FLOW CYTOMETRIC ANALYSIS OF ACUTE LEUKEMIA

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Leukemic cell characteristics

– Immature
– Proliferative
– Self renewal
– Maturation arrest

Morphologically, acute leukemia is defined as:

> 20% blasts in blood or bone marrow

Diagnostic methods
Microscopic examination
Cytochemistry
Flow cytometry
Cytogenetic
FISH
Molecular genetics

Leukemia

Morphologic “blasts” include:

• Stem cells and progenitors (CD34+)
• Promyelocytes
• Promonocytes
• Immature B cell precursors
• Immature T cell precursors

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Acute leukemias reflect normal hematopoietic differentiation, but always demonstrate abnormal antigen expression. Flow cytometry is mainly used for diagnosis, classification and detection of leukemic cells.

Cytogenetics and molecular genetics are the most important for identifying entities with distinct prognoses and clinical behavior.

Currently, every acute leukemia is analyzed by flow cytometry and cytogenetics for appropriate diagnosis, classification and therapy.

WHO classification of AML

- AML with recurrent genetic abnormalities
  - t(8;21)(q22;q22) (AML1/ETO)
  - inv(16)(p13q22) (CBFβ/MYH11)
  - t(15;17)(q22;q12) (PML/RARα) and variants
  - 11q23 (MLL)

- AML associated with dysplasia and therapy
- AML not otherwise categorized
  - Classified according to differentiation (M0-M7)
- Other
  - Biphenotypic, bilinear

Useful antigens in acute leukemia

- B-cell
  - CD45, CD34, CD19, CD20, CD22, slg, CD10, TdT, HLADR

- T-cell
  - CD3, CD2, CD5, CD7, CD4, CD8, CD1a, TdT, CD45, CD34, HLADR

- AML
  - CD45, CD34, CD13, CD33, CD15, MPO, CD117, HLADR, CD14, CD64, CD11b, CD71, glycoporphin A, CD41/61

Immunophenotypic classification of acute leukemia

- Precursor B-cell ALL
- Mature B-cell ALL
- Precursor T-cell ALL
- AML
  - With granulocytic differentiation
    - Promyelocytic
  - With monocytic differentiation
  - With erythroid or megakaryocytic differentiation
  - Acute Leukemias of ambiguous lineage
Acute leukemias of lymphoid precursors (lymphoblastic)

Acute lymphoblastic leukemia

Precursor B lymphoblastic leukemia

Precursor T lymphoblastic leukemia

Precursor B lymphoblastic leukemia

Precursor T lymphoblastic leukemia
Acute leukemias of non-lymphoid precursors (AML)

Acute myeloblastic leukemia (M0-M1)

Acute promyelocytic leukemia (M3)

Acute myeloblastic leukemia (M2)

Acute promyelocytic leukemia (M1)
Acute monocytic leukemia (M4-M5)

Non specific esterase

Erythroleukemia (M6)

- 2 types

  1. With increased myeloblasts and associated erythroid hyperplasia (50% or more erythroid precursors)
     - No erythroid antigen expression on the myeloblasts
  2. Markedly increased abnormal erythroid precursors
Megakaryoblastic Leukemia

Biphenotypic Leukemia
Acute leukemia whose blasts have both myeloid and lymphoid immunophenotypic features

Minimal residual disease (MRD)
- Assess adequacy of response
- Predict relapse
- Guide therapy
  - More intensive therapy
  - Change in therapy

Relative sensitivities of MRD detection methods
- Morphology: $10^0$
- Cytogenetics: $10^0$
- FISH: $10^0$
- Southern blot: $10^6$
- Flow cytometry: $10^0$
- Polymerase chain reaction
  - Antigen receptor rearrangement: $10^{-3}$
  - Translocations: $10^{-6}$

Biphenotypic acute leukemia
Myeloid and T cell lineages
Advantages of flow cytometry for MRD detection

- Universal
- Sensitive
- Only viable cells evaluated
- Rapid

Detection of MRD in acute leukemia

- Patients in clinical remission may have residual malignant cells.
- Studies in large series of patients showed a good correlation between MRD levels and treatment outcome.
- Sensitive techniques to detect MRD may estimate better the leukemia burden and help therapeutic strategies.
- Flow cytometry and PCR are the most promising methods for detecting submicroscopic levels of leukemia.
- Flow cytometric detection of MRD is based on the identification of antigens expressed on leukemic cells but not on normal hematopoietic cells.
- Flow cytometry can be applied to the majority of patients with acute leukemia and can detect 1/10000 leukemic cells among normal bone marrow cells.

Detection of MRD in acute leukemia

- Flow cytometry is capable of minimal residual disease detection
  - Rapid
  - Cost effective
  - Quantitative
  - Applicable to wide range of hematopoietic neoplasms
- Requires
  - Consistent flow cytometric technique
  - Knowing normal patterns of antigen expression
  - Knowing common abnormal patterns

- Blast counts by flow cytometry may not agree with those obtained by microscopy
  - Specimen processing - Red blood cell lysing
  - Variable degree of peripheral blood dilution
  - Not a problem for peripheral blood

Stability of leukemia-associated immunophenotypes

There may be changes at relapse but new abnormalities are usually detected