

Leukemia diagnostics with flow cytometry

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Plan de l'exposé

Hematopoiesis and leukemias

Flow cytometry

Leukemias classification and Medical diagnosis

Mathematical Models for diagnosis and classification
of acute myeloid leukemia

Hematopoiesis and leukemias

Description

Antigen expression

Cluster of Differentiation

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- ▶ **Hematopoiesis** : development of blood cells,
which occurs in marrow and lymphatic tissues.

Immature cells (called **blasts**) ➡ functional **mature**
cells

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Acute lymphoblastic leukemia, **ALL** : lymphoid cells

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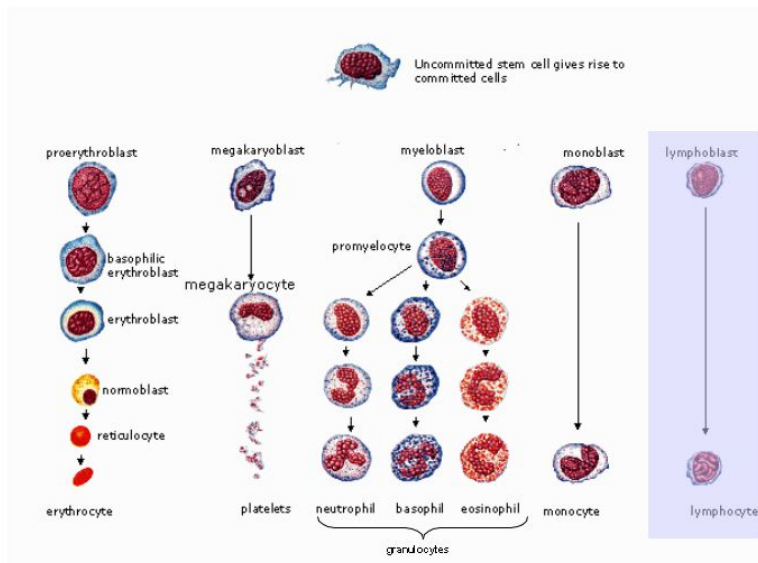
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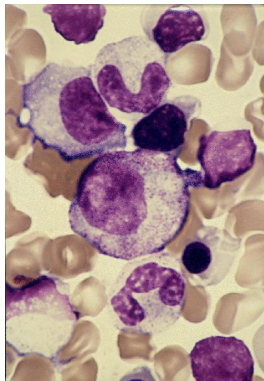
Stages of Hematopoiesis



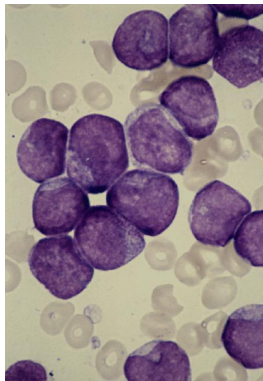
Normal and leukemic marrow

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Normal marrow



Leukemic cells

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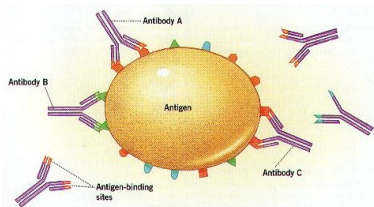
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Antigen expression

- ▶ During the process of differentiation and maturation, some genes are turned on while others are disabled
- ▶ The use of multiple cellular markers simultaneously allows to distinguish one type of cell as distinctly different from another



Antigen-antibody reaction

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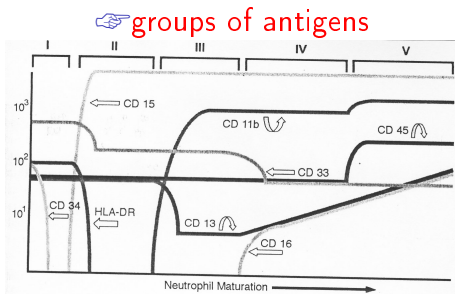
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Cluster of Differentiation

Cluster of Differentiation (CD) molecules are markers on the cell surface, as recognized by specific sets of antibodies, used to identify the cell type, stage of differentiation and activity state of a cell



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Principle

Flow cytometry is a technique for **analyzing large populations of single cells.**

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Flow cytometry is a technique for **analyzing large populations of single cells**.

1. Cells are labeled with a **fluorescent dye** which is coupled to a monoclonal antibody and binds to those cells coated with the antigen for which the antibody is specific.

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👉 Flow cytometers can be considered to be **specialised fluorescence microscopes**.

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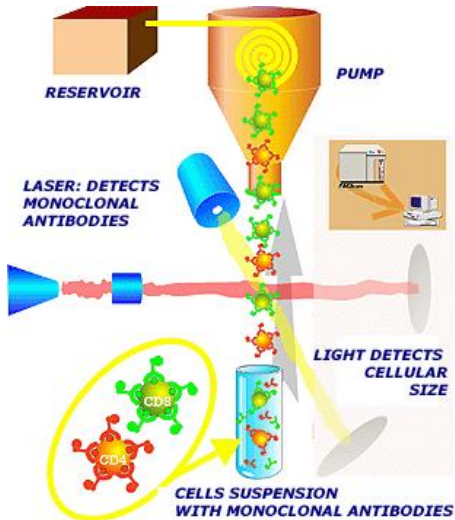
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Each particle is characterized by different variables

- ▶ **FCS** : Forward Angle Light Scatter related to the cell size

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- ▶ **SSC** : Side Scatter related to the cell granularity and complexity

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☞ For each biological sample, flow cytometer produce a file (FCS normalized format) which contain **6 variables** per particle.

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Leukemias classification

Accurate classification of AML at diagnostic is **critically important** to the management of patient :

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👉 **FAB classification** (French American British) :
correlation with morphologic classification

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- ☞ **FAB classification** (French American British) : correlation with morphologic classification
- ☞ **WHO classification** : the main difference with the FAB classification is that this new classification take into account cytogenetic abnormalities

FAB AML classification

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Table 1: Antigens frequently analyzed in the immunophenotyping of hematopoietic malignancies

Antigens with broad expression	
Panmyeloid antigens	CD13, CD33, CDw65, MPO
Pan-B-cell antigens	cyCD22, CD19, cyCD79a
Pan-T-cell antigens	cyCD3, CD2, CD7, CD5
Antigens associated with immaturity or activation	
Immaturity	TdT, CD34, HLA-DR ^a
Antigens with lineage specific and maturation dependent expression	
Myeloid cells	CD14, CD15, glycophorin A, CD41, CD61
B-cells	CD20, CD23, FMC7, cylgμ, slg
T-cells	CD1a, CD4, CD8
NK cells ^b	CD16, CD56, CD57

^aconstitutive expression on monocytes and B-cells, ^bin absence of CD3
cy cytoplasmic, slg surface membrane immunoglobulins

FAB AML classification

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Table 7: Immunological characteristics in acute myeloid leukemia

Antigen	FAB classification						
	M0	M2 t(8;21)	M3 t(15;17)	M4Eo Inv16	M5	M5 t(9;11)	M7
MPO	+/-	+	+	+	-/+	-	-
CD2	-			+/-			
CD13	+/-	+	+	+	+/-	-	+/-
CD14	-	-	-	+/-	+/-	-	-
CD15	-	+/-	-/+	+/-		+	-
CD19	-	+/-					
CD33	+/-	+/-	+	+	+	+	+/-
CD34	+/-	+/-	-	-/+			
CD56		+/-					
CD61	-	-	-	-	-	-	+
CD64	-	-	+/-	+	+	+	
CDw65	-/+	+/-	-/+	+	+/-	+	+/-
CD117	+/-	+/-	-/+	+/-	-/+		
HLA-DR	+/-	+	-	+	+	+	+/-

-: Antigen not expressed, -/+ antigen expressed in less than 50 % of patients, +/- antigen expressed in majority of patients, +: antigen expressed, open fields represent partial expression without specificity for diagnosis or lack of reliable data

Medical diagnosis

Differents analysis on biological sample of peripheral blood or marrow to obtain a valid diagnosis :

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Differents analysis on biological sample of peripheral blood or marrow to obtain a valid diagnosis :

- ▶ Morphologic
- ▶ Cytochemic
- ▶ Flow cytometry
- ▶ Cytogenetic

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Differents analysis on biological sample of peripheral blood or marrow to obtain a valid diagnosis :

- ▶ Morphologic
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For **Flow cytometry**, the diagnostician has to analyze multiple bivariate dot plots from the cytometer.

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Manual analysis of cytometrical datas : control sample

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Used of CD45 which characterize **the maturation of cells** to evaluate the **particular autofluorescence** of the sample

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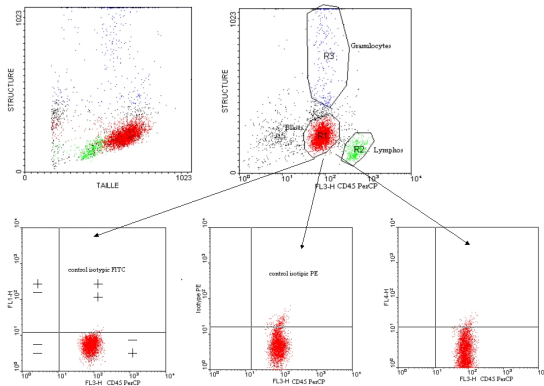
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Manual analysis of cytometrical datas : antigens expression

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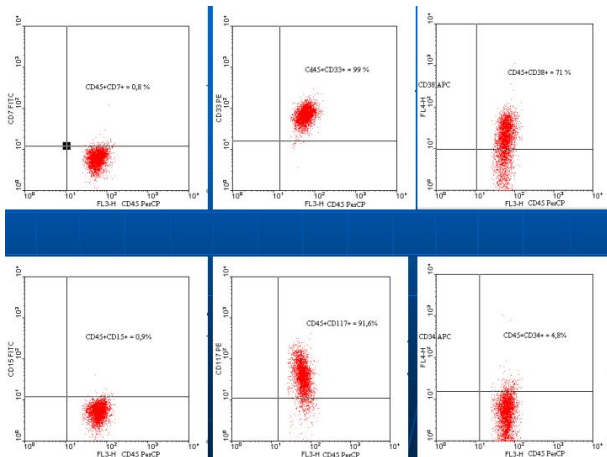
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Goals of this work

1. Diagnosis help and statistical approach

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1. Diagnosis help and statistical approach

Leukemia diagnostics
with flow cytometry

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Hematopoiesis and
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Antigen expression
Cluster of
Differentiation

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Flow cytometer data

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classification and
Medical diagnosis

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- ▶ Find a **methodology** to help the clinician to analyze the cytometer datas more systematically

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- ▶ COmparison between this result and the exiting and emergent classifications.

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Data treatment

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1. Data conversion :

- ▶ Conversion of FCS to ASCII format file
- ▶ Conversion of fluorescence data in logarithm scale

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3. **Determination of all CD positive rate** for the blasts sub-population

Statistical analysis

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1. Principal Component Analysis

- ▶ show that the variables are not correlated
- ▶ allow the detection of singular data

1. Principal Component Analysis

2. Ascendant Hierarchical Classification

- ▶ try to group patient in terms of similarity
- ▶ First results give a encouraging dendrogramm where we can detect some groups form the FAB classification

