RECENT CASE

Lymph Node
Submitted history:
• F/U Kaposi Sarcoma, very concerned for vascular tumor

NOTICE OF FACULTY DISCLOSURE

In accordance with ACCME guidelines, any individual in a position to influence and/or control the content of this ASCP CME activity has disclosed all relevant financial relationships within the past 12 months with commercial interests that provide products and/or services related to the content of this CME activity.

The individual below has responded that he has the following relevant financial relationship with commercial interest to disclose:

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Adjunct Associate Professor
MD Anderson Cancer Center/University of Texas
WHAT MAKES A SPLEEN PATHOLOGIST?

- Blood cells
- Lymph nodes
- Infectious disease pathology

WHAT MAKES A SPLEEN PATHOLOGIST?

SPLENIC FUNCTIONS

1. Filtration
   a. Cutting erythrocyte (or other blood cell) destruction
   b. Physiologic (as red blood cells age)
   c. Pathologic
      i. Associated with blood cell abnormalities
      ii. Associated with primary splenic changes
   d. Pitting ("swelling") of erythrocytes

2. Removal of erythrophagocytosis
   a. Physiologic
   b. Pathologic

3. Destruction of abnormal and broken cells
   a. Associated with physiologic
   b. Associated with pathologic

4. Removal of other particulate material (e.g., bacteria, cellular fragments)

- Immunology
  a. Trapping and processing of antigen
  b. "Homing" of lymphocytes
  c. Lymphocyte transformation and proliferation
  d. Antibody and lymphokine production
  e. Macrophage activation

- Reservoir
  a. Storage or normal sequestration of platelets, granulocytes
  b. Recapturing of cells
  c. Red blood cell storage (rhein in humans)

- Hemostasis
  a. Erythropoiesis, monoperoxidase
  b. Physiologic function of red blood cells in heart or small spleen
  c. Lymphocyte and macrophage production

HOW THE SPLEEN WORKS...
VASCULAR ARCHITECTURE OF SPLEEN

CLOSED CIRCULATION

Artery → Arteriole → Capillary bed → Venule → Vein

Sinusoid

OPEN CIRCULATION

Arterioles

Sinus lining cell (littoral cell)

Intestinal space/macrophages

ARCHITECTURE OF THE SPLEEN

Special stains and immunos can help define histologic compartments of the spleen that are subtle

Because of its unique nature, unusual combinations of antibodies are used to highlight various features

Actin: defines cords and circumference of WP

CD8: highlights splenic littoral cells as well as larger subset of T-cells (cords)

Bcl-2: marginal zone cells, mantle and T-cells

IgD: mantle zone positive; MZ negative

CD34: arterioles positive, but not sinuses

ARCHITECTURE OF THE SPLEEN

CD8:

- highlights splenic littoral cells
- larger subset of T-cells (cords)

NOTE: Littoral cell angioma (LCA), a common benign vascular neoplasm of spleen is purported to be of splenic littoral cell origin. Surprisingly, LCA is also typically CD8 negative!

CD8 stain

Bcl-2 staining in normal marginal zone

HINTS TO BETTER DIAGNOSIS

Touch imprints

Sectioning
- Approximately 1 cm
- Sections 5 mm thick

When possible, submit splenic hilar lymph nodes

Thin sections
- Blotted free of blood before fixation

GROSS EXAMINATION OF SPLEEN

Examine capsule, note any alterations

Section unfixed spleen at 1-2 cm intervals

Note any focal lesions

Note normal pattern versus miliary or beefy

Sections for histologic examination
- Thinner sections of involved, interface and unaffected spleen
- Capsule should be included if any abnormalities are noted
- Splenic hilar lymph node should be included, if present
- Thinner sections (5 mm)
- Blot to remove blood*
- Place into adequate amounts of fixative (at least 10x volume of tissue

* Blood will interfere with fixation and make interpretation more difficult.
AN ORGANIZED APPROACH TO DIAGNOSIS IN THE SPLEEN

GROSS EXAMINATION
- DIFFUSE
- FOCAL
  - WHITE PULP
  - RED PULP
  - MASS
  - CYST

MICROSCOPIC EXAMINATION

WHITE PULP
- R&W
- DIFFUSE

Lymphoma
- Hyperplasia
- DIFFUSE
- WP
- MASS

GROSS APPEARANCE
- "beefy"
- "miliary"
- Focal - nodular - mass

APPROACH TO LYMPHOID LESIONS OF SPLEEN
Generally, sort disorders into compartments
- White pulp diseases
- Red pulp diseases
- Focal diseases
APPROACH TO LYMPHOID LESIONS OF SPLEEN

White pulp lymphomas – “The usual suspects”

- Splenic marginal zone lymphoma
- CLL
- Follicular lymphoma
- Mantle cell lymphoma

SPLENIC MARGINAL ZONE LYMPHOMA

Small B cells
Predominantly in white pulp (increased white pulp decreased red pulp)
Expansion of B cells into PALS regions
May have increased amounts of cytoplasm
May be present in a marginal zone pattern or as uniform nodules
Follicular colonization may be present

SMZL CYTOLOGY

Uniform white pulp nodules
Marginal zone pattern white pulp nodules

Follicles interrupted by low proliferation, Marginal Zone

SMZL

Ki-67

MZL
**SMZL: BLOOD AND BONE MARROW**

- Vitous lymphocytes
- Hassall's corpuscles, subtle involvement in bone marrow

**SPLENIC HYPERPLASIA**

**MARGINAL ZONE**

- Expansion of outer (third) layer of splenic follicle structure

**CLINICAL:** Variety of causes. Notably autoimmune phenomena (e.g., ITP, autoimmune hemolytic anemias)

**Cytology:**
- Small lymphocytes, with increased cytoplasm (monocytoid)
- Occasional plasma cells or plasma cells with lymphocytes (Russel bodies may be present; Dutcher bodies vary rarely)
- Occasional larger, transformed cells

Hyperplasia defined as increased of layer thickness to >12 cells

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**Features**

<table>
<thead>
<tr>
<th></th>
<th>MZ Hyperplasia</th>
<th>MZ Lymphoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expanded MZ (&gt;12 cell layers)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Numerous B-cells in PALS</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Follicles</td>
<td>3 layers</td>
<td>Loss of layers; often uniform single layer</td>
</tr>
<tr>
<td>Red Pulp</td>
<td>No increase in red pulp B-cells</td>
<td>Often has clusters of red pulp B-cells</td>
</tr>
<tr>
<td>Clonality</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Kappa/lambda</td>
<td>Polyclonal</td>
<td>Monoclonal</td>
</tr>
<tr>
<td>KI-67</td>
<td>Normal secondary follicles with high proliferation</td>
<td>Collected follicles have low proliferation in neoplastic cells</td>
</tr>
</tbody>
</table>

**APPROACH TO LYMPHOID LESIONS OF Spleen**

Red pulp (diseases with a circulating component)
- Hairy cell leukemia
- Diffuse red pulp small B-cell lymphomas
- Hepatosplenic T-cell lymphoma
- T-cell Large Granular Lymphocytic Leukemia
- Sezary Syndrome
- Rare variants of CLL, MCL

**HAIRY CELL LEUKEMIA**

Small B-cells, abundant cytoplasm
- Diffuse red pulp involvement
- Blood lakes, lined by tumor cells, not sinus cells
- PB, BM, and splenic involvement
- Immunophenotype: CD103, CD22, CD19
- IHC: TRAP, DBA.44, Annexin A1, BRAF
- Monocytopenia, other cytopenias
- BRAF mutation seen in most cases

**Distinctive Splenic Lymphoma/Leukemias**

**RED PULP**

- Hairy Cell Leukemia
- Hepatosplenic T-cell lymphoma
- Splenic Diffuse Red Pulp Small B-cell Lymphoma, Undifferentiated
- T-LGL leukemia
HAIRY CELL LEUKEMIA

PERIPHERAL BLOOD FINDINGS

Cytopenias
- Especially monocytopenia
Cytoplasmic projections
- Will often distort adjacent red blood cells

HAIRY CELL LEUKEMIA

MARROW FINDINGS

Often hypocellular
Aggregates are most often interstitial
May be deceptively extensive
Fried-egg appearance
- Round nucleus with central placement, surrounded by moderate to large amounts of clear cytoplasm
Severe fibrosis (DRY TAP)

HAIRY CELL LEUKEMIA

BRAF MUTATION IN HAIRY CELL LEUKEMIA

Greater than 90% of HCL patients have evidence of BRAF V600E mutations
While current therapies are effective, this raises the possibility of anti-BRAF therapies ( vemurafenib)
Provides diagnostic confirmation of HCL
Excludes almost all other lymphomas

HAIRY CELL LEUKEMIA

SPLEON

Small, "blood lakes"

GENE ARRAY STUDIES OF HAIRY CELL LEUKEMIA

Distinctive pattern of expression in HCL versus normal B cells and other lymphoma types

SPLENIC B CELL LEUKEMIA/LYMPHOMA, UNCLASSIFIABLE

Red pulp splenic B cell lymphomas
Poorly characterized
Very rare
Previously, a subset were identified as diffuse variant of splenic marginal zone lymphoma
Some were identified as hairy cell leukemia

TYPES
- Splenic diffuse red pulp small B cell lymphoma
- Hairy cell leukemia variant
### Hairy Cell Variant

<table>
<thead>
<tr>
<th>Summary of Flow Cytometric Findings</th>
</tr>
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<tbody>
<tr>
<td><strong>Splenomegaly</strong></td>
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<tr>
<td><strong>SMZL</strong></td>
</tr>
<tr>
<td><strong>HCL</strong></td>
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<tr>
<td><strong>HCL-V</strong></td>
</tr>
<tr>
<td><strong>SDRPL</strong></td>
</tr>
<tr>
<td>CD20</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>CD5</td>
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<td>+/−(50%)</td>
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<td>+</td>
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<tr>
<td>+</td>
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<tr>
<td>+</td>
</tr>
<tr>
<td>CD7</td>
</tr>
<tr>
<td>+</td>
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<td>+/−(55%)</td>
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<tr>
<td>+</td>
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<tr>
<td>+</td>
</tr>
<tr>
<td>CD8</td>
</tr>
<tr>
<td>+/−(5%)</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>+</td>
</tr>
<tr>
<td>CD10</td>
</tr>
<tr>
<td>−</td>
</tr>
<tr>
<td>Rare</td>
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<tr>
<td>+</td>
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<tr>
<td>+</td>
</tr>
</tbody>
</table>

### Hepatosplenic T-Cell Lymphoma

- Clinical: young males, post-transplant
- Sites: spleen, marrow, liver, +/- PB
- Often small, mature appearing; can occasionally be larger, more 'blastic'
- Red pulp infiltration
- Intrasinusoidal pattern
  - Can also see intrasinusoidal pattern in marrow and liver

CD2, CD3, & TIA-1 positive, CD56+/
CD5 negative
CD4/CD8 double negative
TCR γδ+/
Isochromosome 7q, +8
EBV negative

### Summary

- Red pulp involvement: slightly larger cells, occasional prominent nuclear inclusion

Red pulp: note the characteristic intravascular localization
HSTCL
Atypical cells, medium sized, pale cytoplasm, strongly CD3+

MARROW FINDINGS
Histology:
Marrow hypercellular
Medium size neoplastic cells within BM sinusoids
Highlighted by CD3

Cytology:
mild nuclear irregularities, loosely condensed chromatin variably inconspicuous nucleoli (clusters)

T CELL LARGE GRANULAR LYMPHOCYTIC LEUKEMIA
Proliferation of cytotoxic T lymphocytes
Seen in blood, marrow and spleen
Association with autoimmune disease (especially rheumatoid arthritis)
Often associated with neutropenia and other cytopenias
Increased small lymphocytes in red pulp of spleen, sparing of white pulp

Immunophenotype:
CD2+CD3+CD8+, frequent expression of CD87, CD16, dim expression or loss of CD5 by flow cytometry
Clonal T cell gene rearrangements by PCR

T LGL LEUKEMIA
Expansion of red pulp by small lymphocytes
Mildly increased amount of cytoplasm
Increased CD8 positive T cells in cords and sinuses

Mass
Hematopoietic
Myeloid
Hematologic
NHL
Splenoid
Epithelioid

Non-hematopoietic

Distinctive Splenic Lymphoma/Leukemias
Focal/Nodulare
Hodgkin Lymphoma
Diffuse Large B-cell Lymphoma
**APPROACH TO LYMPHOID LESIONS OF SPLEEN**

Focal - 'tumoral' masses with no specific location
- Diffuse large B-cell lymphoma (DLBCL)
- De novo
- Arising from pre-existing low-grade lymphoma of spleen or other site
- Hodgkin lymphoma

**SECONBDARY INVOLVEMENT BY HODGKIN LYMPHOMA**

Gross:
Solitary or multiple tumor masses in the spleen
Splenic involvement is generally detectable grossly but may be subtle, only a few millimeters in size

**HODGKIN LYMPHOMA**

Early lesions are found in the PALS or in the marginal zones
As the disease progresses, effacement of the lymphoid follicles and involvement of the red pulp
Sarcoid-type granulomas
Hodgkin cells/R-S cells seen

**Splenic Hyperplasia Overview**

- Follicular hyperplasia
- Marginal zone hyperplasia
- T-cell hyperplasia
- Specific Entities
- Castleman Disease
- Autoimmune lymphoproliferative disorder
**SPLENIC HYPERPLASIA**

**FOLLICULAR**

Typical Follicular Hyperplasia
- Similar to lymph node
- Similar histology
- Similar criteria regarding benign vs. lymphoma
- **DIFFERENTIAL DIAGNOSIS:** follicular lymphoma, other B-cell/white pulp lymphomas

Most often seen in ITP
Commonly observed in other autoimmune disorders (e.g., SLE, RA/Felty syndrome, AIHA)
Infections (AIDS)
Miscellaneous

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Follicular Lymphoma</th>
<th>Follicular Hyperplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase in follicle density</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Back-to-back follicles</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Lack of tingible-body macrophages</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Mantle zone</td>
<td>Poorly formed or absent</td>
<td>Present</td>
</tr>
<tr>
<td>Follicle polarization</td>
<td>Not present</td>
<td>Often present</td>
</tr>
<tr>
<td>Dysplastic/abnormal cytology</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Ki67 proliferation rate</td>
<td>Low</td>
<td>High</td>
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</tbody>
</table>

**FOLLICULAR HYPERPLASIA: Polarization**

**INFLAMMATORY PSEUDOTUMOR**

A mass-forming lesion of polymorphous cytologic composition, which is the morphologic manifestation of diverse processes spanning from reactive, inflammatory, infectious, to neoplastic
**Inflammatory pseudotumor**
- Reactive, most likely secondary to infectious causes.
- Prominence of inflammatory and sclerotic changes.
- Clinical history.
- Spindle cells are CD34+ /CD45+ positive, CD30+.

**Inflammatory pseudotumor-like follicular dendritic cell tumor**
- Neoplasm of follicular dendritic cells; EBV-associated.

**Inflammatory myofibroblastic tumor**
- Neoplasm of myofibroblastic cells.
- Increased smooth muscle actin/b-actin positive spindle cells. Occasionally ALK positive.
- Vascular invasion/presence of dystrophic calcifications may be present.

**Capillary hemangioma with prominent sclerosis/SANT**
- Benign neoplasm of vascular components and/or reactive vascular process.
- Stimulates IPI T cell with prominent vascular changes. Laminar nature of involvement, & a distinctive stroma. Vascular spaces highlight prominent epithelioid CD3+.

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**SPLENIC HAMARTOMA**
- Disorganized, mature, red pulp elements.
- White pulp structures are rare.
- Stroma may become densely fibrotic.
- Rare cases with a lobular pattern overlap capillary hemangiomas.
- Sinus structures CD8+/CD68+.
- Frequent EMH.

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**NON-HEMATOPOIETIC: VASCULAR LESIONS**

**Littoral Cell Angioma**
- Clinical: RELATIVELY COMMON
  - Often clinically silent, asymptomatic mass, discovered incidentally.
  - Wide age range.
  - Association with other malignancies, poorly understood.
- Gross:
  - Red pulp.
  - Mass or multinodular, firm or "spongy", bloody.
- Microscopic:
  - Anastomosing, patent vascular spaces.
  - Lined by plump, spenic endothelial-derived cells.
  - Occasionally papillary projections.
  - Luminar macrophages.
  - Micoses rare.
  - Macrophages may be prominent in cords.
  - Variable fibrosis and sclerosis.

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**VASCULAR LESIONS OF SPLEEN
DIFFERENTIAL DIAGNOSIS**

- Sclerosing Capillary Hemangioma
- Splenic Hamartoma
- Mycobacterial Spindle Cell Pseudotumor
- Bacillary Angiomatosis
- Kaposi Sarcoma
- Angiosarcoma

---

**Diagnosis**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Features</th>
<th>Immunostains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-neoplastic</td>
<td>Palisades, more common in spleen, قد تظهر على الشاشة في حالة غير ناضجة</td>
<td>CD34+ /CD45+ positive for SCL/TdT in B-Z Y</td>
</tr>
</tbody>
</table>
LITTORAL CELL ANGIOMA

Immuno
- Positive for vascular markers CD31/WF/Ulex/WT1
- NEGATIVE for CD34
- Typically negative for CD8
- Often positive for CD21 and CD68

LITTORAL CELL ANGIOMA

<table>
<thead>
<tr>
<th></th>
<th>CD34</th>
<th>CD8</th>
<th>WT1</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>-</td>
<td>+</td>
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QUESTIONS?

A HUMBLE REQUEST...
Gross photos of splenic pathology are hard to come by.
If you have any gross photos of spleen IN ANY FORMAT (digital, kodachromes, etc), I would be most appreciative.
These will be handled in a HIPAA-compliant manner
They will be returned promptly!
It is quite likely they would be published eventually, so please include your name with materials.

domalley@clarientinc.com
THAT, IN A SLEEP, UNFOLDS BOTH HEAVEN AND EARTH...

Wm. Shakespeare
A Midsummer Night's Dream