COMPARISON BETWEEN MICROSCOPY AND FLOW CYTOMETRY IN THE ASSESSMENT OF MARROW INVOLVEMENT BY LYMPHOPROLIFERATIVE AND PLASMA CELL NEOPLASIAS

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Procedures to determine involvement of bone marrow by lymphoma or myeloma

Aspirate
- Smears - cytospins
- Flow cytometry
- Cytogenetics / F.I.S.H.
- Molecular genetics

Biopsy
- Conventional histology
- Immunohistochemistry
- Cell disaggregation

Focal bone marrow involvement
Follicular Lymphoma

Lymphoma in bone marrow

Immunohistochemistry

Antibody-Enzyme-Colored substrate
**Immunohistochemistry**

- Allows the recognition of antigens in tissues even after fixation, decalcification and histologic processing.
- Fixed tissue immunohistology is very attractive since it allows the direct correlation of the expression of antigens with morphologic changes.
- With an increasing number of useful antibodies the utilization of immunohistochemistry has grown significantly.

**CD20 (brown) + Factor VIII (red)**

**Cyclin D1**

**Bone marrow – Large B-cell lymphoma**

**IMMUNOHISTOLOGY (IH) vs. FLOW CYTOMETRY (FCM)**

<table>
<thead>
<tr>
<th></th>
<th>IH</th>
<th>FCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>MORPHOLOGIC CORRELATION</td>
<td>Good</td>
<td>Limited</td>
</tr>
<tr>
<td>QUANTITATION OF ANTIGEN EXPRESSION</td>
<td>Poor</td>
<td>Good</td>
</tr>
<tr>
<td>SIMULTANEOUS DETECTION OF MULTIPLE ANTIGENS</td>
<td>Poor</td>
<td>Excellent</td>
</tr>
<tr>
<td>BACKGROUND STAINING</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>POTENTIAL CELL LOSS</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>
**Does flow cytometry increase the sensitivity of detection of lymphoma in bone marrow?**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>No.</th>
<th>Results distribution</th>
<th>% Discord</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naughton &amp; al</td>
<td>1998</td>
<td>273</td>
<td>BM+ FC+</td>
<td>37</td>
</tr>
<tr>
<td>Dunphy &amp; al</td>
<td>1998</td>
<td>188</td>
<td>BM+ FC+</td>
<td>75</td>
</tr>
<tr>
<td>Hansson &amp; al</td>
<td>1999</td>
<td>175</td>
<td>BM+ FC+</td>
<td>49</td>
</tr>
<tr>
<td>Duggan &amp; al</td>
<td>2000</td>
<td>227</td>
<td>BM+ FC+</td>
<td>49</td>
</tr>
<tr>
<td>Palacio &amp; al</td>
<td>2001</td>
<td>79</td>
<td>BM+ FC+</td>
<td>16</td>
</tr>
<tr>
<td>Mazur &amp; al</td>
<td>2004</td>
<td>53</td>
<td>BM+ FC+</td>
<td>21</td>
</tr>
<tr>
<td>Perea &amp; al</td>
<td>2004</td>
<td>380</td>
<td>BM+ FC+</td>
<td>102</td>
</tr>
</tbody>
</table>

- BM, Bone marrow; FC, flow cytometry; FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; LPL, lymphoplasmacytic lymphoma.

**Flow Cytometry in Clinical Practice**

- Whether morphologic examination or FC alone is adequate for the detection of all cases of B-lymphoid neoplasm bone marrow involvement. FC failed to detect bone marrow involvement in those B-NHL cases having focal paratrabecular infiltration, but proved to be more sensitive than histology in detecting small clonal B-cells.

**Authors**


**Clinical Usefulness of Flow Cytometry**

- FC is just as sensitive or slightly more sensitive than histology in the detection of bone marrow involvement in FL and DLBCL. The clinical relevance of the small clonal B-cell population in patients without histologic bone marrow involvement remains an open question.

**Sample Results**

- FL: 43 BM+ FC+ | 95 BM– FC– | 24 BM– FC+ | 26 BM+ FC– | Total (188) 27%

- DLBCL: 7 BM+ FC+ | 36 BM– FC– | 3 BM– FC+ | 12 BM+ FC– | Total (58) 26%

- MCL: 25 BM+ FC+ | 26 BM– FC– | 3 BM– FC+ | 12 BM+ FC– | Total (57) 11%

- LPL: 12 BM+ FC+ | 6 BM– FC– | 4 BM– FC+ | 4 BM+ FC– | Total (25) 28%

- MZL: 12 BM+ FC+ | 16 BM– FC– | 2 BM– FC+ | 1 BM+ FC– | Total (31) 10%

- Burkitt: 1 BM+ FC+ | 5 BM– FC– | 1 BM– FC+ | 1 BM+ FC– | Total (7) 14%

- Others: 2 BM+ FC+ | 12 BM– FC– | 0 BM– FC+ | 0 BM+ FC– | Total (14)

- Total: 102 BM+ FC+ | 196 BM– FC– | 36 BM– FC+ | 46 BM+ FC– | Total (380) (22%)

- BM, Bone marrow; FC, Flow cytometry; FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; LPL, lymphoplasmacytic lymphoma; MZL, marginal zone lymphoma.

**CD20 Immunohistochemistry**

- Large B-cell lymphoma

**Results**

- Most samples showed concordance between morphological and flow cytometric results. Flow cytometry identified BM involvement in the absence of morphological infiltration. Discordance seems to have no influence on the outcome of FL patients.
• Microscopy of bone marrow biopsies and flow cytometric analysis of aspirates are both superior to microscopy of marrow aspirate smears in the detection and classification of lymphoma.

• Immunohistochemistry is useful for distinguishing general cell lineage in lymphoid infiltrates; however, determination of clonality is extremely difficult.

What is the best method to detect malignant plasma cells in the bone marrow?
Bone marrow biopsy (H&E)

Patterns of plasma cell infiltrates in myeloma (CD138 stain)

Bone marrow I.S.H.

Kappa RNA

Lambda RNA

Percentage of plasma cells - Aspirate smears

Correlation: 0.85

$r^2$: 0.72

Percentage of plasma cells - H&E stained sections

Correlation: 0.88

$r^2$: 0.77

Percentage of plasma cells - CD138 stain

Correlation: 0.92

$r^2$: 0.96
• Extent of plasma cell infiltration in the marrow is best estimated on CD138 stains of bone marrow core biopsies

• Does flow cytometry increase the sensitivity of detection of myeloma in bone marrow?

• For reasons that are not completely clear, the number of plasma cells detected by flow cytometry is always less than those observed in marrow aspirate smears.

Arch Path Lab Med (2007) 131: 951
Smock KJ et al. Quantitation of Plasma Cells in Bone Marrow Aspirates by Flow Cytometric Analysis Compared With Morphologic Assessment.

POLYCLONAL PLASMA CELLS

Cytoplasmic Ig

Kappa

Lambda
Although the percentage of plasma cells detected by flow cytometry in bone marrow aspirates is usually inaccurate, the ratio between normal and abnormal plasma cells is informative.

Levels of normal and neoplastic plasma cells immediately after transplantation provide a powerful prediction of both progression-free and overall survival.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at transplantation, older than 55 years</td>
<td>0.426</td>
<td>NT</td>
</tr>
<tr>
<td>Sex, male</td>
<td>0.078</td>
<td>NT</td>
</tr>
<tr>
<td>Presentation creatinine, greater than 130 µM</td>
<td>0.267</td>
<td>NT</td>
</tr>
<tr>
<td>Presentation hemoglobin, less than 12 g/dL</td>
<td>0.021</td>
<td>0.442</td>
</tr>
<tr>
<td>Beta2-m, greater than 4.0 mg/L</td>
<td>0.016</td>
<td>0.050</td>
</tr>
<tr>
<td>Immunofixation-negative after transplantation</td>
<td>0.002</td>
<td>0.786</td>
</tr>
<tr>
<td>Neoplastic plasma cells at 3 months after transplantation</td>
<td>0.003</td>
<td>0.014</td>
</tr>
</tbody>
</table>

Perez-Persona E. et al. New criteria to identify risk of progression in monoclonal gammopathy of uncertain significance and smoldering multiple myeloma based on multiparameter flow cytometry analysis of bone marrow plasma cells.

### Aberrant phenotype in smoldering MM & MGUS

<table>
<thead>
<tr>
<th>CD38</th>
<th>CD45</th>
<th>CD19</th>
<th>CD56</th>
<th>Cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>42 (50%)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>20 (24%)</td>
</tr>
<tr>
<td>+</td>
<td>-/dim</td>
<td>-</td>
<td>+</td>
<td>9 (11%)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>7 (8%)</td>
</tr>
<tr>
<td>+</td>
<td>dim</td>
<td>-</td>
<td>-</td>
<td>4 (5%)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>dim</td>
<td>++</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

### Flow cytometry (FCM) in plasma cell dyscrasias

- Useful in cases of plasmacytosis of uncertain nature
- Cannot be applied to quantitate plasma cells in the marrow
- Helpful in recognizing and following up MGUS and smoldering Myeloma
- May be useful in assessing therapeutic response