

Current Practice of Cytology in the Diagnosis of Acute Leukemias in Madagascar

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Abstract

Introduction - Acute leukemias are clone proliferation of cells blocked at a stage of maturation. These pathologies are frequent in Western countries with an incidence of 2.5 to 3.5 / 100 000 inhabitants/year. Their frequency is about 25.5% in Madagascar. In Madagascar, diagnosis and classification of acute leukemias is cytological because our laboratories haven't immunophenotyping, molecular biology and karyotype. We share through this study our daily practice in front of acute leukemia.

Method - We conducted a prospective descriptive study of cases of acute leukemia diagnosed between January and April 2017 at hematological laboratory of Joseph Ravoahangy Andrianavalona Antananarivo hospital (Madagascar)

Results - During this period, 16 cases of acute leukemia were diagnosed in our unit. Blood and bone marrow smear cytological study revealed 6 cases (37.5%) of ALL2, 4 cases (25.0%) of AML2, 2 cases (12.5%) of AML3, 2 cases (12.5%) of AML4, 1cas (6.25%) of AML5 and 1 case (6.25%) of ALL1.

Conclusion - Cytology is crucial in malignant hemopathies, particularly in low resource countries, although the need for molecular biology and immunophenotyping is well established.

Keywords - Acute Leukemia, Clone Cells Proliferation, Cytology, Diagnosis, Classification.

I. INTRODUCTION

Acute leukemia (AL) is heterogeneous group of malignant hematological malignancies characterized by clonal and uncontrolled proliferation of hematopoietic precursors blocked in their differentiation(1). Acute leukemias account for between 10% and 15% of hematological malignancies(2). Their frequency is about 25.5% in Madagascar (3). Although immunophenotyping, molecular biology and cytogenetics are currently essential, these new techniques are a complement to the morphological examination of cells under the microscope remains unavoidable. Through the cases diagnosed between January and April 2017 in our unit, we performed a cytological description of acute leukemias.

II. METHOD

We performed a descriptive prospective study of myelograms done in hematological laboratory of Joseph Ravoahangy Andrianavalona Antananarivo hospital (Madagascar) between January and April 2017. Cytological and cytochemical study was carried out on blood and bone marrow samples.

Patients with blood and / or bone marrow blastosis greater than 20% were selected.

For each patient, 7 blood smears and 7 marrow smears were performed. Blood and bone marrow smears were stained with May Grunwald Giemsa (MGG) while others were used for a possible cytochemical study. Concerning cytochemistry we studied blasts reaction to myeloperoxidase

in case doubt about the myeloid or lymphoid nature of blasts arose. Criteria of French-American-British group were used for classification of acute leukemias (table 1) (4).

Otherwise, we studied blood count data as well as clinical data. Indeed, these data have helped us in some cases to guide the diagnosis and classification.

Table 1: French-American-British (FAB) acute myeloid leukemia classification

FAB class	Name of subtype
M0	Dedifferentiated
M1	Myeloblastic
M2	Myeloblastic with maturation
M3	Promyelocytic
M4	Myelomonocytic
M5	Monocytic
M6	Erythroleukemic

III. RESULTS

During this period, 120 myelograms were performed, 16 cases (13.3%) of leukemia have been diagnosed with 7 cases (43.7%) of acute lymphoblastic leukemia (ALL) and 9 cases (56.3%) acute myeloid leukemia (AML). Age of patients

ranged from 9 to 69 years old with an average of 32 years old. Sex ratio was 1. Seven patients were in bad general condition, 11 patients (68.8%) presented anemic syndrome, 6 patients (37.5%) hemorrhagic syndrome, and 7 patients (43.8%) a tumor syndrome. Complete Blood Count (C) showed anemia and thrombocytopenia in 93.8% of cases (n = 15). Anemia was normocytic (93.8%, n = 15) normochromic (93.8%, n = 15) in the majority of cases. Hyperleukocytosis or leukocytosis was found in 93.8% of cases (n = 15). It was sometimes associated with neutropenia (31.1%, n = 5) or agranulocytosis (25%, n = 4).

Blood smear study found blood blastosis in 87.5% of cases (n = 14) with an average blast percentage of 42.3%.

Bone marrow smear study showed richly cellular bone marrow in 81.3% of cases (n = 13) (Figure 1). All patients had significant marrow blasts associated with erythroid hypoplasia (100%, n = 16), granulocytic hypoplasia (100%, n = 16) and megakaryocytic hypoplasia (absence of megakaryocytes in 50% of rare cases and megakaryocytes in 50% of case).

Cytological and cytochemical classification blasts allowed to diagnose 6 cases (37.5%) of ALL2, 4 cases (25%) of AML2, 2 cases (12.5%) of AML3 with one hypercellular case, 2 cases of AML4 (12.5%), 1 case (6.3%) of AML5 and 1 case (6.3%) of ALL1.

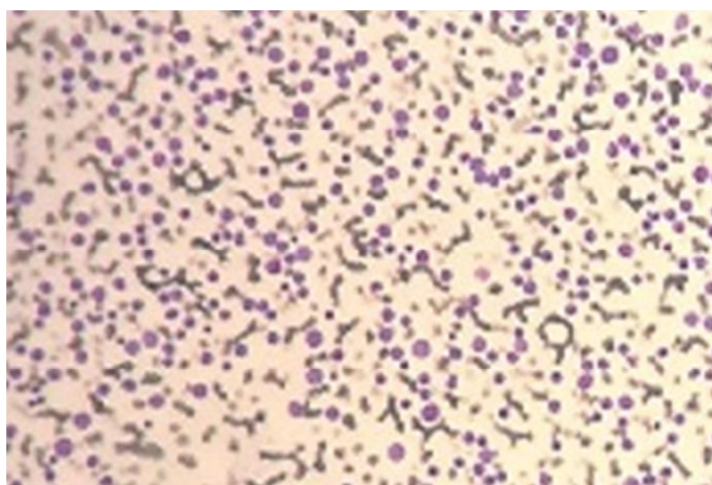


Figure 1 : rich marrow seen in optical microscopy during ALL2 (objective x10)

Source : Hematology Laboratory of JRA Antananarivo Hospital

IV. DISCUSSION

Diagnosis of acute leukemia is based on bundle of arguments. Medical practitioner is often alert by classic signs showing medullary insufficiency (pallor, exercise

dyspnea, infections, purpura or bruises), or tumoral syndrome (5). Although these signs are fickle, their presence shows the severity of acute leukemia (2). In our series, study of complete blood count and blood smear showed cytopenia associated with circulating blastosis in the majority of cases.

These results confirm those reported by some studies (5) (6). Myeloid type was more frequent than the lymphoid type, which is consistent with findings from other studies(7). ALL2 was the most common cytologic type. Currently, ALL classification is immunophenotypic but no longer based on cytology. Therefore, we could not compare our data to literature. On the other hand, our AML data are similar with data from a Malagasy study conducted in 2015 (5). In addition, we were able to make a cytological description only for some types of acute leukemia because during this period, there were no cases of ALL3, AML0, AML1, AML6 and AML7.

In the case of ALL1, the patient had presented bicytopenia with leukocytosis and blood blastosis that accounted for 10% of the leukocyte count. Myelogram showed a rich marrow with hypoplasia of the 3 lines and a

medullary blastosis at 93%. Non-granular blasts were predominantly small in size, with a high nucleo-cytoplasmic ratio, basophilic cytoplasm and chromatin sometimes dense with single pale nucleolus often not visible, making it difficult to distinguish with mature lymphocytes (Figure 2a).

In the case of ALL2, all patients had bicytopenia associated with leukocytosis (83.3%, n = 5) and sometimes very large blood blastosis (94% of leukocytes). All patients had bone marrow hypoplasia often associated with very significant blastosis. Morphologically, blasts were of variable size, non-granular, nucleo-cytoplasmic ratio was variable but high in large number of blasts, nucleoli are multiple, in variable numbers, usually well visible (Figure 2b and 2c). Moreover, to avoid missing myeloid blasts, we performed reaction to myeloperoxidase.

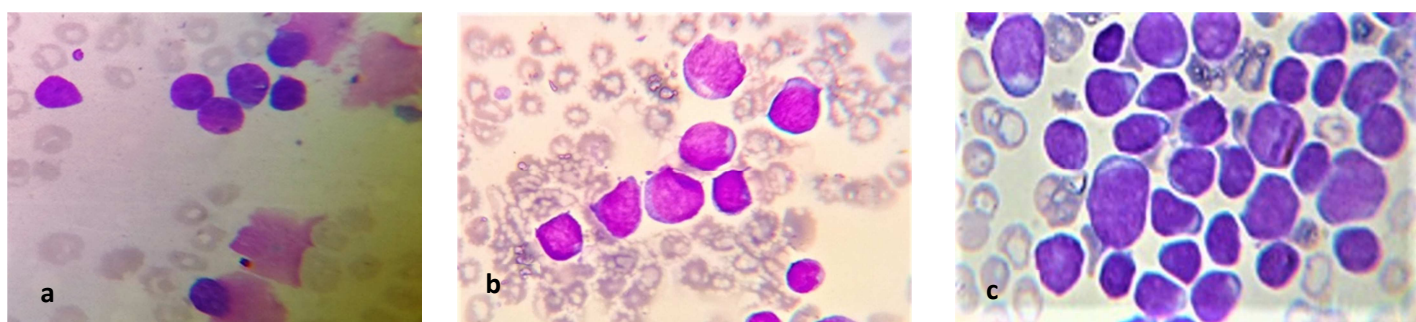


Figure 2: Acute Lymphoblastic Leukemia (ALL)

- a. Medullary smear during ALL1: small blasts difficult to differentiate from mature lymphocytes
- b. Blood smear during ALL2: variable size blasts
- c. Medullary smear in ALL2: fine chromatin blasts

Source: Hematology Laboratory of JRA Antananarivo Hospital

For AML2 or acute myeloid leukemia with maturation, all patients had bicytopenia with leukocytosis (75%, n = 3) and blood blastosis. Bone marrow study had shown bone marrow hypoplasia with sometimes significant blastosis (84% of the medullary formula) on a rich marrow. The blasts were often large, with a high nucleo-cytoplasmic ratio, granular cytoplasm sometimes with mature grains. In blood and especially in marrow we have found discreet maturation in the form of some promyelocytes and myelocytes. There were rarely Auer bodies in the blasts (Figure 3a and 3b).

In the case of AML3 or acute promyelocytic leukemia, one patient had presented classic form with pancytopenia. Marrow was rich with hypoplasia of 3 lines associated with at 84%. Blasts had promyelocyte appearance sometimes with normal morphology but most had dystrophic nuclei.

Their cytoplasm was richly granular with Auer bodies in large numbers, forming in the cell "bundles" of reddish twigs (Figure 3c).

The other patient had a richly cellular variant form with 83.3G / L bicytopenia and leukocytosis associated with significant blood blastosis (98% of leukocytes).

Patient had bone marrow hypoplasia with significant blastosis (82% of medullary cells) in poor marrow. Blasts were dysmorphic promyelocytes, poor in granulation with Auer bodies and sometimes notched nuclei (in "pseudo-Pegler") (Figure 3d).

With respect to AML4 or acute myelomonocytic leukemia, patients had bicytopenia associated with leukocytosis and blood blastosis. Marrow was hypoplastic for both patients, with significant blastosis in both cases.

There were 2 blast populations. Blasts were often dystrophic. Myeloblasts had irregular nuclei and immature granulations. Monocytic cells were in different stages of maturation (monoblasts, promonocytes and monocytes), with an often-vacuolated cytoplasm. We performed a myeloperoxidase reaction to differentiate the 2 blast populations to establish their percentage (Figure 4a and 4b).

In the case of AML5 or acute monocytic leukemia, the patient had anemia associated with blood blastosis. The patient had a medullary hypoplasia on rich marrow. Blasts were monoblasts, large cells with irregular nuclei and nucleoli, dusty cytoplasm and very often vacuolated. More than 80% of the medullary cells were monocytes or promonocytes (Figure 4c and 4d).

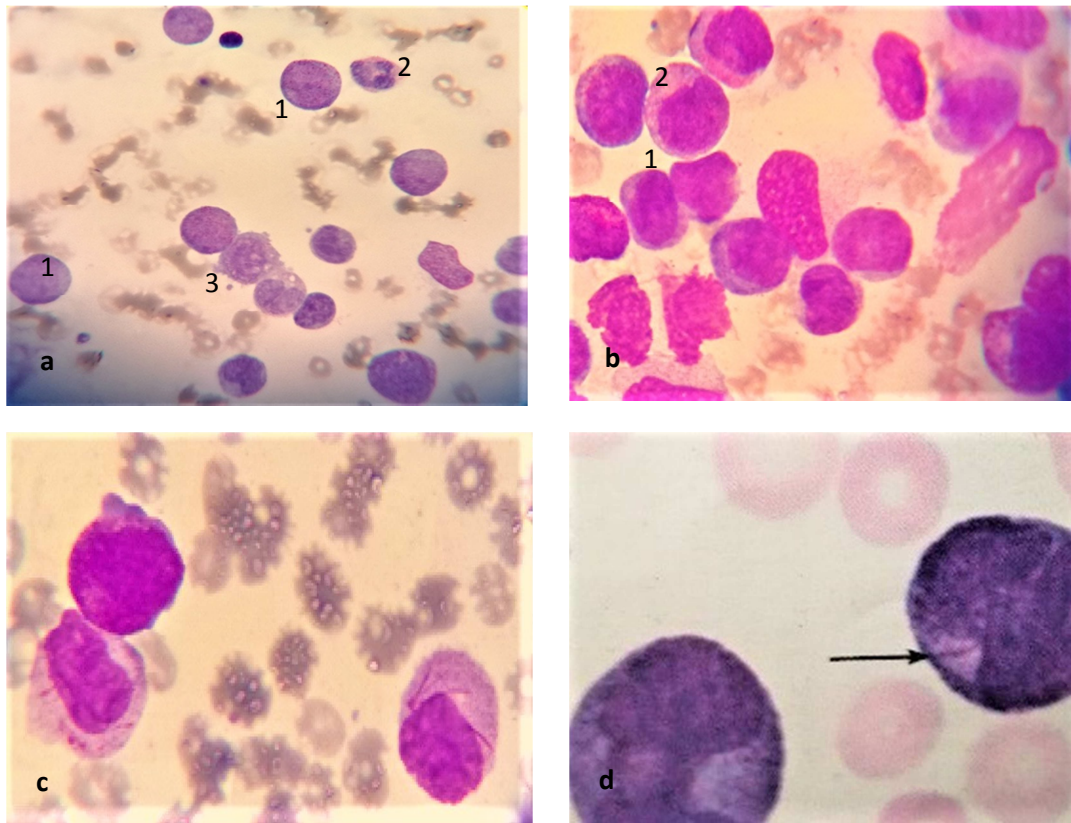


Figure 3: Acute myeloid leukemia (AML): AML2, AML3

- a. Blood smear in matured AML (AML2): myeloblasts (1), polymorphonuclear neutrophils (2), erythroblasts (3)
- b. Medullary cytology during AML2: myeloblast (1), promyelocyte (2)
- c. Medullary smear of AML3: promyelocytic blasts with Auer body
- d. blasts with a dumbbell-shaped core, an Auer body (arrow) in the nuclear notch

Source: Hematology Laboratory of JRA Antananarivo Hospital

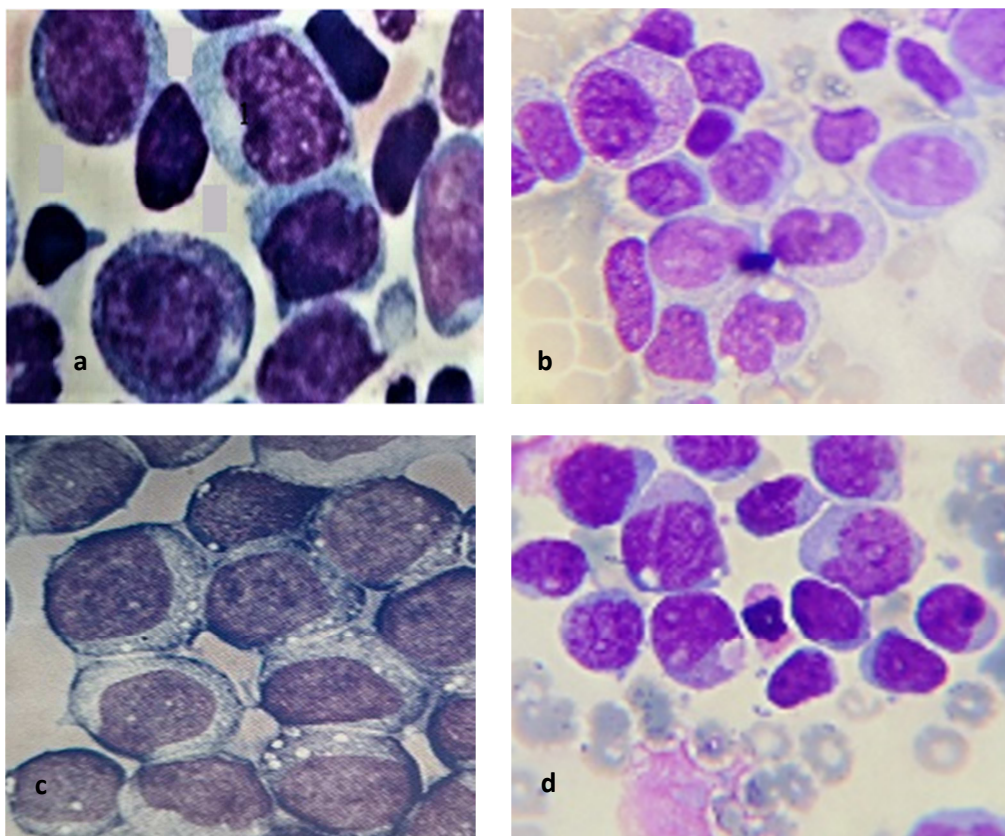


Figure 4: Acute myeloid leukemia (AML): AML4, AML5

a and b. cytology of a myelomonocytic LA

c and d. cytology of monocytic LA

Source: Hematology Laboratory of JRA Antananarivo Hospital

V. CONCLUSION

Diagnosis of acute leukemia is based on a bundle of clinical and biological arguments. The clinical signs and the data of the hemogram can make suspect acute leukemia. Cytology is crucial in malignant hemopathies, particularly in low resource countries, although the need for molecular biology and immunophenotyping is well established.

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