ACCURATE DIAGNOSIS OF SOME DIFFICULT CASES OF ACUTE LEUKEMIA. CYTOCHEMISTRY AND IMMUNOPHENOTYPE AS IMPORTANT METHODS IN THIS DIAGNOSIS

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Abstract

Acute leukemias are divided into myeloid (or nonlymphocytic) and lymphoid according to the predominant neoplastic cell line. According to morphology, cytochemical staining and flow cytometry immunophenotyping they are classified according to FAB and WHO classification. In this study we represent some difficult cases of acute leukemia diagnosed on the basis of the interpretation of three methods, morphology with Giemsa romanowsky smear, cytochemistry with citochemical reactions and immunophenotyping by flow cytometry. None of this methods alone can give an accurate diagnosis of the type of leukosis, especially in difficult cases not readily determinable which exhibit nonspecific and aberrant morphological and immunophenotypic features. With morphology, immunophenotyping, and cytochemical tests together, the lineage of differentiation (ALL or AML) can be reproducibly identified in more than 95% of acute leukemias. ALL can be further subclassified based on T versus B precursor differentiation and ALL1-ALL3 according to FAB. AML can be further subclassified in MO-M7 classes according to FAB.

Background

Leukemia is a neoplastic disease characterized by the uncontrolled proliferation of hemopoetic cells. This proliferation is followed by the predominance in the bone marrow and in the peripheral blood of immmature lymphoid or myeloid cells (bllasts).

Acute leukemias are classificated according to FAB (French-American-British cooperative group) by finding bllasts in bone marrow e"30% of cells (2).

In 2001, a group convened by the World Health Organization (WHO) published a new classification of hematopoietic and lymphoid neoplasms, including the acute leukemias. The directive for the classification was that it be up to date, incorporate genetic and immunophenotypic as well as morphologic information, and that it also be usable throughout the world, not just in Western academic medical centers. The AML classification includes four groups: AML with recurrent cytogenetic abnormalities, AML with multilineage dysplasia, therapy-related AML and MDS, and AML not otherwise specified, the latter being a modification of the FAB AML classification. The WHO classification does not group the acute lymphoid leukemias together, but separates them under three broader categories of lymphoid disease: precursor B-cell and precursor T-cell neoplasms, and mature B-cell neoplasms (7,8).

The WHO classification uses >20% blasts in the marrow or peripheral blood as a diagnostic criterion (8).

Classification FAB

Acute leukemias are divided into myeloid (or nonlymphocytic) and lymphoid according to the predominant neoplastic cell line (2).

According to morphology, cytochemical staining and flow cytometry immuno phenotyping they are also subclassification by FAB (3). 70-80% of cases can be classified only according to morphology. In 10-15% of cases it is necessary to use even cytochemia for correct classification. In about 10% of the cases (undifferentiated leukemia) accurate diagnosis is achieved by immunophenotypic and cytogenetic methods. Biological molecular methods (PCR, FISH) have shown that all types of leukemias are characterized by specific molecular disorders (3,5,6).

1. Acute lymphoblastic leukemia

FAb classification	Morphology
L1	monomorphic small blasts
L2	heterogeneous large blasts
L3	Burkit like cell

2. Acute non lymphoblastic (myeloblastic) leukemi

FAB	Morphology
M0	minimally differentiated
M1	without maturation
M2	with maturation
M3	promyelocytic
M4	myelomonocytic
M5	a.monoblastic; b.monocytic
M6	erythroleukemia
M7	megakaryoblastic

3. Mixed leukemias
Aim
Morphology, cytochemical tests and immunophenotyping, can be applied for the lineage differentiation of leukemia klone cell.

Precisely this was the purpose of this publication, to present some cases of acute leukemia diagnosed on the basis of the interpretation of the above three methods applied in the context of FAB and WHO criteria.

Methods
Morphology
In our everyday work we use examination of Giemsa-Romanowski stained smears from pheripheral blood and bone morrow, to evaluate morphologic alterations of cells. Cytologic features: cell dimentions, nuclear chromatin, nuclear shape, nucleoli, cytoplasmic size, cytoplasmic basophilia, cytoplasmic granularity, and vacuoli, ect, are observed. Experienced morphologists can accurately classify 70 to 80% of acute leukemia (as ALL or AML) on Romanovsky-stained smears (5,1).

Cytochemistry
Cytochemical stains may be used to demonstrate myeloid differentiation in leukemic blasts (6). Useful stains are myeloperoxidase (MPO), Sudan black B (SBB), and nonspecific esterase (NSE). The MPO reaction is positive in cells of the granulocytic series and may be weakly positive in monocytes. Lymphocytes and erythroid precursors are negative. MPO is useful for distinguishing AML from ALL. A threshold of 3% positivity is frequently used to separate these two forms of leukemia (1,3).

NSE (non specific esterase) activity is found in monocytes. Several substrates are available, but the most commonly used are alpha naphthyl butyrate (ANB) and alpha naphthyl acetate (ANA). This is generally unnecessary with the ANB stain, but the ANA stain also reacts with megakaryoblasts with a coarse granular pattern. In megakaryoblasts, the NSE activity is partially resistant to NaF inhibition.

Megakaryoblasts are negative with ANB staining. These features may be used to identify megakaryoblasts, although if possible this should be corroborated with immuno phenotyping.

The substrate for SE (specific esterase) staining is naphthol AS-D chloroacetate. SE is present in neutrophils and their precursors, but may also be present in monoblasts and monocytes. In the neutrophil series, it is only found in specific granules; hence, the reaction is typically negative in poorly differentiated AML. SE has been used to demonstrate myeloid precursors.

PAS reacts primarily with glycogen. ALL lymphoblasts often have prominent PAS staining in the form of coarse granules or blocks. Maturing neutrophils are diffusely PAS positive. Erythroid precursors are

normally PAS negative. A coarsely granular staining pattern may be seen in pronormoblasts and basophilic normoblasts in myelodysplastic syndrome (MDS)-related AML. Other enzyme stains, such as acid phosphatase, may be useful in diagnosis of specifik acute leukemia.

Cytochemical reactions
Mieloperoxidase reaction (Graham-Knoll method)

PAS reaction (periodic acid of Schiff)
Chloroacetate esterase reaction
á-naphthyl actetate esterase reaction
Naphthol AS acetate esterase reaction
Perls reaction
Alkaline leukocyte phosphatase reaction
Acid phosphatase reaction
Immunocytochemical reactions (metoda APAAP method)
Ect (5)

Cytochemical reactions		FAB(3)
MPO		
	-	M0, M7, L1, L2, L3
	+	M1, M2, M3, M4
Chloroacetate esterase (CAE)	+	M2, M3, M4
Nonspecific esterases (NSE)	+	M5, M4
Acid phosphatase	+	M6, M5
PAS	+	M6, M5 (±), L1, L2
Perls	+	MDS (RARS)

Immunophenotyping by Flow Cytometry.

Immunophenotyping greatly facilitates diagnosis of the acute leukemias (6). The antibodies used recognize surface and cytoplasmic antigens expressed by human hematopoietic cells and their leukemic counterparts. Most of the antigens are lineage associated, rather than lineage specific, but expression of groups of antigens is accurate in assignment of lineage.

In difficult cases, a limited number of cytoplasmic antigens are currently considered lineage specific [cytoplasmic CD22 (cCD22) or cCD79a for B differentiation, cCD3 for T, cCD41 or cCD61 for megakaryoblastic, and cMPO for myeloid] (1,3,6).

The principle of this method is recording surface markers and/or cytoplasmic specific cell lines or cell differentiation stages through monoclonal antibodies (McAB). An antigen or cell differentiation molecular structure was called CD (cluster of differentiation), followed by a serial number example: CD3. For the same CD can be used different McAB (6,5).

Flow cytometry or citofluorometry (CFM).

The principle of this cytometric method is the digitalisation of signals emitted during the contact to each cell with an excited light (laser source) associated with: physical characteristics of the cell and fluorochrome presence on McAB, which relate to Ag differentiating this cell.

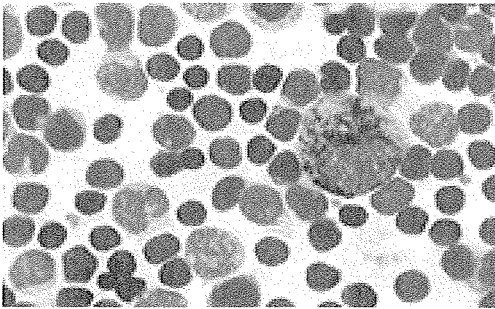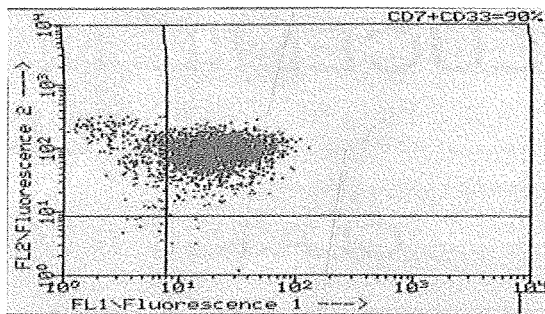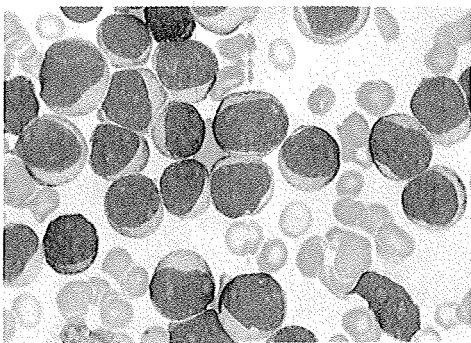| FAB (2,3)	CD
M0	CD34,CD13,CD33,HLA-DR,anti-MPO
M1, M2	CD11,CD13,CD33,HLA-DR
M3	CD13,CD33,CD2,CD34
M4, M5	CD11, CD13,CD33,CD14,HLA-DR
M6	glycophorin
M7	CD41,CD42a,CD42b,CD61

Case 1

LAM0 Lymphoid blast type

LAM0 Cytochemistry with peroxidase
Positive for only one large central element different from other small blasts cells
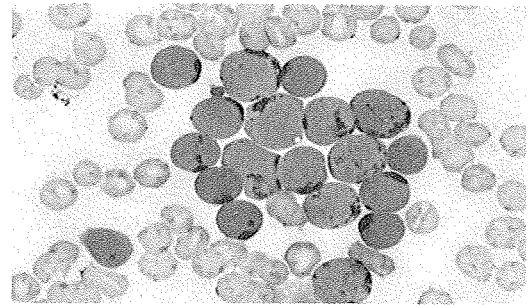

Combined expression of CD7 and CD33 in bone marrow bllasts
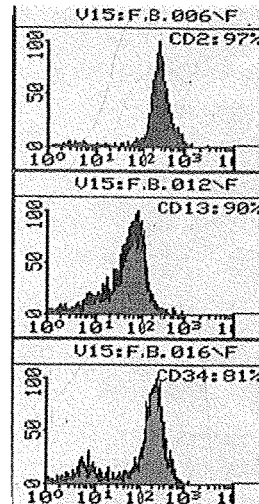
Case 2

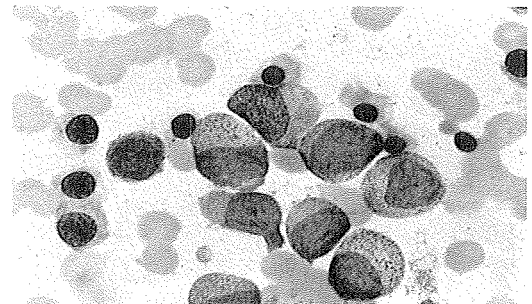LA M1 Typical myeloid blast with less cytoplasm and without granules

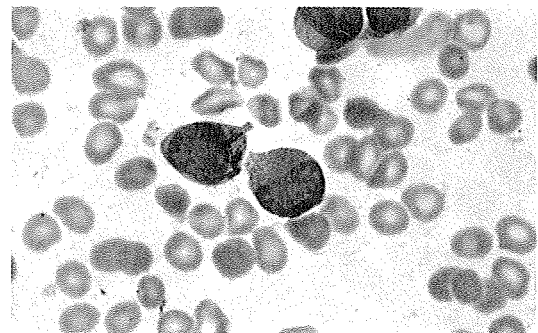LAM1, Cytochemistry, positive peroxidase activity in all blasts

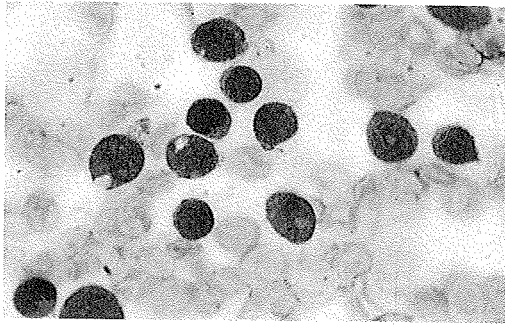Leukemic cells express CD13, CD34 but also CD2. The diagnosis will be determined by the positivity over 3% of cells per peroxidase

Case 3

LA M2 bone marrow blasts with protoplasm and azurophilic granules

LAM2 Cytochemistry in periphery One blast is positive for peroxidase, the other cell with peroxidase granules is negative (leukemic cell)
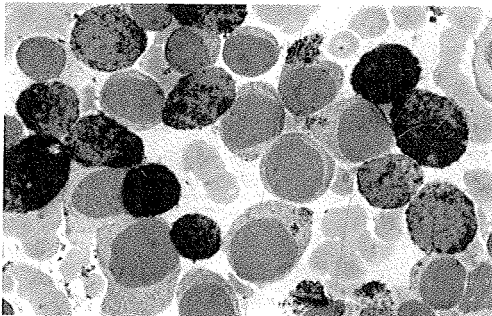
LAM2 positive Pas reaction in bone marrow blasts. Is rare in acute leukemia
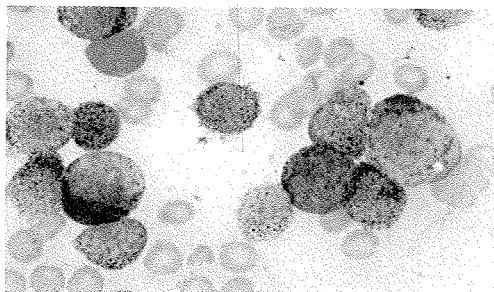
Case 4

LAM4. Some element have proto plasm with azurophilic granules, probable myeloid line
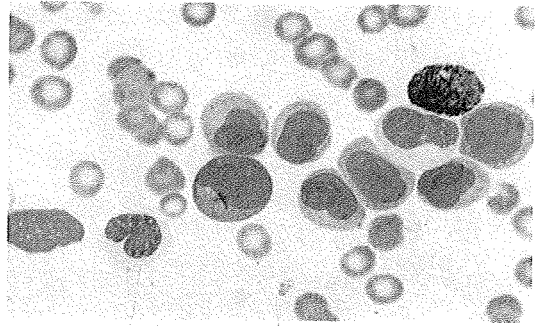

LAM4. Peroxidase positive in blastic elements of granular line. Weak or completely lacking peroxidase in monoblasts
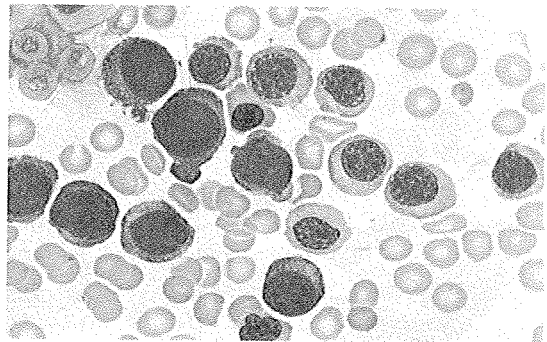

LAM4. Blasts positive for CAE, monocytic blasts positive for blu non specific esterase.
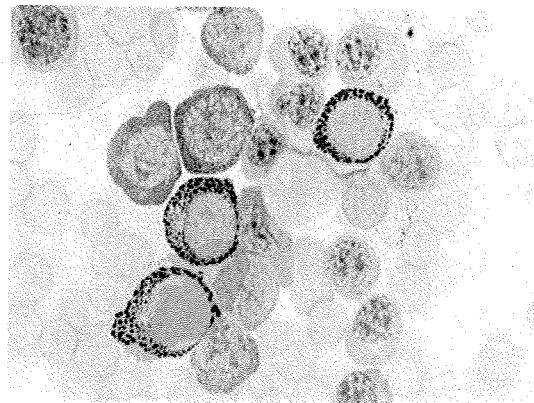
Case 5

LAM5 B.citochemistry shows only one blast with Auer Rods peroxidase positive, and two granulocyte one positive and the other neg for peroxidase
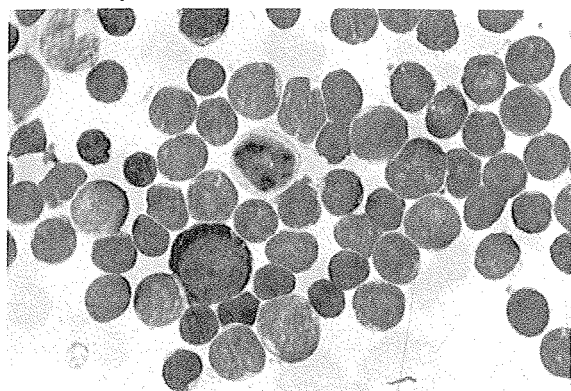
Case 6

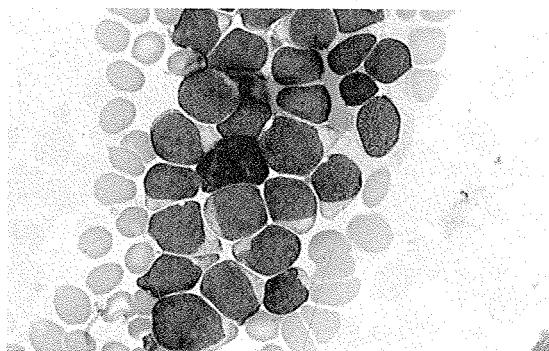LAM6. Bone marrow morphology. Erythroid leukemic elements in different stages

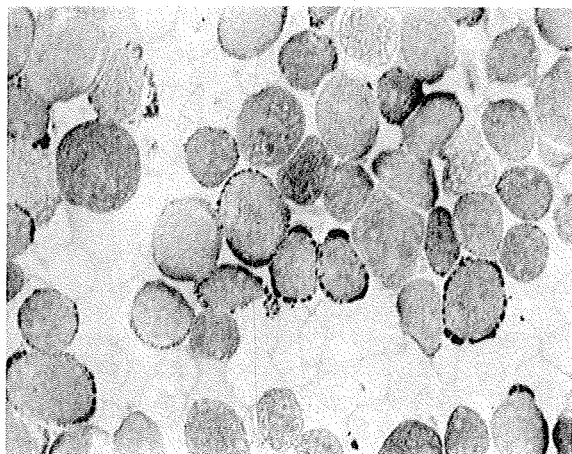LAM6. Positive PAS in almost all erythroid elements

Case 7

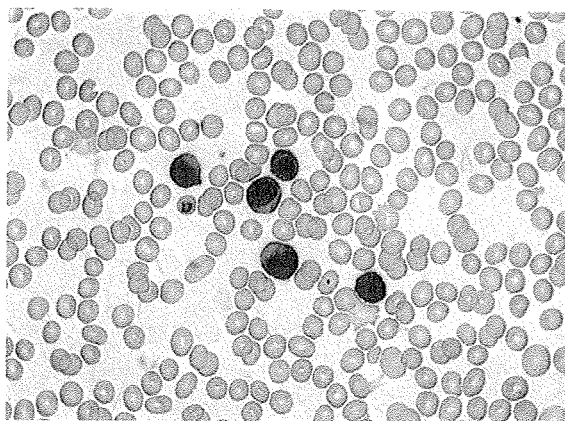LAL1.bone marrow morphology, elements are small with less cytoplasm and no nucleolus

Peripheral blood of the same case. Expressing positivity for CD10 (immunohyistochemistry with APAAT)

All the blasts are negative for PAS. In the centre a PAS positive, eosinophyl

Conclusion

With morphology, cytochemical tests and immunophenotyping, the lineage of differentiation (ALL or AML) can be reproducibly identified in more than 95% of acute leukemias. ALL can be further subclassified based on T versus B precursor differentiation and ALL1-ALL3 according to FAB. AML can be further subclassified in MO-M7 classes according to FAB.

None of the above methods on its own can give an accurate diagnosis of the type of leukosis, especially in difficult cases not readily determinable which exhibit nonspecific and aberrant morphological and immunophenotypic features.

PAS positive in some blasts, with peripheral placement like crown (typical for LAL)

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