The spectrum of flow cytometry of the bone marrow

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Disclosure of speaker’s interests

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• (Potential) conflict of interest

• None
FLOW CYTOMETRY

is a procedure used as a part of integrated hematopathological diagnostics that includes:

• blood and bone marrow smear cytology,
• bone marrow biopsy morphology
• immunophenotyping by flow cytometry and immunohistochemistry
• cytogenetics
• and molecular genetics
Immunofluorescence

Direct

Indirect

Cell

Primary Antibody
Secondary Antibody
Fluorophore

Antigen
Data analysis

Display Plots → Create Gates → Display Statistics → Analyze Statistics

<table>
<thead>
<tr>
<th>Plot Types:</th>
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<tbody>
<tr>
<td>Histogram</td>
<td>Polygon</td>
<td># of Events</td>
<td>% positive for</td>
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<tr>
<td>Dot</td>
<td>Ellipse</td>
<td>% of Gated</td>
<td>particular markers:</td>
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<tr>
<td>Contour</td>
<td>Histogram</td>
<td>% of Parent</td>
<td>viable cells</td>
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<tr>
<td>Density</td>
<td>Quadrant</td>
<td>% of Total mean</td>
<td>immunophenotype</td>
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<tr>
<td></td>
<td></td>
<td>geometric mean</td>
<td>mean fluorescence intensity</td>
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<td></td>
<td></td>
<td>standard deviation</td>
<td>DNA content</td>
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<td></td>
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<td>absolute counts</td>
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Normal Bone Marrow
Four- and Five-Color Flow Cytometry Analysis of Leukocyte Differentiation Pathways in Normal Bone Marrow: A Reference Document Based on a Systematic Approach by the GTLLF and GEIL

Christine Arnolet, Marie C. Béné, Françoise Durrieu, Jean Feuillard, Chantal Fossat, Bernard Husson, Hélène Jouault, Marc Maynadié, and Francis Lacombe

http://www.leukemia-net.org/content/diagnostics/diagnostics/flow_cytometry_atlas/index_eng.html
NORMAL BM SAMPLE
(B-CELL ANALYSIS)
MANTLE ZONE  CD19, CD20, CD22, CD79, slg, FMC7, bcl-2, CD23+ (CD5, CD10, CD43, bcl-6 neg)

NODAL MALT

CLL  CD19, CD20, CD43, CD23, slg+/-, CD5 (CD79 CD10, CD22, FMC7 neg)

HCL  CD19, CD20, CD22, slg++, CD11c, CD25, CD103, CD123 (CD5, CD10, CD23 neg)

BURKITT  CD19, CD20, CD22, CD79, slg, CD10, DR, bcl-6 (CD5, CD23, Tdt neg)

DIFFUSE LARGE B CELL LYMPHOMAS, slg+, cIg+, CD19, CD20, CD22, CD79, CD22++, CD79, CD10+ (CD5 rare cases, bcl-2, bcl-6, MUM-1 can be useful for further classification)

FOLLICULAR  CD19, CD20, CD22, slg+, CD79, CD10, bcl-2 (CD5, CD23, CD43 neg)

MANTLE ZONE  CD19, CD20, CD22, CD79, FMC7, slg, bcl-1, CD5, CD43, (CD10, CD23 neg)

T LYMPHOMAS AND ANGIOIMMUNOBLASTIC  CD5+/CD10+/CD3+/CD4+

SEZARY  CD3, CD2, CD5, CD4, CD7+/- (CD8 neg)

LARGE CELLS ANAPLASTIC  CD2, CD4, CD3+, CD30

PERIPHERAL  CD2, CD3, CD5, CD4

ANGIOCENTRIC  CD2, CD5, CD4 ou CD8, CD56

INTESTINAL  CD3, CD7, CD103

ATLL  CD3, CD4, CD25, CD45RO, CD7neg

INTESTINAL OR LUNG MALT

Follicular, mantle zone, marginal zone

SLVL

circulating marginal zone cells

WALDENSTROM  CD19, CD20+, CD22, CD79 slg+, cIg, FMC7 (CD5, CD10, CD23 neg)

MYELOMA  cIg, CD56, CD45+, CD38, CD138 (CD19, CD20, CD22 neg)
Chronic lymphocytic leukemia

MRD detection 0.1%
Hairy cell leukemia
Mb Waldenström

Follicular lymphoma
B-cell maturation in the bone marrow
B-ALL
Plasma cells: CD138++/CD38++

Myeloma

- Cyt. KAPPA FITC
- Cyt. LAMBDA PE
- CD56 ECD
- CD138 PC5.5
- CD34 PC7
- CD117 APC
- CD19 A700
- CD38 A750
- CD20 PB
- CD45 KO

MRD: CD27, CD81
Analysis of T-Cells

Normal bone marrow
T-ALL tube: CD7, CD1a, CD8, CD3, CD34, CD2, CD10, CD4, CD5, CD45

Normal Bone Marrow
CD7+ cells

T-ALL
CD7++ CD34+ CD3-CD4-CD8-
Angioimmunoblastic T-cell lymphoma
NK cell and Large granular lymphocyte-related phenotypes

Normal Bone marrow
Case 46 BM Workshop EAHP Lisbon 2012: LGL leukemia

Abnormal Population:
- CD2+/CD3+/CD5-/dim/CD7+dim/CD4-/CD8-
- TCR A/B-/TCR D/G+
- CD16+/CD57+/CD56-/CD94-
What is in the “blast region” CD45 dim?

Maturation of granulopoiesis
Classic acute promyelocytic leukemia (PML-RARα)

Leukemic promyelocytes are in the granulocyte region and are positive for CD117 but do not express neutrophil markers CD16, CD11b, CD10, CD15

Low CD38
HLA-DR neg
Acute promyelocytic leukemia hypogranular variant

“monocytic” scatter, subset CD34+ cells, expression of CD2+
AML with t(8;21) 
(RUNX1-RUNX1T1)
AML with inv.16 (CBFB-MYH11)
AML with monocytic differentiation

- Often CD56, CD4
- CD34 negative
- CD14 variable
- HLA-DR variable
- MPO can be positive or negative
Blastic plasmacytoid dendritic cell neoplasm:
CD123+, CD56+, CD33+, CD36+, CD4+, DR+, CD38
Changes in various blood and bone marrow cell compartments detected by FCM in MDS patients

Blasts
- Abnormal expression of CD34, CD117
- Abnormal expression of HLA-DR, CD38
- Abnormal expression of lymphoid markers
- Abnormal expression of myeloid maturation markers

Granulopoiesis
- Abnormal scatter
- Abnormal maturation patterns
- Abnormal expression of lymphoid markers
- Abnormal expression of progenitor markers

Monocytes

Erythropoiesis
- Abnormal expression of CD36 and CD71
- Abnormal maturation patterns

Megakaryocytes and platelets
- Abnormal expression of CD41, CD42c, and CD42b, CD61
- Abnormal response to platelet activation

Decreased B-cell progenitors in BM
Increased T-reg in blood

Dendritic cells
- Both plasmacytoid and myeloid subsets markedly decreased
4-parameter screening panel (Ogata score) consists of:
1. % CD34+ myeloid progenitor cells among all nucleated cells (<2%)
2. % CD34+ B cell precursors among all CD34+ cells (>5%)
3. SSC 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;94:1066-74;
Della Porta MG, et al., Haematologica 2012;97:1209-17
Rajab & Porwit, Clin Cytometry, 2015 Feb.9
Bardet et al. Haematologica, 2015 Apr;100(4):472-8
Examples of aberrant findings in Blast/progenitor region

Normal

[Blasts] CD7 APC700 / CD34 PC7

Aberrant

[CD34] HL-DR PB / CD38 APC750

[AQ : 0.36%]

[Blasts] CD33 PC5.5 / CD34 PC7

[AM : 3.56%]

[Blasts] CD7 APC700 / CD34 PC7

CA : 0.76%

AR : 0.04%

CD34 PC7

CD7 APC700

[CD38] HL-DR PB / CD38 APC750

AY : 0.54%

Al : 0.42%

AX : 0.01%

CD34 PC7

HL-DR PB

[Blasts] CD33 PC5.5 / CD34 PC7

[AQ : 16.00%]

[AN : 0.00%]

[AM : 0.18%]

CD33 PC5.5

CD34 PC7
Abnormal findings in neutrophils

Normal

Aberrant
Advantages of FCM

• FCM is rapid, sensitive, gives quantitative results and allows several antigens to be assessed simultaneously.
• Various subpopulations of cells can be analyzed separately with high sensitivity.
• Small abnormal cell populations can be detected in a reactive background.
• Allows detection of antigen expression on the cell surface, which is of importance when planning antibody-based therapy such as Rituximab (CD20) or Campath (CD52), as the antigens have to be expressed on cell-surface for effective therapy.
Disadvantages of FCM

• It may be difficult to assess, which cells on cytologic/histologic preparations correspond to different populations detected by FCM

• Inadequate sampling, fibrosis, and necrosis may render non representative samples.

• If neoplastic cells are fragile as in many high-grade NHL, they may be destroyed during preparation for FCM analysis.
Questions?