Current Issues in Practical Hematopathology: Diagnosis of Bone Marrow Lymphomas

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Disclosures

I have no disclosures relevant to the content of this lecture

Outline of lecture

Overview the approach to the use of bone marrow sampling in lymphoma diagnosis and staging
Review key diagnostic features of lymphomas that involve the bone marrow
  – Primary marrow/blood lymphomas/leukemias
  – Secondary marrow involvement by extramedullary lymphomas
Emphasize differential diagnosis and the appropriate use of ancillary studies

Clinical scenarios for bone marrow sampling in lymphoma diagnosis

To establish a diagnosis and classify a lymphoid leukemia
  – Clinical manifestation: peripheral lymphocytosis
To stage a lymphoma diagnosed on biopsy of an extramedullary tissue
To establish a diagnosis of a lymphoma suspected clinically, but not yet proven
  – Unexplained lymphadenopathy, splenomegaly, extramedullary mass, and/or paraprotein
**Important data in marrow evaluation for lymphoma**

- Bone marrow biopsy
  - Disease burden
  - Pattern of lymphomatous involvement
- CBC findings, lymphadenopathy/splenomegaly
  - Establish presence of ‘neoplastic cell mass’
- Flow cytometry of aspirate and/or blood
- Cytogenetics/FISH*
- Molecular diagnostic studies*
- Bone marrow aspirate often not very helpful
  - May be falsely negative
  - Cytomorphology usually better in blood

*In special cases

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**The bone marrow biopsy in lymphoma/leukemia diagnosis**

- Large sample important
  - Suggested minimum length of 1.2 cm
  - Bilateral is probably more sensitive, but most clinical outcome studies based on unilateral
- Gentle decalcification
  - Enhances morphology and immunogenicity
- H&E (at least 2 levels) and reticulin stains
  - Focal reticulin increase can draw attention to paratrabecular aggregates missed on casual review of the H&E


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**Quantifying marrow lymphomatous involvement**

- Estimate percentage of involvement
  - ‘Minimally involved’, ‘focally involved’, ‘extensively involved’ too subjective
- Important to establish baseline involvement prior to therapy
- Two methods of expressing
  - Percentage of cellularity (excluding adipocytes)
  - Percentage of intertrabecular marrow space (including adipocytes)
Non-paratrabecular nodules: reactive or neoplastic?

**Reactive**
- Usually few in number ($\leq 3$)
- Small size
- Located only in hemopoietic marrow
- Smooth borders with surrounding fat
- T-cells usually predominate; may have B-cell follicles

**Neoplastic**
- More frequent
- Large size
- May be present in subcortical fatty marrow
- Infiltrate surrounding fat and marrow
- B-cells usually predominate

Immunohistochemistry often unhelpful to distinguish reactive from neoplastic lymphoid aggregates
Reactive germinal centers and increased reticulin may be present in both
Interstitial pattern (HCL, CD20)

Intrasinusoidal pattern (SMZL, CD20)

Diffuse pattern (CLL)

Chronic lymphocytic leukemia (CLL)
- Low-grade B-cell leukemia involving bone marrow and blood with characteristic immunophenotype
- Immunophenotype and genetics generally allow clear distinction from other low-grade B-cell NHLs
CLL versus SLL versus MBL

- **CLL-like cells in blood**: CD20dim+, CD5+, CD23+, CD10-, monotypic light chain
  - Monoclonal B-cell count >=5 x 10^9/L*
  - Lymphadenopathy with biopsy showing SLL
  - CLL
- Monoclonal B-cell count <5 x 10^9/L
  - No lymphadenopathy
  - SLL
  - **MBL**

*Can also diagnose as CLL if patient has splenomegaly and/or cytopenias related to bone marrow infiltration

**No level of bone marrow involvement defined that would establish CLL (vs SLL or MBL)

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B-cell prolymphocytic leukemia (B-PLL)

- **Aggressive de novo B-cell leukemia**
  - Marked and rapidly increasing leukocytosis
  - Splenomegaly and systemic symptoms
    - Usually lack significant lymphadenopathy
  - Median survival only 2-4 years
- **Prolymphocytes >55% of all circulating lymphoid cells**
  - CD20bright+, FMC7+, CD5-/+, CD23-/+
  - Del(17p) and del(13q)common, no t(11;14)

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The spectrum of CLL lymphocytes

**Prolymphocytes**

- At least 1.5-2x the diameter of small lymphocytes
- Prominent central nucleoli
- Moderately dispersed chromatin
- Moderately abundant pale basophilic cytoplasm

**Small lymphocytes**
CLL vs PLL

- Generally stable or slowly increasing WBC
- Prolymphocytes may be increased, but <55%
- CD20dim+, sIgdim+, FMC7-, CD5+, CD23+

Appearance in bone marrow and lymph nodes may be indistinguishable

Rapidly increasing WBC
- Prolymphocytes are >55%
- CD20br+, sIgbr+, FMC7+, CD5/23 often -

Lymphoplasmacytic lymphoma

- Post-germinal center B-cell lymphoma with plasmacytic differentiation
  - IgM protein in >90% of cases (Waldenstrom's)
    - Often have hyperviscosity
    - Association with hepatitis C
  - Bone marrow usually heavily involved
    - Interstitial, nodular, or diffuse pattern of involvement

- Spectrum of small lymphocytes, 'lymphocytes', and plasma cells in biopsy and aspirate
  - CD20+, monotypic IgM, usually CD5/23-
**LPL: Differential diagnosis**

- **CLL may have plasmacytic differentiation**
  - CD5+, CD23+, CD20dim unlike LPL
  - IgM paraprotein, if any, is usually low-level
- **Splenic marginal zone lymphoma**
  - Intrasinusoidal marrow involvement
  - Usually less prominent plasmacytic differentiation
  - IgM paraprotein, if any, is usually low-level
- **Small-cell plasma cell myeloma (PCM)**
  - *MYD88* point mutation recently identified in ~90% of LPL; rare in myeloma and MZL

*Treon NEJM 2012; 367: 826-833*

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**LPL versus small cell PCM**

- IgM paraprotein
- Monotypic surface immunoglobulin
- CD138 subpopulation
- CD19+, CD45+, PAX5+ subpopulation
- CyclinD1-
- *MYD88* mutated

- Non-IgM paraprotein
- Few or no cells with surface Ig
- All cells CD138+
- Neoplastic cells are CD45-, CD19-, PAX5-
- Often CyclinD1+
- *MYD88* wild-type

*Small cell PCM is often CD20+*
Hairy cell leukemia (HCL)

- Mature B-cell lymphoma involving blood, bone marrow and spleen
  - Symptoms related to cytopenias (monocytopenia nearly ubiquitous at diagnosis)
- Hairy cells in blood are often rare
  - Leukocytosis very uncommon
  - Interstitial bone marrow infiltration pattern
  - Diffuse pattern in advanced cases; nodules are rare
- CD20bright+, CD5-, CD10-
  - CD11c+, CD103+, CD25+
  - Also CD123, TRAP, DBA.44, annexinA1, cyclinD1 (weak)
HCL in bone marrow aspirate

HCL in blood

HCL in subtle interstitial pattern
Diagnostic issues with HCL

- May be missed if diagnosis is not considered
  - Monocytopenia is a helpful clue
  - Consider performing CD20 on bone marrow in cases of unexplained cytopenia
  - Can be misdiagnosed as MDS
- Critical to distinguish from other low-grade B-cell lymphomas, as treatment is distinct
  - \textit{BRAF} mutation highly specific for HCL, but rarely needed
- Integrate all available diagnostic information
  - CBC findings
  - Interstitial bone marrow infiltration pattern
  - Usual presence of splenomegaly
  - Characteristic immunophenotype

Tiacci E et al. NEJM 2011; 364: 2305

Large granular lymphocyte leukemia (LGL)

- Indolent T-cell leukemia involving bone marrow and peripheral blood
  - Cytopenic (usually neutropenic)
  - Associated with autoimmune diseases
- Increased circulating clonal LGL (>2 x 10^9/L)
- CD3+, CD8+, CD57+, CD16+, TCRαβ+
  - Express cytotoxic markers (TIA1, granzymeB)
  - Variants may be CD4+, CD4-/CD8-, or TCRγδ+
- Interstitial and intrasinusoidal bone marrow patterns; non-paratrabecular reactive B-cell follicles also common

Tiacci E et al. NEJM 2011; 364: 2305
Diagnostic issues with LGL leukemia

- Distinction from reactive increase in LGLs
  - Post-splenectomy
  - Post-transplant (organ or BMT)
  - Viral infections or paraneoplastic
  - Autoimmune diseases and Felty’s syndrome
- LGL leukemia cells are morphologically identical to normal/reactive LGLs
- Apply diagnostic criteria!
  - LGL increase should be documented for >6 months
  - Proof of TCR clonality by PCR
  - Immunophenotypic aberrancy helpful
    - Uniformly strong CD57, often weak CD5, CD7, and/or CD8
  - Cytopenias +/- splenomegaly

Ohgami RS Leukemia 2011; 25: 1439

General issues in lymphoma staging

- Positive marrow should be histologically evident disease
  - Clone only detected by flow cytometry and/or PCR is not considered as a positive staging marrow
- Marrow lymphoma appearance may differ from primary
  - Review the extramedullary lymphoma for comparison
- Biopsy much more sensitive than aspirate at detecting lymphoma

Problems in trying to primarily classify lymphoma on a bone marrow sample

- Infiltration pattern is usually non-specific
  - Paratrabecular nodules tend to exclude CLL
- Significant overlap in immunophenotypes
  - CD5-, CD10-, CD23- small B-cell lymphoma can be LPL, MZL, FL, or DLBCL (discordant)
  - CD5+ MZL, LPL, and HCL may occur
- Marrow often discordant from lymph node
  - DLBCL or grade 3 FL in node may show small cell involvement of marrow

Arber DA, George TI. AJSP 2005
Mantle cell lymphoma (MCL)

- PB involvement in almost all patients
  - 30% have $>5 \times 10^9/L$ circulating MCL cells
  - 5-10% have frank leukemic presentation
  - MCL cells may have more prominent nucleoli than in tissue sections, resembling prolymphocytes
- BM involvement in almost all patients
- Nodular (including paratrabecular), interstitial, and/or diffuse patterns

Cohen PL Br J Haematol 1998
**Follicular lymphoma (FL)**

- Rarely can present as leukemia with marked leukocytosis mimicking CLL
  - Concurrent splenomegaly and lymphadenopathy are almost always present
  - Cells more irregular and clefted than CLL cells
- Bone marrow usually performed to evaluate newly diagnosed clinically Stage I/II FL
  - BM involved in 40-70% of cases
- Paratrabecular involvement in 85% of cases
  - Non-paratrabecular nodules are also common

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**Paratrabecular aggregates**

- Can occur in any lymphoma except CLL
- Elongate along bone trabecula, often only 2-3 cells thick at ends
  - Non-paratrabecular aggregates may touch trabecula, but are spherical
- Reticulin stain can reveal subtle paratrabecular aggregates
- May be under-sampled or missed entirely in aspirate (e.g. flow cytometry)
Almost all SMZL patients have some circulating neoplastic cells in blood
- Most have absolute lymphocytosis, but marked leukocytosis is uncommon
Involves bone marrow in ~100% of cases
- Lymphocytosis may precede splenomegaly
Intrasinusoidal and nodular non-paratrabecular
- Nodules may contain reactive germinal centers
- Post-splenectomy pattern more nodular

Intrasinusoidal pattern
- Linear arrays or chains of 3-5 of lymphocytes
  - May not be able to clearly discern vascular space
  - Usually not clearly evident on H&E and must be revealed by immunostains
- Not specific for SMZL
  - Can also occur in LGL, HCL, FL, CLL, IVLBCL

Diffuse large B-cell lymphoma

- Bone marrow is usually performed, as it influences IPI and prognosis
  - Likelihood of involvement is very low in Stage I/II disease
  - Marrow involved in 11-27% of DLBCL cases
  - May rarely present as primary marrow disease in elderly or HIV+ patients
- Can have any pattern of involvement

Concordant marrow involvement in DLBCL

- About 50% of positive staging marrow cases
- Marrow lymphoma is composed predominantly of large cells resembling the extramedullary DLBCL
- Associated with poorer prognosis and increased risk of CNS relapse than discordant involvement

Kremer M et al. Lab Invest 2003

Discordant marrow involvement in DLBCL

– About 50% of positive marrow staging cases
– Marrow lymphoma is composed of small neoplastic cells
  - Often resembles FL, with paratrabecular aggregates
– 1/3 of cases are clonally unrelated to the extramedullary DLBCL
– Discordant vs concordant involvement should be specified in the report

Kremer M et al. Lab Invest 2003

Intravascular large B-cell lymphoma

- Rare, highly aggressive lymphoma in which tumor cells grow within vascular lumina of various organs
  - Bone marrow, spleen, liver, skin, CNS
- ‘Asian’ variant usually has bone marrow involvement and is often CD5+
  - Presentation as FUO, cytopenias, hepatosplenomegaly
  - Hemophagocytic syndrome in ~60% of cases
Hodgkin lymphoma

- Bone marrow staging usually performed in newly diagnosed classical Hodgkin lymphoma cases
  - Positive in 5-10% of adult and 2% of pediatric cases
    - <1% positive in clinical Stage IA/IIA disease
    - More frequently positive in HIV+ patients and in lymphocyte-depleted subtype (up to 75%)
- NLPHL only rarely involves the bone marrow (<2% of cases)

Hodgkin lymphoma

- Diffuse pattern, often with nodules
- RS cells and variants in typical background
  - If prior known CHL, only need RS variants
- Adjacent uninvolved marrow frequently shows reactive changes
  - Eosinophilia, plasmacytosis, lymphoid aggregates, granulomas

Ponzoni M et al. Mod Pathol 2002
Differential diagnosis

- Lymphomas
  - ALCL
  - T-cell/histiocyte-rich DLBCL
  - EBV+ DLBCL of the elderly

- Myeloid neoplasms
  - Primary myelofibrosis
  - Hyperesoinophilic syndrome

- Metastatic carcinoma, melanoma, other

- Usually can be resolved by IHC
Other findings in bone marrow from lymphoma patients

- Dysplasia of hemopoietic elements
  - HCL, T-cell lymphomas, and DBLCL
  - Marrow may or may not be involved by lymphoma (‘paraneoplastic’ dysplasia)
- Increased reticulin deposition
- Granulomas
- Hemophagocytic syndromes
  - Particularly with EBV+ lymphomas

Nardi et al. Mod Pathol 2010 (abstract); Auger et al. J Clin Pathol 1986

Conclusions

- The bone marrow sample is one tool used in the diagnosis and classification of lymphoid lymphomas and leukemias
  - It is NOT always the ‘gold standard’ answer!
- Clinical context is critical in classifying lymphoid leukemias
- Correct diagnosis can usually be achieved by appropriate use of ancillary studies and stepping back to look at the overall clinicopathologic picture

Diagnosis of lymphoid leukemias

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<thead>
<tr>
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<th>Important diagnostic modalities</th>
<th>Bone marrow recommended?</th>
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<tbody>
<tr>
<td>CLL</td>
<td>PBL morphology &amp; flow FISH for prognosis</td>
<td>No, only as baseline prior to therapy</td>
</tr>
<tr>
<td>LPL</td>
<td>Paraprotein evaluation, biopsy of involved tissue</td>
<td>Yes</td>
</tr>
<tr>
<td>HCL</td>
<td>PBL morphology &amp; flow</td>
<td>Yes</td>
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<tr>
<td>LGL</td>
<td>PBL morphology &amp; flow TCR clonality testing</td>
<td>Usually not</td>
</tr>
<tr>
<td>T-PLL &amp; B-PLL</td>
<td>PBL morphology &amp; flow</td>
<td>Usually not</td>
</tr>
<tr>
<td>Aggressive NK leukemia</td>
<td>PBL morphology &amp; flow EBV testing</td>
<td>Yes</td>
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<tr>
<td>Adult T-cell leukemia/lymphoma</td>
<td>PBL morphology &amp; flow HTLV1 serology</td>
<td>Usually not</td>
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