Using peripheral blood smear review to triage specimens for flow cytometry

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Financial disclosure

• No conflicts
Objectives

• Describe peripheral blood smear findings that could be used to recommend flow cytometric immunophenotyping.

• Describe clinical situations where peripheral blood flow cytometric studies might be of interest, despite absence of peripheral blood smear findings.

• Discuss the pros and cons of flow cytometric immunophenotyping for borderline lymphocytosis.
Why perform PB flow cytometry?

- T-cell subset enumeration for immunodeficiency monitoring, such as CD4 count in HIV
- RBC evaluation for hemolytic anemia, such as EMA for hereditary spherocytosis
- HLA-B27 for ankylosing spondylitis
- Fetal hemoglobin for fetomaternal bleed
- Immunophenotyping (FCI) for hematolymphoid neoplasms:
  - Distinguish normal and neoplastic through phenotypic aberrancy or restriction
  - Phenotype characteristic of a disease entity
Medical indications for FCI

- Bethesda consensus panel 2016:
  - Circulating blasts
  - Atypical / abnormal lymphoid cells
  - Circulating plasma cells
  - Pancytopenia, and other cytopenias
  - Leukocytosis:
    - Lymphocytosis, monocytosis, eosinophilia
    - But NOT basophilia and neutrophilia

Davis BH et al., Cytometry Part B (Clinical Cytometry) 2007; 72:S5-13
Experience suggesting flow studies on peripheral blood smear review

- Period November 2014 – October 2015
- Retrieved all PB smear reviews where pathologists comments were available (n = 5,228)
- Identified specimens where flow cytometric evaluation was recommended or suggested (n = 211, 4.0%)
- Determined if flow cytometric studies performed (n = 96, 45.5%)
- Reviewed flow cytometric findings
Experience suggesting flow studies on peripheral blood smear review

<table>
<thead>
<tr>
<th>Comment</th>
<th>Total</th>
<th>Flow performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>211</td>
<td>96 (45.5%)</td>
</tr>
<tr>
<td>Recommend FC if clinically indicated.</td>
<td>22</td>
<td>7 (31.8%)</td>
</tr>
<tr>
<td>Absolute lymphocytosis consistent with CLPD or peripheralized NHL.</td>
<td>11</td>
<td>4 (36.4)</td>
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<tr>
<td>Recommend FC if clinically indicated.</td>
<td>99</td>
<td>39 (39.4)</td>
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<tr>
<td>Borderline absolute lymphocytosis. Recommend FC to exclude CLPD if finding persists without explanation.</td>
<td>51</td>
<td>27 (52.9)</td>
</tr>
<tr>
<td>Absolute lymphocytosis suggestive of CLPD. Recommend FC if clinically indicated.</td>
<td>28</td>
<td>19 (67.9)</td>
</tr>
</tbody>
</table>
Can PB smear review help triage for FCI?

1. Combined morphology & FCI diagnostic
2. Combined morphology & FCI suggest next steps
3. FCI alone informative, without morphology
4. Morphology alone informative, without FCI
5. Combined morphology & FCI findings of undetermined significance
6. Combined morphology & FCI findings misleading
Case 1: 66 year old man.
WBC: 116.5 x 10^9/L;
  75% blasts, 17% monocytes
Hemoglobin: 8.3 g/dL, MCV 92.5 fL
Platelets: 30 x 10^9/L
Case 1:
Combined morphology & FCI diagnostic

- Acute myeloid leukemia:
  - Requires > 20% blasts (or equivalents) by morphology
  - Cytogenetic (classical and FISH) and molecular studies can be performed on PB
  - PB not recommended for minimal residual disease assessment

- Chronic lymphocytic leukemia:
  - BM not necessary for diagnosis of overt disease

Arber DA et al., Arch Pathol Lab Med, 2017 [Epub ahead of print]
Monocytosis

- Reactive monocytosis vs. CMML vs. AML
- Often morphology and flow not definitive
- Monoblasts and promonocytes often lack immunophenotypic markers of immaturity
- Immunophenotypic “aberrancy” is frequent, but also seen in reactive monocytosis
- CMML reported to have higher proportion of classical (CD14+, CD16-) monocytes, versus intermediate (CD14+, CD16+) and non-classical (CD14dim+, CD16+), but requires confirmation

Matarraz S et al., Cytometry B Clin Cytom. 2015 [Epub ahead of print]
Selimoglu-Buet D et al., Blood 2015;125(23):3618-26
Case 2: 57 year old woman
WBC: 82.7 x 10^9/L
Hemoglobin: 10.3 g/dL, MCV 95.8 fL
Platelets: 131 x 10^9/L
Case 2:
Combined morphology & FCI diagnostic

• Follicular lymphoma may be CD10 negative by FCI, perhaps due to cells from different compartments

• Higher grade follicular lymphoma cannot be distinguished from diffuse large B-cell lymphoma with a germinal center phenotype

• If higher grade, consider allocation for cytogenetic studies (classical and FISH) for MYC and/or BCL-2 and BCL-6 gene rearrangements

Maeshima AM et al., Leuk Lymphoma. 2015; 56:2000-4
Case 3: 58 year old woman
WBC 19.7 x 10^9/L; 84% lymphocytes
Hemoglobin 12.7 g/dL
Platelets 327 x 10^9/L
Case 3:
Combined findings suggest next steps

- CD5-, CD10- B-cell lymphoid neoplasm
- Lymphocytes with villous projections raise the possibility of splenic marginal zone lymphoma
- Recommend evaluation for splenomegaly, lymphadenopathy or other extranodal disease
Case 4:
Case 4: 43 year old woman
WBC 116 x 10^9/L
Hgb: 8.1 g/dL
Platelets: 61 x 10^9/L
History of B lymphoblastic leukemia, NOS
FCI alone informative, without morphology

• B lymphoblastic leukemia, with some caveats:
  • Reports of circulating hematogones
  • CML with low level lymphoblasts uncertain significance

• Hairy cell leukemia

• PB staging for known disease e.g. myeloma

• Hypereosinophilic syndrome, lymphocyte variant:
  • Abnormal flow cytometry (T-cells lacking CD4 or CD8, or lacking CD3, sometimes with lack CD7 or increased CD5, and activation markers CD25 or HLA-DR)
  • Or clonal T-cell receptor gene rearrangement

Soma L et al., Cytometry B Clin Cytom 2016; 90:440-8
Case 5: 21 year old woman
WBC 12.7 x 10^9/L
Hemoglobin 12.7 g/dL
Platelets 327 x 10^9/L

History of fatigue and sore throat
Monospot positive
Morphology alone informative, without FCI

- Utility of abnormal lymphocytosis in establishing diagnosis of infectious mononucleosis:
  - Presence of atypical lymphocytosis significantly increased likelihood of mononucleosis
  - Absolute lymphocyte count $\geq 4 \times 10^9$/L helpful in diagnosing adolescents
- Immunophenotype associated with viral infection:
  - Increased CD8 positive, cytotoxic T-cells
  - CD7 negative T-cells in infectious mononucleosis
  - Clonotypic T-cells in cytomegalovirus infection

Ebell MH et al, JAMA 2016;315(14):1502-9
Stephen JR. Blood 2008; 112:4367-68
### Outcome of recommended flow studies

<table>
<thead>
<tr>
<th>Comment</th>
<th>Flow Positive</th>
<th>Flow Negative</th>
<th>Flow Uncertain</th>
</tr>
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<tbody>
<tr>
<td>All (n=96)</td>
<td>62 (64.6%)</td>
<td>22 (22.9%)</td>
<td>12 (12.5%)</td>
</tr>
<tr>
<td>Recommend FC if clinically indicated (n=7)</td>
<td>5</td>
<td>2</td>
<td>0</td>
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<td>Absolute lymphocytosis consistent with CLPD or peripheralized NHL.</td>
<td>4</td>
<td>4</td>
<td>0</td>
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<td>Recommend FC if clinically indicated (n=4)</td>
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<td></td>
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<tr>
<td><strong>Borderline absolute lymphocytosis. Recommend FC to exclude CLPD if finding persists without explanation. (n=39)</strong></td>
<td>12 (30.8%)</td>
<td>16 (40.0%)</td>
<td>11 (28.2%)</td>
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<tr>
<td>Absolute lymphocytosis suggestive of CLPD.</td>
<td>22</td>
<td>4</td>
<td>1</td>
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<tr>
<td>Recommend FC if clinically indicated (n=27)</td>
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<td></td>
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<tr>
<td>Absolute lymphocytosis consistent with CLPD.</td>
<td>19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Recommend FC if clinically indicated (n=19)</td>
<td></td>
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</tbody>
</table>
Outcome of recommended flow studies

- Acute myeloid leukemia
- B lymphoblastic leukemia
- Mantle cell lymphoma
- B-cell lymphoma, CD5(-), CD10(-)
- Peripheral T-cell lymphoma
Monoclonal B lymphocytosis

- Absolute lymphocyte count $\leq 5 \times 10^9/L (> 0.5)$
- No history of lymphoid neoplasm, or clinical evidence of overt disease (beware limited history)
- Exclude hairy cell leukemia
- Most known about MBL with an immunophenotypic characteristic of chronic lymphocytic leukemia (CLL):
  - 1% per year progression to CLL
  - Increased incidence of infection and other neoplasms
  - Immune senescence or persistent immune stimulation

Rawstron AC et al., Cytometry B Clin Cytom 2010;78:S19-23
Case 6: 89 year old man
WBC 8.4 x 10⁹/L; ALC 5.36 x 10⁹/L
Hemoglobin 13.2 g/dL
Platelets 183 x 10⁹/L
Case 6:

- Increased LGL
- Increased CD8+ T-cells
- Immunophenotype not characteristic of T-cell LGLL
- Would molecular studies help?
T-cell clonopathy of undetermined significance

- Age-related T-cell restriction (most > 60 years old)
- Immune senescence:
  - Impaired immunity to specific antigens e.g. CMV
  - Reduced repertoire of T-cells, CD8+ or CD8+ & CD4+
  - Often clonal
- What are minimal criteria for T cell LGLL?:
  - Appropriate clinical context
  - Persists without explanation

Ghia P et al., Br J Haem 2007; 139:780-90
Neff JL et al., Surgical Pathology 2013; 6:631-639
Case 7: 22 year old woman
WBC 9.5 x 10^9/L; ALC 3.62 x 10^9/L
Hemoglobin 10.4 g/dL
Platelets 33 x 10^9/L
Case 7:

- Increased LGL
- Increased natural killer cells
- History of CML
- Failure TKI therapy
- s/p stem cell Tx
- Receiving dasatinib therapy
Borderline lymphocytosis of uncertain clinical significance

- Clonotypic T-cells of undetermined significance:
  - Immune senescence
  - Post-transplantation (autologous and allogeneic), with cumulative incidence of 20% at 2 years

- Chronic NK cell lymphocytosis; at least some clonal:
  - Post-transplantation
  - Dasatinib therapy

Cao F et al., Int J Lab Hem 2015;37:783-90
Wolniak KL et al., Am J Clin Pathol 2013;139:231-241
Shimura Y et al., Int J Hematol 2015;102:426-33
Results of suggested flow studies on peripheral blood smear review

Flow cytometric findings of uncertain significance:

- **Absolute lymphocyte count \( \leq 5.5 \times 10^9/L \) (n=7):**
  - Monoclonal B-lymphocytosis (\( n = 5, \text{ALC} 3.57, 3.93, 4.19, 4.41, 4.59 \times 10^9/L \))
  - Increased CD4 positive T-cells, subsequent diagnosis of DLBCL (\( \text{ALC} 3.91 \times 10^9/L \))
  - Increased NK-like T-cells (\( \text{ALC} 5.36 \times 10^9/L \))

- **Absolute lymphocyte count \( > 5.5 \times 10^9/L \) (n=5):**
  - Increased gamma-delta T-cells (\( \text{ALC} 5.67 \times 10^9/L \))
  - Increased CD8+ T-cells s/p transplant (\( n = 3, \text{ALC} 6.25, 8.28, 8.32 \times 10^9/L \))
  - Increased NK cells, s/p transplant for AML
Case 8: 68 year old woman
WBC $15.2 \times 10^9$/L; ALC $7.63 \times 10^9$/L
Hemoglobin 14.3 g/dL
Platelets $271 \times 10^9$/L
Case 8:
Case 8:

Thymectomy
Thymoma, type A
PB smear and FCI findings misleading

- Thymoma-associated lymphocytosis:
  - Polyclonal mature T cells
  - Immunoregulatory / autoimmune disorder

- Angioimmunoblastic T-cell lymphoma with polyclonal plasmacytosis
  - Prominent polytypic plasmacytosis

- Do the peripheral blood smear review and/or flow cytometric results explain the clinical findings?

Barton AD. Cancer 1997; 80:1409-17
Using peripheral blood smear review to triage for flow cytometry

- Flow cytometry can assist in the diagnosis and classification of hematolymphoid neoplasms.
- A proactive PB smear reviewer can obviate the need for repeat PB or bone marrow collection.
- Flow cytometry doesn't always add value, particularly for borderline lymphocytosis.
- It’s important to correlate PB smear findings with the clinical information i.e. a laboratory medicine consult.