Analysis of bone marrow for MDS-related aberrancies

According to
International/ELN Flow Cytometry Working Group (IMDSFlow)
Anna Porwit
Lund, Sweden

Approach used at Hematopathology, Lund

- 1. new patients with cytopenia and <10% cells in blast region: start with Screening tube
- 2. if blast region>10%: full MDS panel
- 3. if “Ogata score” >2: full MDS panel
- 4. patients where previous bm showed dysplasia : full MDS panel
- 5. patients with >20% cells in blast region : full AML panel

Screening tube

<table>
<thead>
<tr>
<th>Ab</th>
<th>Clone</th>
<th>Titre (ul)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC</td>
<td>CD4</td>
<td>13B8.2</td>
</tr>
<tr>
<td>Kappa</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>PE</td>
<td>CD8</td>
<td>B9.11</td>
</tr>
<tr>
<td>Lambda</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>ECD</td>
<td>CD3</td>
<td>UCHT1</td>
</tr>
<tr>
<td>CD14</td>
<td>RMO52</td>
<td></td>
</tr>
<tr>
<td>PC5.5</td>
<td>CD33</td>
<td>D3HL60.251</td>
</tr>
<tr>
<td>PC7</td>
<td>CD20</td>
<td>B9E9</td>
</tr>
<tr>
<td>CD56</td>
<td>N901</td>
<td></td>
</tr>
<tr>
<td>APC</td>
<td>CD34</td>
<td>581</td>
</tr>
<tr>
<td>A700</td>
<td>CD19</td>
<td>J3</td>
</tr>
<tr>
<td>A750</td>
<td>CD10</td>
<td>ALB1</td>
</tr>
<tr>
<td>KO</td>
<td>CD5</td>
<td>BL1a</td>
</tr>
<tr>
<td>PB</td>
<td>CD5</td>
<td>BL1a</td>
</tr>
<tr>
<td>CD45</td>
<td>J.33</td>
<td></td>
</tr>
</tbody>
</table>

Excludes abnormal B-cell population
Gives orientation in T-cell subsets

4-parameter screening score consists of:
1. % CD34+ myeloid progenitor cells among all nucleated cells (<2%)
2. % CD34+ B cell precursors among all CD34+ cells (>5%)
3. 1.3 ratio of granulocytes (ratio to lymphocytes >6)
4. CD45 expression of myeloid progenitor cells (ratio to lymphocytes 4-7.5)

Ogata et al., Blood, 2006;108:1037-1044; Ogata et al., Haematologica, 2009;94:1066-1074; Della Porta MG, et al., Haematologica, 2012;97:1209-1217
Bardet et al. Haematologica, 2015 Apr;100(4):472-478

Example of comprehensive 10 color acute leukemia/MDS panel

https://www.leukemia-net.org/content/diagnostics/diagnostics/flow_cytometry_atlas/index_eng.html

### Immature myeloid and monocytic progenitors

<table>
<thead>
<tr>
<th>Recommended analysis</th>
<th>Aberrancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of cells in nucleated cell fraction</td>
<td>Increased percentage</td>
</tr>
<tr>
<td>Expression of CD45</td>
<td>Lack of/decreased/increased</td>
</tr>
<tr>
<td>Expression of CD16</td>
<td>Lack of/decreased/increased</td>
</tr>
<tr>
<td>Expression of CD17</td>
<td>Homogeneous under/expression</td>
</tr>
<tr>
<td>Expression of HLA-DR</td>
<td>Lack of/increased expression</td>
</tr>
<tr>
<td>Expression of CD13</td>
<td>Lack of/decreased/increased</td>
</tr>
<tr>
<td>Asynchronous expression of CD13</td>
<td>Presence of mature markers</td>
</tr>
<tr>
<td>Asynchronous expression of CD5</td>
<td>Presence of mature markers</td>
</tr>
<tr>
<td>Expression of CD10</td>
<td>Presence of lineage infidelity markers</td>
</tr>
<tr>
<td>Expression of CD117</td>
<td>Presence of lineage infidelity markers</td>
</tr>
<tr>
<td>Expression of CD19</td>
<td>Presence of lineage infidelity markers</td>
</tr>
<tr>
<td>Expression of CD33</td>
<td>Presence of lineage infidelity markers</td>
</tr>
<tr>
<td>Expression of CD34</td>
<td>Presence of lineage infidelity markers</td>
</tr>
<tr>
<td>Expression of CD56</td>
<td>Presence of lineage infidelity markers</td>
</tr>
<tr>
<td>Expression of CD10</td>
<td>Lack of/decreased/increased</td>
</tr>
<tr>
<td>Aberrant SSC signal</td>
<td>Altered</td>
</tr>
</tbody>
</table>

**Example of aberrant precursors**

**Visualization of all 10 markers by radar analysis**

### Maturing neutrophils

<table>
<thead>
<tr>
<th>Recommended analysis</th>
<th>Aberrancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of cells as ratio to lymphocytes</td>
<td>Decreased</td>
</tr>
<tr>
<td>SSC as ratio vs SSC of lymphocytes</td>
<td>Decreased</td>
</tr>
<tr>
<td>Relationship of CD13 and CD11b</td>
<td>Altered pattern</td>
</tr>
<tr>
<td>Relationship of CD13 and CD16</td>
<td>Altered pattern</td>
</tr>
<tr>
<td>Relationship of CD15 and CD10</td>
<td>Altered pattern; for example, lack of CD10 on mature neutrophils</td>
</tr>
</tbody>
</table>
Examples of aberrant neutrophils

Examples of aberrancies in monocytes

Erythropoietic tube on non-lysed BM

- 2.5 μl CD71-FITC,
- 2.5 μl CD13-PE,
- 5 μl CD117-ECD,
- 5 μl CD105-PE-Cy7,
- 5 μl CD36-PB,
- 2.5 μl CD45-KO
- DRAQ5 gating

Monocytes

Recommended analysis | Aberrancy
--- | ---
Percentage of cells | Decreased/increased
Distribution of maturation stages | Shift towards immature
Relationship of HLA-DR and CD11b | Altered pattern
Relationship of CD36 and CD14 | Altered pattern
Expression of CD13 | (Homogenous)
Expression of CD33 | (Homogenous)
Expression of CD56 | Presence of lineage infidelity marker
Asynchronous expression of CD34 | Presence of immature

Erythroid compartment

Recommended analysis | Aberrancy
--- | ---
Percentage of nucleated erythroid cells | Increased
Relationship CD71 and CD235a | Altered pattern
Expression of CD71 | Decreased
Expression of CD71 | Increased CV
Expression of CD36 | Decreased
Expression of CD36 | Increased CV
Percentage of CD117-positive precursors | Increased
Expression of CD105 | Altered expression
Expression of CD105 | Percentage

Analysis
Normal bone marrow
Radar pattern of erythroid maturation:
see Poster 031 for details

Abnormal patterns in MDS

Other

Relation of cell compartments

Percentage of mDCs in relation to total WBC: low or absent
Percentage of pDCs in relation to total WBC: low or absent
Percentage of basophils in relation to total WBC: absent or increased
Percentage of eosinophils within neutrophil compartment: absent or high

How to report FCM findings?
Guidelines of the MDsFlow WG on FCM in MDS 2015

• A:
  FCM analysis: NO MDS-related features

• B:
  FCM analysis: some changes often seen in MDS

• C:
  FCM analysis: consistent with MDS

Integrated Flow Cytometric diagnostic approach
Scoring system

Diagnostic Flow score (Ogata et al.)

- Dysplasia by FC: myeloid progenitors
- Dysplasia by FC: Neutrophils (SNC or two or more other aberrancies)
- Monocytes (CD16 or two or more other aberrancies)
- Erythroid precursors (CD36 or CD71)

Conclusion: A, B/C, C

Case 1: Male 59 years old

- Previously healthy
- Developed increasing fatigue about 2 months before presentation
- 6 weeks before presentation GP found anemia
- Patient went on vacation to Barbados
- Felt even more fatigue after coming back
- No fever, night sweats or weight loss
Status and Lab

- No lymphadenopathy or organomegaly
- No bruising or rash, no neurological deficit
- Hb 70g/L, MCV 115, reticulocytes 33x10⁹/L
- WBC 24.4x10⁹/L, no eosinophilia or basophilia
- Neutrophils 17.5x10⁹/L, Monocytes 6.1x10⁹/L
- Platelets 148x10⁹/L
- LDH and creatinine borderline
- Bone marrow biopsy with flow cytometry was performed

CD45 vs SSC

A. Blasts are not increased
B. Monocytes are increased
C. Granulopoiesis shows abnormal scatter
D. Lymphocytes are within normal limits

Which is NOT true?????
Bone marrow smear, blasts 5%

Bone marrow biopsy

Cytogenetics, FISH, molecular studies

- t(11;19)(q23;p13.1), MLL-ELL
- FISH confirmed MLL rearrangement
- JAK-2 mutation negative
- BCR-ABL1 negative

- 11q23 abnormalities
- leading to the MLL gene
- rearrangement are most common in AML

Diagnosis and Follow-up

- Chronic myelomonocytic leukemia (CMML-1)
- One month later blasts were 9%
- Due to cytogenetics this patient was at risk of rapid progression to AML
- Induction with FLAG-IDA
- Consolidation with 2 cycles of intensification protocol Dana-Farber
- Doing well after BMT with 10/10 matched unrelated donor

Reference

**Case 2**

- 52 year old male presented with pancytopenia, fatigue, bleeding gums
- Hb 107 g/L, MCV 99,
- WBC 1.5x10^9/L, ANC 0.3x10^9/L, Plt 72x10^9/L
- Smears 6-10% blasts in various areas
- Erythropoiesis 48%
- Dysplasia

**Screening tube on lysed sample**

**Screening tube: Ogata score**

**Flow cytometry on lysed BM sample**

**Flow cytometry on non-lysed sample**

**Aberrant pattern of erythroid maturation**

Erythropoiesis: 23%
Early: 62%
CD117+ blasts: 7%
erythroid: 8.6%
Case ctd

IHC

CD34 and CD117 counted approx. 11-13%
P53 overexpressed

Cytogenetics/Molecular

- 41-44, X, -Y, -4, -5, der(7)(t;7;5;p175;q31), der(17)(t;7;17)(q31;p11), -18, der(20)(t;5;20)(q31;p11), -22, +1-4mar [cp21]/46XY[4]
- Illumina TruSight S4 genes
- TP53 c.747G>T Tier 1 57%

Azacytidine treatment started but progressed to AML within 3 months

Summary of recommendations for FCM in Myelodysplastic syndromes

- For FCM application for MDS diagnostics
  - Follow International MDS Flow methodological recommendations
  - For screening purposes
    - Follow a mini-panel based on the so-called Ogata score
  - For extended analysis: perform FCM in all cell compartments following ELN recommended antigen combinations
    - Myeloid and lymphoid progenitor cells
    - Maturing myelomonocytic cells
    - Immature and mature erythroid cells
  - Generate integrated flow score (A;B;C)
- Integrate flow cytometry findings in the bone marrow report (morphology, cytogenetics, flow cytometry, molecular methods)

Publications

References


6. Poster 031, Violidaki et al. ESCCA 2017