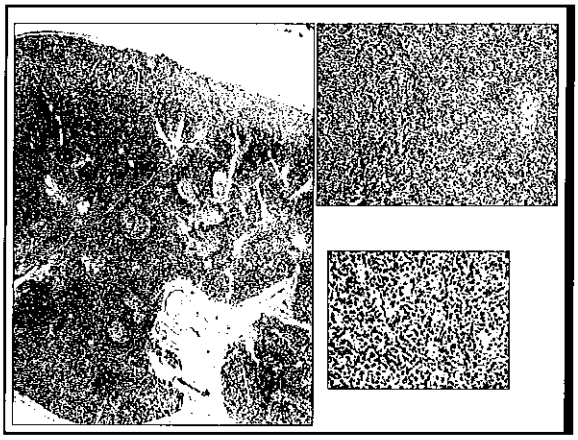
## RECENT CASE

Lymph Node

**Submitted history:**

- R/O Kaposi Sarcoma, very concerned for vascular tumor



What IHC stain is this? What is the diagnosis?

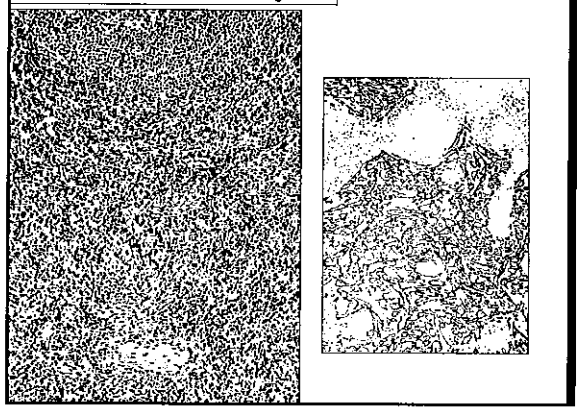


PLATE LX SPLENECTOMY

7

# AN OVERVIEW OF SPLENIC PATHOLOGY

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Adjunct Associate Professor
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EPS

## 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:**

**DENNIS P. O'MALLEY, MD –**

**CLARIENT/GE HEALTHCARE - SALARY/EMPLOYEE**

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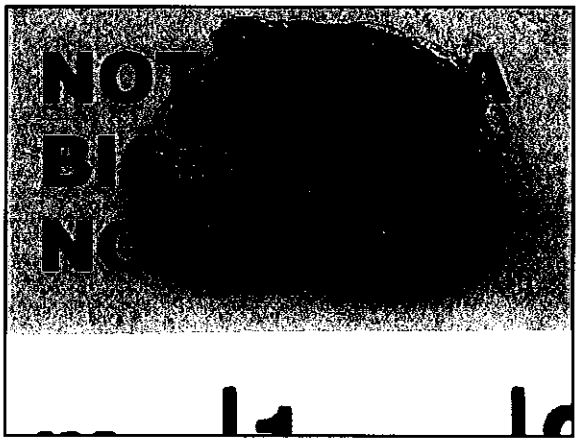
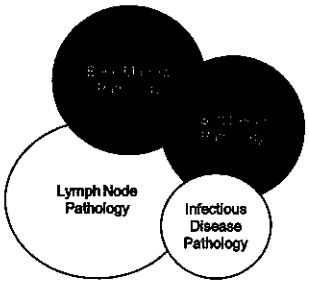


## WHAT MAKES A SPLEEN PATHOLOGIST?




## WHAT MAKES A SPLEEN PATHOLOGIST?

## SPLENIC FUNCTIONS

I. Filtration

A. Culling-erythrocyte (or other blood cell) destruction
1. Physiologic (as red blood cells age)
2. Pathologic
a. Associated with blood cell abnormalities
b. Associated with primary splenic changes

B. Pitting ("facelifting" of erythrocytes)
1. Removal of cytoplasmic inclusions
2. Remodeling of cell membranes

C. Erythroclasis—destruction of abnormal red blood cells with liberation into circulation of erythrocyte fragments

D. Removal of other particulate material (e.g., bacteria, colloidal particles)

II. Immunologic

A. Trapping and processing of antigen

B. "Homing" of lymphocytes

C. Lymphocyte transformation and proliferation

D. Antibody and lymphokine production

E. Macrophage activation

III. Reservoir

A. Storage or normal sequestration of platelets, granulocytes

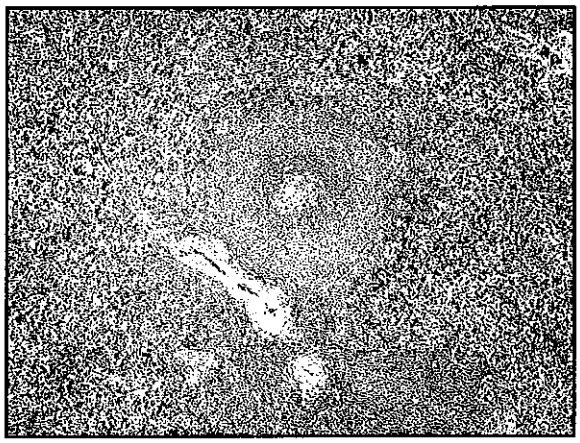
B. Recycling of iron

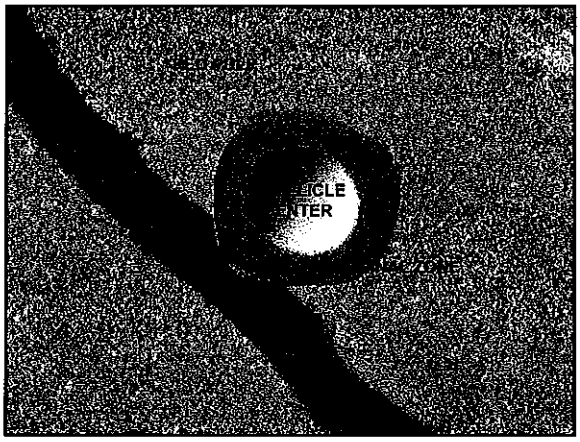
C. Red blood cell storage (minor in humans)

IV. Hematopoietic

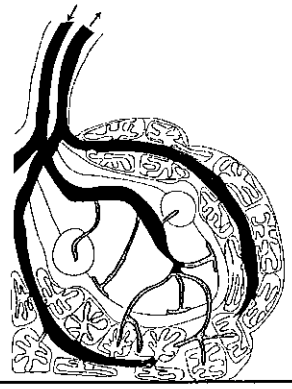
A. Erythropoiesis, granulopoiesis, megakaryopoiesis (not a normal function in fetal or adult spleens)

B. Lymphocyte and macrophage production

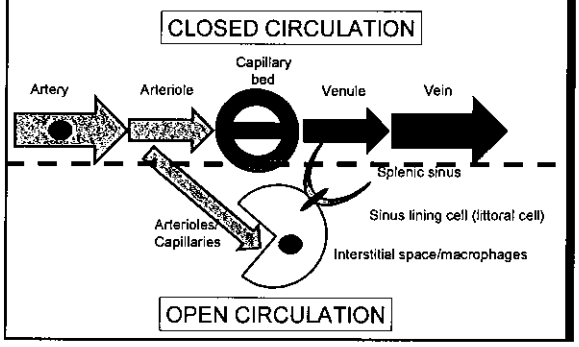





## HOW THE SPLEEN WORKS...



## VASCULAR ARCHITECTURE OF SPLEEN




## 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**

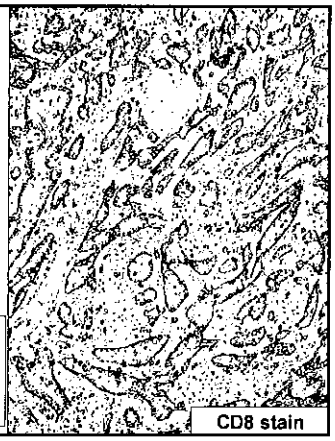
**CD34: arterioles positive, but not sinuses**

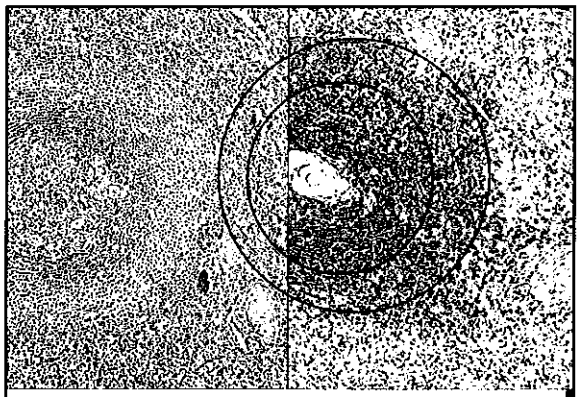
## ARCHITECTURE OF THE SPLEEN

**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!**



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**

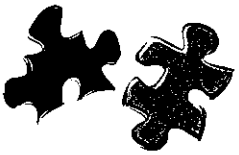
**Note normal pattern versus miliary or beefy**

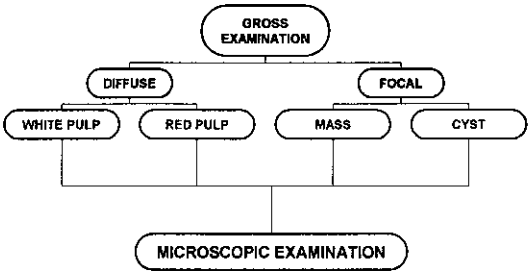
**Sections for histologic examination**

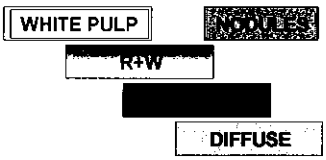
- Thinner sections of involved, interface and unaffected spleen
- Capsule should be included if any abnormalities are noted
- Splenic hilar lymph nodes should be included, if present
- Thinner sections (5 mm)
- Blot to remove blood*
- Place into adequate amounts of fixative (at least 10x volume of tissue

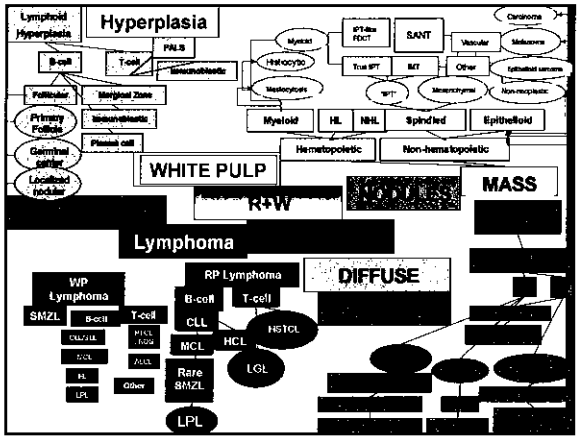
* Blood will interfere with fixation and make interpretation more difficult.

# AN ORGANIZED APPROACH TO DIAGNOSIS IN THE SPLEEN

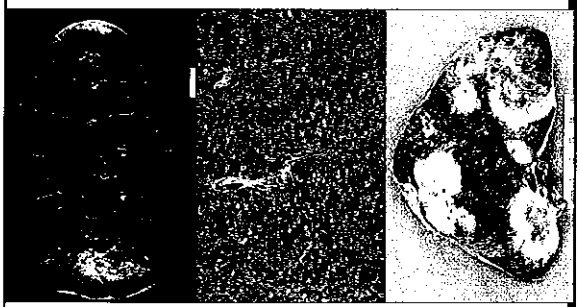





## GROSS APPEARANCE



RP – "beefy" WP – "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 Lymphomas

WHITE PULP

Other "small B-cell lymphomas"

- Follicular lymphoma
- Mantle cell lymphoma
- Lymphoplasmacytic lymphoma

Splenic Marginal Zone Lymphoma

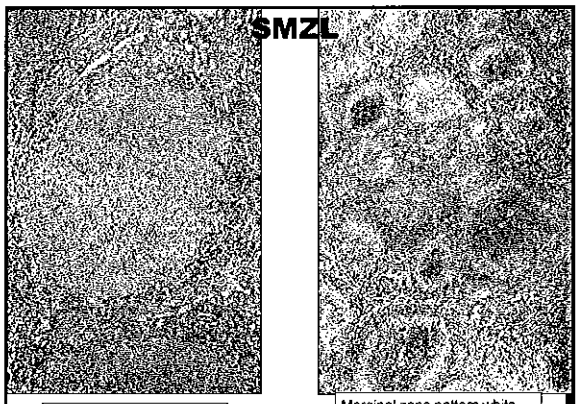
Everything Else

## 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

Miliary Pattern

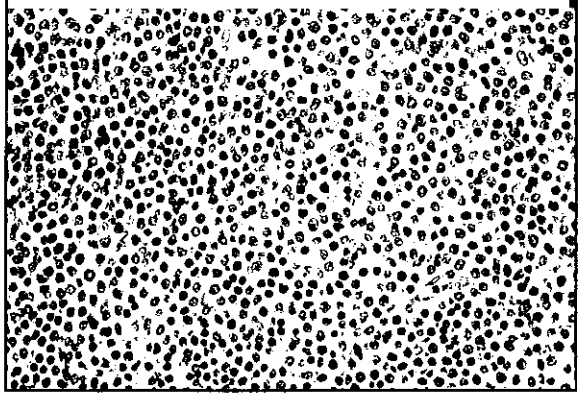
## SMZL

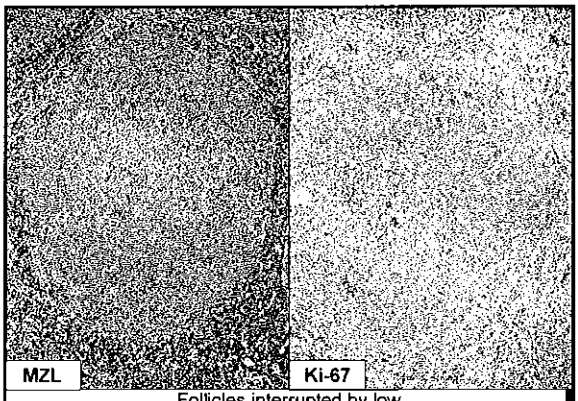


Uniform white pulp nodules

Marginal zone pattern white pulp nodules

## SMZL CYTOLOGY



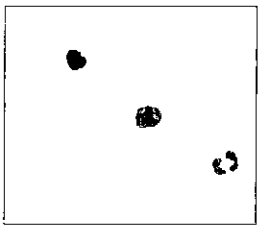


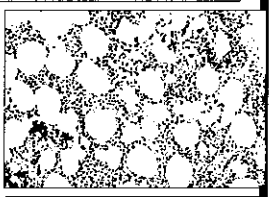
MZL

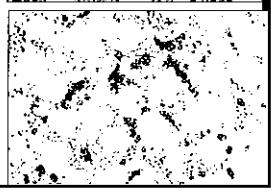
Ki-67

Follicles interrupted by low proliferation, Marginal Zone

## SMZL: BLOOD AND BONE MARROW







- Villous lymphocytes
- Intrasinusoidal, subtle involvement in bone marrow

## SPLENIC HYPERPLASIA MARGINAL ZONE

**Expansion of the outer (third) layer of splenic follicle structure**

**CLINICAL: variety of causes. Notably autoimmune phenomenon (ex. ITP, autoimmune hemolytic anemias)**

Cytology

- Small lymphocytes, with increased cytoplasm (monocytoid)
- Occasional plasma cells or plasmacytoid lymphocytes (Russell bodies may be present; Dutcher bodies very rare)
- Occasional larger, transformed cells

**Hyperplasia defined as increased of layer thickness to >12 cells***

Farhi DC. AJCP 1995.
Kroft SH. Mod Pathol 1997.

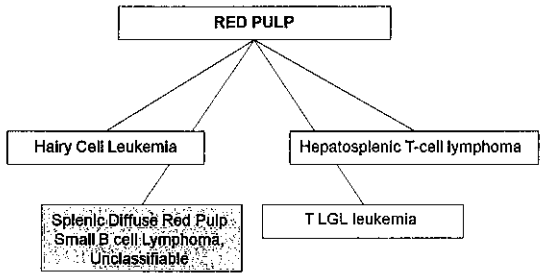
| Features | MZ Hyperplasia | MZ Lymphoma |
|---|---|---|
| **Expanded MZ (>12 cell layers)** | Yes | Yes |
| **Numerous B-cells in PALS** | No | Yes |
| **Follicles** | 3 layers | Loss of layers; often uniform single layer |
| **Red Pulp** | No increase in red pulp B-cells | Often has clusters of red pulp B-cells |
| **Clonality** | No | Yes |
| **Kappa/lambda** | Polyclonal | Monoclonal |
| **KI-67** | **Normal secondary follicles with high proliferation** | **Colonized follicles have low proliferation in neoplastic cells** |

## 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

## Distinctive Splenic Lymphoma/Leukemias




## HAIRY CELL LEUKEMIA

**Small B-cells, abundant cytoplasm**

**Diffuse red pulp involvement**

**Blood lakes, lined by tumor cells, not sinus cells**

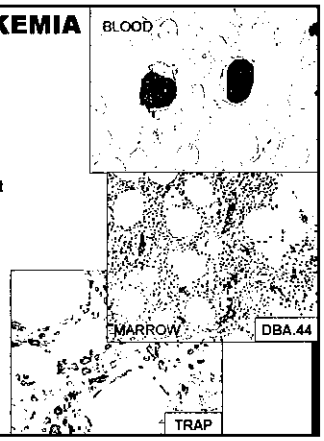
**PB, BM and splenic involvement**

**Immunophenotype: CD103, CD22, CD11c**

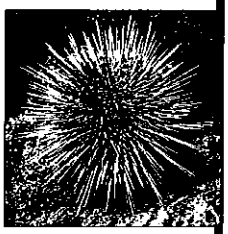
**IHC: TRAP, DBA.44, Annexin A1, BRAF**

**Monocytopenia, other cytopenias**

**BRAF mutation seen in most cases**



## HAIRY CELL LEUKEMIA PERIPHERAL BLOOD FINDINGS

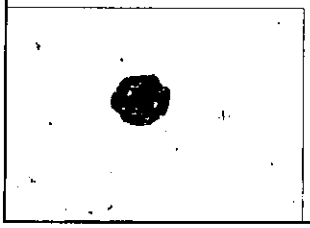


**Cytopenias**

- Especially monocytopenia

**Cytoplasmic projections**

- Will often indent adjacent red blood cells



## HAIRY CELL LEUKEMIA MARROW FINDINGS

**Often hypocellular**

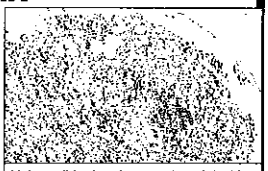
**Aggregates are most often interstitial**

**May be deceptively extensive**

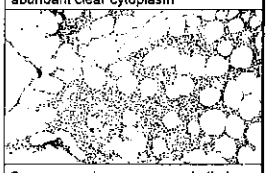
**Fried-egg appearance**

- Round nucleus with central placement, surrounded by moderate to large amounts of clear cytoplasm

**Severe fibrosis (DRY TAP)**



Hairy cell leukemia: round nuclei with abundant clear cytoplasm



Some cases have a more spindled appearance

## HAIRY CELL LEUKEMIA

SPLEEN

Small, "blood lake"

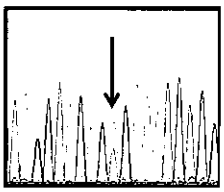
## BRAF MUTATION IN HAIRY CELL LEUKEMIA

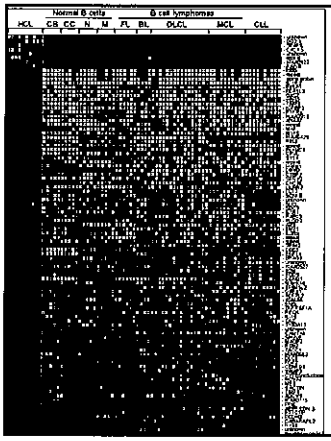
**Greater than 90% of HCL patients have evidence of *BRAF* V600 E mutations**

**While current therapies are effective, this raises the possibility of anti-BRAF therapies (vemurafenib)**

**Provides diagnostic confirmation of HCL**

**Excludes almost all other lymphomas**






## GENE ARRAY STUDIES OF HAIRY CELL LEUKEMIA

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

From Basso 2004, J Exp Med

## 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

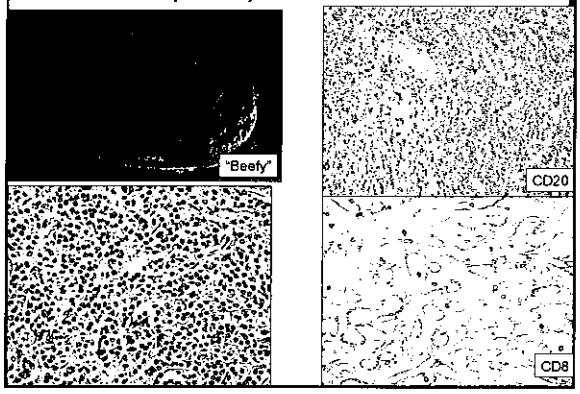
Some were identified as hairy cell leukemia

TYPES

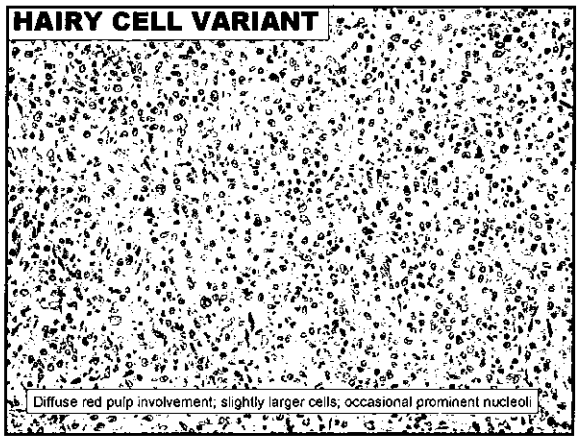
**Splenic diffuse red pulp small B cell lymphoma**

**Hairy cell leukemia-variant**

## SPLENIC DIFFUSE RED PULP SMALL B CELL LYMPHOMA (SDRPL)




## HAIRY CELL VARIANT




| HCL | HCL-V |
|---|---|
| WBC not elevated | Elevated WBC |
| Moderate-severe diffuse bone marrow fibrosis | No marrow fibrosis |
| Positive for TRAP staining | Rare positivity for TRAP staining |
| Neutropenia | Neutropenia no typical |
| Pancytopenia | Cytopenias may be present but not usual |
| Monocytopenia | Monocytopenia rare |
| Response to cladribine/interferon alpha | Minimal or no response to cladribine/interferon alpha |
| BRAF V 600E mutation (~90%) | No evidence of BRAF mutation |

## SUMMARY OF FLOW CYTOMETRIC FINDINGS SPLENIC SMALL B CELL LYMPHOMAS

| Flow | SMZL[b] | HCL | HCL-V[c] | SDRPL[d] |
|---|---|---|---|---|
| CD22 | + | + | + | |
| CD11c | +/- (50%) | + | -/+ (15-25%) | + (97%) |
| CD25 | -/+ (up to 33%)[a] | + (99%) [a] | - | - (3%) |
| CD103 | -/+ (15-25%)[b] | + | +/- (66%)[a] | -/+ (38%) |
| CD5 | -/+ (20%) | - | - | -/+ (14%) |
| CD123 | -/+ (3%) | + (95%) | - (9%) [a] | -/+ (16%) |
| FMC7 | + | + [a] | + | |
| CD23 | -/+ (30%) | - [a] | - | - |
| CD10 | "infrequent" | Rare [c] | | - |

a. Matutes 2006; b. Matutes 2008; c. Cessna 2005; d. Traverse 2007; e. Robbins 1993

## 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

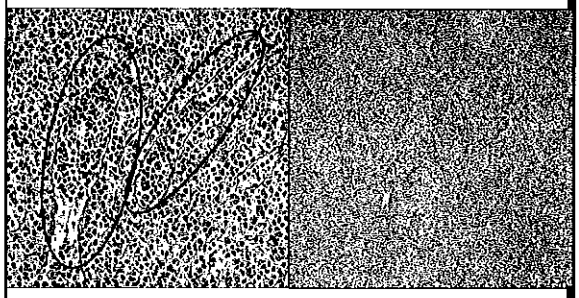
CD4/CD8 double negative

TCR γ/δ+

Isochromosome 7q, +8

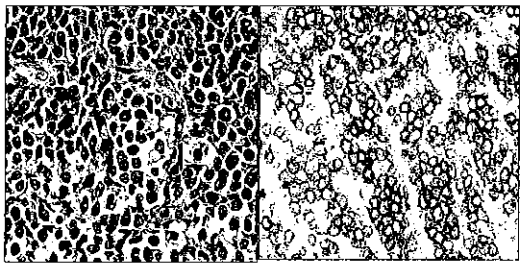
EBV negative

## HSTCL



Red pulp:note the characteristic intravascular localization

## HSTCL



Atypical cells, medium sized, pale cytoplasm, strongly CD3+

## MARROW FINDINGS

Histology:

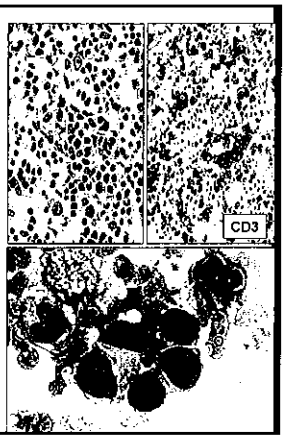
Marrow hypercellular

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

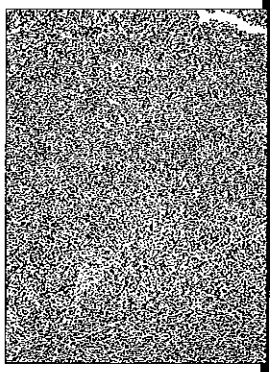
Association with autoimmune disease (especially rheumatoid arthritis)

Often associated with neutropenia and other cytopenias

Increased small lymphocytes in red pulp of spleen, sparing of white pulp

Immunophenotype: CD2+/CD3+/CD8+, frequent coexpression of CD57, CD16, dim expression or loss of CD5 by flow cytometry

Clonal T cell gene rearrangements by PCR

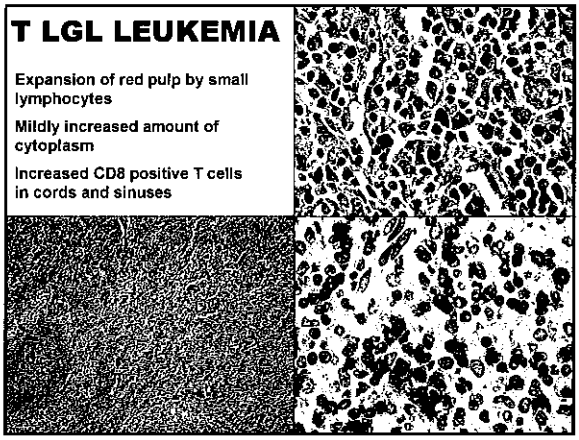


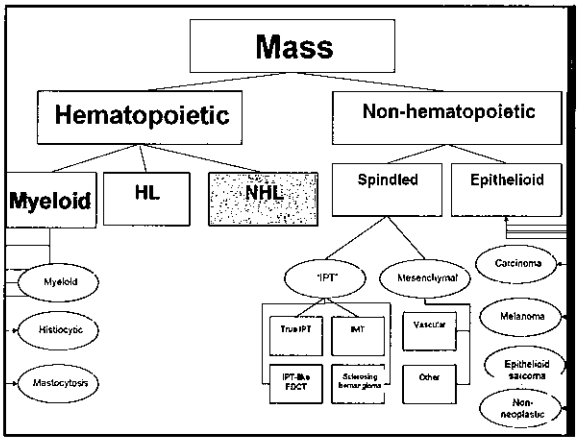
## T LGL LEUKEMIA

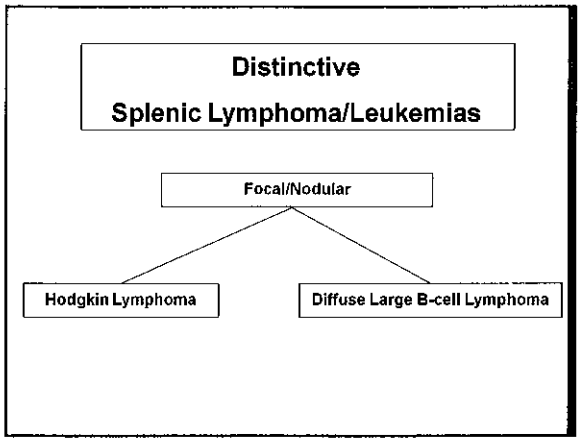
Expansion of red pulp by small lymphocytes

Mildly increased amount of cytoplasm

Increased CD8 positive T cells in cords and sinuses



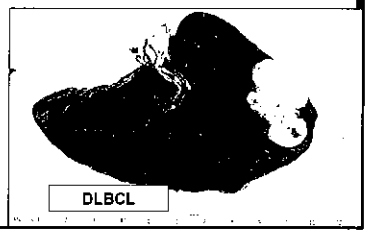


## APPROACH TO LYMPHOID LESIONS OF SPLEEN

**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*




## A FEW WORDS ABOUT HODGKIN LYMPHOMA IN SPLEEN

**Is it common?**

- *Yes, depending on stage*

**Why don't we see spleens with Hodgkin very often?**

- *Used to be part of laparoscopic staging; now done by radiologic staging*

**Is it easy to subtype CHL in spleen?**

- *No, and probably shouldn't be subtyped in this location*

**Does Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) occur is spleen?**

*Very rarely involves spleen. Only high stage disease, which is very rare.*

## SECONDARY 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**

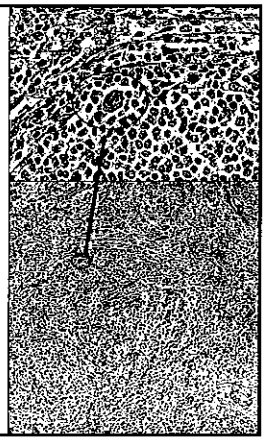
## HODGKIN LYMPHOMA

**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**

**Sarcoidal-type granulomas**

**Hodgkin cells/R-S cells seen**



## SPLENIC HYPERPLASIA OVERVIEW

**Follicular hyperplasia**

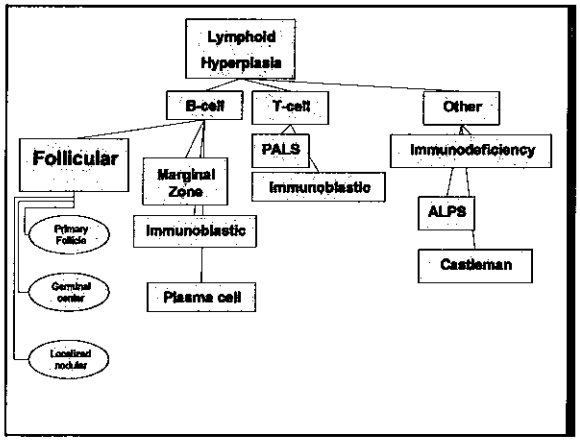
**Marginal zone hyperplasia**

**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 versus lymphoma
- DIFFERENTIAL DIAGNOSIS: follicular lymphoma, other B-cell/white pulp lymphomas

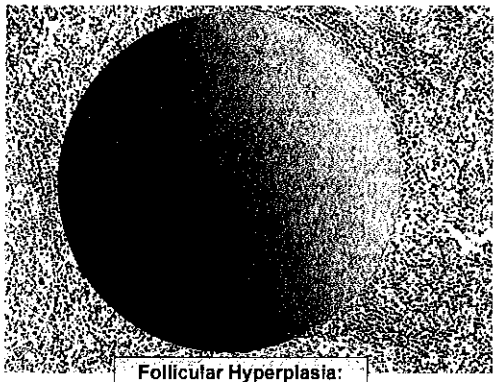
**Most often seen in ITP**

**Commonly observed in other autoimmune disorders (e.g. SLE, RA/Felty syndrome, AIHA)**

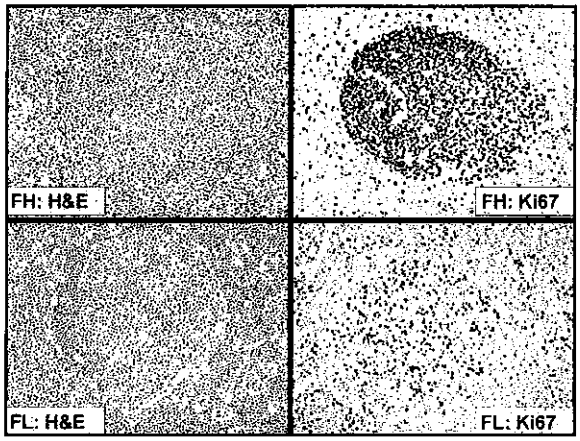
**Infections (AIDS)**

**Miscellaneous**

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

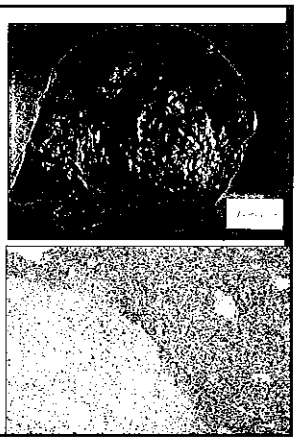


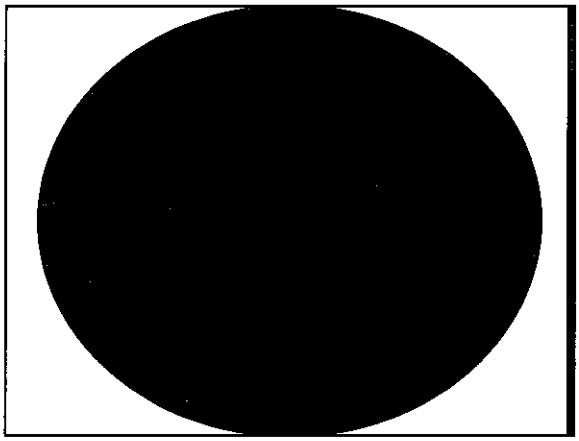
Follicular Hyperplasia: Polarization




## 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**





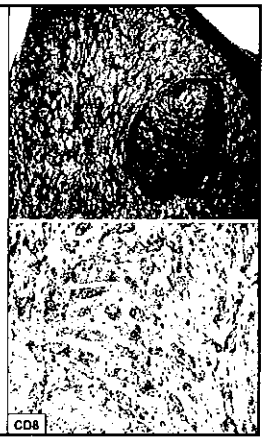
| | Etiology | Distinguishing Features |
|---|---|---|
| Inflammatory pseudotumor | Reactive, most probably secondary to infectious causes. | Prominence of inflammatory and/or sclerotic changes. Clinical history. |
| Inflammatory pseudotumor-like follicular dendritic cell tumor | Neoplasm of follicular dendritic cells; EBV-associated. | Spindle cells are CD21/CD35 positive. EBV positive. |
| Inflammatory myofibroblastic tumor | Neoplasm of myofibroblastic cells. | Increased smooth muscle actin/HHF-35 positive spindle cells. Occasionally ALK positive. Cytogenetic abnormalities mat be present. |
| Capillary hemangioma with prominent sclerosis/SANT | Benign neoplasm of vascular components and/or reactive vascular process | Simulates IPT but with prominent vasculature. Lobular nature of involvement is a diagnostic clue. Vascular markers highlight prominent capillaries (CD34+). |

## VASCULAR LESIONS OF SPLEEN
## DIFFERENTIAL DIAGNOSIS

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

## 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




| | Diagnosis | Features | Immunos |
|---|---|---|---|
| Non-neoplastic | Peliosis | Rare. Unknown etiology. Ectatic sinusoids and blood-filled spaces. | Endothelial lining positive for CD31/FVIII/Ulex/WT1 |
| | Hamartoma | Red pulp, very little or no white pulp seen. | CD31+, FVIII+, CD8+, WT1 |
| Benign neoplasms | Hemangioma | Most common vascular neoplasm of spleen. Cavernous or capillary. Single or multiple. Similar features to other body sites. | CD31+, FVIII+ CD34+ |
| | Lymphangioma | Thin lining, not keratin positive. Filled with proteinaceous fluid. Rarely single, more often part of multisystem lymphangiomatosis | Must rule out mesothelial cyst which are keratin +. Lined by CD31+ FVIII+WT1+D240+ cells. |
| | Littoral cell angioma | Neoplasm of sinus lining cells. Larger, plump cells. May have papillary features and form cystic spaces. Focal hemophagocytosis | CD31+, FVIII+, WT1+, CD34-, CD68+, often CD21+ |
| Malignant neoplasms | Angiosarcoma | Vascular malignancy. May have solid areas. Anaplasia, mitoses, necrosis not uncommon. | CD31/FVIII/Ulex/WT1/Flt variably positive. CD34+ in 50%. CD6 variable |

## NON-HEMATOPOIETIC: VASCULAR LESIONS

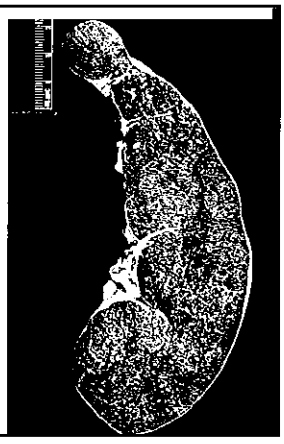
**Littoral Cell Angioma**

**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



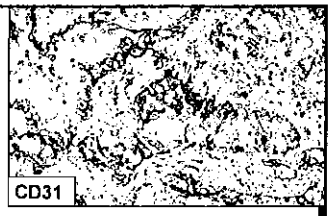
## Littoral Cell Angioma

- Microscopic
  - Anastomosing, patent vascular spaces
  - Lined by plump, splenic endothelial-derived cells
  - Occasionally papillary projections
  - Luminal macrophages
  - Mitoses rare
  - Macrophages may be prominent in cords
  - Variable fibrosis and sclerosis

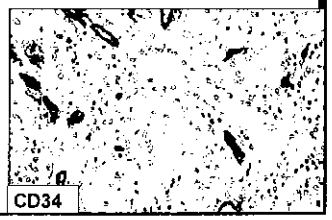
**LITTORAL CELL ANGIOMA**

**Immuno**

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



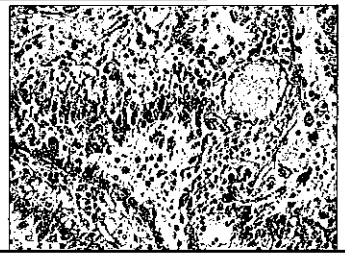
CD31



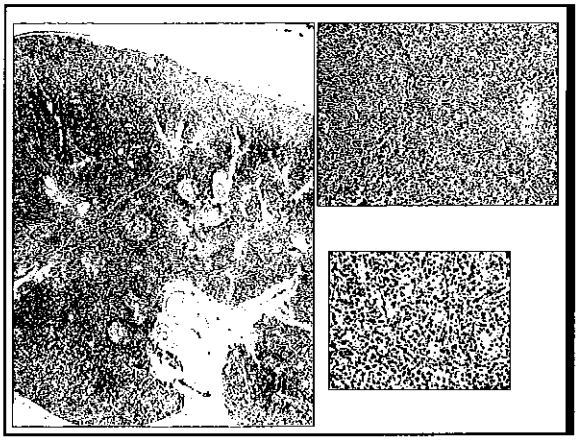
CD34

**LITTORAL CELL ANGIOMA**

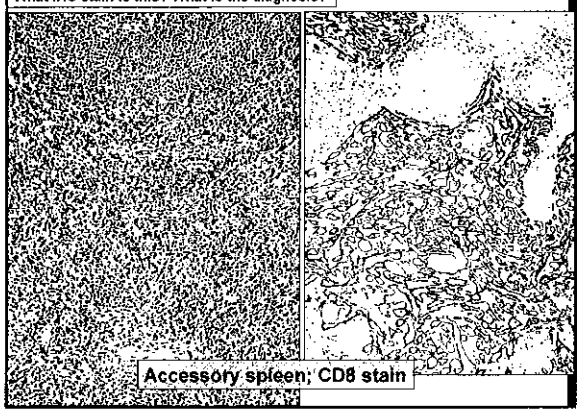
| | | | |
|---|---|---|---|
| CD34 | - | + | - |
| CD8 | - | - | + |
| WT1 | + | + | + |



***QUESTIONS?***



What IHC stain is this? What is the diagnosis?



Accessory spleen; CD8 stain

**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 you name with materials.

domalley@clarientinc.com

THAT, IN A SPLEEN, UNFOLDS BOTH HEAVEN AND EARTH...

*Wm. Shakespeare*

*A Midsummer Night's Dream*

domalley@clarientinc.com