Monoclonal B Cell Lymphocytosis in Blood Donors... What???

Jane M. Rachel, MLS, MA
Director, Education and Scientific Development
Manager, Molecular Diagnostics and Flow Cytometry
- MBL origins and history
- First blood donor study
- Other MBL studies
- Second blood donor study
- Clinical significance of MBL
Chronic Lymphocytic Leukemia (CLL)

Characteristics
- Most common leukemia in adults; ~25% of all leukemias
- Annual incidence in U.S. - 3/100,000; higher in midwest
- Cause unknown; suspect genetic component
- Accumulate long-lived dysfunctional B cells

Diagnosis
- Median age ~70
- ~50% asymptomatic
- B cell population; κ:λ clonality
- Abnormal B cell phenotype: CD19 + CD5 (T cell marker)

Prognosis
- Clinically heterogeneous
- Prognostic markers, staging
- Psychological burden
Monoclonal B cell Lymphocytosis (MBL) 
Origins and History - 1990s

Clonal B cell populations

- CLL immunophenotype
- First degree relatives of CLL patients
- Residents near hazardous waste sites

Evolution of flow cytometry

- Increased utilization
- Multicolor reagents
- Multiparameter analysis
Flow Cytometry for Blood Bankers

Analyze surface antigens on WBCs

- All high-frequency antigens
- Presence/absence characterizes WBC maturity and lineage
- No systems with cute names
- CD whatever

Immunophenotyping

- Mix whole blood with typing sera
  - Fluorescent-labeled anti-CD MoAb
  - Can use 6-8 anti-CDs per tube
  - Labeled with different fluors (colors)
- Incubate
- Lyse RBCs
- Wash, resuspend
- Detect antibody binding by flow cytometry
Flow Cytometry for Blood Bankers

- 1000s cells/sec
- Cells pass laser
- Laser displacement
  - Forward scatter – size
  - Side scatter – granularity
- Excites fluorochromes
- Emitted light
  - Bound anti-CD
  - Specific wavelengths
  - Fluorochromes = data
Flow Cytometry for Blood Bankers

Size ↑

Granularity →

Fluorescence Intensity →
Flow Cytometry Laboratory
SLH – 1990s

- Reagent quality control
- All CD typing reagents
- No commercial WBCs
- Normal blood donor sample
- Community Blood Center
- Occasionally not normal
Not Normals - 1995

January 5 - 45 year old male – Case 1

CD19  68%
CD20  66%
HLA-DR 78%
CD19/5 67%
κ:λ ratio 68.0 (weak)

October 24 – 55 year old male – Case 2

CD19  51%
CD20  45%
CD22  45%
CD23  53%
HLA-DR 54%
CD19/5 45%
κ:λ ratio 49.0 (weak)
Normal
What To Do????!!!!!

- Identified donors
- CBC recalled for counseling
- Samples retested to confirm
- Results provided to physician
- Donors permanently deferred
- Donor units presumably transfused
Not Normal - 1997

June 25 – 69 year old male – Case 3

CD19 68%
CD20 59%
CD23 6%
HLA-DR 62%
CD19/5 10%
CD10 0%
κ:λ ratio 55.0 (strong)
Case 1 - CLL

Case 3 – not CLL
Clonal B cells in blood donors

- 656 blood donor samples
- 3 with clonal B cell populations
- Prevalence 0.5%
- Poster at 1999 AABB

Plapp FV, Rachel JM, Zucker ML
Chronic B lymphocytic leukemia in blood donors
Transfusion 39: 10S, 1999

Gerald E. Marti, MD, PhD
- MBL origins and history
- First blood donor study
- Other MBL studies
- Second blood donor study
- Clinical significance of MBL
First Blood Donor Study
June 2000-July 2002

- Saint Luke’s Hospital Foundation grant
- Prevalence of CLL in blood donors
- Samples de-identified
- Age and gender
- Analyzed within 48 hrs
- EDTA sample for CBC
- Sodium heparin for flow cytometry
Study Design

- Screening criteria
  - FW vs SS lymphocyte gating; total lymphs
  - $\geq 40\%$ CD19$^+$ B cells (ref 4-23%) or
  - $>15\%$ CD19$^+$5$^+$ B cells (ref 0-4%)

- Rationale
  - Intentionally insensitive
  - Transfusion considerations
  - Establish screening costs

- Targeting CLL; MBL not yet defined
Data Analysis

- Initial screening – 5,141 samples
  - FW vs SS lymphocyte gating; total lymphs
  - $\geq 40\%$ CD19$^+$ B cells (ref 4-23%) or
  - $> 15\%$ CD19$^+$5$^+$ B cells (ref 0-4%)

- Clonality assessment – 14 samples – 0.27%
  - $\kappa:\lambda$ ratio $>5.5$ or $<0.5$

- Full workup – 7 samples – 0.14%
  - CLL immunophenotyping
  - IgH by PCR – FDA/CBER – Abbasi and Marti
## Data Summary

<table>
<thead>
<tr>
<th>Donor</th>
<th>Age Gender</th>
<th>WBC 10⁹/L</th>
<th>Lymp 10⁹/L</th>
<th>B cell 10⁹/L</th>
<th>CD19 %</th>
<th>CD20 %</th>
<th>CD19/5 %</th>
<th>CD23 %</th>
<th>κ:λ - ratio intensity</th>
<th>IgH PCR</th>
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<tr>
<td>1</td>
<td>79 M</td>
<td>9.7</td>
<td>5.2</td>
<td>2.9</td>
<td>56</td>
<td>37</td>
<td>37 str</td>
<td>10</td>
<td>1:59 - 0.03 mod</td>
<td>MC</td>
<td>MBL</td>
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<td>2</td>
<td>69 M</td>
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<td>46</td>
<td>42</td>
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<td>37</td>
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<tr>
<td>3</td>
<td>39 F</td>
<td>7.0</td>
<td>3.6</td>
<td>2.0</td>
<td>56</td>
<td>36</td>
<td>37 wk</td>
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<td>33</td>
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<td>63</td>
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<td>CLL</td>
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<tr>
<td>6</td>
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<td>6.1</td>
<td>3.2</td>
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<td>59</td>
<td>53</td>
<td>45 wk</td>
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<td>MBL</td>
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<td>52 F</td>
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<td>34</td>
<td>27</td>
<td>27 wk</td>
<td>22</td>
<td>31:1 - 31.0 wk</td>
<td>OLC</td>
<td>MBL</td>
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</tbody>
</table>


- MBL origins and history
- First blood donor study
- Other MBL studies
- Second blood donor study
- Clinical significance of MBL
Monoclonal B Cell Lymphocytosis (MBL) 2005

- Monoclonal B cell population in peripheral blood:
  - Overall κ:λ ratio >3:1 or <0.3:1 or
  - >25% B cells with weak/absent sIg or
  - Disease-specific immunophenotype

- Exclusion criteria:
  - Lymphadenopathy and organomegaly
  - Autoimmune/infectious disease
  - B lymphocyte count >5x10⁹/L

- Sub-classification:
  - CLL-like – CD5+23⁺; most cases
  - Atypical-CLL - CD5⁺20br23neg
  - Non-CLL – CD5neg

- Stable over 3-month period

Marti GE, Rawstron AC, Ghia P, et al
Diagnostic criteria for monoclonal B-cell lymphocytosis
Br J Haematol. 2005;130:325-332
<table>
<thead>
<tr>
<th>Year</th>
<th>PI and Country</th>
<th>Population</th>
<th>Age Range</th>
<th>N</th>
<th>Flow Colors</th>
<th>Events Collected</th>
<th>Prevalence %</th>
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<td>40-76</td>
<td>1926</td>
<td>2-4</td>
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<tr>
<td>2009</td>
<td>Nieto - Spain</td>
<td>Healthy</td>
<td>&gt;40</td>
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<td>5,000,000</td>
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<td>Dagklis - Italy</td>
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<td>18-102</td>
<td>1725</td>
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<td>500,000</td>
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</table>
- MBL origins and history
- First blood donor study
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- Second blood donor study
- Clinical significance of MBL
Specific aims:

- Determine prevalence of MBL in blood donors aged 45 years and older
- Utilize six-color flow cytometry to achieve 0.01% sensitivity
- Characterize IgHV gene mutation status for detected MBL cases

Collaborators:

- Community Blood Center, Kansas City MO:
  - Geri Venable, Jay Menitove
- Saint Luke’s Hospital, Kansas City MO:
  - Jane Rachel, Jeff Boren, Fred Plapp
- FDA/CBER, Bethesda MD:
  - Fatima Abbasi, Gerald Marti
- Università Vita-Salute San Raffaele/Istituto Scientifico San Raffaele, Milan, Italy
  - Antonis Dagklis, Paolo Ghia
- ATSDR/CDC, Atlanta GA:
  - Youn Shim, Robert Vogt
Study Design

- Obtain residual blood samples from >2,000 donors age 45 and older
- Label samples and data sheet with unique random 5-digit numbers
- Record age, gender, transfusion history, cancer history on data sheet
  - Samples to SLH
  - Data sheets to ATSDR/CDC - Atlanta
- Complete blood counts
- MBL screening
  - Six-color flow cytometry
  - 500,000 events
- Positive samples
  - Extract DNA for IgHV analysis - Milan
  - Send to FDA/CBER for MBL immunophenotyping - Bethesda
MBL Criteria

Cluster of 50 or more B lymphocytes:

- Clonality:
  - κ:λ ratio >3:1 or <0.3:1
  - monotypic

- Immunophenotypic MBL subtypes:
  - classical CLL (CD5⁺)
  - atypical CLL (CD5⁺/CD20br)
  - non-CLL (CD5neg)
MBL Analysis - Negative

![CD20 APC-H vs CD19 PerCP-Cy5-5-H](image1)

![KAPPA PE-H vs LAMBDA FITC-H](image2)

<table>
<thead>
<tr>
<th>Population</th>
<th>#Events</th>
<th>%Parent</th>
<th>%Total</th>
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<tbody>
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<td>CD19+/CD20+</td>
<td>12,778</td>
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<tr>
<td>Q1-9</td>
<td>7,456</td>
<td>58.4</td>
<td>####</td>
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<td>Q2-9</td>
<td>50</td>
<td>0.4</td>
<td>####</td>
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<td>Q3-9</td>
<td>13</td>
<td>0.1</td>
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<tr>
<td>Q4-9</td>
<td>5,259</td>
<td>41.2</td>
<td>####</td>
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</table>
MBL Analysis - Negative
MBL Analysis – Negative

CD19-/-CD5+
CD19+/CD5+
CD19+/CD5-

Q1
Q2
Q3
Q4

CD19+/CD5+

Q1-1
Q2-1

Q3-1
Q4-1

CD19+/CD5-

Q2-2
Q4-2
MBL Analysis – Negative
MBL Analysis – Negative

<table>
<thead>
<tr>
<th>Population</th>
<th>#Events</th>
<th>%Parent</th>
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<tbody>
<tr>
<td>Q1-8</td>
<td>35</td>
<td>62.5</td>
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<tr>
<td>Q2-8</td>
<td>0</td>
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<tr>
<td>Q3-8</td>
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<td>0.0</td>
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<tr>
<td>Q4-8</td>
<td>21</td>
<td>37.5</td>
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<table>
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<th>%Parent</th>
<th>%Granularity</th>
<th>%Total</th>
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<tbody>
<tr>
<td>CD19+/CD5+/CD20 DIM</td>
<td>3</td>
<td>####</td>
<td>0.00</td>
<td>0.00</td>
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<tr>
<td>Q1-10</td>
<td>3</td>
<td>100.0</td>
<td>####</td>
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<td>Q2-10</td>
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<td>####</td>
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<td>Q3-10</td>
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<td>0.0</td>
<td>####</td>
<td>####</td>
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<tr>
<td>Q4-10</td>
<td>0</td>
<td>0.0</td>
<td>####</td>
<td>####</td>
</tr>
</tbody>
</table>
MBL Positives – CLL phenotype

- MBL STUDY-23231-Tube_001
  - 207 cells
  - 0.04% of total
  - CD19+/CD5+/CD20 DIM

- MBL STUDY-28845-Tube_001
  - 5,378 cells
  - 1.08% of total
  - CD19+/CD5+/CD20 DIM

- MBL STUDY-99421-Tube_001
  - 139,441 cells
  - 27.89% of total
  - CD19+/CD5+/CD20 DIM
MBL Positives – CD 5 negative
## Preliminary Data Summary

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Prevalence Estimate (95% CI)</th>
<th>45-54 yrs</th>
<th>55-64 yrs</th>
<th>&gt;64 yrs</th>
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</thead>
<tbody>
<tr>
<td>Overall</td>
<td>1090</td>
<td>7.7% (6.2-9.5)</td>
<td>4.9%</td>
<td>8.6%</td>
<td>11.5%</td>
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<tr>
<td>Men</td>
<td>637</td>
<td>10.2% (8.0-12.8)</td>
<td>7.1%</td>
<td>9.3%</td>
<td>16.6%</td>
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<tr>
<td>Women</td>
<td>450</td>
<td>4.2% (2.6-6.5)</td>
<td>2.4%</td>
<td>7.7%</td>
<td>1.3%</td>
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</tbody>
</table>
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Evolving thoughts on MBL

Prevalence estimates
- Population studied – age, gender, health, location
- Sensitivity of flow cytometry – reagents, events
- Consensus protocol not developed

MBL precursor to CLL
- CLL is preceded by MBL – Landgren et al
- Prevalence higher than CLL
- May regress, remain stable, progress to CLL
- 1% per year progress to CLL – Rawstron et al
- Analogous to MGUS and myeloma

MBL sub-classification:
- CLL phenotype – CD5^+23^+
  - high count – clinical MBL (cMBL)
  - low count – population MBL (pMBL)
- Atypical-CLL - CD5^+20^{br}
- Non-CLL – CD5^{neg}

Landgren O, Albitar M, Ma W et al
B-cell clones as early markers for chronic lymphocytic leukemia

Rawstron AC, Bennett FL, O'Connor SJ et al
Monoclonal B-cell lymphocytosis and chronic lymphocytic leukemia
Significance of MBL in blood donors

Donor considerations
- Healthy, asymptomatic
- CLL-like, mutated, low-count pMBL
- Regress or remain stable
- Anxiety, insurance, employment
- Longitudinal study population needed

Recipient considerations
- Leukocyte depletion; irradiation
- No published data on MBL/CLL transmission
- Recent meta-analysis of transfusion and NHL
  - Increased risk of NHL
  - Highest for CLL/SLL
- Related allogeneic SCT to CLL patients

Castillo JJ, Dalia S, Pascual SK
Association between red blood cell transfusions and development of non-Hodgkin lymphoma: a meta-analysis of observational studies
Blood 2010;116:2897-2907
The only normal people
are the ones
you don't know very well

Joe Ancis