine individual fibers
- 2+: Fine fiber network throughout section, no coarse fibers
- 3+: Diffuse fiber network with scattered thick coarse fibers, no collagen
- 4+: Diffuse often coarse fiber network with areas of collagenization

- Reticular fibers formed by fibroblasts
- Normally few, primarily perivascular and periendosteal
- Increased in many conditions, may be associated with collagen
- Cause “dry tap” aspirate
- Evaluated by Gordon-Sweet and trichrome stain
- Interpretation must avoid areas of crush artifact and perivascular regions

Bone Marrow Artifacts

<table>
<thead>
<tr>
<th>Bone Marrow Aspirate</th>
<th>Bone Marrow Biopsy</th>
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<tbody>
<tr>
<td>Poor staining</td>
<td>Aspiration artefact</td>
</tr>
<tr>
<td>Inadequate particles</td>
<td>Suboptimal sectioning</td>
</tr>
<tr>
<td>Cell crushing and distortion</td>
<td>Poor staining</td>
</tr>
<tr>
<td>Contaminated stains</td>
<td>Biopsy of previous biopsy site</td>
</tr>
<tr>
<td>Thick smears</td>
<td>Subcortical specimen</td>
</tr>
<tr>
<td>Uneven cell distribution</td>
<td>Crushed specimen</td>
</tr>
<tr>
<td>Clotted specimen</td>
<td>Inadequate fixation</td>
</tr>
<tr>
<td></td>
<td>Excessive decalcification</td>
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Bone Marrow Artifacts

Cytochemical Stains

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<th>Stain</th>
<th>Primary Reactivity</th>
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<tr>
<td>Myeloperoxidase (MPO)</td>
<td>Myeloid primary granule enzyme, best granulocyte marker, relatively unstable, fades</td>
</tr>
<tr>
<td>Sudan black</td>
<td>Lipid in myeloid primary granules, good granulocyte marker, very stable, does not fade</td>
</tr>
<tr>
<td>Naphthol ASD chloroacetate esterase</td>
<td>Myeloid primary granule enzyme, mast cells, less sensitive and specific than MPO</td>
</tr>
<tr>
<td>α-Naphthyl acetate esterase</td>
<td>Enzyme in monocytes/macrophages (fluoride-inhibited), megakaryocytes (fluoride-resistant), some T-cell subsets</td>
</tr>
<tr>
<td>β-Naphthyl butyrate esterase</td>
<td>Enzyme in monocytes/macrophages (diffuse), T lymphocytes (focal, paranuclear)</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>Ubiquitous distribution, tartrate-resistant in HCL (TRAP)</td>
</tr>
<tr>
<td>Periodic acid-Schiff</td>
<td>Glycogen stain, useful in diagnosis of ALL and erythroblastemia</td>
</tr>
<tr>
<td>Giemsa/toluidine blue</td>
<td>Metachromatic stain, mast cells and basophils</td>
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<tr>
<td>Prussian blue</td>
<td>Erythroblast and storage iron, loss during decalcification</td>
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Immunophenotypic Analysis

Cytospin or Tissue Section

Immunohistochemical Stains

Flow Cytometry
Flow Cytometry

- Cells are incubated with fluorochrome labeled MoAbs
- Cells are passed in “single file” through highly focused laser beam
- Different fluorochromes emit light at different wavelengths
- Emitted light analyzed by computer and plotted on a histogram
- Data analysis shows number and immunophenotypic characteristics of the cell population

Cluster Designations

- International Workshops on Human Leukocyte Differentiation Antigens
- Sponsored by World Health Organization
- Hybridoma technology, antibodies shared, common reactivity identified, antigens defined
- 8th Workshop - Adelaide, Australia, 2004
- CD1 - CD247
- General conclusions
  - Complex interrelationships
  - Few lineage-specific antigens

Immunophenotypic Analysis