Flow cytometry in the diagnosis of myelodysplastic syndromes

Marciano Reis, MD, FRCPC
Chief, Dept. of Clinical Pathology
Sunnybrook Health Sciences Centre
Women’s College Hospital
Chief, Dept. of Laboratory Hematology
University Health Network
Associate Professor
University of Toronto

HIAE
São Paulo, November 2012
WHO 2001

“Immunophenotype is not relevant in the diagnosis of myelodysplastic syndromes”
MDS AND IMMUNOPHENOTYPING
PubMed Search 130 publ.
(Flow cytometry, English)

Number of publ

MDS and flow cytometry – WHO 2008

- Multiple aberrant features (>3) in maturation patterns of erythroid and myeloid lineage are highly specific for MDS, single aberrancies are not diagnostic
- Emerging pathological CD34 and or CD117 positive populations are suggestive of transformation
Strategies in used in published studies

- Immunophenotyping of blasts (Ficoll or CD45 gating)
- Immunophenotyping of isolated CD34+ cells (MACS or CD34/SSC gating)
- Scoring systems (results from many separate antigens)
- Pattern recognition (multicolor analysis and comparison with normal/reactive blood and bone marrow)
Erythroid lineage

Erythroid dysplasia:

function based on expression of H-ferritin, CD71 and CD105 in GPA positive nucleated cells

Malcovati L. et al., Leukemia, 2005, Della Porta MG et al, Leukemia, 2006
Erythroid lineage

- PROSPECTIVE VALIDATION
  - A correct diagnosis was made in 98% of cases showing morphological erythroid dysplasia (n=53, 98% sensitivity)
  - Among 65 controls (10 healthy, 55 anemic, no dysplasia) one was incorrectly classified (98.5% specificity)
  - 60% of MDS patients without convincing morphological erythroid dysplasia had pathological findings in flow cytometry analysis of erythroid lineage (n=15)

Della Porta MG et al, Leukemia, 2006
Granulopoietic lineage
Stetler Stevenson, 2001

Figure 1. Myeloid abnormalities in MDS demonstrated by CD45 versus side light scatter. Data from patients with straightforward myelodysplastic syndrome, diagnosed by morphology. (A) Healthy donor bone marrow: normal granulocytes and precursors in the boxed region. (B) MDS patient bone marrow (ungated): hypogranular neutrophils with low side scatter (arrow) and a discrete blast population (oval) are demonstrated. (C) MDS patient bone marrow (ungated): hypogranular neutrophils with low side scatter (arrow) and a discrete blast population (oval) are demonstrated.
Granulopoietic lineage
Wells, 2003

- Hypogranulation
- Increased DR on myeloid cells
- Abnormal expression of CD56 on granulocytes and monocytes
- Aberrant expression of CD7 on myeloid cells
- Proposal for a scoring system
Granulopoietic lineage
Brent Wood’s group

Normal patterns of granulocytic and monocytic maturation in bone marrow by 4-color flow cytometry
Kussick et al. Am J Clin Path 2005
**Granulopoietic lineage**

**Table 6**
Distribution of Cases by Cytogenetic, Morphologic, and Flow Cytometric Findings

<table>
<thead>
<tr>
<th>Cytogenetics</th>
<th>Morphologic Studies</th>
<th>Flow Cytometry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Normal*</td>
<td>Normal</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Indeterminate</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>3</td>
</tr>
<tr>
<td>Abnormal*</td>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Indeterminate</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Abnormal</td>
<td>0</td>
</tr>
</tbody>
</table>

*The Fisher exact test showed a highly significant association ($P < .001$) between the morphologic and flow cytometric results within the cytogenetically normal and abnormal groups.

Flow abnormal:
At least three aberrant features present

Granulopoietic lineage: Low grade MDS

Refractory anemia: decreased CD33 and HLA-DR on myeloid precursors, abnormal CD38 expression on CD34+ blasts
Granulopoietic lineage: Low grade MDS

Analysis of CD34+ cells by four color flow cytometry

Table 6. Association between FCM score and cytogenetics in LGw/oRS patients

<table>
<thead>
<tr>
<th>Cytogenetics, no.*</th>
<th>Abnormal</th>
<th>Normal</th>
<th>No metaphases</th>
<th>+8</th>
<th>del(20q)</th>
<th>der(1;7)</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>16</td>
<td>10</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>FCM score</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3 or higher</td>
<td>10</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

Data are number of patients. Patients with +8, del(20q), and der(1;7) included those with additional cytogenetic aberrations.

*Abnormal vs normal, $P = .530$ (proportions of patients with FCM scores of 3 or higher are compared).

†+8 or del(20q) vs others, $P = .002$ (proportions of patients with FCM scores of 3 or higher are compared).

Ogata et al., Blood 2006
**CD34+ cells by flow cytometry vs blasts on smears**

- In many MDS cases there is a very good concordance between the percentages of blasts on smears and CD34+ cells in bone marrow. **BUT**
- There are cases with CD34−/CD117+ pathological populations.
- Cases with fibrosis where samples obtained for flow cytometry are not representative.
- Haemodiluted samples (second or third pull) where there may be a significant difference.

Percentages of CD34+ cells cannot replace differential counts on smears but flow cytometry may give additional information if pathological phenotypes of CD34+ cells are found.
Lymphoid precursors
Sternberg, 2005

- Anomalies of B lineage progenitors in low risk MDS
- Consistent with other studies showing decreased lymphoid progenitors in RA or 5q-syndrome
Conclusions from literature survey

- Flow cytometry results correlate well with morphology and cytogenetics in MDS.
- Flow cytometry can give additional information in MDS work-up of patients with borderline features but large studies focused on that issue are scarce.
- Megakaryocytic lineage dysplasia is better assessed by morphology/IHC than by flow cytometry.
Efforts towards standardization

- Normal bone marrow atlas:
  by Marie-Christine Béné (Nancy) et al
  ELN Website
  http://www.leukemia-net.org/content/diagnostics/diagnostics/flow_cytometry_atlas/

A systematized 4 colours approach

- GTLLF: Groupe de Travail sur les Leucémies & Lymphomes Francophone
- Issued from AFC (Association Française de Cytométrie) and GEIL (Groupe d’Etude Immunologique des Leucémies)
- 9 centres
- Two phases
- 65 normal bone marrow samples

Marie-Christine Béné, 2008
First: mature cells

- Positive identification of mature cells in the bone marrow
- Color code
- CD45 bright: *lymphocytes*
- CD14/CD11b: *monocytes*
- CD16/CD11b: *granulocytes (NK, B cells)*
- The rest: « bermudes »

Marie-Christine Béné, 2008
Marie-Christine Béné, 2008
Reproducibility

Marie-Christine Béné, 2008
Myelodysplasia
granulocytes CD11b/CD16
CD11b/CD117/CD34/CD45

SSC Height

CD45 APC

Granulocytes

Gating CD11b+

Gating CD117+

Marie-Christine Béné, 2008
Myelodysplasia
granulocytes CD11b/CD117

Marie-Christine Béné, 2008
Multilineage dysplasia: three lineages evaluated by CD34/CD38

MDS

normal
Diagnostic and prognostic significance of FCM in MDS

Figure 3. MDS flow-scores in healthy controls and patients with MDS classified by morphology. The MDS flow-score represents the presence of dysplastic features in myeloid blasts, granulocytes, and monocytes as detected by flow cytometry. Flow-scores were calculated according to the scoring system as proposed by Wells et al\(^a\) (Tables 2, 4); individual scores for the patients with MDS are depicted in Table 3. Spearman \(r = .813, P < .001\). The few patients that were classified as MDS-U, MDS/MPD, or hypoplastic MDS are not included in this graph. Horizontal bars are medians.\(^a\)\(P < .05; **P < .001\).

Van de Loosedrecht et al, Blood

2008 111: 1067-1077
Diagnostic and prognostic significance of FCM in MDS

Figure 5. MDS flow-scores in relation to clinical parameters. (A) Flow-scores in relation to transfusion dependency (nonflow versus dependent) or disease progression toward at least RAEB-1 (Spearman r = .448; P = .002); patients with RAEB-2 were excluded. (B) Patients are subdivided by WPSS. Spearman r = .513; P < .001. Horizontal bars are medians.

Van de Loosedrecht et al, Blood
2008 111: 1067-1077
Flow cytometry and response to treatment in MDS

Table 2. Epo levels and immunophenotype of myeloid blasts at start of Epo/G-CSF treatment

<table>
<thead>
<tr>
<th>Epo &lt; 100 U/L</th>
<th>Epo &gt; 100 U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>nFCM</td>
<td>aFCM</td>
</tr>
<tr>
<td>Responders</td>
<td>13</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>1</td>
</tr>
<tr>
<td>Response rate</td>
<td>94%</td>
</tr>
</tbody>
</table>

aFCM indicates aberrant immunophenotype of myeloid blasts; Epo, erythropoietin; G-CSF, granulocyte-colony-stimulating factor; and nFCM, normal immunophenotype of myeloid blasts.

Figure 2. Endogenous Epo levels and flow cytometry of myeloid blasts as biomarkers in the prediction of response to Epo/G-CSF treatment in low-/intermediate-1-risk MDS. Points granted for Epo level in this model are exactly as in the validated model of Hellstrom-Lindberg et al. Normal and aberrant FCM score 0 and −2 points, respectively. Applying this new model defines 3 subgroups with 94%, 17%, and 11% probability to respond to growth factor treatment.

Table 3. Multivariate logistic regression analysis of prediction of response to Epo/G-CSF

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aberrant flow cytometry</td>
<td>.001</td>
<td>0.035</td>
<td>0.005-0.274</td>
</tr>
<tr>
<td>Serum Epo at study entry</td>
<td>.019</td>
<td>0.245</td>
<td>0.076-0.795</td>
</tr>
<tr>
<td>Pretreatment RBC transfusions</td>
<td>.291</td>
<td>0.294</td>
<td>0.030-2.850</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; Epo, erythropoietin; G-CSF, granulocyte-colony-stimulating factor; and RBC, red blood cell.

Westers et al., BLOOD, 2010 VOL 115, 1779
Prognostic relevance of cytometric quantitative assessment in patients with myelodysplastic syndromes


Table 4 Impact of flow cytometric parameters on leukemia-free survival in univariate and multivariate analyses

<table>
<thead>
<tr>
<th></th>
<th>P</th>
<th>HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34⁺, &lt;3 vs. ≥3%</td>
<td>&lt;0.0001</td>
<td>9.98</td>
<td>5.76–16.69</td>
</tr>
<tr>
<td>CD117⁺, &lt;5% vs. ≥5%</td>
<td>&lt;0.0001</td>
<td>4.87</td>
<td>2.58–9.16</td>
</tr>
<tr>
<td>CD11b⁻/CD66b⁻, &lt;5% vs. ≥5%</td>
<td>&lt;0.0001</td>
<td>6.20</td>
<td>3.36–11.44</td>
</tr>
<tr>
<td>CD11b⁺/CD66b⁺, &lt;15% vs. ≥15%</td>
<td>&lt;0.0001</td>
<td>0.32</td>
<td>0.18–0.56</td>
</tr>
<tr>
<td>CD11b⁺/CD66b⁺, &lt;25% vs. ≥25%</td>
<td>0.0002</td>
<td>0.35</td>
<td>0.20–0.60</td>
</tr>
<tr>
<td><strong>Multivariate analysis, cytometric parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34⁺, &lt;3 vs. ≥3%</td>
<td>0.0003</td>
<td>6.83</td>
<td>2.41–19.30</td>
</tr>
<tr>
<td>CD117⁺, &lt;5% vs. ≥5%</td>
<td>0.322</td>
<td>1.48</td>
<td>0.68–3.22</td>
</tr>
<tr>
<td>CD11b⁻/CD66b⁻, &lt;5% vs. ≥5%</td>
<td>0.393</td>
<td>1.57</td>
<td>0.56–4.44</td>
</tr>
<tr>
<td>CD11b⁺/CD66b⁺, &lt;15% vs. ≥15%</td>
<td>0.092</td>
<td>0.56</td>
<td>0.29–1.10</td>
</tr>
<tr>
<td>CD11b⁺/CD66b⁺, &lt;25% vs. ≥25%</td>
<td>0.602</td>
<td>0.83</td>
<td>0.41–1.67</td>
</tr>
<tr>
<td><strong>Multivariate analysis, cytometric parameters, and IPSS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34⁺, &lt;3 vs. ≥3%</td>
<td>0.0007</td>
<td>6.23</td>
<td>2.16–17.95</td>
</tr>
<tr>
<td>CD117⁺, &lt;5% vs. ≥5%</td>
<td>0.966</td>
<td>1.01</td>
<td>0.42–2.40</td>
</tr>
<tr>
<td>CD11b⁻/CD66b⁻, &lt;5% vs. ≥5%</td>
<td>0.666</td>
<td>1.27</td>
<td>0.43–3.77</td>
</tr>
<tr>
<td>CD11b⁺/CD66b⁺, &lt;15% vs. ≥15%</td>
<td>0.214</td>
<td>0.65</td>
<td>0.33–1.28</td>
</tr>
<tr>
<td>CD11b⁺/CD66b⁺, &lt;25% vs. ≥25%</td>
<td>0.581</td>
<td>1.24</td>
<td>0.58–2.63</td>
</tr>
<tr>
<td>IPSS</td>
<td>0.001</td>
<td>1.99</td>
<td>1.31–3.02</td>
</tr>
</tbody>
</table>

Boldface type represents statistical significance.

IPSS, International Prognostic Scoring System.
Prognostic significance in various IPSS groups

Figure 2: Overall survival (A) and leukemia-free survival (B) according to the number of flow cytometric prognostic factors (CD34+, CD117+, and CD11b+/66b++) in low and intermediate-1 IPSS (II), and intermediate-2 and high IPSS (III) subgroups.

Efforts towards standardization

1st European Leukemia Net Workshop on Flow Cytometry Diagnostics in MDS: March 2008, Amsterdam


Flow Cytometry in MDS ELN Workshop

- Erythroid dysplasia by flow:
  - Increased percentage after lysis
  - Abnormal scatter and CD45 expression
  - Abnormal expression of H- and M-ferritin
  - Abnormal pattern of CD71/CD235a (glycophorin A)
  - Abnormal expression of CD105, CD34, CD36, CD117
Flow Cytometry in MDS ELN Workshop

- Abnormal Features of Blasts:
  - Increased percentage
  - Abnormal scatter and CD45 expression
  - Abnormal expression of stem cell markers CD34 and CD117
  - Abnormal expression of HLA-DR, CD11b, CD15
  - Lineage infidelity markers CD7, CD2, CD5, CD56
  - Abnormal expression of CD13, CD33, TdT, CD36, CD4
Granulopoietic dysplasia by flow:

- Abnormal scatter and CD45 expression
- Abnormal CD11b/CD13 pattern
- Abnormal CD13/CD16 pattern
- Persisting expression of CD34
- Abnormal expression of CD33, CD15, CD10, CD36, CD64
- Expression of HLA-DR
- Lineage infidelity markers CD7, CD2, CD5, CD19, CD5, CD56
Flow Cytometry in MDS
ELN Workshop

- Abnormal Features of Monocytes:
  - Decreased/increased percentages
  - Abnormal scatter and CD45 expression
  - Abnormal expression of CD33, CD13, CD36
  - Abnormal CD11b/HLA-DR pattern
  - Expression of CD34Abnormal expression of CD14, CD64, CD11c, CD15
  - Overexpression of CD56
  - Lineage infidelity markers CD7, CD2, CD19
Implementation of flow cytometry in the diagnostic work-up of myelodysplastic syndromes in a multicenter approach: Report from the Dutch Working Party on Flow Cytometry in MDS

Westers et al. Leukemia Research online Sept 5th

- Standardization between 8 Dutch centers: 4 color
- Analysis of pitfalls:
  - Large numbers of dead cells
  - Discrepancies between labs in enumeration of erythroid and monocytic populations
  - Overestimation of number of aberrant features
  - Overestimation of lineage infidelity markers (CD56, CD7)

Learning and workshops diminished interlaboratory variability in enumeration of various populations of BM cells
Highest variability: neutrophils
8-color FC panel
for MDS diagnostics,
Karolinska Hospital Stockholm

Tube 1                Tube 2

- FITC              CD36            CD56
- PE               GPA              CD7
- PerCP-Cy5         CD34            CD117
- PE-Cy7           CD13            CD33
- APC              CD117           CD34
- APC-Cy7          CD11b           HLA-DR
- PacBl            CD16            CD14
- AmCy             CD45            CD45
Normal Bone Marrow
MDS patient
Future prospects

- There is accumulating evidence that FCM has become an important part of integrated diagnostics of MDS.
- Technical progress in multicolor flow cytometry and new analysis programs, when combined with ongoing efforts to standardize the methodology, will make it possible to apply FCM in individual risk-assessment and choice of best therapy for MDS patients.
- 10-color panel is being tested and will be in clinical use at TGH 2012
Santo Antonio da Platina - PR