Lessons Learned- Mimics of Lymphoma

DR L. JEFFREY MEDEIROS
Lessons Learned
Mimics of Lymphoma

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Outline

- Infectious mononucleosis
- Kikuchi-Fujimoto lymphadenitis
- Castleman disease
- Intravascular lymphocytosis
- Seminoma
- Nasopharyngeal carcinoma
- Thymoma
- Myeloid sarcoma

Infectious Mononucleosis
Basic Facts

- Caused by Epstein-Barr virus (HHV-4)
- Spread by contact with human secretions
  (Saliva to oral epithelium to B-cells)
- Age of contact depends on living conditions
  - Poor - < 3 years
  - Good - 10-19 years
- Incubation period is 2-5 weeks
  - First week: Humoral antibody response
  - Second week: Cellular immune response
Infectious Mononucleosis
Clinical and Laboratory Features

Clinical Presentation
- Usually adolescents and young adults
- Flu-like illness is common
- Fever, pharyngitis, fatigue

Physical Findings
- Lymphadenopathy
- Hepatosplenomegaly
- Tonsillitis, skin rash

Laboratory Findings
- Thrombocytopenia
- Anemia
- PB lymphocytosis with atypical lymphocytes

This case is not too difficult
Small lymph node
Architecture is partially preserved

Spectrum of cells

Acute EBV+ Lymphadenitis (Inf Mono) Looks Like Large Cell Lymphoma

Tougher case
No architecture
Necrosis
Many large cells

Acute EBV+ Lymphadenitis (Inf Mono) Histologic Features
Marked expansion/distortion of the architecture
Partial preservation in some cases (when lucky)
Spectrum of cells
Many immunoblasts
Histiocytes, plasmacytoid lymphs, plasma cells
Reed-Sternberg-like cells +/-
Follicular hyperplasia is common
Necrosis is common
Capsular infiltration +/-
Vasculitis +/-

Acute EBV+ Lymphadenitis (Inf Mono) Immunohistochemistry and In Situ Hybridization
CD20
CD3
CD30
EBER

7

8

9
**Differential Diagnosis**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV lymphadenitis</td>
<td>Resembles inf. mono. histologically CMV inclusions +/- EBV absent</td>
</tr>
<tr>
<td>Large B-cell lymphoma</td>
<td>Architecture replaced Monotonous cell population EBV negative (usually) Monoclonal</td>
</tr>
<tr>
<td>Anaplastic large cell lymphoma</td>
<td>Sinusoidal (common) or diffuse pattern Hallmark cells, ALK+ Monoclonal</td>
</tr>
<tr>
<td>Classical Hodgkin lymphoma</td>
<td>No spectrum of cell types RS+H cells: CD15+/- CD45-, EBV +/-</td>
</tr>
</tbody>
</table>

**Kikuchi-Fujimoto Lymphadenitis**

**Clinical Features**

First described in 1972 in Japan  
A.K.A. histiocytic necrotizing lymphadenitis  
Median age 30 years (wide range)  
Female predominance  
Cervical LNs # 1  
Patients present with:  
Moderate fever, chills  
Myalgias +/-

**Histologic Features**

Paracortical and wedge-shaped infiltrate
Kikuchi-Fujimoto Lymphadenitis

Proliferative Phase

- Overall architecture preserved
- Paracortical; patchy necrosis +/-
- Increased histiocytes; often C-shaped
- Increased plasmacytoid dendritic cells
- No granulocytes; no (or rare) plasma cells
- Follicular hyperplasia +/-
- 3 phases: Necrotizing, Proliferative, Xanthomatous

Necrotizing and Xanthomatous Phases

- Necrotizing
- Xanthomatous
Kikuchi-Fujimoto Lymphadenitis
Immunohistochemistry

Numerous histiocytes
CD68+, lysozyme+, MPO+
Plasmacytoid dendritic cells
CD123+, TCL1+
Many T-cells
CD8 > CD4
CD30+ immunoblasts
Ki-67 high

Kikuchi-Fujimoto Lymphadenitis
Differential Diagnosis

<table>
<thead>
<tr>
<th>Disease</th>
<th>Differential Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE lymphadenitis</td>
<td>Can be identical to K-F Hematoxylin bodies +/-</td>
</tr>
<tr>
<td>Infectious lymphadenitis</td>
<td>Different quality of necrosis (coagulative with polys)</td>
</tr>
<tr>
<td>Infarcted lymphoma</td>
<td>Ghosts of tumor cells Immunostains highlight dead cells</td>
</tr>
<tr>
<td>Large B-cell lymphoma</td>
<td>Only proliferative phase of K-F Immunophenotype helps</td>
</tr>
</tbody>
</table>
Castleman Disease
Classification

<table>
<thead>
<tr>
<th>Clinical</th>
<th>Pathological</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unicentric</td>
<td>Hyaline-vascular variant</td>
</tr>
<tr>
<td></td>
<td>Plasma cell variant, HHV8-</td>
</tr>
<tr>
<td>Multicentric</td>
<td>Plasma cell variant, HHV8+</td>
</tr>
<tr>
<td></td>
<td>HIV, Endemic</td>
</tr>
<tr>
<td></td>
<td>Plasma cell variant, HHV8-</td>
</tr>
<tr>
<td></td>
<td>Idiopathic, TAFRO</td>
</tr>
<tr>
<td></td>
<td>POEMS</td>
</tr>
</tbody>
</table>

Hyaline-vascular Castleman Disease
Clinical Features

- ~75% of all cases of unicentric CD
- Any age (8-70 yrs)
- Usually asymptomatic
- Small or very large mass (up to 16 cm)
- Usually above the diaphragm
  - Mediastinum is #1 site
- Surgical excision is optimal therapy
Hyaline-vascular Castleman Disease

Histologic Features

Follicular
- Large follicles but small germinal centers
- “Twinning”
- “Onion-skin” mantle zones
- Lymphocyte depletion of germinal centers
- Hyaline-vascular lesions

Interfollicular
- This can be predominant (stroma-rich)
- Numerous high endothelial venules
- Actin+/-, CD68+, CD21+/−
Hyaline-vascular Castleman Disease
Differential Diagnosis

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular hyperplasia</td>
<td>No hyaline-vascular lesions</td>
</tr>
<tr>
<td></td>
<td>No lymphocyte depletion</td>
</tr>
<tr>
<td></td>
<td>No interfollicular vascularity</td>
</tr>
<tr>
<td>Follicular lymphoma</td>
<td>Many follicles</td>
</tr>
<tr>
<td></td>
<td>Uniform cell population</td>
</tr>
<tr>
<td></td>
<td>No lymphocyte depletion</td>
</tr>
<tr>
<td></td>
<td>No interfollicular vascularity</td>
</tr>
<tr>
<td>Mantle cell lymphoma, mantle</td>
<td>CD5+ cyclin D1+</td>
</tr>
<tr>
<td>zone pattern</td>
<td></td>
</tr>
<tr>
<td>Plasma cell variant CD</td>
<td>Marked plasmacytosis</td>
</tr>
<tr>
<td></td>
<td>Can have H-V follicles</td>
</tr>
</tbody>
</table>

Plasma Cell CD (Unicentric)
Clinical Features

~25% of cases of unicentric CD
Almost any age
Small lymph node(s) at one site
Systemic symptoms in a small subset
? Misclassified multicentric cases
Plasma Cell CD (Unicentric)
Histologic and Immunophenotypic Features

- Interfollicular sheets of plasma cells
- Sinuses usually patent
- Follicles have some H-V lesions +/-
- Polytypic plasma cells and B-cells
- Human herpes virus 8 (KSHV) -

Plasma Cell CD (Unicentric)
Differential Diagnosis

<table>
<thead>
<tr>
<th></th>
<th>Rheumatoid arthritis</th>
<th>Multicentric CD</th>
<th>Plasmacytoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grossly smaller</td>
<td></td>
<td>Multiple LN groups</td>
<td>Replaces of LN</td>
</tr>
<tr>
<td>No H-V lesions</td>
<td></td>
<td>~50% HHV-8+, HIV+</td>
<td>Monoclonal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~50% idiopathic</td>
<td></td>
</tr>
</tbody>
</table>

Multicentric Castleman Disease
Clinical Features

- Usually associated with systemic symptoms
- Often associated with HIV infection
- Lymphadenopathy – 100% of patients
- Hepatosplenomegaly, effusions, skin rash +/-

Laboratory
- Elevated ESR, anemia, thrombocytopenia
- Polyclonal hypergammaglobulinemia
**Multicentric Castleman Disease**  
**HHV8 (+)/HIV**

- Interfollicular sheets of plasma cells
- Atypical plasma cells/plasmablasts
- Follicles show H-V changes
- Blurring of boundary between germinal centers and mantle zones
- Plasma cells can be monotypic lambda

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**Multicentric Castleman Disease**  
**HHV8 Positive (HIV +)**

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**Multicentric Castleman Disease**  
**Presence of “Microlymphoma”**

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Multicentric Castleman Disease
HHV8 Negative/Idiopathic

Interfollicular sheets of plasma cells
+/- Follicles with H-V changes
No atypical plasma cells/plasmablasts
Plasma cells can be monotypic (lambda)

Multicentric Castleman Disease
Idiopathic

Multicentric Castleman Disease
Idiopathic

Must exclude
Infectious and autoimmune diseases, lymphomas, FDC sarcoma, and POEMS syndrome

Blood 129: 1646, 2017
TAFRO Syndrome
Thrombocytopenia, Anasarca, Fever, Reticulin fibrosis in BM, and Organomegaly
Variant of idiopathic multicentric Castleman disease (CD)
Etiology unknown; adults; median age in 6th decade
Symptoms related to cytokine storm, but not IL-6
TAFRO versus idiopathic multicentric CD
Thrombocytopenia, anasarca, and low IgG levels only in TAFRO

POEMS Syndrome
Polyneuropathy, Organomegaly, Endocrinopathy, M protein, Skin changes
Paraneoplastic syndrome caused by elevated angiogenic and inflammatory cytokines
Associated with underlying plasma cell dyscrasia
95% lambda
Often osteosclerotic
50% of patients have multicentric Castleman disease, plasma cell variant, HHV8 -

Multicentric Castleman Disease
Multiple types

- POEMS-associated
- HHV8 (+) Idiopathic MCD
- HHV8 (-) MCD
- TAFRO-associated
- Others
- HIV
- HHV8
Intravascular Lymphocytosis

A 51-year-old woman who had a perforated appendix

Intravascular Lymphocytosis

CD20

CD3

100 appendectomy specimens
50 perforated and 50 unperforated
20 appendices from right hemicolectomy specimens (control)

IVL significantly more common in
Younger patients (<38 years)
Laparoscopy specimens

IVL did not correlate with perforation or location of inflammation

Possible explanations
Grasping or squeezing during the procedure
Compression when brought out via laparoscopic incision

Histopathology 55: 660, 2009
Intravascular Lymphocytosis
Differential Diagnosis

<table>
<thead>
<tr>
<th>Intravascular large B-cell lymphoma</th>
<th>Monotonous large cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD20+, CD3-, CD5-</td>
</tr>
<tr>
<td>Chronic lymphocytic leukemia</td>
<td>Small lymphocytes</td>
</tr>
<tr>
<td></td>
<td>No large cells</td>
</tr>
<tr>
<td></td>
<td>CD20+, CD5+, CD23+, LEF1+</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
<td>TdT+</td>
</tr>
<tr>
<td></td>
<td>Immature B- or T-cell lineage</td>
</tr>
</tbody>
</table>

Intravascular Large B-cell Lymphoma
3 Clinical Variants

Classical (Western)
- Fever of unknown origin
- Night sweats, weight loss
- Skin lesions ~40%
- Neurological symptoms ~ 30%
- Endocrine insufficiency
- Lung disease (ground glass or nodules on chest film)

Cutaneous
- One or multiple skin lesions
- Normal complete blood count
- Almost all patients are women
- Best prognosis

Hemophagocytic syndrome-associated (Asian)
- Bone marrow involvement almost universal
- Fever, hepatosplenomegaly, thrombocytopenia
- Worst prognosis

Blood 132:1561, 2018
Intravascular Large B-cell Lymphoma
Skin

Seminoma
Clinical Features
Most common germ cell tumor of testis
Age: 30-45 years
80-90% have a palpable mass
Often no symptoms; testicular pain ~20%
Laboratory tests: ↑LDH
↑HCG (~10%)
AFP negative
75% of pts have stage I (localized) disease
Metastases to: retroperitoneal LNs, lungs
Metastatic Seminoma to LN
Many Granulomas

Mediastinal Seminoma

Primary Mediastinal Seminoma
Clinical Features
3-4% of tumors in the mediastinum
Mean age: 32 years (range, 19-56)
> 90% of patients are men
Usually associated with the thymus
Ectopic germ cells or thymic cells with germ cell potential?
Present as mass
+/- compressive
**Seminoma**

**Immunohistochemistry**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOX17</td>
<td>&gt; 95%</td>
</tr>
<tr>
<td>OCT3/4</td>
<td>~ 90%</td>
</tr>
<tr>
<td>SALL4</td>
<td>~ 90%</td>
</tr>
<tr>
<td>CAM5.2 (low mw keratin)</td>
<td>80-90%</td>
</tr>
<tr>
<td>PLAP</td>
<td>80-90%</td>
</tr>
<tr>
<td>CD117/KIT</td>
<td>80-90%</td>
</tr>
<tr>
<td>MAGEC2</td>
<td>80-90%</td>
</tr>
<tr>
<td>CD3</td>
<td>Negative</td>
</tr>
<tr>
<td>CD20</td>
<td>Negative</td>
</tr>
<tr>
<td>CD30</td>
<td>Negative</td>
</tr>
</tbody>
</table>

**Metastatic Seminoma**

**Differential Diagnosis**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse large B-cell lymphoma</td>
<td>Not cohesive</td>
</tr>
<tr>
<td></td>
<td>No abundant pale cytoplasm</td>
</tr>
<tr>
<td></td>
<td>CD20+, CD45/LCA+</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
<td>Reed-Sternberg/Hodgkin cells</td>
</tr>
<tr>
<td></td>
<td>CD15+/-, CD30+, PAX5+</td>
</tr>
<tr>
<td>Anaplastic large cell lymphoma</td>
<td>Hallmark cells</td>
</tr>
<tr>
<td></td>
<td>T-cell; ALK+</td>
</tr>
<tr>
<td>Granulomatous lymphadenitis</td>
<td>No tumor cells</td>
</tr>
<tr>
<td></td>
<td>Necrotizing granulomas</td>
</tr>
<tr>
<td></td>
<td>Evidence of organism</td>
</tr>
</tbody>
</table>

**Nasopharyngeal Carcinoma**

**Clinical Features**

- Rare in US; 72x more common in SE China
- Men > women
- Median age: 30-50 yo
  - ~15% in children
- Presentation
  - Nasal symptoms
  - Obstruction, discharge, cranial nerve palsies
  - Asymptomatic posterior cervical mass
- Metastases
  - LNs, lungs, bones, liver
Nasopharyngeal Carcinoma
Pathologic Features

Two general pathologic types of NPC
Keratinizing (linked to HPV)
Non-keratinizing (linked to EBV)
  - Differentiated
  - Undifferentiated (lymphoepithelioma)

Undifferentiated type more common in children

Nasopharyngeal Carcinoma Metastatic to LN


Nasopharyngeal Carcinoma Metastatic to LN
Eosinophil Rich
### Differential Diagnosis of Metastatic Nasopharyngeal Carcinoma

<table>
<thead>
<tr>
<th>Classical HL</th>
<th>Fibrous bands and RS + H cells CD15+/−, CD30+, PAX5+ Keratin+</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLBCL - NOS</td>
<td>Sheets of large cells CD20+ CD45/LCA+ CD15− Clonal IGH, IGK, or IGL rearrangements</td>
</tr>
<tr>
<td>Peripheral T-cell lymphoma</td>
<td>Cytologic atypia of T-cells Aberrant immunophenotype +/- Clonal TRG and TRB rearrangements</td>
</tr>
</tbody>
</table>

### Thymoma
#### Clinical Features
- **Median age:** 30–40 years (up to elderly)
- Men and women equally affected
- **Anterior mediastinal mass**
  - 30–50% Asymptomatic
  - 30% Local compression
  - 20% Myasthenia gravis

#### Pathology
- Epithelial cell rich (spindle cell or epithelioid)
- Thymocytes and epithelial cells (B1 or B2)
Thymoma

**Immunophenotype**

**Immunohistochemistry**

- Thymic epithelial cells
  - CK5/6, CK903, pankeratin, p63
- Thymocytes
  - Immature T-cells: TdT(+), CD4/8(+)

**Flow Cytometry**

- Thymocytes show maturation (smear)

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**Thymoma vs T Lymphoblastic Lymphoma**

**Flow Cytometry**

- Thymoma
- T-Lymphoblastic lymphoma

(J Clin Pathol 2018)
Differential Diagnosis of Thymoma

<table>
<thead>
<tr>
<th>Condition</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-LBL</td>
<td>Younger patients&lt;br&gt;Often PB and BM involvement&lt;br&gt;No/very few CK+ cells&lt;br&gt;Tight clusters by flow cytometry</td>
</tr>
<tr>
<td>DLBCL - NOS</td>
<td>Sheets of large cells&lt;br&gt;CD20+ CD45/LCA+ CD15-</td>
</tr>
<tr>
<td>Nodular sclerosis HL</td>
<td>Fibrous bands&lt;br&gt;RS+H cells&lt;br&gt;CD15+/-, CD30+, PAX5+, CK-</td>
</tr>
</tbody>
</table>

Myeloid Sarcoma

Clinical Features

Three scenarios:
1. Concurrent evidence of AML in blood and bone marrow
2. History of AML (first sign of relapse)
3. Precedes systemic AML

Can also occur in pts with myelodysplastic syndrome (MDS), myeloproliferative neoplasm (MPN) or MDS/MPN

Myeloid (Granulocytic) Sarcoma
Myeloid (Granulocytic) Sarcoma

Myeloid (Monocytic) Sarcoma

Uterine Cervix

Myeloid Sarcoma
Histologic Features

Diffuse pattern
Often paracortical
Blasts or promonocytes

Immature chromatin
Thin nuclear membranes
Small nucleoli
Mitoses
**Myeloid Sarcoma**

**Immunohistochemistry**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysozyme</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>CD137 (c-kit)</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>CD41</td>
<td>&gt;95%</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
<td>80-90%</td>
</tr>
<tr>
<td>CD45/LCA</td>
<td>70-80%</td>
</tr>
<tr>
<td>CD15</td>
<td>40-50%</td>
</tr>
<tr>
<td>CD99</td>
<td>30-40%</td>
</tr>
<tr>
<td>TdT</td>
<td>30-40% (dim)</td>
</tr>
<tr>
<td>CD34</td>
<td>30-40%</td>
</tr>
<tr>
<td>CD56</td>
<td>30-40%</td>
</tr>
<tr>
<td>PAX5</td>
<td>+ in cases with t(8;21)</td>
</tr>
<tr>
<td>CD20</td>
<td>Rare</td>
</tr>
<tr>
<td>CD3 or CD5</td>
<td>Negative</td>
</tr>
</tbody>
</table>

**Myeloid Sarcoma**

**Differential Diagnosis**

<table>
<thead>
<tr>
<th>Diffuse large B-cell lymphoma</th>
<th>Thicker nuclear membranes</th>
<th>More prominent nucleoli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burkitt lymphoma</td>
<td>Thicker nuclear membranes</td>
<td>Multiple basophilic nucleoli</td>
</tr>
<tr>
<td>Anaplastic large cell lymphoma</td>
<td>Hallmark cells</td>
<td>T-cell; ALK+</td>
</tr>
<tr>
<td>Lymphoblastic lymphoma</td>
<td>TdT+</td>
<td>Immature B- or T-cell lineage</td>
</tr>
<tr>
<td>Ewing sarcoma</td>
<td>CD99 +/-, keratin +/-</td>
<td>Myeloid antigens -</td>
</tr>
</tbody>
</table>

**When You Have The Fresh Specimen**

**Don’t Forget**

- Look for the green color
- Do a touch prep
- Consider cytochemistry
  - Myeloperoxidase
  - Butyrate esterase
- Triage for cytogenetics and molecular
Infectious Mononucleosis
Tonsillitis

Type 2 Downey cells in blood smear

Infectious Mononucleosis
Lymphocytosis

Acute EBV+ Lymphadenitis (Inf Mono)
3 More Cases that Mimic Lymphoma
Kikuchi-Fujimoto Lymphadenitis
Necrotic and proliferative stages

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32 cases analyzed by HUMARA assay
25 / 32 cases were monoclonal
   22 / 29 hyaline vascular variant
   3 / 3 plasma cell variant
3 cases had clonal karyotypes
No IGH or TRG or TRB rearrangements
Hyaline vascular CD may be a neoplasm of stromal cells

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Multicentric Castleman Disease
Differential Diagnosis

<table>
<thead>
<tr>
<th>Unicentric plasma cell variant</th>
<th>Unicentric HHV-8-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No HIV infection</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hyaline-vascular variant</th>
<th>HV lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Big follicles</td>
</tr>
<tr>
<td></td>
<td>Interfollicular vascularity</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Peripheral T-cell lymphoma</th>
<th>Architecture effaced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monoclonal T-cell population</td>
</tr>
</tbody>
</table>

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TAFRO Syndrome

A 62-year-old woman with obstruction of the ileum and underwent right hemicolectomy.

Intravascular Lymphocytosis

A 62-year-old woman with obstruction of the ileum and underwent right hemicolectomy.

Intravascular Large B-cell Lymphoma

Bone Marrow
Mediastinal Mass in 18 yo

Dx: Seminoma
B-Chronic Lymphoproliferative Neoplasms in Blood and Bone Marrow

DR KATHRYN FOUCAR
B-Chronic Lymphoproliferative Neoplasms in Blood and Bone Marrow

2019 Hawaii Hemepath Conference
Kathryn Foucar
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Disclosures:
No relevant disclosures

Outline
• Classification of B-CLPN
• WHO Criteria
• Prognosis Assessment
• Key Problem Areas
**B-Chronic Lymphoproliferative Neoplasm**

**Definition:**
Clonal proliferation of morphologically and immunophenotypically mature B-lymphocytes. These cells are generally characterized by a low proliferation rate and prolonged cell survival.

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**Approach to CLPN**

- Clinical - Age, physical exam
- CBC - all lineages
- Morphology of lymphocytes
- IP profile, genotype
- BM findings
- Prognostic factors (esp. CLL)
- Key new genetic features relevant to classification/prognosis/therapy

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**Incidence of Leukemic Lymphoproliferative Neoplasms**

<table>
<thead>
<tr>
<th>Type of Leukemia</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic Lymphocytic Leukemia</td>
<td>&gt; 85%</td>
</tr>
<tr>
<td>Hairy Cell Leukemia</td>
<td>~ 5%</td>
</tr>
<tr>
<td>Mantle Cell, SMZL, Other Lymphomas</td>
<td>&lt; 5%</td>
</tr>
<tr>
<td>Prolymphocytic Leukemia (B,T)</td>
<td>&lt; 2%</td>
</tr>
<tr>
<td><strong>Normal CBC</strong></td>
<td></td>
</tr>
<tr>
<td>Monoclonal B lymphocytosis</td>
<td>5-8%</td>
</tr>
<tr>
<td>(detected by flow cytometry)</td>
<td></td>
</tr>
</tbody>
</table>
WHO 2016/17 and Other New Features

- No changes to CLL criteria
- Better delineation of MBL: High (≥ 500) vs Low Count (< 500) for CLL type MBL
- More information regarding histologic progression but unresolved issue of “expanded proliferation centers” in LN
- New IP markers integrated in case work-up: LEF1, CD200, CD49d

Monoclonal B Lymphocytosis (<3%)

Absolute monoclonal B cell = 300

Monoclonal B Lymphocytosis

- High count (clinical) (≥ 500) and low count (< 500)
- Predisposes to CLL; 100 times more common
- 12% of healthy subjects (8 color flow)
- Borderline with CLL unclear; same genetics as CLL
- Overlap with blood involvement with SLL key issue. (BM criteria for MBL not defined)
- Must know CBC data and clinical to interpret flow
- About 1% progression per year to CLL for high count MBL
Chronic Lymphocytic Leukemia

Clinical: Middle age to elderly

Hemogram: Sustained (3 mos) monoclonal lymphocytosis, (≥5,000) with CLL IP; variable cytopenias

Morphology: Small lymphocytes, condensed chromatin, inconspicuous nucleoli
Prolymphocytes, other forms

Bone Marrow: Pattern of infiltration nodular, mixed, diffuse

WHO 2016/2017: No significant revisions in diagnostic criteria

CLL: Preserved Hematopoiesis

CLL, smudge cell
CLL: Diagnostic Immunophenotypic Profile

Mature B Monoclonal SIg (dim)

B-cell antigens CD19, dim CD20, dim CD22

Coexpression CD5, CD23

Absent CD10, FMC7, CD11c (dim to neg.), CD79b, CD103

Prognosis CD49d‡, CD38, ZAP 70 (≥ 30% = ⊗)

‡ CD49d overall most significant IP feature

CLL: Flow Cytometry

Prognostic Factors in CLL

Source: Gribben JG. Blood 2018; 132:31-39
**Progression Risk in CLL**

![Graph showing progression risk in CLL]


---

**Impact of CD49d on Survival in CLL**

- Increased hazard of death (2.3)
- Lower OS at 5 years: 87% vs 90%
- Lower OS at 10 years: 62% vs 84%
- Most robust prognostic marker

![Graph showing impact of CD49d on survival in CLL]

Source: Bullian, et al. JCO 2014; 32:897

---

**CLL: Morphology – IP – Genotype**

- **del(13q)** linked to typical morphology and IP (key microRNA deletion)
- **tri 12** linked to atypical morphology, atypical IP
- **del(11q)** linked to high stage, aggressive disease course
- **del(17p)** advanced stage, chemo resistance
- **miRNA** deletions lead to post-transcriptional abnormalities; tumor suppressor deletion

---
Genetics and Prognosis in CLL

Favorable
- del13q as sole abnormality

Intermediate
- Normal
- Trisomy 12

Unfavorable
- p53 deletion (17p)
- ATM deletion (11q)
- Complex karyotype


When to assess prognostic factors?
- At initial diagnosis
- When patient requires therapy
- Discuss with clinician
- Assessment in patients >75 years may not impact outcome (still recommended)

Refs: Shanafelt, Cancer 2010, Parikh, Blood 2016
Progression of Chronic Lymphocytic Leukemia
• Prolymphocytoid (clonal)
• Large cell lymphoma (Richter’s) (2/3 clonal evolution; 1/3 EBV-ass’t 2° neoplasm)
• Clonal DLBCL PD-1⁺, may respond PD-1 blocking antibodies

CLL with ≥ 10% Prolymphocytes
• Independently associated with NOTCH1 mutations,
• High CD38 expression,
• Unmutated IGHV, and increased risk of Richter Syndrome (LCL)

CLL: Large cell lymphoma (Richter)

Prolymphocytes predominant

Mature B, NOS

KL: Kappa

CD 20

CD 19

CD 22
**B-PLL: Key Issues**

- Does it exist? Diagnosis of Exclusion
- MCL often misdiagnosed as B-PLL
- Transformations of CLL misdiagnosed as B-PLL
- Confusion in literature “B-cell PLL: a specific subgroup of mantle cell lymphoma”

_Blood 2014; 124:412_

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**MCL Mimicking B-PLL**

61 year old male with lymphocytosis

---

**Hairy Cell Leukemia**

**Clinical:** Middle age to elderly, splenomegaly

**Hemogram:** Pancytopenia, monocytopenia

**Morphology:** Oval to round nuclei with “spongy” chromatin; abundant cytoplasm with hairy projections

**Cytochemistry:** TRAP (strong)

**Bone Marrow:** Distinct pattern of infiltration

**Molecular:** _BRAF_ mutation in all cases

---
HCL: Rare cells in blood
High index of suspicion

Hairy Cell Leukemia – Marked Monocytopenia
67-year-old male with lymphocytosis

HCL: Sensitivity of Flow Cytometry
0.3% of cells
HCL: Immunohistochemistry

- Annexin
- CD20
- TRAP
- DBA 44

HCL: IHC and Caveats

- Annexin 1—Myeloid cells+, T cells+
- CD123—Dendritic cells+
- DBA 44—Only subset+
- TRAP—Histiocytes TRAP+
- Cyclin D1-weak—Confuse with MCL
- CD103—often weak
- BRAF—often weak

Hypocellular HCL

- 10-20% of cases
- TNFα production by hairy cells
- Misdiagnosis of aplastic anemia
- Very subtle infiltrates
- HCL infiltrates highlighted by CD20 (More IHC can be used)
Hypocellular HCL; rare cell in blood

Hypocellular HCL; rare cell in blood

Splenomegalic B-CLPN with Leukemic Manifestations

<table>
<thead>
<tr>
<th>Lymphoma</th>
<th>Leukemic Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCL</td>
<td>Variable WBC, often low</td>
</tr>
<tr>
<td>HCLv</td>
<td>Leukocytosis</td>
</tr>
<tr>
<td>MCL</td>
<td>Leukocytosis; may be SOX-11 negative, CD200 positive</td>
</tr>
<tr>
<td>SMZL</td>
<td>Leukocytosis, villous lymphocytes</td>
</tr>
<tr>
<td>B-PLL</td>
<td>Leukocytosis (exclude MCL)</td>
</tr>
<tr>
<td>Other splenic red pulp lymphomas</td>
<td>Villous lymphocytes in blood (overlap with HCLv)</td>
</tr>
</tbody>
</table>
Case

Hx: 69 year old female with splenomegaly and thrombocytopenia
Case

CBC:
- WBC 5.7 ANC: 3.5
- RBC 5.16 ALC: 1.8
- H/H 14.3/43.5 AMC: 0.3
- MCV 84
- Plt 94 (L)

Blood

Rare cell

BM biopsy

Normal Retic Stain
**BM: Flow Cytometric IP**

- CD5 neg
- CD10 neg

---

**Differential Dx:**

- Monoclonal B lymphocytosis, non CLL
- Hairy cell leukemia (HCL)
- Splenic marginal zone lymphoma (SMZL)
- HCL variant
- CD5- mantle cell lymphoma (MCL)
- Other

---

**BM Core Bx: CD20**

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**Key Findings**

- Splenomegaly
- CD103⁺, CD25(-), CD11c⁺, CD22⁺
- Occult in blood and by H+E in BM
- Sinusoidal by CD20
- Normal monocyte count

**HCL variant: Key Features**

- Lymphocytosis, variably prominent nucleoli
- Splenomegaly; red pulp
- Aspirable BM (often sinusoidal)
- Lack CD25, CD123, Annexin, TRAP, BRAF (all positive in HCL)
- Unresponsive to 2-CDA alone (may respond 2-CDA with Rituximab)
- *BRAF* wild type

**Key Tips in HCLv Diagnosis**

- Consider HCLv when BM infiltration is exclusively sinusoidal
- MBL unlikely in patient with splenomegaly and thrombocytopenia
- SMZL may express CD103 but does not typically exhibit bright CD22/CD11c
- SMZL usually exhibits nodular and sinusoidal BM infiltrates
B-CLPN: Key Tips WHO 2016/2017

- Recognize morphology, IP features of distinct entities
- B-CLPN have characteristic IP profile (not single marker)
- Identification of biologic subtypes of CLL important (morphology, IP, genetic clues)
- HCL: Pancytopenia (monocytopenia)
  - Very subtle BM infiltrates
  - Hypocellular (!) / BRAF mutations
- Overlap disorders: (Atypical CLL and lymphomas)
  - (B-PLL and MCL)
- Utility of genetics in MCL, CLL, other lymphomas
Plasma Cell Neoplasms

DR ROBERT MCKENNA
Notice of Faculty Disclosure

In accordance with ACCME guidelines, any individual in a position to influence and/or control the content of this CME activity has disclosed all relevant financial relationships within the past 12 months with commercial interests that provide products and/or services related to the content of this CME activity.

The individual below have responded that they have no relevant financial relationship with commercial interest to disclose:

Robert W. McKenna, MD

Plasma Cell Neoplasms

- Monoclonal Gammopathy of Undetermined Significance (MGUS)
- Plasma Cell Myeloma
- Solitary Plasmacytoma
- Immunoglobulin Deposition Diseases
- Plasma Cell Neoplasms with Associated Paraneoplastic Syndrome

Plasma Cell Neoplasms (PCN)

- Diagnostic Studies for plasma cell neoplasms
- Flow cytometry
- Genetics
- Issues related to revised WHO criteria in diagnosis of PCN
  - Modified criteria for diagnosis of plasma cell myeloma
  - Changes in the classification of MGUS
  - Issues in diagnosis of solitary plasmacytoma
  - Issues in diagnosis of amyloidosis
Diagnostic Testing for Suspected PCN

- Assessment for monoclonal immunoglobulin
- Radiographic Studies
- Biopsy—Morphologic Assessment

Uses of Serum-Free Light Chain Assay (Quantification and K/L ratio)

- Screening in combination with SPE and IFE
- Baseline values are prognostic
  - MGUS
  - Smoldering myeloma
  - Symptomatic myeloma
  - Plasmacytoma
  - AL amyloidosis
- Hematologic responses to treatment
  - AL amyloidosis; oligosecretory - non-secretory myeloma; LCDY
  - Stringent complete response in plasma cell myeloma


Imaging Studies

- Radiological Skeletal Survey
  - Bone lesions in ~70% of PCM
  - More frequent by PET-CT and MRI
- Lytic lesions in ~70%  
- Osteoporosis in 10-15%
- Compression fractures
- Pathological fractures
- Most common sites:
  - Vertebrae, ribs, skull, shoulders, pelvis, long bones
Morphologic Assessment

- Bone Marrow Biopsy
- Aspiration and trephine
- Quantity and atypicality of plasma cells
- Directed biopsy

IgA Plasma Cell Myeloma

Convoluted Plasma Cell Myeloma

Plasmablastic Myeloma

Cytoplasmic Crystalline Inclusions
Patterns of Marrow Involvement in PCM

Interstital  Focal  Diffuse

---

Immunohistochemical Assessment of PCN

- Assessment of quantity of plasma cells on bone marrow sections
- Identification of a monoclonal plasma cell proliferation
- Identification of aberrant antigen expression
- Distinction of myeloma from other neoplasms

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Quantification of Plasma Cells in Plasma Cell Myeloma

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Kappa  Lambda
Low Level of Bone Marrow Involvement in Plasma Cell Myeloma

Identification of Clonal Plasma Cells

Kappa Lambda

Polytypic Plasma Cell Proliferation in the BM
Differentiating Myeloma From Other Neoplasms

Genetic Analysis of Plasma Cell Neoplasms

Conventional Cytogenetics
- Standard for detecting genomic abnormalities and outcome discrimination in PCN
- Only ~40% of PCM have identifiable abnormalities
- Deletion of 13 and hypodiploidy are prognostic karyotype changes

Karyotypes are courtesy of Michelle Dolan, MD, Univ. of MN, Cytogenetic Lab.

Genetic Analysis of Plasma Cell Neoplasms

Fluorescent In Situ Hybridization (FISH)
- >90% of cases have detectable abnormalities
- Employed for establishing risk-based stratification
- Cell sorting or cIg FISH improves yield

FISH illustrations are courtesy of Michelle Dolan, MD, Univ. of MN Cytogenetics Lab.
Genetic Analysis of Plasma Cell Neoplasms

Gene Expression Profiling (GEP)
- Powerful technique in patient stratification
- GEP signature distinguishes high- and low-risk myeloma
- Most sensitive and specific for identification of high-risk PCM


Gene Expression Profiling (GEP)
  - Hyperdiploid (45%)
  - Non-hyperdiploid (40%)
    - Cyclin D translocation-15%
    - t(11;14)(q13;q32)-16%
    - t(6;14)(p25;q32)-2%
    - t(12;14)(p13;q32)<1%
    - NSD2 translocation-15%
    - t(4;14)(p16;q32)
    - MAF translocation-10%
    - t(14;16)(q32;p23)-5%
    - t(14;20)(q32;q11)-2%
    - t(8;14)(q24;q32)-1%
  - Unclassified (other) (15%)

References
- Bergsagel PL and Kuehl WM. Oncogene 2001; 20: 561

Cytogenetics of Non-IgM MGUS
- Numerical and structural abnormalities by FISH in most patients
- Hyperdiploidy in ~ 40% with trisomies similar to those in myeloma
- ≥ 50% have 14q32 translocations t(11;14)-15%-25%, t(4;14) - 2%-9%, t(14;16) - 1%-5%
- 40% to 50% have del(13)
- No obvious clinical or biologic correlations
Factors in Progression of Plasma Cell Neoplasms

- Secondary genetic events
  - Gains of chromosome 1q and loss of 1p
  - Secondary IgH or IgL translocations
  - Translocations involving MYC or N-MYC
  - Deletion or mutation of TP53
  - Activating mutations of K- or NRAS
- Bone marrow microenvironment

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Mayo Stratification of Myeloma and Risk-Adapted Therapy (mSMART)

<table>
<thead>
<tr>
<th>Standard Risk (60%)</th>
<th>Intermediate Risk (20%)</th>
<th>High Risk (20%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(11;14)</td>
<td>t(4;14)</td>
<td>del 17p</td>
</tr>
<tr>
<td>t(6;14)</td>
<td>del 13</td>
<td>t(14;16)</td>
</tr>
<tr>
<td>Hyperdiploid</td>
<td>Hypodiploid</td>
<td>t(14;20)</td>
</tr>
<tr>
<td>All Others</td>
<td>GEP High Risk</td>
<td></td>
</tr>
<tr>
<td>(OS=8-10yrs)</td>
<td>(OS=4-5yrs)</td>
<td>(OS=3yrs)</td>
</tr>
</tbody>
</table>

Chesi M and Bergsagel PL. Int J Hematol 2013; 97: 313

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Revisions in the WHO Classification of PCN (2017)

- Monoclonal Gammapathy of Undetermined Significance
  - Non-IgM (Plasma cell) MGUS (Includes light chain MGUS)
  - IgM (lymphoplasmacytic) MGUS
- Plasma Cell Myeloma (New criteria)
  - Molecular cytogenetic categories (IMWG)
  - Clinical Variants
    - Smoldering (asymptomatic) myeloma (High-risk symptomatic myeloma)
    - Non-secretory myeloma
    - Plasma cell leukemia
- Plasmacytoma (Changes in radiographic requirements)
  - Solitary plasmacytoma of bone (SP with minimal BM involvement)
  - Extramedullary plasmacytoma
- Immunoglobulin Deposition Diseases
  - Primary amyloidosis (Recommendations on amyloid testing)
  - Systemic light and heavy chain deposition diseases
- Plasma Cell Neoplasms with Associated Paraneoplastic Syndrome
  - POEMS syndrome (Changes in criteria for diagnosis)
  - TEMPI Syndrome (Provisional)
Diagnostic Criteria for Plasma Cell Myeloma (Revised 4th edition-2016)

- Clonal BM plasma cells > 10% or biopsy-proven plasmacytoma and
- End organ damage attributable to the plasma cell proliferative disorder (CRAB)
  - C: high calcium levels (>11 mg/dl)
  - R: renal dysfunction (Cr >2 mg/dl)
  - A: anemia (Hgb < 10 g/dl)
  - B: bone destruction (CT or PET-CT)
- One or more specific biomarkers of malignancy

Diagnostic Criteria for Smoldering (Asymptomatic) Myeloma

- Serum M-protein (IgG or IgA) >3.0g/dL or urinary M-protein >500mg per 24 h and/or clonal BM plasma cells at myeloma levels
- Absence of myeloma defining events (CRAB) or amyloidosis
  (Progression to symptomatic myeloma is ~10%/yr.)

High Risk Smoldering Myeloma

- Extreme bone marrow plasmacytosis (>60%)
- Extremely abnormal serum free light chain ratio (>100)
- >1 focal bone lesion detected only by MRI
- 2-year time to progression rates >60%
- Clinical trials have shown that smoldering myeloma with high risk features benefit from treatment
  - Delayed time to progression and improved overall survival
Biomarkers of Malignancy in PCM

- Clonal bone marrow plasma cells > 60%
- Involved:uninvolved serum free light chain ratio >100
- >1 focal lesion on MRI studies

Monoclonal Gammopathy of Undetermined Signif. (MGUS)

- MGUS is a potentially malignant clonal plasma cell expansion (precursor lesion)
- Defined by:
  - Marrow plasma cells < 10% and low level of infiltration in biopsies
  - M-protein in serum < 3.0g/dl
  - No myeloma related end organ damage (CRAB) or paraneoplastic syndrome

Monoclonal Gammopathy of Undetermined Signif. (MGUS)

- ~3% to 4% of persons >50 years of age
- A significant minority progress to a malignant plasma cell neoplasm (~1%/year)
- Can not be certain at diagnosis which will remain stable (risk predictors)
### Types of MGUS (WHO-2017)

- IgM or lymphoplasmacytic MGUS (15%)
- Non-IgM or plasma cell MGUS (85%)
  - 60%-IgG, 15%-IgA, 1%-IgD, 1%-IgE, 3%-biclonal, 20%-light chain only
- Distinct biologic and clinical entities

### Differences Between IgM MGUS and Non-IgM MGUS?

<table>
<thead>
<tr>
<th>Non-IgM MGUS</th>
<th>IgM MGUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cell</td>
<td>Lymphoplasmacytic</td>
</tr>
<tr>
<td>Genetics similar to myeloma</td>
<td>MYD88 L265P mutation in ~50% of cases</td>
</tr>
<tr>
<td>Rate of progression is 1.0%/yr.</td>
<td>Rate of progression is 1.5%/yr.</td>
</tr>
</tbody>
</table>

### Differences Between IgM MGUS and Non-IgM MGUS

- **Non-IgM MGUS**
  - Progression to plasma cell myeloma or primary amyloidosis
- **IgM MGUS**
  - Progression to lymphoplasmacytic lymphoma (WM) or other lymphoproliferative dis.
Light Chain MGUS

- Abnormal free light chain ratio (<0.26 or >1.65)
- Increased level of the involved free light chain
- No immunoglobulin heavy chain on IFE
- Clonal plasma cells <10%
- Urinary M-protein <500mg/24hr
- Absence of end-organ damage (CRAB) or amyloidosis
- Up to 20% of MGUS
- Rate of progression is 0.3%/year

Solitary Plasmacytoma

- Solitary lesion of bone or soft tissue consisting of clonal PCs
- Normal random BM biopsy without clonal PCs
- Normal skeletal survey and MRI or CT except for the solitary lesion
- Absence of end-organ damage
- Solitary plasmacytoma with minimal bone marrow involvement:
  - Same as above plus clonal PCs of <10% in random BM biopsy
  - 60% vs. 10% progression in 3 yrs.

Extraosseous Plasmacytoma

- Most Common Primary Site –
  - ~80% in upper respiratory track
    (Spread to cervical nodes ~15%)
- Less Common Sites
  - Lymph nodes (primary), salivary glands, thyroid, breast, GI track, CNS, etc.
- ~25% local recurrence, occasional spread to other sites
- ~15% progress to PCM
Differential Diagnosis of Extraosseous Plasmacytoma

- Other lymphoid neoplasms that exhibit marked clonal plasma cell differentiation
  - Marginal zone lymphoma
  - Lymphoplasmacytic lymphoma
  - Plasmablastic lymphoma

Extraosseous Plasmacytoma vs. Lymphoma with Extreme Plasma Cell Differentiation

- Presence of areas in tissue section of typical lymphoma
- IgM monoclonal protein
- Detection of clonally related lymphocytes
- PC Immunophenotype by flow cytometry
  - CD19(−), CD56(+) more likely plasmacytoma
  - CD19(+), CD56(−) more likely lymphoma
  - CD20(+) more likely lymphoma

Diagnostic Criteria For Amyloidosis

- Tissue biopsy showing typical morphology
- Apple green birefringence under polarized light after Congo Red stain
- Typical fibrillar ultrastructure
Characterization of Amyloid in Patients with M-Protein at MGUS Levels

- MGUS is a common finding (3-4% of people >50 years of age; 5% of those >70)
- Can not assume amyloid is AL (light chain) amyloid simply because a patient has a MGUS
- Amyloid should be characterized in that setting
Criteria for Diagnosis of Myeloma in Patients with Amyloidosis

- Criteria for a diagnosis of plasma cell myeloma in patients with light chain amyloidosis
  - 10% or more plasma cells or an M-protein at myeloma levels
- If plasma cell number and M-protein are not at myeloma levels a diagnosis of myeloma should not be made

Thank you! Questions?
FLOW CYTOMETRIC DIAGNOSIS OF COMPLEX HEMATOLOGY CASES

DR HORATIU OLTEANU
53-year-old M with history of MDS

- Diagnosed with MDS-MLD in 04/2017
- Treated with 2 cycles of hypomethylating agents
- PB in 10/2017: 31% blasts
- Follow-up BM biopsy with flow cytometry
• Two blast populations:
  - Red (5-8%): CD2 partial (+), CD13 (+), CD33 (+), CD34 (+), CD38 variably (+), CD45 dim (+), CD117 bright (+), HLA-DR partial (+)
  - Cyan (20-25%): CD2 partial (+), CD7 variably (+), CD13 variably (+), CD33 partial dim (+), CD34 variably (+), CD36 (+), CD38 variably (+), CD45 moderately (+), CD117 variably dim (+)

Cytogenetics

- 44-47,XY,add(3)(p25),-5,-7,i(8)(q10),add(11)(p15), del(12)(p11.2),-17,-20, i(21)(q10), +2-5mar[18]/ 45,XY,add(3)(q29),-5, del(7)(q31), add(12)(p12),-17, +i(21)q10[2]

- Each metaphase cell examined has a large number of clonal abnormalities including monosomy 5, monosomy or deletion 7, trisomy 8q (18 cells), monosomy 17, monosomy 20 (18 cells), and other structural abnormalities involving chromosomes 3, 8, 11, 12, 21, and there are 2-5 unidentified markers. These abnormalities are distributed among two related clones. As such this is a highly complex karyotype, and would be expected to carry a relatively unfavorable prognosis.
Diagnosis:
Acute Myeloid Leukemia with Myelodysplasia-Related Changes

Blasts with erythroid differentiation
- Routine AML panel screens for CD36(+), CD34(-), CD45(-), CD117(+) blasts of potential erythroid lineage:
  - CD36/CD56/CD64/CD11b/CD34/CD16/CD2/CD45
  - CD34/CD117/CD15/CD33/HLA-DR/CD14/CD7/CD45
- CD36 and CD64 co-expression is found in monocytes
  - CD11b bright (+) also establishes monocytic lineage
- CD36(+)/CD45(-) blasts of megakaryocytic lineage
  - Also express CD41 and CD61
- Additional tubes to confirm erythroid lineage:
  - CD71, Glycophorin A (not specific)

Bonus Case
29-year-old M with pancytopenia

- History of DM and possible autoimmune disease
- Normocellular bone marrow with trilineage hematopoiesis and no morphologic evidence of a hematologic malignancy
- Normal male karyotype and normal MDS FISH
- Abnormal flow cytometry findings

Absence of CD36 expression on monocytes (blue) and erythroid precursors (cyan)
- Likely absence of CD36 expression on platelets [no shift towards CD36 of granulocytes (green)]
- Normal myeloblasts (red) and hematogones (yellow)

Diagnosis:

**Congenital CD36 Deficiency, Type I**
Congenital CD36 deficiency

- Two types are recognized:
  - Type I: neither platelets, nor monocytes express CD36
  - Type II: CD36 is expressed in platelets, but not in monocytes
- Some studies have observed abnormalities of glucose and lipid metabolism in type I CD36 deficiency
- There is no literature data on the association of CD36 deficiency and increased risk of aplastic anemia or MDS

Case #2

58-year-old F with history of renal transplant and sickle cell disease

- ESRD, status post renal transplant 1982
  - Presented with worsening anemia and thrombocytopenia
- CBC: Normal WBC (9,200/uL) and 4% blasts
- PB flow cytometry performed in 01/2017
Red (4% blasts): Small to medium sized cells, CD1a (-), CD2 (-), surface CD3 (-), cytoplasmic CD3 (-), CD4 (-), CD5 (-), CD7 (+), CD8 (-), CD10 (-), CD11b (-), CD13 bright (+), CD14 (-), CD15 (-), CD16 (-), CD19 (-), CD20 (-), CD22 (-), CD33 variably (+), CD34 (+), CD38 bright (+), CD41 (+), CD45 dim to moderately (+), CD45RO (-), CD56 dim (+), CD61 (+), CD64 (-), CD79a (-), CD117 (-), HLA-DR (+), MPO (-), TdT (-), Glycophorin A (-), and surface immunoglobulin (-).

BM biopsy: 46% blasts; moderate to severe increase in reticulin fibrosis (MF grade 2-3)
Cytogenetics

- 42,XX,add(2)(q37),-4,del(7)(q32),-9,del(12)(p12),der(14)(q44;14)(q12;p11.2),-16,add(17)(p11.2),-20,17(14)del(12)(q12),14,del(17)(p11.2),-21,del(22)(q12)[14]/46,XX[4]

- 14 cells each have a large number of clonal structural and numerical abnormalities in a complement of 42 chromosomes. Each metaphase cell examined has monosomies involving chromosomes 9 and 16, and structural abnormalities involving chromosomes 2, 7q, 12p, 14, 17, 20, 21, and 22. Deletion of 7q and 12p are consistent with a myeloid disorder. Translocation involving 17p likely deleted p53. Monosomal karyotypes are associated with relatively unfavorable outcomes in AML, as is deletion of p53.

Diagnosis:

Acute Myeloid Leukemia with Megakaryocytic Differentiation

Acute megakaryoblastic leukemia

- <5% of AML; can occur at any age
- Morphology: variably-sized blasts
  - May show cytoplasmic projections or blebs
  - Associated fibrosis may cause “dry tap”
- Immunophenotype:
  - Blasts are positive for CD41, CD61, CD35, CD13, CD33
  - Usually negative for CD34, CD45, MPO, Tdt
  - May be positive for CD7
- Genetics:
  - MDS-associated complex karyotypes
  - t(1;22)(p13;q13), inv(3)(q21q26.2), t(3;3)(q21;q26.2), or +21
Case #3

66-year-old F with h/o AML, s/p allo SCT; 2% blasts on PB smear

Other results
- CBC: WBC=4,100/uL, Hb=11.4 g/dL, Plt=160,000/uL
  - Differential count: 2% blasts, 62% segs, 31% lymphocytes, 2% monocytes, 3% eosinophils; 0 nRBCs
- Morphology:
  - PB: 2% blasts; frequent large/giant platelets; rare meg
- Cytogenetics:
  - 46,XX[4]/46,XY[16] (host and donor metaphases)
Peripheral blood smear

Giant platelets/megakaryocytes in Acute Myeloid Leukemia (AML)

Platelets immunophenotype
- Normal platelets/megakaryocytes:
  - Positive: CD36, CD41, CD61
  - Negative: CD11b, CD34, CD45, CD64, CD117, CD235 (Gly A), HLA-DR
- Abnormal megakaryocytes (in MDS/MPN):
  - CD34(+)
- Differential diagnosis:
  - Spurious CD36 expression on blasts
  - Erythroid precursors
  - Immature monocytes
Case #4

70-year-old M with history of MDS

- Diagnosed with MDS-EB-2 in 03/2015
  - Treated with 3 cycles of hypomethylating agents and CLAG-M, and worked up for allogeneic SCT
- Follow-up BM biopsies in 2017: CR
  - Patient declined allogeneic SCT
- Progressed to AML with MRC in 10/2017
  - Treated with hypomethylating agents and clinical trials x2
- Follow-up BM biopsy in 03/2018
Bone marrow flow cytometry

- Two aberrant populations:
  - **Red (6-8% myeloblasts)**: CD13 uniformly (+), CD33 partial (+), CD34 bright (+), CD38 variably dim (+), CD45 dim (+), CD117 variably (+), HLA-DR (+), CD25(+)
  - **Cyan (3-5% mast cells)**: CD13 uniformly (+), CD33 (+), CD34 variably (+), CD38 (+), CD45 moderately (+), CD117 bright (+), HLA-DR variably dim (+), CD25 dim (+)

- By morphology: 33% blasts; 30% mast cells

Cytogenetic / Molecular analysis

- 47,XX, der(13)(q11;p11.2), +21, der(21)(q11;p11.2) [cp16]/46,XX[4]
  - Multiple structural & numerical abnormalities including trisomy 21 in all cells, and either a der(13)(q11;p11.3) or der(21)(q1;21) in all of the abnormal cells. All derivative chromosomes result in a gain of 1q and either loss of 13p or 21p.
  - Published data indicate a possible association of unbalanced 1q rearrangements with a highly proliferative phenotype in myeloproliferative neoplasms with a propensity of disease transformation.

- FISH analysis performed on the same specimen also revealed trisomy 21 in 48% of interphase cells analyzed.
  - KIT (D816V) mutation by allele-specific PCR
  - DNMT3A, NRAS, RUNX1, SRSF2 mutations by NGS
  - Also, variants of unknown significance: CDH1, INPP4B, MITF, MLL2, MSH6
Diagnosis:
1. Persistent AML-MRC
2. Mast Cell Leukemia

Mast cell leukemia
- Mast cells ≥20% of nucleated cells in aspirate smear
- Neoplastic mast cells often show marked atypia
  - Hypogranular cytoplasm
  - Irregularly shaped nuclei (monocytoid or bilobed)
  - Prominent nucleoli
- Typical cases show ≥10% circulating mast cells
  - If <10%: “Aleukemic” variant of mast cell leukemia
- Meets other criteria of systemic mastocytosis (SM)
  - CD2 and/or CD25 expression
  - KIT D816V mutation

Case #5
46-year-old M with history of cutaneous mastocytosis and diverticulitis

- Diagnosed with cutaneous mastocytosis (CM) 16 years prior
  - Treated with aspirin and antihistamines for recurrent skin rashes
- CT abdomen/pelvis performed due to LLQ pain
  - New sclerotic hip and spine lesions
- BM biopsy performed in 08/2017

Two aberrant populations:
- Red (5-7% plasma cells): CD38 bright (+), CD138 (+), CD19 (-), CD56 (+), CD45 (-), cytoplasmic lambda restricted
- Cyan (0.8% mast cells): CD117 bright (+), CD25 (+)

By morphology: 35% plasma cells; 15% mast cells

Cytogenetics and Molecular analysis

- 46, XY – Normal male karyotype
- FISH positive for gains of chromosome 1q21 and normal results with the t(4;14), t(14;16), t(14;20), and 17p probe sets
  - Duplication of 1q in multiple myeloma is considered a secondary aberration associated with tumor progression and advanced disease. It is often translocated to different chromosome partners and it suggests gene amplification.
- KIT (D816V) mutation by allele-specific PCR
**Diagnosis:**

Systemic Mastocytosis (SM) and Plasma Cell Neoplasm, consistent with SM with an Associated Hematological Neoplasm
Systemic mastocytosis with associated hematological neoplasm

- Meets other criteria of systemic mastocytosis (SM)
  - Clusters of mast cells with abnormal morphology
  - CD2 and/or CD25 expression
  - KIT D816V mutation
- Concomitant presence of hematologic neoplasms that represent a distinct entity (WHO classification)
  - Most common: myeloid neoplasms (AML, MDS)
  - Less common: lymphoma, plasma cell neoplasms

Case #6

71-year-old M with history of PCM

- Diagnosed with plasma cell myeloma in 2014
  - Status post autologous SCT in 2016
  - Maintenance therapy with lenalidomide
- Presents with anemia and 2% circulating blasts
  - Normal WBC and platelet counts
- BM biopsy performed in 04/2018

71-year-old M with history of PCM

- Diagnosed with plasma cell myeloma in 2014
  - Status post autologous SCT in 2016
  - Maintenance therapy with lenalidomide
- Presents with anemia and 2% circulating blasts
  - Normal WBC and platelet counts
- BM biopsy performed in 04/2018
• Two aberrant populations:
  - **Red (17% myeloblasts):** CD13 variably (+), CD33 slightly bright (+), CD34 (+), CD38 dim (+), CD45 moderately (+), CD117 slightly bright (+), HLA-DR variably (+)
  - **Cyan (0.75% plasma cells):** CD38 bright (+), CD19 (-), CD56 bright (+), CD45 moderately (+), CD200 (+), intracellular kappa restricted (previously established immunophenotype is shown)

• By morphology: 20% blasts; 3% plasma cells

**Bone marrow flow cytometry**

**Cytogenetics**


• Two cells were normal, while 18 cells each have a complex karyotype with monosomy 5, deletion 7q, unknown rearrangements of chromosomes 9q, 12p, 18p and 19p. Four of these abnormal cells were tetraploidized. A complex karyotype, and abnormalities of both 5 and 7 together, would be expected to be associated with an unfavorable prognosis. Given previous treatment for myeloma this may represent a therapy-related secondary myeloid disorder.
Diagnosis:
1. Therapy-related AML
2. Low-level persistent PCM

Case #7
80-year-old M with history of fatigue

- Found to have worsening creatinine with hypercalcemia
  - SPEP with IFE: IgG kappa = 3.2 g/dL
  - Serum free light chains: kappa = 2,327 mg/L

- BM biopsy performed in 03/2016
  - Mild anemia (9.7 g/dL) and lymphocytosis (4,800/μL)
  - Normal WBC and platelet count
  - Aspirate diff: 65% plasma cells; 9% lymphocytes

- CT chest/abdomen: Axillary and abdominal LAD
What is your diagnosis?

- A. Lymphoplasmacytic lymphoma/ Waldenstrom macroglobulinemia
- B. Plasma cell myeloma and chronic lymphocytic leukemia/small lymphocytic lymphoma
- C. Plasma cell myeloma and mantle cell lymphoma
- D. Plasma cell myeloma and reactive polymorphous lymphohistiocytic lesions
Bone marrow flow cytometry

- Two aberrant populations:
  - **Red (1.2% plasma cells):** CD38 bright (+), CD19 (-), CD56 (+), CD45 dim (-), CD20 variably dim (+), CD200 (-), intracellular kappa restricted
  - **Blue (11% B cells):** CD19 (+), CD20 variably dim (+), CD5 (+), CD10 (-), CD38 partial (+), CD23 variably (+), FMC-7 (-), dim surface lambda restricted, intracellular lambda restricted

Cytogenetics

- **47,XY – Normal male karyotype**

- Myeloma FISH: In 200 interphase cells analyzed extra signal for FGFR3 (indicating trisomy 4p); deletion or monosomy 16 (MAF), and trisomy 11 (CCND1) were observed in 57-65% of plasma-cell enriched cells.

- CLL/SLL FISH: Biallelic 13q deletions and intragenic IGH deletion observed in 32.5-43% of 200 cells analyzed.

Diagnosis:

1. Plasma Cell Myeloma
2. CLL/SLL
Concomitant plasma cell and B-cell clones
• When found in BM and with similar light chain restriction, the differential diagnosis includes B-cell NHL with plasmacytic differentiation
  • May be unrelated
  • CD19 and CD45 expression on plasma cells are useful in making the differential diagnosis
• When the two clones show different light chain restriction – likely unrelated

Case #8

78-year-old F with cytopenias and LAD
• CBC: WBC=29,000/uL, Hb=10.7 g/dL, plt=72,000/uL
• Morphology:
  • PB: neutrophilia with hypogranular, hypolobated segs; eosinophilia; monocytosis; rare lymphoma cells
  • BM: 4% blasts; small, hypolobated megakaryocytes; focal clusters of lymphoma cells in the core biopsy
• Immunophenotype:
  • 0.67% aberrant T cells: CD3(+), CD2(-), CD4(-), CD5(-), CD7(-), CD8(-), CD16(-), CD56(-), CD57(-)
Cytogenetic results
• Karyotype: 46,XX
• Genetics:
  • MDS FISH (-)
  • PDGFRA, PDGFRB, FGFR1 FISH (-)
  • Clonal TCR gene rearrangement

Diagnosis:
Chronic Myelomonocytic Leukemia (CMML) and Peripheral T-cell Lymphoma, NOS (PTCL)
CMML and PTCL, NOS

- Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRα, PDGFRβ, or FGFR1
  - T-lymphoblastic leukemia/lymphoma
  - Chronic myelomonocytic leukemia
  - CMML and PTCL, NOS (?)

- Other unusual features:
  - Less common IP (CD4/CD8 double-negative; r/o T-ALL and HSTCL)

Summary

- Flow cytometry effectively discriminates multiple, normal and abnormal population
- Synchronous or metachronous, related or independent clonal populations may be seen in the same specimen
- Integration of morphologic, immunophenotypic, genetic, molecular and clinical information is critical for arriving to the correct diagnosis

Thank you for participating!
Blood and Bone Marrow Diagnosis: A Challenge at any Patient Age

DR KATHRYN FOUCAR
Blood and Bone Marrow
Diagnosis: A Challenge at Any Patient Age

2019 Hawaii Hemepath Conference
Kathryn Foucar
kfoucar@salud.unm.edu

Objectives
• Appreciate age-based normal lymphocyte parameters
• Identify clues to unique blood and bone marrow disorders in children and adults
• Review pancytopenia causes and clues to diagnosis based on age

Age-Related Variations in Blood Lymphocytes
• Lymphocytes predominate in blood by 2-3 weeks of age
• Sustained lymphocyte predominance in blood throughout childhood
• Neutrophils predominate by late childhood, early adolescence
**Absolute Lymphocyte Count 9,000**

- 3 yr old—normal
- 65 yr old—CLL

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**Absolute Lymphocyte Count by Age**

<table>
<thead>
<tr>
<th>Age</th>
<th>Lymphocytosis</th>
<th>Lymphopenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn</td>
<td>&gt; 10,000</td>
<td>&lt; 2,500</td>
</tr>
<tr>
<td>&lt; 1 year</td>
<td>&gt; 9,000</td>
<td>&lt; 4,000</td>
</tr>
<tr>
<td>Child</td>
<td>&gt; 7,000</td>
<td>&lt; 2,800</td>
</tr>
<tr>
<td>Adult</td>
<td>&gt; 4,000</td>
<td>&lt; 1,500</td>
</tr>
</tbody>
</table>

*Conventional units per µL

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**Age, Clinical Features Key**

- Morphology: Non-Activated

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Non-Activated Lymphs: CD4’s predominate

Age, Clinical Features Key

Child-Pertussis
Adult- Leuk/Lymphoma

Lymphs: Child vs. Adult

Blood: 10-month-old male; “Kiddie Lymphs”

Lymphs in Young Child: Distinct Vacuoles/Granules

Sialic Acid/Storage Disease
Vacuolated Lymphocytes in Adult

Mantle Cell Lymphoma

Discrete vacuoles in lymphocytes as a subtle clue to mantle cell lymphoma

Ref: Lynch DT, Foucar K. Blood 2016; 127: 3292

Lymphocytosis: Key Tips

- Dramatic age-related variations in normal
- Assess lymphocytes: morphologic heterogeneity (viral infection) vs. homogeneity (possible neoplasm); “kiddie” lymphs, pertussis
- Assess other lineages
- Sustained lymphocytosis in adult often neoplastic
- Isolated lymphocytosis in child more likely non-neoplastic
- Flow cytometric IP valuable on a limited basis (Flow may be misleading)
Lymphocytes in Normal BM

<table>
<thead>
<tr>
<th></th>
<th>Neonates, Infants, Young Children</th>
<th>Children (&gt;4 years)</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Lymphocytes</td>
<td>May exceed 40%</td>
<td>~ 15-20%</td>
<td>&lt; 5-15%</td>
</tr>
<tr>
<td>Morphology of lymphocytes</td>
<td>Hematogones may be abundant</td>
<td>Variable proportion of hematogones</td>
<td>Usually mature</td>
</tr>
<tr>
<td>Distribution of lymphocytes</td>
<td>Diffuse, small clusters</td>
<td>Diffuse, small clusters</td>
<td>Diffuse, small clusters</td>
</tr>
<tr>
<td>IP of lymphocytes</td>
<td>B’s predominate (HG’s)</td>
<td>Variable</td>
<td>T’s predominate</td>
</tr>
</tbody>
</table>

BMA in 2 year old with thrombocytopenia

Marked increase in lymphocytes > 50%

Morphology

Hematogones vs. Lymphs
Hematogones

• Benign B-cell precursors showing spectrum of maturation
• Key morphologic feature: homogeneous, dense chromatin
• Diffuse, small clusters on biopsy, clot section
• Spectrum of maturation confirmed by flow cytometry, IHC
• Key clinical settings: young patient (variety of pediatric conditions), BM recovery
ALL, Precursor B

Cytopenias

• Single, bicytopenia, pancytopenia
• “Explained” vs “Unexplained”

Age-Based Approach to Pancytopenia

<table>
<thead>
<tr>
<th>Neonate:</th>
<th>Infection, germline disorder, maternal therapy/underlying illness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant:</td>
<td>Infection, nutritional deficiency, neoplasm, germline disorder</td>
</tr>
<tr>
<td>Child:</td>
<td>Infection, systemic illness, nutritional deficiency, neoplasm</td>
</tr>
<tr>
<td>Adult:</td>
<td>Infection, systemic illness, medications, nutritional def., neoplasms, homeopathic remedies</td>
</tr>
</tbody>
</table>
Case: Pancytopenia

History:
83 year old female with fatigue and bruising

CBC:
- WBC 5.5
- MCV 98
- H/H 9.4/27
- Plt 10

Blood: Pancytopenia
- Absolute Neutrophil ct.: $0.1 \times 10^9 /L$
- Platelet Count: $10 \times 10^9 /L$
- Moderate N/N Anemia

Hematopoietic Failure
Flow Cytometry

Flow Cytometry Cytospin

FISH for PML-RARA
**Additional Studies**
- Quantitative PCR for *PML-RARA*
  - Positive – high copy number (typical at diagnosis)

**Final Diagnosis:**
Acute Promyelocytic Leukemia (confirmed molecularly)

**Acute Promyelocytic Leukemia**
- Rapid diagnosis essential
- Morphologic features are highly characteristic; rapid MPO cytochemical stain valuable
- Flow cytometry features are highly characteristic
- *PML-RARA* fusion gene may be cryptic by FISH (6% of cases)
- PCR can detect fusion gene in false negative FISH cases

**Case: Bicytopenia**
78-yr-old female with neutropenia, anemia and progressive functional decline.

**CBC:**
- WBC 1.5, Hgb 9.1, Hct 28%, MCV 98 Fl, RDW 16.7%, Plt 177
Case: Diagnosis

Anemia and neutropenia secondary to zinc-induced copper deficiency
Case: Disease Course

• Searched EMR re dentures (found in Nurses notes)
• Alerted hematologist
• Additional history: patient never removed dentures even at night
• Serum zinc 166 (H)
• Serum copper < 10(L)
• Complete resolution of CBC and functional deficits after copper therapy

Features of Copper Deficiency

• Neurologic syndrome mimics subacute combined degeneration of cobalamin deficiency
• Patients may also have ataxic myelopathy
• Copper deficiency from zinc ingestion, prolonged parenteral nutrition, enteric feeding, following gastrectomy, liver disease (alcoholism) denture pastes (formerly)
• Cause of deficiency may not be apparent

Case: Pancytopenia

42-yr-old male with 2 week history SOB, gum bleeding, tingling sensation upper and lower extremities, weight loss, malaise, weakness.
**Case**

<table>
<thead>
<tr>
<th>CBC</th>
<th>3.2 (↓ ANC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>1.19</td>
</tr>
<tr>
<td>Hgb</td>
<td>4.7</td>
</tr>
<tr>
<td>Hct</td>
<td>15%</td>
</tr>
<tr>
<td>MCV</td>
<td>122</td>
</tr>
<tr>
<td>RDW</td>
<td>30.7%</td>
</tr>
<tr>
<td>Plt</td>
<td>27</td>
</tr>
<tr>
<td>Retic</td>
<td>5.9%</td>
</tr>
<tr>
<td>4 NRBC/100WBC</td>
<td></td>
</tr>
</tbody>
</table>

**Blood-Pancytopenia**

**NRBC at Feather**
Case: Diagnosis

Clinical dx: TTP
Blood smear dx: ?

Additional Labs

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute retic</td>
<td>0.07</td>
<td>Nl</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>&lt; 8</td>
<td>↓</td>
</tr>
<tr>
<td>LDH</td>
<td>1600</td>
<td>↑</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>93</td>
<td>↓</td>
</tr>
</tbody>
</table>
**Case: Diagnosis**

Megaloblastic anemia mimicking TTP

Reference

**Follow Up Labs**

ADAMTS13: 71 Nl
MMA: 3.08  
IF ab: positive
Shiga toxin: negative

**Case: Key Tips**

- Megaloblastic anemia can manifest with pancytopenia
- Oval macrocytes broken in spleen (frags, ↑ RDW)
- MCV 122 — not due to retics (Note MCV may be normal in Meg An)
- Retic % was elevated but not absolute retic count
Case: Pancytopenia
14-year-old African-American female with no past medical history; presents with emesis x 3 days, excessive fatigue, decreased appetite

**CBC:** WBC 1.9, RBC 1.4, Hgb 4.7, Hct 13.6%, MCV 99, MCHC 34.8, RDW 34%, Plt 67
**Blood: 14-year-old girl**

**Bone Marrow Biopsy**

**Case: Differential Diagnosis**

- Congenital dyserythropoietic anemia
- Other constitutional RBC disorder
- Myeloid neoplasm (pancytopenia, packed bone marrow)
- Megaloblastic anemia (?? normal MCV)
**Case: Additional Lab Data**

- Low serum cobalamin
- Increased homocysteine
- Increased methylmalonic acid
- Positive intrinsic factor

**Case: Final Diagnosis**

*Megaloblastic anemia, pernicious anemia*

**Follow-up:**
Patient responded to cobalamin therapy

**Megaloblastic Anemia Caveats**

- Highly variable blood picture (normal MCV, lack of “classic” features)
- Risk of vitamin B<sub>12</sub> deficiency-associated neurologic impairment significant, especially in infants
- If cobalamin level is borderline but not below lower limit, still pursue other tests—MMA, IF ab, etc.
Summary

• Clinical information, CBC data, blood smear and bone marrow morphology essential
• Correlation with age-related normal parameters essential
• Appreciation of the full spectrum of findings in megaloblastic anemia and copper deficiency is key to distinction from a neoplasm or other condition
• Pancytopenia may have non-neoplastic or neoplastic cause
Flow cytometric evaluation of plasma cell dyscrasias

DR HORATIU OLTEANU
Flow Cytometric Evaluation of Plasma Cell Dyscrasias

Horatiu Olteanu, MD, PhD
Professor and Medical Director of Flow Cytometry
Mayo Clinic, Rochester, MN

General Considerations
- The flow cytometric diagnosis of plasma cell and lymphoproliferative disorders relies on two broad approaches:
  - Demonstration of clonality
  - Demonstration of immunophenotypic aberrancies (i.e. differential antigen expression from a normal counterpart)
- In mature B-cell LPDs, demonstration of clonality is accomplished through surface immunoglobulin light chain analysis
  - Often represents the primary method of flow cytometric diagnosis

General Considerations
- In plasma cell myeloma (PCM) and related disorders, the primary approach to the flow cytometric diagnosis relies on the identification of an aberrant immunophenotype
  - In the presence or absence of distinct cytoplasmic light chain restriction
  - Particularly in the MRD analysis setting
- CD38 is brightly expressed on normal PCs and, in conjunction with CD138, used as a lineage-defining antigen
  - Other maturing bone marrow elements also express CD38
Normal vs. Abnormal PCs

- The most common IP of normal PCs may be described as CD38 bright (+), CD138 bright (+), CD19 (+), CD20 (-), CD27 bright (+), CD28 (-), CD56 (-), CD81 (+), CD117 (-), CD200 predominantly (-), and polytypic cytoplasmic immunoglobulin.

- The typical IP of PCM shows various combinations of aberrancies, including CD38 (+) (often dimmer than normal PCs), CD138 bright (+), CD19 (-), CD20 (-) (may be positive in a subset of PCM), CD27 (-), CD28 (+), CD56 (+), CD81 (-), CD117 (-) (may be positive in a subset of PCM), CD200 (+), and monoclonal light chain expression.

- Usually defined as a cytoplasmic kappa:lambda ratio >5 or <0.5.

Frequency of IP Aberrancies in PCM

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Frequency of expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19 (-)</td>
<td>~ 95%</td>
</tr>
<tr>
<td>CD20 (+)</td>
<td>15-20%</td>
</tr>
<tr>
<td>CD27 (+)/dim (+)</td>
<td>45-65%</td>
</tr>
<tr>
<td>CD28 (+)</td>
<td>15-45%</td>
</tr>
<tr>
<td>CD45 (-)</td>
<td>45-80%</td>
</tr>
<tr>
<td>CD56 (+)</td>
<td>75%</td>
</tr>
<tr>
<td>CD81 (-)</td>
<td>45-55%</td>
</tr>
<tr>
<td>CD117 (+)</td>
<td>35%</td>
</tr>
<tr>
<td>CD200 (+)</td>
<td>75-80%</td>
</tr>
</tbody>
</table>
Normal PCs

Aberrant PCs in PCM

Normal and Aberrant PCs
Case #1

36-year-old M with massive splenomegaly
- Several month history of abdominal pain
  - Also, night sweats, weight loss, increasing fatigue
  - Splenomegaly (25 cm) on CT abdomen/pelvis
  - Diffuse marrow uptake on PET-CT
  - Pancytopenia
- BM biopsy performed for further work-up:
  - Hypercellular core (95%) with 60% plasma cells
  - No JAK2 mutation detected
  - Normal male karyotype

- Yellow (1.1% plasma cells): 0.56% intracellular kappa (+) (blue) and 0.48% intracellular lambda (+) (cyan); ic kappa/lambda = 1.2:1
- Violet (0.75% immature B cells / hematogones)
Bone marrow flow cytometry

- Two aberrant populations of plasma cells:
  - Yellow and blue (0.56%): CD38 bright (+), CD19 (-), CD56 (+),
    CD45 (+), CD200 (-), intracellular kappa restricted
  - Yellow and cyan (0.48%): CD38 bright (+), CD19 (-), CD56 (+),
    CD45 (+), CD200 (-), intracellular lambda restricted

- Splenectomy:
  - 2,170 g spleen with multifocal myeloma deposits
  - Fibrocongestive splenomegaly with multiple old infarcts

Diagnosis:
Biclonal Plasma Cell Myeloma
PCM Unusual Findings

- Immunophenotypic aberrancies are more important in discriminating normal from abnormal plasma cells, than intracytoplasmic light chain restriction
- PCM may be diagnosed in younger patients and may show organomegaly, due to plasmacytomas
- PCM may be preceded by neutrophilia or eosinophilia, mimicking a MPN

General Technical Issues

- PCs show low recovery by flow cytometry
- Neoplastic PCs have variable light scatter properties and CD45 expression
- PCs have increased propensity to form complexes with granulocytes
- PCs show high autofluorescence

Low PC Recovery by FC

- PCs are under-represented in FC analysis
  - Only 1-30% recovery, compared to morphology
- Potential contributing factors:
  - Patchy, heterogeneous distribution of PCM cells in the BM
  - Relative higher degree of hemodilution in the specimen processed for FC
  - Differential distribution of PCs in lipid-enriched particles in the morphology slides, compared to the lipid-depleted liquid BM aliquot analyzed by FC
  - Further loss during processing, staining, and acquisition on the flow cytometer, related to particular physical characteristics of PCM cells, which render them potentially more susceptible to mechanical damage
Low PC Recovery by FC

- Accurate quantification of BM PCs has prognostic value in the MRD setting
- There also is literature data on the prognostic value of flow cytometric enumeration of PCM cells at diagnosis
- The percentage of PCs used to establish a diagnosis of PCM is generated from morphology slides (gold standard)

Variable Light Scatter and CD45 Properties in PCs

- Traditional FC data analysis employs gating approaches using FSC/SSC and CD45/SSC 2D-plots
- This strategy offers a predictable and often reproducible pattern of normal cell subsets that can be found in BM specimens
- PCM cells tend to vary in size (FSC) and cytoplasmic complexity (SSC), and show variable CD45 expression, which renders these fluorescence parameters inadequate for gating
- Current combinations of recommended markers: CD38, CD138, and CD45
PCs Tend to Form Complexes with Granulocytes

- This phenomenon may be responsible for both unusual light scatter properties (high FSC and SSC on a subset of PCs), as well as for over-interpretation of CD45.

High Autofluorescence in PCs

- May lead to erroneously ascribing positive expression of certain antigens to PCM cells.
- Use of an isotype control, multivariate computational tools, and visualization plots may circumvent this issue.

Added Value of FC in the Diagnostic Work-Up of PCM

- In general, PCM can be diagnosed by a combination of clinical, morphologic, radiologic, and laboratory criteria, and the contribution of FC in the initial evaluation is limited.
- FCIP plays a more important role in the DD of PCM.
  - Unusual morphologic variants of PCM vs. LPL
  - PCM vs. prominent reactive plasmacytosis
  - Coexistent PCM and unrelated B-cell clone of similar light chain restriction vs. low-grade B-cell lymphoma with extensive plasmacytic differentiation
  - PCM vs. MGUS or SMM
PCM with Lymphoplasmacytoid Morphology

Florid Reactive Plasmacytosis

Lymphoplasmacytic Lymphoma/Waldenstrom Macroglobulinemia
PCM and Clonally Unrelated B-Cell NHL with Similar Light Chain Restriction

PCM vs. MGUS or SMM
- Clonal PCs found in MGUS, SMM, and PCM are immunophenotypically aberrant and can be readily distinguished from normal ones.
- The presence of a large (>95%) proportion of abnormal PCs/BM PCs has been associated with a higher risk of progression of MGUS and SMM to PCM.
- Similar prevalence of common antigen expression (CD19, CD56, CD20, CD45, CD117) in clonal PCs from MGUS and PCM, with perhaps the exception of CD200.
  - More frequently expressed in PCM (73%) compared to MGUS (54%).

Prognostic Utility of FC in PCM
- Qualitative prognostic markers:
  - CD19, CD28, CD45, CD56, CD81, CD117, CD200
- Quantitative prognostic markers:
  - Percentage of PCs (of total bone marrow cells)
  - Percentage of clonal PCs (of bone marrow PCs)
  - Number of circulating clonal PCs
  - Evaluation of PC DNA ploidy
Qualitative Prognostic Markers

- Expression of CD19 and CD28, and lack of CD117 was associated with worse PFS and OS
- Lack of CD45 or CD56 expression has been associated with less favorable outcome
- CD81 expression has been identified as an independent prognostic factor for worse PFS and OS
- Lack of CD200 expression was associated with a favorable outcome in most reports

Quantitative Prognostic Parameters

- The overall percentage of PCs (calculated as proportion of nucleated BM cells) and the relative percentage of myeloma cells (reported as the proportion of BM PCs) were identified as quantitative FC parameters with prognostic significance
- A relative higher proportion of normal PCs (>5% of BM PCs) at diagnosis has been associated with a better PFS and OS

Quantitative Prognostic Parameters

- The number of circulating PCs has also prognostic significance in PCM
- A subset of patients with SMM and high levels of circulating PCs (≥400) have an elevated risk of progression within the first 2-3 years following diagnosis
- Patients with newly diagnosed PCM may be stratified in a higher risk group (with worse OS), based on a cut-off of ≥400 clonal PCs
- Amongst patients with recurrent PCM, a higher number (≥100) of circulating clonal PCs may also predict for worse survival

Quantitative Prognostic Parameters

- FC can assess PC DNA content and proliferation

G0/G1 – Quiescent phase/pre-DNA synthesis phase; cells in these phases have normal (diploid or 2N) DNA content.
S-phase – DNA synthesis phase; DNA content increases from 2N toward 4N.
G2/M – Post-DNA synthesis phase/mitosis phase; cells have doubled their DNA content to 4N and are ready to divide.

DNA index Ploidy

- ≤0.35 Hypodiploid
- 0.35-1.05 Diploid
- 1.06-1.74 Hyperdiploid
- >1.74 Tetraploid
PC Proliferation Assessment
- Has several reportables with diagnostic and/or prognostic information:
  - PC clonality – diagnostic
  - Clonal PC DNA index (DNA ploidy) – diagnostic & prognostic
  - Clonal PC proliferation (S phase) – prognostic
  - Percent polyclonal PCs of total PCs – prognostic

DNA Ploidy Facilitates Detection of Clonal PCs
- Non-diploid PCs = abnormal
- Can establish clonality in a background of polytypic PCs
- May detect bi-clonal PC populations
- Increased level of detection of very small clonal population - analytic sensitivity $10^{-4} - 2 \times 10^{-5}$

Case #2
Kappa clone confirmed by hyperdiploidy

DNA index (kappa): 1.15
- Hyperdiploidy

Case #3

DNA index (kappa): 1.23
- Hyperdiploidy
DNA index (lambda): 0.93
- Hypodiploidy

Biclonal PCs confirmed by non-diploid DNA content
Case #4

DNA index (kappa) : 1.47
- Hyperdiploidy

Case #4 (LOD: 0.002%)

Hyperdiploidy is a Good Prognostic Factor


Hyperdiploidy

Hyperdiploidy

High S phase is a Poor Prognostic Factor

- S-phase ≥ 3.0 – Worse prognosis
- S-phase < 3.0 – Good prognosis


- With the current version of the test, the best cutoff has been established at the S-phase of 2%

Mayo Stratification for Myeloma and Risk-adapted Therapy (mSMART)

mSMART 3.0: Classification of Active MM

High-Risk
- FISH
  - Del 17p
  - t(4;14)
  - t(14;16)
  - t(14;20)
- ISS Stage 3
- High Plasma Cell S phase
- DEP: High risk signature

Standard-Risk
- All others including:
  - Trisomies
  - t(11;14)
  - t(16;14)

MRD FC Analysis in PCM

- MRD analysis is likely to exert the most impact in evaluating the effectiveness of new therapeutic drugs and in potentially identifying subsets of patients at risk for progression and in need for more intensive treatment

- PCM has well-defined and nuanced response criteria and it is estimated that up to 33% of patient in CR, and 10% of those in near CR or VGPR may be considered potentially cured (i.e. relapse-free at 10-year follow-up)
MRD FC Analysis in PCM

- This data implies that a better definition for CR is necessary, since 40% of patients with PCM in CR will relapse, and 20% will succumb to their disease within 4 years of initial treatment.
- Accurate estimation of the depth of the response appears to correlate with better outcome in PCM, which supports the addition of MRD analysis to the definition of CR.

Prognostic influence of sequential MRD status by MFC before and after ASCT. (A) PFS and (B) OS (n = 157).

Test Design (EuroFlow Group)

- 10 color – 2 tubes with 6 anchor antigens:
  - Tube 1 CD38 CD138 CD45 CD19 CD56 CD27 CD81 CD117
  - Tube 2 CD38 CD138 CD45 CD19 CD56 CD27 kappa lambda
- CD38 is a multi-epitope antibody
- 10 million cells per tube, collect 5 million each tube.
- Infinicyt software for analysis
- Sensitivity of at least $10^{-5}$ if 2 million events are collected
### Multiple Myeloma FISH by Flow, BM

<table>
<thead>
<tr>
<th>Marker Name</th>
<th>Normal Range</th>
<th>Value</th>
<th>%</th>
<th>Result Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Abnormal Plasmacytoid Occ</td>
<td>0-5%</td>
<td>6%</td>
<td>6%</td>
<td>Normal</td>
</tr>
<tr>
<td>% Abnormal Plasma Cells (Blast-like)</td>
<td>0-1%</td>
<td>1%</td>
<td>1%</td>
<td>Normal</td>
</tr>
<tr>
<td>Non-Apoptotic Events</td>
<td>0-185</td>
<td>18</td>
<td>18</td>
<td>Normal</td>
</tr>
<tr>
<td>Total Plasma Cell Events</td>
<td>0-185</td>
<td>185</td>
<td>185</td>
<td>Normal</td>
</tr>
<tr>
<td>Polyclonal Lymphocytes</td>
<td>0-185</td>
<td>185</td>
<td>185</td>
<td>Normal</td>
</tr>
<tr>
<td>Monoclonal Lymphocytes</td>
<td>0-185</td>
<td>185</td>
<td>185</td>
<td>Normal</td>
</tr>
</tbody>
</table>

**FISH Diagnoses:**
- Bone marrow, flow cytometric immunophenotyping

**Interpretation:**
- Monoclonal kappa plasma cells identified (ratio: 0.29:185)
- FISH normal: 185

**Comment:**
This assay is intended for post-therapeutic assessment of patients with plasma cell neoplasms, when in complete remission. Consultation with clinical and other laboratory studies is recommended.

Plasma cell analysis was performed with antibodies to the following antigens: Monoclonal BPC panel: CD38, CD138, CD20, CD56, CD19, CD138, CD20, CD19, kappa and lambda immunoglobulin light chains.

**Quality Assurance:** Specimen received within validated guidelines.
Summary

- Routine flow cytometry in PCM requires careful consideration of a number of technical aspects related to plasma cell analysis.
- Flow cytometry has applications in the differential diagnosis, prognosis, and treatment of PCM.
- Minimal residual disease analysis by flow cytometry is a powerful predictor of outcome in myeloma, in the clinical trial setting.

Thank you for participating!