CLONAL LYMPHOCYTOYSIS: APPROACH FOR THE PRIMARY PROVIDER

AND

DECIPHERING “MONOCLONAL B-CELL LYMPHOCYTOYSIS”

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OUTLINE

- Lymphocytosis
  - Benign and Reactive
  - Clonal

- Fundamentals of Flow Cytometry (Immunophenotyping)

- Monoclonal B-cell Lymphocytosis, Chronic Lymphocytic Leukemia
23 year-old grad student with fever, pharyngitis.
- CBC: WBC 14.3
- Differential: ALC 8.8. Lab notes variant/atypical forms

55 year-old undergoing routine annual H&P.
- CBC: WBC normal
- Differential: ALC 4.9

78 year-old with fatigue, enlarged lymph nodes.
- CBC: WBC 26.0
- Differential: ALC 21.0
LYMPHOCYTES 101

- Main component of the adaptive immune system
  - Humoral immunity
    - B-cells
  - Cellular immunity
    - T-cells

- Important component of innate immune system
  - NK-cells
  - T-cell subset

- Circulating blood lymphocytes
  - T-cells (CD3+ cells): 60-80% $\rightarrow$ CD4 > CD8
  - B-cells (CD20+ cells): 10-20%
  - NK-cells (CD56+ cells): 5-10%
**APPROACH TO LYMPHOCYTOYSIS**

- **LYMPHOCYTOYSIS**
  - Definition: Elevated *absolute* lymphocyte count (ALC) in the peripheral blood (>4.0 K/ul in adults)

- Frequent cause (or contributor) of high WBC (leukocytosis)
  - Always review the WBC differential!
ETIOLOGY OF LYMPHOCYTOSIS

- **Polyclonal** causes (transient, reactive, benign)

- **Clonal** causes (i.e. lymphoproliferative disorders)
  - Monoclonal B-cell lymphocytosis
  - Chronic neoplasm of lymphocytes
    i.e. chronic lymphocytic leukemia
  - Aggressive malignant lymphomas
POLYCLONAL LYMPHOCYTOSIS
General approach to the workup of lymphocytosis.

Paolo Strati, and Tait D. Shanafelt Blood 2015;126:454-462

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APPRAOCH TO LYMPHOCYTOSIS

- **Polyclonal lymphocytosis**
  - Transient
    - Stress, injury, surgery, acute illness
  - Infectious
    - Viral infections, atypical bacterial infections (e.g. mycoplasma)
    - Pertussis (characteristic morphology)
  - Reactive
    - Reactive lymphocytosis (atypical lymphocytosis)
      - Infectious mononucleosis, drug reaction
    - Large granular lymphocytes (s/p malignancy, transplant)
- **Benign persistent**
  - Persistent polyclonal B-cell lymphocytosis
    - Young to middle-aged women smokers
  - Splenectomy
  - Thymoma
WHEN TO INVESTIGATE LYMPHOCYTOSIS

- Abnormal findings
  - High lymphocyte count (>15.0)
  - Absolute count > 4.0-5.0 on more than one occasion
  - Increasing lymphocyte counts
  - Cytopenia(s) (neutropenia, anemia, thrombocytopenia)
  - Organomegaly/lymphadenopathy

- Next steps
  - Serial CBC(s) *with* differential
  - Peripheral blood smear review *with or without*
  - Immunophenotyping by flow cytometry
  (Hematology referral if worrisome features present)
FLOW CYTOMETRY

Addition of antibody

Sheath fluid

Sample (stained cells in suspension)

Nozzle

Hydrodynamic Focusing
Cells pass through 'single file'

Fluorescence emitted from stained cells detected

Forward and side scattered light from all cells detected

Laser light source
FLOW CYTOMETRY

- Simultaneous detection of many surface and/or cytoplasmic markers and physical characteristics of large numbers of cells
  - 10-20 parameters clinically

- Need *fresh* sample and cell suspension of *non-cohesive* cells

- Not useful for evaluating most solid tumors

- Excellent for interrogating complex, heterogeneous mixtures of cells
  - E.g. bone marrow, lymph nodes, blood

- Clonality assessment
Immunoglobulin molecule

lambda light chain-restricted (monotypic ~ monoclonal)

polytypic ~ polyclonal

CLONALITY

lambda kappa

polytypic ~ polyclonal
CATEGORIES

- 23 year-old grad student with fever, pharyngitis.
  - CBC: WBC 14.3
  - Differential: ALC 8.8. Lab notes variant/atypical forms
  - Flow cytometry: Normal

- 55 year-old undergoing routine annual H&P. Provider checks a
  CBC. WBC is normal but lymphocytes are increased slightly (ALC
  4.9).
  - CBC: WBC normal
  - Differential: ALC 4.9
  - Flow cytometry: Clonal B-cells (CD5+)

- 78 year-old with fatigue, enlarged lymph nodes.
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TERMINOLOGY

- Clonal Lymphocyte Populations
  - Not all lymphocyte clones are neoplastic or malignant

- Benign
  - i.e. autoimmune disease, hepatitis C infection, *H. pylori* gastritis

- Lymphoproliferative Disorders
  - Clonal and Dysregulated and Persistent
LYMPHOPROLIFERATIVE DISORDERS

- Border between benign and malignant lymphoid populations is ambiguous and difficult to draw
  - Clonal expansions of lymphocytes do not remain localized, but disseminate based on intrinsic properties of lymphocytes
- Early, “in-situ” lesions
  - E.g. MGUS (monoclonal gammopathy of undetermined significance)...precursor to myeloma
  - Difficult to precisely define, may be similar genetically to advanced lesions
    - e.g. 14;18 associated with follicular lymphoma but not diagnostic
- (Malignant) Leukemia/Lymphoma
  - Chronic (non-Hodgkin and Hodgkin)
    - Indolent malignant – e.g. CLL, follicular lymphoma
    - Aggressive malignant – e.g. Burkitt lymphoma
  - Acute
    - Blastic: e.g. ALL

There are no “benign” lymphomas!!!
APPREACH TO LYMPHOCYTOSIS

- Monoclonal lymphocytosis
  - “Pre-malignant” lymphoproliferative disorders
    - Monoclonal B-cell lymphocytosis
  - Malignant lymphoproliferative disorders
CLONAL LYMPHOCYTOSIS, CONT. (MALIGNANT)

- Chronic (indolent) lymphoproliferative disorders
  - Chronic lymphocytic leukemia (B-cell)
  - Large granular lymphocytic leukemia (T-cell or NK-cell)
  - Sezary syndrome (cutaneous T-cell lymphoma)

- Malignant peripheralized non-Hodgkin lymphoma
  - Mantle cell lymphoma
  - T-cell leukemia (T-prolymphocytic leukemia, adult T-cell leukemia)
  - Aggressive lymphomas (DLBCL, Burkitt)

- Malignant “pseudo” lymphocytosis
  - Acute lymphoblastic leukemia (ALL)
  - Acute myeloid leukemia (AML) (rare mimicker)
<table>
<thead>
<tr>
<th>Small, round nuclei</th>
<th>CLL MBL</th>
<th>MCL T-PLL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folded or cleaved nuclei</td>
<td>FL MCL Atypical CLL</td>
<td>T-cell lymphomas Pertussis*</td>
</tr>
<tr>
<td>Convoluted nuclei</td>
<td>Sézary syndrome Adult T-cell leukemia</td>
<td></td>
</tr>
<tr>
<td>Villous cytoplasm</td>
<td>HCL SMZL HCLV</td>
<td>T-PLL LPL</td>
</tr>
<tr>
<td>Plasmacytoid</td>
<td>LPL Plasma cell myeloma Plasma cell leukemia</td>
<td></td>
</tr>
<tr>
<td>Granules</td>
<td>T-LGL NK cell leukemia</td>
<td></td>
</tr>
<tr>
<td>Prominent nucleoli</td>
<td>T-PLL B-PLL HCLV MCL</td>
<td></td>
</tr>
<tr>
<td>Large cells</td>
<td>Burkitt Leukemia DLBCL MCL ALCL</td>
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# Immunophenotype of Clonal B-Cell Disorders

<table>
<thead>
<tr>
<th></th>
<th>CD5</th>
<th>CD10</th>
<th>CD20</th>
<th>CD23</th>
<th>Light chain</th>
<th>Genetic</th>
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</thead>
<tbody>
<tr>
<td>CLL</td>
<td>+</td>
<td>-</td>
<td>Dim</td>
<td>+</td>
<td>Dim</td>
<td></td>
</tr>
<tr>
<td>MCL</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>t(11;14)</td>
</tr>
<tr>
<td>MZL</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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</tr>
<tr>
<td>FL</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>t(14;18)</td>
</tr>
</tbody>
</table>

**CLL:** chronic lymphocytic leukemia  
**MCL:** mantle cell lymphoma  
**MZL:** marginal zone lymphoma  
**FL:** follicular lymphoma
General approach to the workup of lymphocytosis.

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CLONAL LYMPHOCYTOSIS WITH CLL PHENOTYPE
CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

- Most prevalent adult leukemia
- Incidence increases with age (median at diagnosis: 70 years)
  - M>F (1.7:1); Caucasian > African > Asian
- Cancer of B-lymphocytes, *usually* indolent
- Wide range of internal medicine problems:
  - Chronic immune deficiency
  - Infections
  - Autoimmune complications
  - Secondary cancers
CLL DIAGNOSIS

Requires **clonal B-cell count greater than 5 x 10^9/L** in peripheral blood

**OR**

Evidence of disease outside the bone marrow

(e.g. enlarged lymph nodes, spleen)

*Previously dependent on absolute [total] lymphocyte count*

Requires flow cytometry immunophenotyping: CD5+/CD23+ expression

***Heterogeneous presentation ➔ likely several different but related diseases genetically***
**CLL STAGING**

- **Low risk**
  - Rai stage 0: Lymphocytosis and no organ enlargement of the lymph nodes, spleen, or liver, and with near normal red blood cell and platelet counts.

- **Intermediate risk**
  - Rai stage I: Lymphocytosis plus enlarged lymph nodes
  - Rai stage II: Lymphocytosis plus an enlarged spleen (and possibly an enlarged liver)

- **High risk**
  - Rai stage III: Lymphocytosis plus anemia (Hgb <11 or Hct<33%)
  - Rai stage IV: Lymphocytosis plus thrombocytopenia (Plts < 100)
Most patients do NOT need Rx at the time of diagnosis
  - Majority, however, **will** ultimately require it

**When to treat?**
  - 2008 International Workshop on CLL (IWCLL) Standard Criteria for “Active disease”:
    - Progressive marrow failure (i.e. cytopenias)
    - Splenomegaly (massive/progressive/symptomatic)
    - Lymphadenopathy (massive/progressive/symptomatic)
      - Blood 2008;111(12):5446-56
    - Progressing disease: increasing lymphocyte count ("doubling time")
    - Constitutional Symptoms
      - Wt loss (10%, 6 months), fatigue (impairing usual activity), fevers (≥2 weeks), night sweats (>1 month)
    - Poorly responsive autoimmune mediated cytopenias
  - Other: Repeated episodes of infection
Can we predict when Rx will be needed?

Prognosis:
- Molecular genetics
  - Mutation status of immunoglobulin heavy chain variable gene (IGHV)
- Lab studies
  - Beta2-macroglobulin (tumor burden)
- Cell surface markers
  - CD49d (replacing CD38, ZAP-70)
- Molecular cytogenetics (FISH)
  - 17p deletion/TP53 mutation; 11q23 (ATM) loss; trisomy 12; 13q deletion
How about catching CLL early?
“EARLY” FORMS OF WELL-DEFINED LYMPHOID NEOPLASMS

- Retrospective studies reveal almost universal evidence of an “early lesion” many years prior to diagnosis
- Analogous to carcinoma in-situ
  - E.g. adenomatous colon polyps, cervical dysplasia
- However, progression is not always the rule and most patients do not
- “Pre-malignant” paradigm does not immediately extend to lymphomas
  - Innate circulating capacity of lymphocytes
    - Indolent lymphoproliferative disorders
    - Aggressive lymphoproliferative disorders
A NEW PLAYER — MONOCLONAL B-CELL LYMPHOCYTOSIS

- First identified in early 1990s. 
  - Br J Haematol 2007;139(5):690-700
  - Cytometry B Clin Cytom 2007;72(5):344-53
- Series of cross-sectional population-based studies to determine health risks of living near hazardous waste sites
- 0.6% of adults older than 40 years found to have a clonal population of CD5+CD19+ B-cells without meeting diagnostic criteria for leukemia/lymphoma
  - no lymphadenopathy, organomegaly, cytopenias, or disease-related symptoms
- “Precursor lesion” for B-cell lymphoma, esp. CLL
  - Similar to MGUS
- Not uncommon incidental discovery on routine lab studies
MONOCLONAL B-CELL LYMPHOCYTOYSIS

- Prevalent!
  - MBL with CLL-like phenotype:
    - Adults > 60 years old: 5%  
    - Adults > 60 years old with lymphocytosis: 14%
    - Progresses to CLL at rate of 1-2% per year (similar rate of MGUS to myeloma)

- Same genetic makeup as CLL

- Appears to be present years prior to Dx in virtually all patients with CLL  

- 2005: International Familial CLL Consortium proposed term “monoclonal B lymphocytosis”
RISK FACTORS FOR MBL

- RISK FACTORS FOR MBL ONSET
  - Genetic predisposition
    - Family history of CLL
    - Genetic polymorphisms
  - Age
  - Infections
    - Hep C, pneumonia, influenza, cellulitis, URIs, herpes zoster
**APPROACH TO MONOCLONAL B-LYMPHOCYTOSIS**

- Diagnosis requires flow cytometry (routine leukemia/lymphoma immunophenotyping)
- Low-level clonal lymphocytosis
  - CLL phenotype MBL: CD5+/CD23+, weak expression of CD20 and surface immunoglobulin
- High-count (clinical MBL) vs low-count
- No evidence of lymphoma, infections, or autoimmune conditions
  
  As defined, MBL is NOT cancer!
- *Non CLL B-cell phenotype: CD5(-) or CD5+ but noncharacteristic overall phenotype
- *T-cell phenotype
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MORBIDITY OF MBL

- Infection risk
  - Partial or complete depletion of normal B-cells
  - Potentially reduced relative survival
    
    Curr Hematol Malig Rep 2013;8:52-9

- Progression to CLL (1-2% per year)
  - Risk factors:
    - CD38 positivity
    - Unmutated IGHV
    - Deletion of 17p (p53 gene)
    - Elevated B-cell count

- Renal failure
  - Monoclonal B lymphocytosis “of renal significance”
    
    Haematologica 2015;100(12):1180-8.
CASES

- 23 year-old grad student with fever, pharyngitis.
  - CBC: WBC 14.3
  - Differential: ALC 8.8. Lab notes variant/atypical forms
  - Dx: **Infectious mononucleosis (benign lymphocytosis)**

- 55 year-old undergoing routine annual H&P. Provider checks a CBC. WBC is normal but lymphocytes are increased slightly (ALC 4.9).
  - CBC: WBC normal
  - Differential: ALC 4.9
  - Flow cytometry: CD5+ B-cells
  - Dx: **Monoclonal B-lymphocytosis**

- 78 year-old with fatigue, enlarged lymph nodes.
  - CBC: WBC 26.0
  - Differential: ALC 21.0
  - Flow cytometry: CD5+ B-cells
  - Dx: **Chronic lymphocytic leukemia/small lymphocytic lymphoma**
PRINCIPLES OF MANAGEMENT FOR MBL

- Rule out lymphoma/systemic disease
  - Clinical history, physical examination, additional laboratory testing (e.g. renal function)
  - If atypical immunophenotype (e.g. CD5-negative, non-CLL phenotype), additional studies and/or hematology referral is prudent

- NO direct treatment. Watch and wait, monitoring
  - Lymphocyte count trending
    - 70% have NO significant change after median 7 yrs
  - Monitor renal function, for serious infection (bacterial)

- *1-2% risk of progression to CLL per year*
  - 10% risk of requiring treatment over 5-year period

*Curr Hematol Malig Rep 2013;8:52-9*
CLONAL LYMPHOCYCTOSIS WITH NON-CLL PHENOTYPE
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NON-CLL PHENOTYPE CLONAL LYMPHOCYTOPSYTHESIS

- MBL, non-CLL type
  - CD5+ ➔ mantle cell lymphoma
  - CD5- ➔ follicular lymphoma, marginal zone lymphoma, lymphoplasmacytic lymphoma, diffuse large B-cell lymphoma

- Lymphomas
  - B-cell
    - Indolent
    - Aggressive
  - T-cell
    - Indolent – e.g. large granular lymphocyte leukemia (RA patients)
    - Aggressive – non-Hodgkin T-cell lymphoma
TAKE-AWAY PEARLS

- Lymphocytosis
  - Common
  - Patient age, H&P, and duration critical
  - Peripheral smear and flow cytometry key

- Monoclonal B-lymphocytosis
  - Not uncommon in older populations
  - B-cell lymphoproliferative disorder with low morbidity (not cancer!)
  - Diagnosis by **flow cytometry** immunophenotyping, usually CD5+
  - No direct treatment; **1-2% risk of progression** to CLL/year
  - Control bacterial **infections**, potential renal impairment
  - Active monitoring could be provided in primary care setting
  - Non-CLL phenotype MBL should be reviewed by specialist
QUESTIONS?

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