Test Utilization:
- Lymphoma Staging
- Chronic Lymphocytic Leukemia
DISCLOSURES:

Relevant Financial Relationship(s)
None

Off Label Usage
None
Test Utilization:
• Lymphoma Staging

Bone Marrow Evaluation
• Flow Cytometry
• Cytogenetics
• Fluorescence in situ Hybridization
• Immunoglobulin Gene Rearrangements
Bone marrow evaluation for lymphoma staging

• Malignant lymphoma
  • Cancer of immune system cells (lymphocytes)
  • Many types—complex classification based on morphology, phenotype and genetics
  • Diagnosed and classified on biopsies of lymph nodes, other non-nodal sites

• Bone marrow evaluation
  • Determine extent of disease (Stage/IPI)
  • Typical evaluation: morphology, flow cytometry, cytogenetics, ± FISH
  • Minimal data about the utility of these studies in this context
What tests would you order on the bone marrow?

• 64 year old female with cervical, axillary and inguinal adenopathy
• Lymph node biopsy performed
• Bone marrow done for staging 2 days after the lymph node biopsy
• No relevant prior history
Diagnosis:
Follicular Lymphoma, Grade 1
Test Menu

- Bone marrow aspirate and biopsy
- Immunohistochemistry
- Flow cytometry
- Cytogenetics
- FISH for B cell lymphoma
- BCR gene rearrangements

Blood and bone marrow
Bone Marrow Evaluation for Lymphoma Staging
Utility of Flow Cytometry

• Assumptions:
  • Lymphoma diagnosis and classification were established on a tissue other than the bone marrow.
  • The bone marrow was performed subsequent to the lymphoma diagnosis for staging, not for primary diagnosis.
  • Bilateral bone marrow biopsies were obtained (at least 2 linear cm of bone marrow examined)

• Question: does routine flow cytometry of B cell lymphoma staging bone marrows add information:
  • About presence or absence of lymphoma?
  • About lymphoma classification?

Practice Data: Bone marrow flow cytometry in staging B cell lymphomas

<table>
<thead>
<tr>
<th></th>
<th>Flow Positive</th>
<th>Flow Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology Positive</td>
<td>49</td>
<td>10</td>
</tr>
<tr>
<td>Morphology Negative</td>
<td>5</td>
<td>111</td>
</tr>
</tbody>
</table>

Concordant: 91.4%  
Discordant: 8.6%

Resolution of Discordant Results

<table>
<thead>
<tr>
<th></th>
<th>Flow Positive</th>
<th>Flow Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphology Positive</strong></td>
<td>49</td>
<td>10</td>
</tr>
<tr>
<td><strong>Morphology Negative</strong></td>
<td>5</td>
<td>111</td>
</tr>
</tbody>
</table>

- Low level disease (0.09-3.0%)
- All IHC negative
- All DLBCL
- All Widespread/Bulky Disease
- 2/2 studied with PB involvement

- 5 DLBCL with discordant involvement
- 5 Follicular lymphoma
- All focally involved
- All IHC positive

Flow Cytometry in Staging Bone Marrows

• Mayo study similar to others in the literature
  • Concordance/discordance rates similar
  • Morphology positive/flow negative discordance due to focal disease
  • Flow positive/morphology negative discordance
    • More frequent in DLBCL
    • Associated with bulky disease/PB involvement
    • Little apparent effect on prognosis
    • Significance uncertain
Flow Cytometry in Staging Bone Marrows

Recommendations

• Collect specimen for flow cytometry on all lymphoma staging specimens

• Perform flow cytometry only to resolve uncertainty about lymphoma involvement in morphologically equivocal cases
  • Given false negative rate of flow cytometry in focally involved bone marrows, consider immunohistochemistry instead

• **Cancel** flow cytometry on all morphologically negative and unequivocally positive cases
Bone Marrow Evaluation for Lymphoma Staging
Utility of Cytogenetic Analysis

• Assumptions:
  • Lymphoma type is definitively established on a tissue other than the bone marrow.
  • The bone marrow is performed subsequent to the lymphoma diagnosis for staging, not for primary diagnosis.

• Question: does routine karyotyping of lymphoma staging bone marrows add information:
  • About presence or absence of lymphoma?
  • About lymphoma classification?
  • About other diseases that might affect the bone marrow?
Practice data: cytogenetics in staging bone marrows

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma diagnosis</td>
<td>574</td>
</tr>
<tr>
<td>Contemporaneous bone marrow</td>
<td>298</td>
</tr>
<tr>
<td>Cytogenetics performed</td>
<td>112</td>
</tr>
<tr>
<td>BM involved by ML</td>
<td>41</td>
</tr>
<tr>
<td>Normal genetics</td>
<td>32</td>
</tr>
<tr>
<td>Abnormal genetics</td>
<td>9</td>
</tr>
<tr>
<td>Expected karyotype</td>
<td>9</td>
</tr>
<tr>
<td>BM negative for ML</td>
<td>71</td>
</tr>
<tr>
<td>Abnormal genetics</td>
<td>9</td>
</tr>
<tr>
<td>Lymphoma karyotype</td>
<td>0</td>
</tr>
</tbody>
</table>
Practice data: bone marrows negative for lymphoma, abnormal karyotype (n=8)

<table>
<thead>
<tr>
<th>Tissue Dx</th>
<th>BM Dx</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>FL</td>
<td>Normal</td>
<td>Single metaphase abn</td>
</tr>
<tr>
<td>DLBCL</td>
<td>Normal</td>
<td>46,XY,del(20q)[1]</td>
</tr>
<tr>
<td>CHL</td>
<td>Normal</td>
<td>46,XX,t(14;22)(q32;q11.2)[1]</td>
</tr>
<tr>
<td>DLBCL</td>
<td>Normal</td>
<td>46,X,-Y[8]</td>
</tr>
<tr>
<td>DLBCL</td>
<td>Normal</td>
<td>46,X,-Y[19]</td>
</tr>
<tr>
<td>MALT</td>
<td>Normal</td>
<td>46,X,-Y[3]</td>
</tr>
<tr>
<td>DLBCL</td>
<td>Normal</td>
<td>46,X,Y,der(13;14)(q10;q10)c[20]</td>
</tr>
</tbody>
</table>
Practice data: bone marrows negative for lymphoma, other (n=8)

<table>
<thead>
<tr>
<th>BM Diagnosis</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancyto/panhyperplasia</td>
<td>Normal</td>
</tr>
<tr>
<td>?MDS</td>
<td>Normal</td>
</tr>
<tr>
<td>Dyserythropoiesis</td>
<td>Normal</td>
</tr>
<tr>
<td>Atypical megakaryocytes</td>
<td>Normal</td>
</tr>
<tr>
<td>RARS</td>
<td>Normal</td>
</tr>
<tr>
<td>Pancytopenia/dyserythro</td>
<td>46,XX,del(7)(q22)[2]</td>
</tr>
<tr>
<td>Polycythemia vera</td>
<td>46,XX,add(12)(q22)[12]</td>
</tr>
<tr>
<td>AML</td>
<td>46-48, X,Y,add(5)(q13),-7, add(12) (p11.2),-19,+0-2mar[20]</td>
</tr>
</tbody>
</table>
Practice data: cytogenetics in *staging* bone marrows, conclusions

- Cytogenetics does not improve the sensitivity for detection of lymphoma involvement over morphology and selected immunophenotyping.
- Cytogenetics does not add useful additional data in lymphoma positive bone marrows.
- Abnormal cytogenetics in morphologically normal specimens are of doubtful significance.
- Abnormal cytogenetics can support the diagnosis of a myeloid neoplasm (AML, MDS, CMPN).
- Pre transplant routine cytogenetics is possibly justified to exclude morphologically occult Rx-related MDS.
Conclusions: FISH testing for lymphoma on blood and bone marrow

• Coupled with morphology and phenotyping on blood specimens, FISH may help define a particular type of lymphoproliferative disorder (mantle cell lymphoma, follicular lymphoma, T prolymphocytic leukemia)

• FISH is insensitive for detection of lymphoma in staging bone marrow specimens (similar to cytogenetics)

• FISH rarely adds information about lymphoma classification in staging specimens

• Coupled with morphology and phenotyping, FISH may help classify lymphoproliferative disorders when the bone marrow is the primary diagnostic specimen
Immune Receptor Gene Rearrangements

• T cell receptor gene rearrangements
  • Clonal pattern present in 90% of T cell lineage lymphoma and leukemia
  • Clonal pattern present in variable percentages of reactive conditions
    • Blood, bone marrow, skin
  • Non-clonal pattern associated with lymphoid hyperplasias (immune reactions)

• Immunoglobulin gene rearrangements
  • Clonal pattern in 90% of B cell lineage lymphoma and leukemia
  • Clonal pattern rare in reactive conditions
  • Non-clonal pattern associated with lymphoid hyperplasias (immune reactions)
Practice Data: immunoglobulin gene rearrangements on bone marrow

• Total: 47 cases
• 14 clonal pattern
  • Morphology plus flow or immunohistochemistry positive in all
• 33 non-clonal pattern
  • 2 myelodysplastic syndrome
  • 1 acute myeloid leukemia
  • 1 LGL leukemia
  • 29 normal morphology

All Unnecessary
Practice Data: T cell receptor gene rearrangements—Bone Marrow

- Total: 172 cases
- 12 non-clonal pattern and false negative
  - 8 Peripheral T cell lymphoma
  - 2 LGL leukemia
  - 1 CD4+ T cell LPD
  - 1 T-lymphoblastic leukemia
- 121 non-clonal pattern and true negative
  - 14 MDS/MPN
  - 7 Acute myeloid leukemia
  - 6 B cell lymphomas
  - 1 Multiple myeloma
  - 93 normal
Practice Data: T cell receptor gene rearrangements—Bone Marrow

- Total: 172 cases
  - 20 clonal pattern and **true positive**
    - 11 LGL leukemia
    - 6 Peripheral T cell lymphoma
    - 2 T lymphoblastic leukemia
    - 1 CD4+ T cell LPD
  - 19 clonal pattern and **false positive**
    - 2 Multiple myeloma
    - 1 Hairy cell leukemia
    - 2 MDS/MPN
    - 1 Acute myeloid leukemia
    - 1 CMML
    - 12 normal
Practice Data: T cell receptor gene rearrangements—Bone Marrow

Conclusions

- Total: 172 cases
  - 20 True positive results
  - 19 False positive results*
  - 12 False negative results
  - 121 Unnecessary studies

*11% as expected
“These tests are dangerous for patient care. Why do you let us order them?”

**Recommendation**

- TCR and Ig gene rearrangement tests are poor screening modalities
- Reserve TCR and Ig gene rearrangement analysis to resolve specific diagnostic problems posed by morphology and phenotyping
Summary of Test Utilization Principles Approved by Lymphoma Disease Oriented Group

• Specimens are obtained for flow cytometry, routine cytogenetic analysis, FISH and molecular genetics on all lymphoma staging bone marrows.

• Samples forwarded for flow cytometry, cytogenetics, FISH and T cell receptor and immunoglobulin gene rearrangements only at the discretion of the hematopathologist based on clinical information, pathology findings and whether or not patient is pre-auto bone marrow transplant.

• All other tests cancelled.

• Report which tests were forwarded for testing or cancelled.
Utilization Decisions Documented in Pathology Report, Examples

• Perform test:
  • “Cytogenetic analysis, bone marrow aspirate: Sample has been forwarded for testing. Results will be reported in an addendum.”

• Cancel test:
  • “Cytogenetic analysis, bone marrow aspirate: Test not indicated per Hematopathology review. Sample saved.”
Test Menu

- Bone marrow aspirate and biopsy
- Immunohistochemistry
- Flow cytometry
- Cytogenetics
- FISH for B cell lymphoma
- BCR gene rearrangements

Blood and bone marrow