Test Utilization:
• Chronic Lymphocytic Leukemia

Initial Evaluation
• Diagnostic Criteria
• Selection of Tests for Prognosis
Response to Therapy
• Challenges
• Assessment for persistent disease

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Chronic Lymphocytic Leukemia
Development of Test Utilization Algorithms

• Close collaboration with CLL disease oriented group
• Typical points for blood / bone marrow analysis in CLL patients
  • Diagnosis
  • Assessment of initial prognosis
  • Assessment of response to therapy
  • Assessment of progressive disease
    • Genetic progression
    • Transformation
  • Assessment of new cytopenias/systemic symptoms
**CLL-Initial Evaluation on Blood**

**Challenge:**
--Correctly diagnose CLL
--Optimize prognostic testing

- **Suspicion of Chronic Lymphocytic Leukemia**
  - Flow Cytometry
    - **B-CLL Phenotype**
      - **Diagnosis**:
        - B cell count > 5000 = CLL
        - B cell count < 5000 = MBL
      - **Prognostic Studies**
        - B-cell count < 1000* AND NO SLL presentation
          - **STOP**
        - B-cell count between 1000* and 5000
          - Or
          - B-cell count < 1000 AND SLL presentation (i.e. lymphadenopathy)
        - B-cell count > 5000*
          - Or
          - B-cell count < 5000 AND outside history of CLL and first time Mayo patient
            - **FISH**: B-CLL Panel
            - **JGVH Sequencing**

- **Hematopathology**
  - Consistent CLL diagnostic criteria

- **Other Phenotype**
  - Other Flow Panels
    - FISH
    - BM Biopsy
    - LN Biopsy
    - Other Tissue Biopsy
    - As indicated

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*Mayo Clinic*
CLL Initial Evaluation on Blood

It is important to get the diagnosis correct right from the beginning

- Establish CLL Dx
  - Lymphocyte morphology
  - Flow cytometry results
  - Lymph node biopsy

- Consider Absolute B cell Count
  - Chronic lymphocytic leukemia
  - Monoclonal B cell lymphocytosis

Suspicion of Chronic Lymphocytic Leukemia

Flow Cytometry

B-CLL Phenotype

Hematopathology Consistent CLL diagnostic criteria

DDx:
B cell count >5000 = CLL
B cell count <5000 = MBL
Diagnostic Criteria for CLL
Flow Cytometry

- **CD5+ B cells**
- **Dim CD20**
- **CD23+**
- **Dim monotypic light chain**
CLL Lymph Node Morphology

Proliferation Center
CLL Lymph Node Immunohistochemistry

- CD20
- CD3
- CD5
- CD43
- CD23
- Cyclin D1
- kappa
- lambda
**CLL Initial Evaluation on Blood**

**Prognostic Studies**

- **B-cell count <1000 AND NO SLL presentation** → **STOP**
- **B-cell count 1000-5000 OR B-cell count <1000 AND SLL presentation (i.e. lymphadenopathy)**
- **B-cell count >5000**
  - Flow Cytometry: CD38, CD49d, Zap-70
  - FISH: B-CLL Panel
  - IGVH sequencing
Typical CLL FISH Panel*

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Breakpoint</th>
<th>Gene</th>
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<tbody>
<tr>
<td>6q</td>
<td>-</td>
<td>MYB/Cen6</td>
</tr>
<tr>
<td>11q</td>
<td>-</td>
<td>ATM/Cen11</td>
</tr>
<tr>
<td>+12</td>
<td></td>
<td>D12Z3/MDM2</td>
</tr>
<tr>
<td>13q</td>
<td>-</td>
<td>D13S319/LAMP1</td>
</tr>
<tr>
<td>17p</td>
<td>-</td>
<td>TP53/Cen17</td>
</tr>
<tr>
<td>t(11;14)</td>
<td></td>
<td>CCND1/IGH</td>
</tr>
</tbody>
</table>

**IGH BAP reflex to:**
- t(14;18), IGH/BCL2
- t(14;18), IGH/BCL3

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**Prognosis**

Exclude Mantle Cell Lymphoma

Unusual CLL Subgroups

*For Prognosis NOT Diagnosis*
Survival Significance of Standard CLL Risk Factors

**IGVH unmutated**
- CD38 hi
- Zap-70 hi
- p53 mutations
- High risk FISH (17p-, 11q-)

Median survival 8-10 years

**IGVH mutated**
- CD38 lo
- Zap-70 lo
- p53 normal
- Low risk FISH (13q-)

Median survival 25 years

CLL Bone Marrow Assessment for Response to Therapy

- Previously diagnosed CLL patients who have been treated
- No absolute lymphocyte count progression
- No progression of adenopathy or hepatosplenomegaly
- Minimal risk for MDS
- Assess bone marrow to determine:
  - Complete response (CR), minimal residual disease (MRD) negative or
  - CR, but MRD positive or
  - Partial response (nodular) or
  - Persistent disease
CLL Bone Marrow Assessment for Response to Therapy—Challenges
Is Minimal Residual Disease Testing Necessary?

- Level of MRD predicts progression free survival and overall survival:
  - After routine chemotherapy
  - After immunochemotherapy (FCR/alemtuzumab)
  - After stem cell transplantation

CLL Bone Marrow Assessment for Response to Therapy—Challenges

What is the optimal test for MRD detection?

<table>
<thead>
<tr>
<th>Technique</th>
<th>Sensitivity</th>
<th>Analytical Difficulty</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR--consensus primers</td>
<td>Low ($10^{-3}$)</td>
<td>Low</td>
</tr>
<tr>
<td>PCR--allele specific oligonucleotide</td>
<td>High ($10^{-6}$)</td>
<td>High</td>
</tr>
<tr>
<td>CD5/CD19 flow cytometry</td>
<td>Low ($10^{-2}$)</td>
<td>Low</td>
</tr>
<tr>
<td>MRD flow cytometry*</td>
<td>High ($10^{-4}$)</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

CLL Bone Marrow Assessment for Response to Therapy

MRD Flow Cytometry Assay Design

- 6 color, one tube
- CD45, CD5, CD19, CD20, kappa, lambda
- Collect 500,000 events
- Gating strategy
  - Exclude doublets/debris
  - Identify lymphocyte events (CD45/side scatter)
  - Gate on CD19 positive B cells
  - Identify dual CD20 (dim) CD5 positive cells
  - Evaluate for kappa and lambda
  - Calculate %MRD:

\[
\%MRD = \frac{\text{monoclonal CLL events/ non-aggregated WBC}}{100}
\]
CLL MRD By Flow Cytometry

MRD = 0.35%

MRD = 0.02%
CLL MRD By Flow Cytometry
MRD Negative

MRD=0.00%
CLL Minimal Residual Disease
Can Immunohistochemistry Substitute for Flow Cytometry?

• 82 patients
• Confirmed CLL
• Treatment with chemoimmunotherapy
• Bone marrow aspirates and biopsies:
  • Morphology assessment
  • MRD flow cytometry
  • Immunohistochemistry (IHC) for CD3, CD5, CD23 and PAX-5
• Compare MRD flow and IHC results

Amador-Ortiz C, et al; 2013
MRD Flow Cytometry vs. IHC Concordance

Amador-Ortiz C, et al; 2013
### CLL Minimal Residual Disease

**MRD flow cytometry vs. Immunohistochemistry**

<table>
<thead>
<tr>
<th></th>
<th>Flow MRD Positive (n=64)</th>
<th>Flow MRD Negative (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC MRD Positive (n=55)</td>
<td>54</td>
<td>1</td>
</tr>
<tr>
<td>IHC MRD Negative (n=24) / Suspicious (n=3)</td>
<td>7/3</td>
<td>17</td>
</tr>
</tbody>
</table>

Concordance = 87%

**Conclusion:** with a slight loss in sensitivity, IHC can substitute for MRD flow cytometry.

- Discrete IHC positive lymphoid aggregate, hemodilute aspirate
- Very low flow MRD: IHC negative: 0.005 - 0.200%
- IHC suspicious: 0.040 - 0.890%

Amador-Ortiz C, et al; 2013
CLL Bone Marrow Assessment for Response to Therapy—Challenges

What is the significance of lymphoid aggregates?

Residual involvement by CLL (nodular partial remission) VS. Reactive lymphoid aggregates VS. Rituximab effect—T cell rich lymphoid aggregates
CLL Bone Marrow Assessment for Response to Therapy

MRD post therapy?

Intent to perform BM transplant within 3 mo of this bone marrow

No

Yes

Cytogenetics to evaluate occult MDS
CLL Bone Marrow Assessment for Response to Therapy

Morphologic involvement by CLL/SLL ≥30%

No

Flow: CLL MRD

Yes

Diagnosis: “Residual involvement by CLL/SLL (%), nodules present/absent”

NO ancillary studies
**CLL Bone Marrow Assessment for Response to Therapy**

**Flow: CLL MRD**

- **Positive**
  - **Positive**
    - BM lymphoid nodules present?
      - **No**
        - Diagnosis: “Involved by SLL/CLL (%), nodules present”
      - **Yes**
        - Concordant % involvement MRD flow and morphology
          - **No**
            - Perform IHC—see next slide
          - **Yes**
            - Diagnosis: “Involved by SLL/CLL (%), nodules present”
  - **Negative**
    - Diagnosis: “Negative for CLL/SLL by morphologic and MRD assessments”

- **Negative**
  - Lymphoid aggregates/interstitial infiltrates
    - **Yes**
      - Diagnosis: “Negative for CLL/SLL by morphologic and MRD assessments”
    - **No**
      - Perform IHC—see next slide
CLL Bone Marrow Assessment for Response to Therapy

? Do the lymphoid aggregates represent CLL missed by the MRD flow assay or are they T cell aggregates in a Rituximab treated patient

Flow: CLL MRD
  - Negative
  - Yes
    - Lymphoid aggregates/interstitial infiltrates
      - Yes

Immunohistochemistry
  - CD3, CD5, CD20, CD23, PAX-5
  - Negative

CLL Phenotype
  - Yes
    - Diagnosis: “Residual involvement by CLL/SLL (%) nodules present/absent”
  - No
    - Diagnosis: “Negative for CLL/SLL by morphologic and MRD assessments”

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CLL Bone Marrow Assessment for Response to Therapy

- Positive
  - Flow: CLL MRD
  - BM lymphoid nodules present?
    - Yes
      - Concordant % involvement MRD flow and morphology
    - No

- ? What is the degree of BM involvement by CLL:
  --CLL involvement underestimated by the MRD flow
  OR
  --MRD with abundant reactive T cells

- Immunohistochemistry
  - CD3, CD5, CD20, CD23, PAX-5

- Nodules rich in CLL B cells
  - No
  - Yes

- Diagnosis: “Minimal residual involvement by CLL(%)”

- Diagnosis: “Residual involvement by CLL/SLL(%) nodules present”
CLL Test Utilization Summary

• Initial diagnosis
  • Get it right
    • Strict flow cytometry criteria (histograms!)
    • LN biopsy
    • Be patient
  • Judiciously order prognostic studies

• Evaluation of Response to Therapy
  • Importance of MRD testing
  • Use a highly sensitive flow cytometry assay possibly supplemented by IHC
  • Recognize the non-specificity of lymphoid aggregates post-therapy
  • Clinician/Hematopathologist joint algorithm development for evaluation of MRD
Collaborative Process for Optimizing Test Utilization

1. Patient Evaluated
2. Differential Diagnosis Generated
3. Tests Ordered
4. Morphology Review
5. New Clinicopathologic Differential Diagnosis
6. Algorithm Consulted Test Order Refined

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Questions?
Discussion?