Bone marrow basics
Red cell diseases
White cell diseases
Other diseases
Bone marrow basics
  Hematopoiesis
  Bone marrow structure
  Obtaining bone marrow
  Interpreting bone marrow

Red cell diseases
White cell diseases
Other diseases
Bone Marrow Pathology

- Bone marrow basics
  - Hematopoiesis
  - Bone marrow structure
  - Obtaining bone marrow
  - Interpreting bone marrow
- Red cell diseases
- White cell diseases
- Other diseases
Bone Marrow Interpretation

- Review patient history, laboratory data, previous specimens
- Examine peripheral blood smear
- Examine bone marrow aspirate
  - Examine at least two films
  - 10x - Cellularity, megakaryocyte #, infiltrates
  - 40-50x - Examine each cell line, search for nonhematopoietic cells
  - 100x - Fine cytologic detail, representative differential count in fragment trails
  - Prussian blue stain for iron content and abnormal sideroblasts
- Examine bone marrow biopsy
  - Examine slides at three levels or more
  - 4x - Evaluate cellularity, megakaryocyte #, bone structure, focal lesions
  - 10x - Evaluate each cell line, bony structure, focal lesions
- Evaluate flow cytometry, immunohistochemical stains etc.
- Assign final diagnosis
**Specimens**
- Peripheral blood smear
- Bone marrow aspirate
- Bone marrow biopsy
- Lymph nodes
- Body fluids
- Other tissues

**Stains**
- Wright-Giemsa
- Hematoxylin and eosin (H&E)
- Perl’s Prussian blue
- Gordon-Sweet reticulin
- Cytochemical stains
- Immunohistochemical stains
Bone Marrow Cellularity

- Aspirated fragments or biopsy specimens
- Determined by patient age, specimen site, and technical factors
- Methods for assessment
  - Subjective estimate
  - Computerized image analysis or histomorphology
- Adequate biopsy required (20-30 mm)
- Aspirate only, evaluate fragments rather than trails

Cellularity of 25-75% is usually normal in patients 20-70 years of age.
Marrow Cellularity with Age

Marrow Cellularity

% Cellularity

Age

Posterior Iliac Crest
Bone Marrow Cellularity
Normal Bone Marrow Composition

- Erythroid Precursors
- Band Neutrophils
- Metamyelocytes
- Promyelocytes
- Myelocytes
- Lymphocytes
- Plasma Cells
- Eosinophils
- Segmented Neutrophils
M:E Ratio

- Best determined in bone marrow aspirate
- Normal ratio = 2:1 to 4:1
- Increased M:E ratio in myeloid hyperplasia or erythroid hypoplasia
- Decreased M:E ratio in myeloid hypoplasia and erythroid hyperplasia

M:E Ratio = \frac{Cells \ in \ myeloid \ series}{Erythroblasts}
Glycophorin-A Stain
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
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>
</tr>
</thead>
<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>
</tr>
<tr>
<td></td>
<td>Inadequate decalcification</td>
</tr>
</tbody>
</table>
Bone Marrow Artifacts
# Cytochemical Stains

<table>
<thead>
<tr>
<th>Stain</th>
<th>Primary Reactivity</th>
</tr>
</thead>
<tbody>
<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>α-Naphthyl butyrate esterase</td>
<td>Enzyme in monocytes/macrophages (diffuse), T lymphocytes (focal, paranuclear)</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>Ubiquitous distribution, tartrare-resistant in HCL (TRAP)</td>
</tr>
<tr>
<td>Periodic acid-Schiff</td>
<td>Glycogen stain, useful in diagnosis of ALL and erythroleukemia</td>
</tr>
<tr>
<td>Giemsa/toluidine blue</td>
<td>Metachromatic stain, mast cells and basophils</td>
</tr>
<tr>
<td>Prussian blue</td>
<td>Erythroblast and storage iron, loss during decalcification</td>
</tr>
</tbody>
</table>
Cytochemical Stains
Immunophenotypic Analysis

- Cytospin or Tissue Section
- Immunohistochemical Stains
- Flow Cytometry
- Single-Cell Suspension
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

- **T-Lymphoblast**
  - CD34
  - CD3
  - TdT
  - CD7

- **T-Lymphocyte**
  - CD4/8
  - CD3
  - CD7

- **B-Lymphoblast**
  - CD34
  - CD10
  - TdT
  - CD19

- **B-Lymphocyte**
  - CD20
  - CD19

- **Myeloblast**
  - CD34
  - CD13
  - CD33

- **Myeloid Cells**
  - CD34
  - CD13
  - CD33

- **Monocytes**
  - CD14
  - CD45

- **All Leukocytes**
  - CD13
  - CD33

- **T-Lymphocyte**
  - CD4/8
  - CD3
  - TdT

- **B-Lymphocyte**
  - CD20
  - CD19

- **Myeloblast**
  - CD34
  - CD13
  - CD33

- **Myeloid Cells**
  - CD34
  - CD13
  - CD33
Other Techniques

- Cytogenetic analysis
- GTG banding
- Spectral karyotypic analysis (SKY)
- Molecular techniques
  - Fluorescent *in situ* hybridization
  - Polymerase chain reaction
  - Restriction Fragment Length Polymorphisms (RFLPs)
Avaunt! and quit my sight!
Let the earth hide thee! Thy bones are marrowless, thy blood is cold; Thou hast no speculation in those eyes which thou dost glare with!

William Shakespeare
MacBeth
Act 3, Scene 4