ANCILLARY TECHNIQUES IN LEUKEMIA AND LYMPHOMA DIAGNOSIS

MICHAEL J BOROWITZ MD PhD
PROFESSOR OF PATHOLOGY
JOHNS HOPKINS UNIVERSITY
SCHOOL OF MEDICINE
TECHNIQUES TO SUPPLEMENT MORPHOLOGIC DIAGNOSIS

• Immunohistochemistry
• Flow Cytometry
• Polymerase chain reaction (PCR)
  – Antigen receptor
  – Translocations
• Cytogenetics and FISH
• New assays
  – Arrays
  – Sequencing
APPLICATIONS OF ANCILLARY STUDIES

• DIAGNOSIS
  – Demonstration of clonality
  – Aberrant antigen expression
  – Immunoarchitectural abnormalities (limited)

• CLASSIFICATION
  – Pattern of antigen expression
  – Presence of specific genetic abnormalities
Key points

• Limited material in cytologic specimens often requires efficient use of markers tailored to problem suggested by morphology
  – Workup of small lymphocytic lesions and lesions with large neoplastic cells is different
CASE 1

• 51 year old female with history of non-Hodgkin lymphoma
• Presents with new neck swelling and leg pain, determined to be deep vein thrombosis
• Fine needle aspirate and biopsy performed
• Replacement by B cells is prima facie evidence of a neoplasm

• Additional markers can help characterize low grade B cell lymphomas
USEFUL MARKERS IN SMALL B NEOPLASMS

- CD5/CD23/cyclin D1: CLL vs mantle cell
- CD10/bcl6/bcl2: follicular lymphoma
  - bcl2 expression informative for follicular lymphomas ONLY when looking at expression on follicles (i.e. CD10+ cells)
- Kappa and lambda stains rarely useful unless plasmacytoid differentiation apparent
- Ki-67 can help in grading
• CD10 and bcl6 indicate follicle center B cells
• Bcl-2 expression on CD10+ and bcl-6+ cells indicates FL
FOLLICULAR LYMPHOMA

Phenotype

• Follicles express B cell markers with numerous T cells surrounding them
• Typical phenotype is CD10+, bcl-2+, bcl-6+
• CD10 or bcl-6 may be lost in diffuse or interfollicular areas
• Dendritic network can be demonstrated with CD21/CD23, particularly useful in extranodal sites
CASE 2

- 65 year old female with sickle cell anemia
- Worsening anemia and renal insufficiency prompted bone marrow examination
- Small clonal B cell population discovered but not large enough to be diagnostic
- Imaging studies revealed lymphadenopathy
- Needle aspirate performed and sent for flow cytometry
FLOW CYTOMETRY

- The measurement of the optical properties of cells as they move individually in a fluid stream through a focused laser beam
- Advantages: quantitative assessment of correlated antigen expression on individual cells
  - Better than IHC in determining light chain restriction in B cell lymphomas
- Disadvantages: Loss of cellular detail.
  - Disaggregation may selectively lose important populations
APPLICATIONS OF FLOW CYTOMETRY TO LYMPHOMA

• Contributions to diagnosis
  • Demonstration of monoclonal B cell populations
  • Demonstration of abnormal antigen expression in T cell malignancies

• Contributions to classification
  • Description of detailed phenotype
  • Definition of scatter (size) of neoplastic cells
  • Assessment of proliferation or activation markers
FLOW CYTOMETRY FINDINGS

- Clonal B cell population
- Kappa light chain restricted
- CD5+
- Ig relatively bright
- Too little material for further characterization
CASE 2 IMMUNOHISTOCHEMISTRY

- Coordinate with flow lab
- Don’t repeat lots of markers already run, especially when material limited

Diagnosis: Mantle cell lymphoma
DISTINGUISHING LOW GRADE B CELL LYMPHOMAS FROM REACTIVE CONDITIONS

• Clonality
  – Ig light chain restriction (flow cytometry preferred technique)
  – Ig gene rearrangement
• Aberrant antigen expression
  – CD5 (CD43) or cyclin D1 on small B cells
  – bcl-2 on follicular B cells
• B cells where they don’t belong
CASE 3

• 69 year old man with history of prostate cancer
• Developed left axillary adenopathy
• Fine needle aspirate and biopsy performed
  – Inaspirable, but touch prep of core obtained
CYTOLOGY OF TOUCH PREP
CASE 3 CORE BIOPSY

• Large pleomorphic cells in mixed background

• Suggestive of Hodgkin lymphoma
HODGKIN LYMPHOMA IN NEEDLE BIOPSY CORE (RS CELLS CD15 AND CD30+)
CASE 3
LARGE CELLS ARE CD30-CD20+
CASE 3 DISCUSSION

• Large cells in polymorphous background can suggest Hodgkin lymphoma
• If cytology and immunophenotype are appropriate a definitive diagnosis of HL can be made on small specimens
• If large isolated cells are B cells care must be taken interpreting small specimens when architecture can’t be assessed
• In this case, required an excisional biopsy to establish a diagnosis of T cell rich large B cell lymphoma
CASE 4

- 41 year old woman with history of ALL with a CNS relapse
- Treated with intrathecal therapy
- Follow-up LP done
A few suspicious lymphocytes noted
APPLICATIONS OF FLOW CYTOMETRY TO LEUKEMIA

• Contributions to diagnosis
  • Demonstration of blasts where they don’t belong
  • Demonstration of abnormal antigen expression on blasts

• Contributions to classification
  • Determination of lineage
  • Expression of differentiation markers
FLOW CYTOMETRY FINDINGS

- Dim CD45, low SSC population characteristic of blasts
- CD19+CD10+ phenotype indicates B precursor ALL
CASE 5

- 55 yo man with a mediastinal mass
- Fine needle aspirate performed and sent for flow cytometry
- No core biopsy could be obtained
FLOW CYTOMETRY FINDINGS

- Major population of CD4+CD8+ (immature) T cells
- Lymphoblastic lymphoma?
MEDIASTINAL MASS
FLOW CYTOMETRY FINDINGS

- Immature T cells show **NORMAL** path of maturation (gain of CD3 and CD5; transition from CD4+8+ to separate 4+ and 8+ populations)
- Normal thymocytes, c/w thymoma
- Epithelial cells not recovered
MOLECULAR DIAGNOSTIC METHODS IN LYMPHOMA

• Immunoglobulin and T cell receptor gene rearrangement studies
  – Major use is to assess clonality
  – However, clonality does not equal malignancy

• Molecular assays for specific translocations
  – Useful in classification of lymphoma
  – FISH and PCR can both be used

• Newer methods
MOLECULAR ANALYSIS OF IGH REARRANGEMENTS BY PCR AND CAPILLARY ELECTROPHORESIS

polyclonal

monoclonal
## COMMON TRANSLOCATIONS IN LYMPHOMA

<table>
<thead>
<tr>
<th>CYTOGENETIC</th>
<th>MOLECULAR</th>
<th>LYMPHOMA</th>
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</thead>
<tbody>
<tr>
<td>t(2;5)(p23;q35)</td>
<td>NPM-ALK</td>
<td>CD30+ ALCL</td>
</tr>
<tr>
<td>t(11;14)(q13;q32)</td>
<td>Cyclin D1-IgH@</td>
<td>Mantle cell</td>
</tr>
<tr>
<td>t(14;18)(q21;q32)</td>
<td>BCL2-IgH@</td>
<td>Follicular</td>
</tr>
<tr>
<td>t(3;v)(q27;v)</td>
<td>BCL6-(Ig+)</td>
<td>Large cell</td>
</tr>
<tr>
<td>t(8;14)(q24;q32)</td>
<td>MYC-IgH@</td>
<td>Burkitt</td>
</tr>
<tr>
<td>t(11;18)(q21;q21)</td>
<td>API2-MLT</td>
<td>MALT</td>
</tr>
</tbody>
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Routine by FISH       Routine by FISH or PCR     Not routine
SUMMARY

- Judicious use of limited tissue essential for optimal diagnosis of lymphoma in cytologic specimens
- IHC on cores useful for correlation with architecture and cytology; flow cytometry on aspirates superior for B cell clonality and quantitative antigen assessment
- Molecular studies can detect clonality or specific genetic abnormalities