2013 AAIM Pathology Workshop

John Schmieg, M.D., Ph.D.

Disclosures

• None
Pathology Workshop Objectives

• Define the general philosophy of reviewing pathology reports
  – Review the various components of
    • Bone marrow aspirate and biopsy
    • Flow Cytometry
    • Cytogenetic Studies
    • Molecular studies
  – Outline a general approach to interpreting pathology reports
    • Define the difference between a non-diagnostic bone marrow biopsy and a negative bone marrow biopsy
• Discuss case studies with relevant pathology reports / bone marrow biopsies in relation to staging and prognosis / mortality risk. If new treatments improve prognosis, briefly outline the details.
  – Review a case of Anemia of Unknown Etiology
  – Review a case of Early Myelodysplastic Syndrome
  – Review a case of MGUS / Multiple Myeloma
  – Review a case of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL)
Bone Marrow Biopsy and Aspirate Report

- Biopsy and aspirate findings usually reported together
- Biopsy: architecture, cellularity, some cytology
- Aspirate: cytology, quantitative data (cell %s)
- Biopsy and aspirate findings oftentimes suffice for a definitive diagnosis
- Flow cytometry, cytogenetic, and molecular findings are sometimes needed for a definitive diagnosis, and are usually incorporated in the biopsy/aspirate report, oftentimes as addenda
Flow Cytometry Report

- Separate report detailing the immunophenotypic features of the various cell populations in a specimen
- Data obtained using fluorescently labeled antibodies specific for various proteins of interest that help identify different cell types and reveal any abnormalities in marker expression
- Important in distinguishing acute myeloid leukemias (AMLs) from acute lymphoblastic leukemias (ALLs)
- Can identify monoclonal B cell populations and phenotypically abnormal T cell populations that aid in the diagnosis of lymphoproliferative disorders (i.e., lymphomas), as well as monoclonal plasma cell populations that aid in the diagnosis of plasma cell neoplasms (e.g., myeloma)
- Have limited utility in the work-up of MDS and MPNs
- Can yield diagnostic, prognostic, and predictive information
- Flow cytometry results are often available before biopsy/aspirate work-up is complete so these results are often incorporated into the biopsy/aspirate report and contribute to the definitive diagnosis seen there
Cytogenetics Report

- Separate report detailing any chromosomal abnormalities detected in the cells of a specimen.
- Two main methods: classic karyotyping and Fluorescence In Situ Hybridization (FISH)
- Very important in the work-up of MDS, AMLs, ALLs, and myelomas
- Also important in the evaluation of some lymphoproliferative disorders/lymphomas
- Occasionally relevant in the work-up of MPNs
- Can yield diagnostic, prognostic, and predictive information
Molecular Report

- Separate report detailing any specific gene mutations or monoclonal T or B cell populations present in a specimen
- Numerous different methodologies and genes can be looked at depending on the situation
- Important in the evaluation of AMLs, MPNs, and lymphomas
- Can yield diagnostic, prognostic, and predictive information

Pathology Workshop Objectives

- Define the general philosophy of reviewing pathology reports
  - Review the various components of
    - Bone marrow aspirate and biopsy
    - Flow Cytometry
    - Cytogenetic Studies
    - Molecular studies
  - Outline a general approach to interpreting pathology reports
    - Define the difference between a non-diagnostic bone marrow biopsy and a negative bone marrow biopsy
- Discuss case studies with relevant pathology reports / bone marrow biopsies in relation to staging and prognosis / mortality risk. If new treatments improve prognosis, briefly outline the details.
  - Review a case of Anemia of Unknown Etiology
  - Review a case of Early Myelodysplastic Syndrome
  - Review a case of MGUS / Multiple Myeloma
  - Review a case of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL)
Approach to Interpreting a Hemepath Report

• Biopsy/aspirate report will generally incorporate flow cytometric, cytogenetic, and molecular information (as they become available), and yield the most comprehensive diagnosis
• If biopsy/aspirate report does not mention flow cytometry, cytogenetics, or molecular, see if there are separate reports for these studies because information in these reports can make an otherwise non-diagnostic biopsy diagnostic

“Non-diagnostic” vs. “Negative” Bone Marrow Biopsies

• “Non-diagnostic” Biopsy: Findings (morphologic, immunophenotypic, cytogenetic, and molecular) not normal but not sufficient in and of themselves to render a definitive diagnosis based on defined criteria, but do not exclude the possibility of certain disorders
• “Negative” Biopsy: Findings (morphologic, immunophenotypic, cytogenetic, and molecular) are normal, and do not support the diagnosis of a hematologic (or any other) disorder
Pathology Workshop Objectives

• Define the general philosophy of reviewing pathology reports
  – Review the various components of
    • Bone marrow aspirate and biopsy
    • Flow Cytometry
    • Cytogenetic Studies
    • Molecular studies
  – Outline a general approach to interpreting pathology reports
    • Define the difference between a non-diagnostic bone marrow biopsy and a negative bone marrow biopsy

• Discuss case studies with relevant pathology reports / bone marrow biopsies in relation to staging and prognosis / mortality risk. If new treatments improve prognosis, briefly outline the details.
  – Review a case of Anemia of Unknown Etiology
  – Review a case of Early Myelodysplastic Syndrome
  – Review a case of MGUS / Multiple Myeloma
  – Review a case of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL)
Anemia of Unknown Etiology

• 55-year old Hispanic female with no significant past medical history presented to her PCP with a primary complaint of fatigue, and was found to have a normochromic normocytic anemia (Hgb 8, Hct 24) with mild anisopoikilocytosis; WBC, ANC, and platelet counts are normal; spleen not enlarged
• Serum iron, ferritin, folate, and vitamin B12 levels are all normal
• LDH, total bilirubin, and haptoglobin all wnl
• Reticulocyte count is decreased; EPO levels are elevated
• Bone marrow biopsy is performed

Anemia of Unknown Etiology (cont.)

Bone Marrow Biopsy
Anemia of Unknown Etiology (cont.)

Bone Marrow Aspirate

- Immunohistochemistry for Parvovirus B19 performed on the biopsy is negative
- Flow cytometry: No phenotypically abnormal cell population detected; no increase in blasts
- Cytogenetic studies: Karyotype: 46,XX [20]; normal MDS FISH panel
- Negative for BCR-ABL1 gene rearrangement and JAK2 V617F mutation
Anemia of Unknown Etiology (cont.)

- Final bone marrow report: Normocellular marrow (40-50%) with marked erythroid hypoplasia (M:E ratio >10:1) with an associated left-shift. Blasts are not increased. No myelodysplasia is identified. No evidence of a lymphoproliferative disorder or plasma cell dyscrasia
- DDx: Acquired pure red cell aplasia secondary to viral infection (not parvovirus B19), drug, autoimmunity, thymoma, or idiopathic
- Example of “non-diagnostic” bone marrow biopsy

Approach to Anemia of Unknown Etiology

- Blood loss
  - GI
  - Menstrual
  - Other
- Increased destruction
  - Hemolysis
- Impaired production
  - Bone marrow dysfunction
Underwriting Concerns

• Would you insure this woman?
  – If so, how would you assess her mortality?
  – If not, what would need to change to consider insuring her?

• Are there any factors present that you would consider positive in terms of mortality?

• Are there any factors present that you would consider negative in terms of mortality?

Questions? Comments?
Pathology Workshop Objectives

• Define the general philosophy of reviewing pathology reports
  – Review the various components of
    • Bone marrow aspirate and biopsy
    • Flow Cytometry
    • Cytogenetic Studies
    • Molecular studies
  – Outline a general approach to interpreting pathology reports
    • Define the difference between a non-diagnostic bone marrow biopsy and a negative bone marrow biopsy
• Discuss case studies with relevant pathology reports / bone marrow biopsies in relation to staging and prognosis / mortality risk. If new treatments improve prognosis, briefly outline the details.
  – Review a case of Anemia of Unknown Etiology
  – Review a case of Early Myelodysplastic Syndrome
  – Review a case of MGUS / Multiple Myeloma
  – Review a case of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL)

Early Myelodysplastic Syndrome (MDS)

• 67-year old Caucasian male with no significant past medical history presents to his PCP for an annual check-up and a routine CBC showed a normochromic normocytic anemia (Hgb 9, Hct 27) with mild anisopoikilocytosis; WBC, ANC, and platelet counts are normal; spleen is not enlarged
• Serum iron, ferritin, folate, and vitamin B12 levels are all normal
• LDH, total bilirubin, and haptoglobin all wnl
• Although serum iron, ferritin, folate, and vitamin B12 levels are all normal, the patient is placed on a multivitamin and iron supplement , and asked to return in 6 months
• A follow-up CBC performed 6 months later shows a persistent normochromic normocytic anemia (Hgb 9, Hct 27) with mild anisopoikilocytosis refractory to multivitamin and iron supplement treatment; WBC, ANC, and platelet counts are still normal
• Reticulocyte count performed at the time is decreased and EPO levels are elevated
• Bone marrow biopsy is performed
Early MDS (cont.)

Bone Marrow Biopsy

Wright-Giemsa stain

Iron stain

Early MDS (cont.)

Bone Marrow Aspirate

Wright-Giemsa stain

Iron stain
Early MDS (cont.)

• Flow cytometry: No phenotypically abnormal cell population detected; no increase in blasts
• Cytogenetic studies: Karyotype: 45,XY,-5[7]/46,XY[13]; MDS FISH panel: -5 detected in 70 of 200 cells analyzed
• Negative for BCR-ABL1 gene rearrangement and JAK2 V617F mutation

Early MDS (cont.)

• Bone marrow report before cytogenetic studies: Mildly hypercellular marrow (~50%) with mild erythroid hyperplasia and mild dyserythropoiesis, pending cytogenetics. Blasts are not increased. DDX includes MDS, megaloblastic anemia, toxin exposure, and medication effect
• Bone marrow report after cytogenetic studies: MDS morphologically consistent with refractory anemia with unilineage dysplasia (good prognostic factor) with isolated chromosome 5 deletion (good prognostic factor) and no increase in blasts (good prognostic factor), IPPS score of 0 (low risk group)
Myelodysplastic Syndrome

- Results from ineffective function or production of myeloid blood cells
- Classification:
  - Refractory cytopenia with unilineage dysplasia
  - Refractory anemia with ringed sideroblasts
  - Refractory cytopenia with multilineage dysplasia
  - Refractory anemia with excess blasts
  - MDS with isolated del(5q)

IPSS-R Cytogenetic Risk Groups

<table>
<thead>
<tr>
<th>Cytogenetic Prognostic Subgroups</th>
<th>Cytogenetic Abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very good</td>
<td>-Y, del(11q)</td>
</tr>
<tr>
<td>Good</td>
<td>Normal, del(5q), del(12p), del(20q) double including del(5q)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>del(7q), +8, +19, i(17q), any other single or double independent clones</td>
</tr>
<tr>
<td>Poor</td>
<td>-7, inv(3)/t(3q)/del(3q), double including -7/del(7q), Complex: 3 abnormalities</td>
</tr>
<tr>
<td>Very poor</td>
<td>Complex: &gt;3 abnormalities</td>
</tr>
</tbody>
</table>
Revised International Prognostic Scoring System (IPSS-R)

<table>
<thead>
<tr>
<th>Prognostic Variable</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Cytogenetics</td>
<td>Very good</td>
</tr>
<tr>
<td>Bone marrow blast (%)</td>
<td>≤5</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>≥10</td>
</tr>
<tr>
<td>Platelets</td>
<td>≥100</td>
</tr>
<tr>
<td>Absolute neutrophil count</td>
<td>≥0.8</td>
</tr>
</tbody>
</table>

IPPS-R: Prognostic Subgroup Clinical Outcomes

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>IPSS-R Score</th>
<th>Median Overall Survival (years)</th>
<th>Median Time to 25% AML Evolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very low</td>
<td>≤1.5</td>
<td>8.8</td>
<td>&gt;14.5</td>
</tr>
<tr>
<td>Low</td>
<td>&gt;1.5 - 3</td>
<td>5.3</td>
<td>10.8</td>
</tr>
<tr>
<td>Intermediate</td>
<td>&gt;3.5 - 4.5</td>
<td>3.0</td>
<td>3.2</td>
</tr>
<tr>
<td>High</td>
<td>&gt;4.5 - 6</td>
<td>1.6</td>
<td>1.4</td>
</tr>
<tr>
<td>Very high</td>
<td>&gt;6</td>
<td>0.8</td>
<td>0.7</td>
</tr>
</tbody>
</table>
Underwriting Concerns

• Would you insure this man?
  – If so, how would you assess his mortality?
  – If not, what would need to change to consider insuring him?
• Are there any factors present that you would consider positive in terms of mortality?
• Are there any factors present that you would consider negative in terms of mortality?

Questions? Comments?
Pathology Workshop Objectives

- Define the general philosophy of reviewing pathology reports
  - Review the various components of
    - Bone marrow aspirate and biopsy
    - Flow Cytometry
    - Cytogenetic Studies
    - Molecular studies
  - Outline a general approach to interpreting pathology reports
    - Define the difference between a non-diagnostic bone marrow biopsy and a negative bone marrow biopsy
- Discuss case studies with relevant pathology reports / bone marrow biopsies in relation to staging and prognosis / mortality risk. If new treatments improve prognosis, briefly outline the details.
  - Review a case of Anemia of Unknown Etiology
  - Review a case of Early Myelodysplastic Syndrome
  - Review a case of MGUS / Multiple Myeloma
  - Review a case of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL)

MGUS/Multiple Myeloma

- 64-year old African American male with no significant past medical history presents to his PCP for an annual check-up
- A CBC with manual differential is performed, which shows normal counts; however the slide is flagged for pathologist review because of mild rouleaux formation
- A complete metabolic panel shows mildly increased total protein (8.5 g/dL) with normal albumin (4.0 g/dL) and normal creatinine (1.0 mg/dL)
- An SPEP is ordered, which shows a small M spike in the gamma region of 1.5 g/dL; serum IFE shows a monoclonal IgG kappa protein
- Bone marrow biopsy is performed
MGUS/Multiple Myeloma (cont.)

Peripheral blood smear showing rouleaux formation

MGUS/Multiple Myeloma (cont.)

Patient’s Bone Marrow Biopsy

A) Hematoxylin-eosin stain
B) Immunostain for CD138+ cells
C) Immunostain for κ light chains
D) Immunostain for λ light chains
MGUS/Multiple Myeloma (cont.)

Bone Marrow Aspirate

MGUS/Multiple Myeloma (cont.)

- Flow cytometry: Small (0.5%) kappa-restricted monoclonal plasma cell population detected
- Cytogenetic studies: Karyotype: 46,XY[20]; Multiple Myeloma FISH panel: 17p13 (TP53) deletion detected in 15 of 200 cells analyzed
- No molecular testing performed
MGUS/Multiple Myeloma (cont.)

- Bone marrow report: Normocellular marrow (~40%) involved by plasma cell myeloma (monoclonal kappa-restricted plasma cells account for ~20% of the bone marrow cellularity)
- Follow-up bone scan shows no bone lesions
- Serum Beta2-microglobulin level: 1.5 mg/L
- Since no evidence of end organ damage or myeloma-related symptoms, clinical diagnosis of low stage smoldering multiple myeloma with 17q13 deletion (poor prognostic factor)

Monoclonal Gammopathy

- Monoclonal Gammopathy of Unknown Significance (MGUS)
  - Monoclonal paraprotein <3g/dl
  - Bone marrow biopsy with <10% plasma cells
  - Absence of any end-organ sequelae
  - Risk of progression to myeloma – 1%/yr
Monoclonal Gammopathy – cont.

- Asymptomatic (smoldering) Myeloma
  - Serum IgA or IgG monoclonal protein ≥ 3.0 g/dl and/or
  - ≥10% more plasma cells in bone marrow
  - No evidence of end-organ sequelae
  - Risk of progression to myeloma 10%/yr for first five years, 3%/yr for next five years, then 1%/yr

Underwriting Concerns

- Would you insure this man?
  - If so, how would you assess his mortality?
  - If not, what would need to change to consider insuring him?

- Are there any factors present that you would consider positive in terms of mortality?
- Are there any factors present that you would consider negative in terms of mortality?
Questions? Comments?

Pathology Workshop Objectives

• Define the general philosophy of reviewing pathology reports
  – Review the various components of
    • Bone marrow aspirate and biopsy
    • Flow Cytometry
    • Cytogenetic Studies
    • Molecular studies
  – Outline a general approach to interpreting pathology reports
    • Define the difference between a non-diagnostic bone marrow biopsy and a negative bone marrow biopsy
• Discuss case studies with relevant pathology reports / bone marrow biopsies in relation to staging and prognosis / mortality risk. If new treatments improve prognosis, briefly outline the details.
  – Review a case of Anemia of Unknown Etiology
  – Review a case of Early Myelodysplastic Syndrome
  – Review a case of MGUS / Multiple Myeloma
  – Review a case of Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL)
CLL/SLL

- 59-year old Caucasian female is referred to a hematologist for work-up of a mild leukocytosis with relative and absolute lymphocytosis (WBC count 14,000, 70% lymphocytes), mild normochromic normocytic anemia (Hgb 10, Hct 30), and thrombocytopenia (platelets 100,000)
- Peripheral blood smear review shows a lymphocytosis consisting mostly of small mature lymphocytes with occasional smudge cells; the red blood cells show occasional microspherocytes and platelets are mildly reduced but morphologically unremarkable
- Physical exam shows no lymphadenopathy and no hepatosplenomegaly
- Flow cytometry, cytogenetics, and molecular studies of the peripheral blood are performed
- Also, because of the anemia and thrombocytopenia a bone marrow biopsy is performed

CLL/SLL (cont.)

- PB Flow cytometry results: 50% (of all WBCs) population of kappa-restricted B cells positive for CD19 (mod), CD20 (dim), CD22 (dim), CD5 (dim), CD23 (mod), CD38 (mod), and ZAP-70 (mod) c/w CLL/SLL
- PB Cytogenetic results: Karyotype: 46,XX,del(17p13)[10]/46,XX[10]; CLL FISH panel: 17p13 (TP53) deletion detected in 100 of 200 cells analyzed, negative for t(11;14)(q13;q32)
- PB Molecular results: Non-hypermutated
CLL/SLL (cont.)

• BM flow cytometry results: No monoclonal B cell or phenotypically abnormal T cell population detected
• BM cytogenetics: Karyotype 46,XX[20]; CLL FISH panel: WNL
• BM molecular studies: No monoclonal B cell population detected (IgH PCR studies)
CLL/SLL (cont.)

- Peripheral blood report: CLL/SLL, CD38 and ZAP-70+ (poor prognostic factors), with 17p13 (TP53) deletion (poor prognostic factor), non-hypermuted (poor prognostic factor)
- Bone marrow report: Normocellular marrow (~40%) with trilineage hematopoiesis; no evidence of involvement by a B or T cell lymphoproliferative disorder; no increase in blasts
- Example of a “negative” bone marrow

CLL/SLL (cont.)

- Follow up lab studies showed elevated LDH, elevated total and indirect bilirubin with normal direct bilirubin, decreased haptoglobin, and a positive DAT
- Given these lab studies, the negative bone marrow biopsy, and the presence of occasional microspherocytes on the PB smear, a diagnosis of CLL/SLL-associated Evan’s syndrome was made to explain the patient’s anemia and thrombocytopenia
# RAI Clinical Staging System

<table>
<thead>
<tr>
<th>Revised Staging System</th>
<th>Original Staging System</th>
<th>Clinical Features at Diagnosis</th>
<th>Median Survival, Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk</td>
<td>0</td>
<td>Blood &amp; marrow lymphocytes</td>
<td>12</td>
</tr>
<tr>
<td>I</td>
<td>Lymphocytosis &amp; enlarged LN</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Intermediate risk</td>
<td>II</td>
<td>Lymphocytosis &amp; enlarged spleen &amp;/or liver</td>
<td>8</td>
</tr>
<tr>
<td>High risk</td>
<td>III</td>
<td>Lymphocytosis &amp; anemia (Hgb &lt;11)</td>
<td>5</td>
</tr>
<tr>
<td>IV</td>
<td>Lymphocytosis &amp; thromocytopenia (Plt &lt;100,000)</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

## Underwriting Concerns

- Would you insure this woman?
  - If so, how would you assess her mortality?
  - If not, what would need to change to consider insuring her?
- Are there any factors present that you would consider positive in terms of mortality?
- Are there any factors present that you would consider negative in terms of mortality?
Questions? Comments?