Current Issues in Practical Hematopathology: Diagnosis of Bone Marrow Lymphomas

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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

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 paratrabeular aggregates missed on casual review of the H&E

Disclosures
- I have no disclosures relevant to the content of this lecture

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 (<=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

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
- Monoclonal B-cell count >=5 x 10^9/L
  - CD20dim+, CD5+, CD23+, CD10-, monotypic light chain

- Monoclonal B-cell count <5 x 10^9/L
  - CD20+, CD5-, CD23-, CD10-, polytypic light chain

- Lymphadenopathy with biopsy showing SLL
- No lymphadenopathy

- CLL
- 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)

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)

The spectrum of CLL lymphocytes

Small lymphocytes

Prolymphocytes
**CLL vs PLL**

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

**Appearance in bone marrow and lymph nodes may be indistinguishable**

- Rapidly increasing WBC
- Prolymphocytes are >55%
- CD20br+, slgbr+, 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, ‘plymphocytes’, 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
  - Intrasinoidal 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

**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)
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
  - 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+, TCR\(\alpha\beta\)+
  - Express cytotoxic markers (TIA1, granzymeB)
  - Variants may be CD4+, CD4-/CD8-, or TCR\(\gamma\delta\)+
- Interstitial and intrasinusoidal bone marrow patterns; non-paratrabecular reactive B-cell follicles also common

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

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 x 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


**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)

**Splenic marginal zone lymphoma**
- 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

Koemar 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 paratrabeclular 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

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**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

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**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)

Ponzoni M et al. Mod Pathol 2002

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**Differential diagnosis**

- Lymphomas
  - A LCL
  - 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

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**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
Hemophagocytic syndrome
- May precede the diagnosis of lymphoma
- Clinicopathologic diagnosis
  - Fever, splenomegaly, cytopenias, ↑ triglycerides, ↓ fibrinogen, ↑ ferritin, hemophagocytosis, ↓ NK activity, ↑ soluble CD25

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

<table>
<thead>
<tr>
<th>Diagnosis of lymphoid leukemias</th>
<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>
</tr>
<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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