BONE MARROW AND SPLEEN EXAMINATION

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HISTOLOGY OF BONE MARROW
Overview

Bone Marrow:
- Bone marrow sample
- Bone marrow overview
- Bone marrow histology in a core biopsy
- Myeloid cells
- Monocytic and dendritic cells
- Erythroid cells
- Megakaryocytes
- Lymphoid histology
- Bone

Spleen:
- An abbreviated version
Bone Marrow in The Pathology Lab

Usual Sample

- Bone marrow smear
- Bone marrow clot
- Bone marrow biopsy (decalcified)

Always add information from

- Clinical history
- Labs (specially CBC)
- Flow cytometry
- Iron stain
Bone Marrow Biopsy

- The biopsy is used for **quantitative** evaluation of the marrow
  - Cellularity
  - M:E ratio, it is considered the best estimation (vs differential from smear)
  - Numbers of megakaryocytes
  - Presence of tumors, granulomas, infiltrates
  - Presence of fibrosis, necrosis
Bone Marrow Clot Section

- May also be used for quantitative evaluation, especially if the biopsy is suboptimal.
- May give a better evaluation of erythropoiesis, since there is no bone present in the specimen, it can be cut thinner, aiding in the evaluation of red cell precursors.
- The best for immunos… if representative.
Bone Marrow Smears

• The smears are used for **qualitative** evaluation of the marrow
  • cell identification
  • dyspoiesis
  • maturation
  • cytologic abnormalities
Bone Marrow Smears

- The smears are used for **qualitative** evaluation of the marrow
  - cell identification
  - dyspoiesis
  - maturation
  - cytologic abnormalities

This bone marrow is from a patient with chronic myelocytic leukemia. It is hypercellular and contains only a small amount of fat. There are a number of megakaryocytes, which are the largest cells of the bone marrow. The number of **megakaryocytes** is estimated in the thickest part of the particle and not in the sinusoidal blood. The normal number is 3-10 per low-power field. More than 15 in one field in the particle is considered increased. If you have to search for them and find only 1-2 per particle, they are decreased.
Bone Marrow Smear – The differential

Practical advices

• Always do your differential with 1,000x magnification.
  
• Count at least 200-300 nucleated cells
  
• Between 40-60 cells can be counted in one field with oil immersion.
  
• It becomes difficult with 100 or more cells, as we tend to skip or to count the same cells twice.
  
• Fewer than 10 cells per field indicates sinusoidal blood rather than actual bone marrow particles I divide the field into imaginary quarters and start at the 12-o’clock position and count clockwise.
  
• Beginners find it easier to count all the granulocytes in the field and then to go back over it and count the lymphocytes, nucleated red cells, plasma cells, and the other types in the same fashion.
Bone Marrow Smear – The differential

• There are 13 granulocytes:
  • two segs
  • four bands
  • four metamyelocytes
  • two myelocytes
  • one promyelocyte (some promyelocytes do not contain granules)

• Four normoblasts (E5)
• Two late erythroblasts (E4) one of which is in mitosis.
  • There are also three naked nuclei, which are disregarded
Basic approach in the biopsy

- **Cellularity**
  - Depending on Pt age
- **Myeloid to Erythroid ratio (ME ratio)**
  - 1:6 in first week of life
  - 2.5-4:1 (adult physiologic range [3:1])

Increase in either component is reported as myeloid/erythroid “predominant” in the presence of a normal fat:cell ratio, and “hyperplasia” when the cellularity of the bone marrow exceeds 70%.

16 yo patient  43 yo patient
How to tell which ones are which?

- Erythroid cells are seen as round dark nuclei without much cytoplasm - “black dots” usually with a halo around them.

- Myeloid cells have lighter staining nuclei and pink cytoplasm, with maturation you can identify polys and bands (paratrabecular distribution).

- Megakaryocytes are large with multilobated nuclei (perisinusoidal).
Myeloid vs. Erythroid

trabecular bone

ganulocytes

megakaryocyte

erythroid island
Erythroid hyperplasia
Bone marrow lexicon

- Blast → least differentiated
- Cyte → more differentiated
- Pro → 2\textsuperscript{nd} cell in maturation sequence
- Meta → 4\textsuperscript{th} cell in maturation sequence (if 4 maturation stages)
Bone marrow, differentiation and cytological features
Fetal hematopoiesis

- First detected in the yolk sac (2nd to 3rd week), and exclusively produces nucleated red blood cells
- Then dorsal aorta, liver (6th week), spleen and bone marrow (14th weeks)

- Hematopoietic stem cells (CD34, c-kit, Thy1) and CD34 receptors
- The mesenchymal component (adipose tissue, bone and fibroblast) comes from primitive mesenchymal stem cells.
Microenvironment Controls Differentiation

• Immature granulocytic cells have paratrabecular arrangement.
• T-cells and macrophages (IL-6, G-CSF, M-CSF) produce several cytokines that regulate the microenvironment
• Specific cytokines promote lineage specific proliferation:
  • G-CSF → Granulocytes
  • M-CSF → Monocytes/macrophage lineage
  • IL-5 → Eosinophil/basophil production
Lineage development: Granulocytic cells

- Granulopoietic cycle within the bone marrow takes 10 to 14 days but can be accelerated by cytokines (G/GM-CSF)
Lineage development: Granulocytic cells

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblasts</td>
<td>0% to 2%</td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>2% to 5%</td>
</tr>
<tr>
<td>Myelocytes (neutrophilic)</td>
<td>9% to 16%</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>7% to 23%</td>
</tr>
<tr>
<td>Band Forms</td>
<td>8% to 15%</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>4% to 10%</td>
</tr>
<tr>
<td>Myelocytes (eosinophilic)</td>
<td></td>
</tr>
<tr>
<td>Band</td>
<td>0% to 2%</td>
</tr>
<tr>
<td>Mature</td>
<td>0% to 2%</td>
</tr>
<tr>
<td>Monocytes/macrophages</td>
<td>0% to 3%</td>
</tr>
<tr>
<td>Basophils</td>
<td>0% to 1%</td>
</tr>
<tr>
<td>Mast Cells</td>
<td>0% to 2%</td>
</tr>
</tbody>
</table>

TABLE 2. Types of myeloid elements and their normal range in the bone marrow

*Advances in Anatomic Pathology, Vol. 10, No. 1, January, 2003*
Lineage development: Granulocytic cells

A. PAS, B. Sudan black, C. Myeloperoxidase, D. Alpha-naphthol AS-D chloroacetate
<table>
<thead>
<tr>
<th>Stage of Maturation</th>
<th>Morphology</th>
<th>Cytochemical/Immunophenotypic Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblast</td>
<td>High nuclear to cytoplasmic ratio, Blastic, dispersed chromatin, Agranular, minimally granular cytoplasm</td>
<td>Myeloperoxidase + or –, Most myeloblasts are CD34+, HLA-DR+, and coexpress myeloid+ lineage antigen such as CD33</td>
</tr>
<tr>
<td>Promyelocyte</td>
<td>Eccentric nucleus with prominent paranuclear hof (pale zone), Sparse, concentrated azurophilic granules</td>
<td>Myeloperoxidase +, Typically CD34 –, HLA-DR –, and myeloid antigen + (e.g., CD33, CD13)</td>
</tr>
<tr>
<td>Neutrophilic myelocyte</td>
<td>Round nucleus with condensed chromatin, Moderate to abundant secondary (specific) granules which give the cytoplasm a finely granular pink appearance</td>
<td>Myeloperoxidase +, leukocyte alkaline phosphatase +, Myeloid antigen + (CD34 –, HLA-DR –)</td>
</tr>
<tr>
<td>Neutrophilic metamyelocyte</td>
<td>Indented nucleus, condensed chromatin, Cytoplasm packed with granules with predominance of secondary granules</td>
<td>Myeloperoxidase +, leukocyte alkaline phosphatase +, Myeloid antigen + (CD34 –, HLA-DR –)</td>
</tr>
<tr>
<td>Band neutrophil</td>
<td>Horseshoe-shaped mature nucleus lacking discrete indentations, Cytoplasm packed with granules with predominance of secondary granules; gelatinous granules also present</td>
<td>Myeloperoxidase +, leukocyte alkaline phosphatase +, Myeloid antigen + (CD34 – HLA-DR –)</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>3-5 discrete nuclear lobes, Highly condensed chromatin, Cytoplasm packed with granules with predominance of secondary granules; gelatinous granules also present</td>
<td>Myeloperoxidase+, leukocyte alkaline phosphatase +, Myeloid antigen + (CD34 –, HLA-DR –)</td>
</tr>
</tbody>
</table>

*Data from references 39, 46, and 62.
Lineage development: Granulocytic cells

Figure 3.5 Granulocyte differentiation.
Neutrophilic Leukocytes

- Maturation is characterized by development of dark-blue (primary) granules that later on is replaced by secondary granules that differ in their size and staining pattern
  - Neutrophil small granules that stain with light blue and pink
    - Also: Gelatinase (tertiary) granule, which lacks both myeloperoxidase and lactoferrin, but contains gelatinase, acetyltransferase, and lysozyme
  - Basophil large basophilic granules
  - Eosinophil
Myeloblast

- **Nuclei**
  - Round
  - Evenly staining
  - 2-3 nucleoli

- **Cytoplasm**
  - No granules*
  - Unevenly staining
  - Perinuclear clearing
Myeloblast

- CD34+, CD117+, CD38+, HLA-DR+

- Also as myeloid cells:
  - CD13+
  - CD33+
Promyelocyte

- A myeloblast when develop distinctive granules
- Nucleous
  - Chromatin is coarser than myeloblast
  - Indistinct nucleoli
  - Oval round nuclei
- Cytoplasm
  - Primary granules are dark-blue or reddish-blue

Becomes a myelocyte once you identify the granules as basophilic, eosinophilic or neutrophilic
Promyelocyte

- A myeloblast when develop distinctive granules
- Nucleous
  - Chromatin is coarser than myeloblast
  - Indistinct nucleoli
  - Oval round nuclei
- Cytoplasm
  - **Primary granules** are dark-blue or reddish-blue
Neutrophilic Myelocyte

- **Nuclei**
  - Round to oval, flattened in one side

- **Cytoplasm**
  - Initially a perinuclear island of ill-defined reddish granules so neutrophilic differentiation

Secondary granule formation begins in the Golgi region highlighted by the paranuclear hof in this early neutrophilic myelocyte (bone marrow aspirate; Wright stain).
Neutrophilic metamyelocyte

- **Nuclei**
  - Slightly indented nuclei

- **Cytoplasm**
  - Small pinkish granules

- May be seen normally on PB but also indicates myeloid hyperplasia

Composite of a neutrophilic myelocyte (center), neutrophilic metamyelocyte (top), and band neutrophil (bottom) in a bone marrow aspirate smear shows the progression of maturation changes of the nucleus and cytoplasm. Right: Electron micrograph of a myelocyte shows primary and secondary granules (bone marrow aspirate; Wright stain).
Neutrophilic Band

- **Nucleus**
  - Characteristic horseshoe nucleus
  - Indentation is greater than half of the hypothetical round nucleus

- **Cytoplasm**
  - Evenly distributed granules that stain shades of pink and blue

- 1-5% of PB in healthy individuals, if increased is called “shift to the left”
And finally... The Neutrophil

- Nuclei
  - Typically lobulated
  - 35% 2 lobes
  - 41% 3 lobes
  - Hyperlobulation → pernicious anemia (6 or more lobes)
  - Hypolobulation → Pelger-Huet anomaly (2 round lobes connected with a short filament [pince-nez form])

- 50-70% of WBC

The neutrophilic metamyelocyte (Mt), band neutrophil (B), and segmented neutrophil (PMN) evident in the center of this bone marrow aspirate smear highlight the nuclear and cytoplasmic features of maturation (Wright stain).
Progressive nuclear maturation from the round eccentric nucleus of a promyelocyte (P) (lower left) through the myelocyte (My), metamyelocyte (Mt), band (B), and segmented neutrophil (PMN) (bone marrow aspirate smear; Wright stain).
STAGES OF NEUTROPHILIC MATURATION

Early promyelocyte (P), neutrophilic myelocyte (My), neutrophilic metamyelocyte (Mt), band neutrophil (B), and segmented neutrophil (PMN). The progression from basophilic to eosinophilic cytoplasm and the acquisition of first, primary, and then, secondary granules in conjunction with gradual and progressive nuclear segmentation and condensation of the nuclear chromatin are evident.
Morphological abnormalities

- Toxic granules: prominent blue-black to purplish granules that resemble primary granules. Mean asynchrony between maturation of nuclei and lysosomes
- Dohle bodies: pale sky-blue cytoplasmic inclusions (Rough endoplasmic reticulum), acute phase reaction and May-Hegglin anomaly
Monocytes

- Monocytes and related dendritic cells play a pivotal role in host defense from microbial pathogens, wound healing, angiogenesis, hematopoiesis, and various inflammatory reactions.

- Monocytic production within bone marrow is estimated to take about 2 to 3 days.

Figure 3.9 Monocyte differentiation.
Monocytes

- Neither monoblasts nor promonocytes are typically evident in normal bone marrow

- Monoblasts:
  - Nuclei: round to oval with dispersed, blastic nuclear chromatin
  - Cytoplasm: abundant and pale blue, with either agranular or subtle, finely granular cytoplasm

Leukemic monoblast has voluminous, slate blue-gray, finely granular cytoplasm and an immature round nucleus
Monocytes

- **Promonocytes:**
  - Nuclei: folded nuclear configuration with a typically prominent nucleolus and fairly dispersed nuclear chromatin
  - Cytoplasm: abundant and pale blue, with either agranular or subtle, finely granular cytoplasm

Leukemic promonocytes have abundant cytoplasm and folded, immature nuclei
Monocytes/Macrophages

• Diffuse cytoplasmic positivity for **alpha-naphthyl butyrate** and **alpha-naphthyl acetate esterase** (non-specific esterases) in all monocyte stages

• **IHC include:**
  • **Lysozyme**, **CD68** (KP1 or PG-M1 epitopes), and **CD163**
Dendritic cells

• Dendritic cells are defined more by their functional activities than by specific morphologic features, although immunophenotypic subsets are well described

• Dendritic cells varies by the specific cell type, and differences in phenotype are based on derivation from either myeloid or lymphoid progenitor cells
Dendritic cells

- Dendritic cells are infrequent in bone marrow, and immunohistochemical techniques (a profile consisting of CD68, CD123-, and CD43-positive, myeloperoxidase-negative cells) are generally required for cell identification.

- This composite shows rare CD1a-positive cells (left) and slightly more numerous S-100 protein-positive cells (right) in a normal bone marrow core biopsy from a 1-year-old female.
Other myeloid components

Eosinophilic myelocyte and mature eosinophil

- Eosinophil granules are large and refractile, and contain major basic protein, eosinophil peroxidase

Mature segmented basophil

- Granules have histamine and heparin (like mast cells)
- Segmented nuclei
Mast Cells

- Round to oval nuclei
- Granules have histamine and heparin
- Tryptase or CD117, highlight perivascular distribution in bone marrow
Erythroid cells

Erythropoiesis occurs in discrete colonies within the hematopoietic cavity. Basophilic RNA-rich cytoplasm in immature forms. Production takes 5 to 7 days.

- Pronormoblast (erythroblast)
- Basophilic normoblast
- Polychromatophilic normoblast
- Orthochromatric normoblast
- Basophilic erythrocyte
- Reticulocyte
- Erythrocyte
Erythroid cells

- Erythropoietin is the dominant lineage specific growth factor
- Controls survival and proliferation
- Binds to a receptor that activates JAK2, initiating the downstream signaling pathways resulting in phosphorylation and activation of GATA1
Erythroid cells

- erythroblast has deeply basophilic cytoplasm and a round nuclear contour
Erythroid cells

- Basophilic normoblasts
Erythroid cells

- Polychromatophilic normoblasts
- Last stage of cellular division
Stages of Erythroid Maturation
Monocytes/macrophages

- Pronormoblast (erythroblast)
- Basophilic normoblast
- Polychromatophilic normoblast
- Orthochromatic normoblast
- Basophilic erythrocyte
- Reticulocyte
- Erythrocyte
Megakaryocytes

- Megakaryoblast
- Promegakaryocyte
- Platelet-shedding megakaryocyte

IHC:
- CD61, CD41, CD42b
## Megakaryocyte Morphology

<table>
<thead>
<tr>
<th>Stage of Maturation</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Megakaryoblast</td>
<td>Difficult to recognize by morphology alone, but tends to be large blast with a high nuclear to cytoplasmic ratio, basophilic cytoplasm, and variable cytoplasmic blebbing</td>
</tr>
<tr>
<td>Promegakaryocyte</td>
<td>Spectrum of large cells with various degrees of nuclear lobulation, Progressive increase in overall size, variable cytoplasmic granules</td>
</tr>
<tr>
<td>Platelet-shedding megakaryocyte</td>
<td>Large multilobulated megakaryocytes with highly condensed nuclear chromatin, reside adjacent to bone marrow sinuses, Voluminous amounts of cytoplasm with abundant cytoplasmic granules</td>
</tr>
<tr>
<td>Stage of Maturation</td>
<td>Cytochemical/Immunophenotypic Properties</td>
</tr>
<tr>
<td>------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Megakaryoblast</td>
<td>Platelet peroxidase evident by immuno-electron microscopic techniques</td>
</tr>
<tr>
<td></td>
<td>Variable CD34 expression</td>
</tr>
<tr>
<td></td>
<td>Expression of lineage-specific antigens such as CD41, CD42b, CD61, and Mpl1</td>
</tr>
<tr>
<td>Promegakaryocyte</td>
<td>Loss of CD34 but retention of the full complement of megakaryocyte-associated antigens</td>
</tr>
<tr>
<td>Platelet-shedding</td>
<td>Expression of some megakaryocyte-associated antigens such as CD31, CD41, vWF increases with maturation</td>
</tr>
<tr>
<td>megakaryocyte</td>
<td></td>
</tr>
<tr>
<td>Metamegakaryocyte</td>
<td></td>
</tr>
<tr>
<td>Thrombocytes</td>
<td></td>
</tr>
</tbody>
</table>
Megakaryocytes

- Early to middle maturation stage megakaryocyte with round nucleus and abundant cytoplasm
Megakaryocytes

- Megakaryocyte maturation is characterized by the progressive doubling of nuclear material, with the multilobulation of a single nucleus, termed **endomitosis**
Megakaryocytes

- Prominent nuclear lobulation with interconnected lobules is evident in this mature megakaryocyte with an adherent large platelet clump
Megakaryocytes

- Thrombopoietin (produced in the liver) is an obligatory lineage specific growth factor

- Binding of thrombopoietin to its ligand (c-Mpl) activates JAK2 → promotes meg differentiation and proliferation

- Platelets have thrombopoietin receptors

Immature megakaryocytes with prominent cytoplasmic blebbing
Megakaryocytes

- Perisinusoidal and intrasinusoidal localization of megakaryocytes is evident in this bone marrow biopsy from an adult.
Lymphoid and Natural Killer Cells

- Origin of B, T, and natural killer (NK) precursor cells from stem cells that give rise to hematopoietic and lymphoid lineages

- Immature T cells migrate to the thymus

- The bone marrow is the site of B-cell development
Lymphoid and NK Cells

- **Hematogones** (immature benign B lymphoid cells), may be abundant in specimens from pediatric patients.

- An immature lymphoid cell with dispersed chromatin and an irregular nuclear configuration is evident in the peripheral blood of a normal infant.
Lymphoid and NK Cells

- BM Hematogones
  - Nuclear chromatin is highly condensed
Lymphoid and NK Cells

Hematogones

- By gating the lymphocyte population by forward and side scatter properties, an admixture of mature polyclonal B cells and surface immunoglobulin-negative, CD10-positive hematogones is evident in a normal bone marrow aspirate.
Lymphoid and NK Cells

- bone marrow clot section from a premature infant with increased hematogones shows a side-by-side comparison of the number of CD3-positive T cells (left) and the number of CD20-positive B cells (right). Because of the increased numbers of hematogones, B cells are more numerous than T cells.
Lymphoid and NK Cells

- Except in specimens with abundant hematogones, **T cells predominate** in normal bone marrow and exhibit a patchy, partially perivascular distribution.

- B cells are less common and are randomly distributed and dispersed, individually or in small clusters.

- The perivascular (left) and patchy interstitial (right) distribution of T cells is evident on the bone marrow core biopsy from an adult.
Lymphoid and NK Cells

• Mature plasma cells
  • Nuclei: eccentric with a "clockface" chromatin pattern
  • Cytoplasm: prominent paranuclear hof, and abundant basophilic cytoplasm that may contain immunoglobulin vacuoles
Lymphoid and NK Cells

- Plasma cells may have perivascular distribution
- CD138 highlights perivascular plasma cells
- Constitute up to 5% of plasma cells
- Benign plasma cells have a polytypic kappa to lambda light chain ratio
NK Cells

- NK cells are surface CD3-negative, CD56-positive, CD16-positive
- Produce immunoregulatory cytokines
- Also mediate cytotoxicity against target cells that lack matching major histocompatibility complex (MHC) ligands
- NK cells have features of large granular lymphocytes, although cytotoxic T cells (surface CD3 and CD8 positive) also share this morphology
- The granules contain perforin and granzymes,
- **IHC:** granzyme and T-cell intracellular antigen 1 (TIA-1)
Lymphoid and NK Cells

• TIA-1 IHC stain highlights cytoplasmic granules in a bone marrow core biopsy with increased large granular lymphocytes.
Bone Elements

3 elements:

- Osteoblasts
- Osteoclasts
- Osteocytes
  - Osteoblasts assemble to form new bone in the lacunar spaces vacated by osteoclasts, a process called coupling
Bone Elements

Osteoclast

- Osteoclasts resorb bone and are derived from a common monocytic/macrophage/dendritic progenitor cell
- Monocytic cells differentiate into osteoclasts in the presence of M-CSF and RANKL

- Discrete nuclei, coarse cytoplasmic "bone sand" in mature cells, and paratrabecular localization often within scalloped spaces
Bone Elements

Osteoblast

- Stromal-derived cells which produce the bony substrate
- Resemble enlarged plasma cells
- Osteoblasts also contain a cytoplasmic pale area (hof) which, unlike plasma cells, is separated from the nucleus
- If active bone remodeling is in progress, osteoblasts rim bony trabeculae in a single file
<table>
<thead>
<tr>
<th>Patient Age</th>
<th>Cellularity</th>
<th>Bony Trabeculae</th>
<th>Cellular Composition</th>
</tr>
</thead>
</table>
| Newborn    | Up to 100%, but may be lower | Very active bone remodeling and incomplete ossification of cortical bone | Blasts up to 5%  
Predominance of myeloid cells except in cases in which hematogones are numerous; myeloid to erythroid ratio of ~ 4:1  
Lymphoid cells, notably hematogones, may be numerous |
| Infant     | Variable, up to 100%, but may be lower | Very active bone remodeling and incomplete ossification of cortical bone | Blasts up to 5%  
Predominance of myeloid elements; myeloid to erythroid ratio: ~ 5-10:1  
Erythroid elements markedly reduced during physiologic nadir  
Lymphoid cells, notably hematogones, may be abundant (up to 50% of cells) |
| Child      | 60-80%      | Active bone remodeling | Blasts up to 5 percent, but usually lower  
Myeloid elements predominate; myeloid to erythroid ratio: ~ 3:1  
Lymphocytes, notably hematogones, may be abundant |
| Young adult| 50-70%      | Bone remodeling may be evident, especially in young males | Blasts generally <5%  
Myeloid elements predominate; myeloid to erythroid ratio: ~ 3:1  
Lymphocytes generally inconspicuous, but may range up to 20% |
| Adult      | 40-60%      | Bone remodeling absent  
Osteoclasts and osteoblasts inconspicuous  
Bony trabeculae may be thinned (osteopenic), especially in females | Blasts generally <3%  
Myeloid elements predominate; myeloid to erythroid ratio: ~ 3-4:1  
Lymphocytes usually inconspicuous, but may range up to 20% |
| Elderly    | 25-40%      | Bone remodeling absent  
Osteoblasts and osteoclasts inconspicuous  
Bony trabeculae may be thinned (osteopenic), especially in females | Blasts <3%  
Myeloid elements predominate; myeloid to erythroid ratio: ~ 3-4:1  
Mild dysplastic features may be noted  
Lymphocytes are generally inconspicuous, but may range up to 20%, especially if hematopoiesis is reduced.  
Lipogranulomas and lymphoid aggregates may be present |
HISTOLOGY OF THE SPLEEN
Gross Anatomy

- Normal weight 150 g
  - SD 25 g
- Hilus, where it is penetrated by vessels and nerves which follow the extensive branching network of fibrous trabeculae.
- Accessory spleens occur in about 10 percent of individuals
- Following traumatic rupture, small nodules of splenic tissue may grow on the peritoneal surface as implants (splenosis)
White Pulp

- Comprises the lymphoid compartment of the spleen and consists of both follicular B-cell-rich areas as well as T-cell-rich periarteriolar lymphoid sheaths.
Periarteriolar T-Cell-Rich Lymphoid Sheaths

• Counterpart to the paracortical region of lymph nodes

• Lymphoid sheath, which surrounds splenic arteries as they exit the fibrous trabeculae
Spleen: Periarteriolar area

The T cells predominate in the periarteriolar lymphoid sheath (labeled red with Leu-22/CD43). The follicles, which tend to occur at arterial branch points, are labeled blue (L26/CD20).
Primary and Secondary B-Cell Follicles

- Located at the periphery of the T zone and have the identical histologic and phenotypic features of primary and secondary follicles of lymph nodes
Primary and Secondary B-Cell Follicles

MARGINAL ZONE

• Surrounds the primary follicle and the mantle zone of secondary follicles
• Consists of a corona of medium-sized lymphoid cells with prominent pale cytoplasm
Primary and Secondary B-Cell Follicles

MARGINAL ZONE

• The nuclear chromatin of the intermediate-sized marginal zone cells is somewhat less condensed than that of small lymphocytes

• Admixed with a variable number of plasma cells, T cells, and macrophages
Red Pulp

- 4 vascular structures:
  - Slender and nonanastomosing **arterial vessels**
  - Reticular meshwork of thin plates of cellular tissue lying between the sinusoids comprising **splenic cords**
  - Large, thin-walled venous vessels called **sinusoids**
  - **Pulp veins** which drain the sinusoids

Cords

- Reticular meshwork consists of a branching system of cords lying between the sinuses
- Includes the reticular meshwork and may run through a sheath of macrophages with may run through a sheath of macrophages
- Clearance functions are also handled by marginal zone and red pulp macrophages
Sinusoids

- Are lined by tapered endothelial cells separated by slit-like spaces and surrounded by distinctive ring fibers and bridging fibers

- Stain endothelial markers (FVIII) and CD8

- PAS stain highlights the distinctive ring fibers and bridging fibers
Splenic macrophages

Macrophages are preferentially located in the marginal zone and red pulp cords of the spleen (labeled brown with KP-1/CD68).
Spleen, physiology

- Differentiation of reticulocytes, platelets, and monocytes
- Removal of abnormal erythrocytes
- Major site of antibody production, particularly in response to blood-borne antigens.
Useful References

Leukemias

- WHO 2008
- For a single author perspective:
  - Bone marrow -> Dr. Foucar or Dr. Bain books
  - Online free AFIP book:

Lymphomas

- WHO 2008
- For benign lymph node → Ioachim’s Lymph node pathology
Based on…

• AFIP Atlas of non-tumor pathology – Bone marrow by Dr. Foucar.