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Epidemiological, Clinical, Cytologic and Immunophenotypic Aspects of Acute Leukemia: The Experience at the Hematology Laboratory of Avicennahospital.

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Abstract

Background: The diagnosis of leukemia is biological by morphological examination of peripheral blood films, the Bone marrow study, and cytochemical staining, and advanced diagnostic procedures like immunophenotyping, cytogenetic analysis, and molecular genetics for unclassified case.

Methods: The study was conducted in Avicenna Hospital in Marrakech from june 2010 to June 2016. Consecutive patients diagnosed with acute leukemia were included in the study. Details were collected as regarding demographic factors, symptoms, signs, laboratory parameters. The morphological typing and sub-typing of leukemia was based on peripheral smear examination, bone marrow studies (aspiration and biopsy) employing the FAB criteria, and immunophenotyping.

Results: In our study of 60 patients with acute leukemia, 37 cases had AML, 16 cases had ALL and 7 cases remained as unclassified acute leukemia. Pallor is the most common physical sign in 74%. Anemia is the most common hematological abnormality in 90%. In peripheral, 61,6% were ALM against 26,6% were ALL and 11,7% remained unclassified after bone marrow.

Conclusion: A complete blood count with a careful reading of blood and marrow smears supplemented by cytochemical reactions still allows the classification of most acute leukemia.

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Keywords: Acute leukemia; acute myeloid leukemia; acute lymphoid leukemia, cytology; epidemiology; clinical, immunophenotyping.

Introduction:

Acute leukemia (AL) constitute a heterogeneous group of clonal hematologic disorders, characterized by malignant proliferation in the bone marrow of an abnormal cell clone of the hematopoietic tissue , and blocked at a specific stage of differentiation with expansion of immature cells (blasts) that may be present in the peripheral blood [1,2].In addition, there is a deficit of mature cell production, resulting in the establishment of an array of bone marrow failure, which includes febrile neutropenia, anemic syndrome and hemorrhagic syndrome, and their clinical consequences [3,4].Thus requiring rapid and accurate diagnosis to establish an appropriate therapeutic approach [5].

According to the origin of the precursor involved, we distinguish: Acute lymphoblastic leukemia (ALL) especially observed in children [6], but also in adults after 50-60 years, and Acute myeloid leukemia (AML) whose frequency increases with age (median around 63 years) [7]; exceptionally malignant cells can express markers of two lines; it is biphenotypic acute leukemia.

The classification of leukemia into a series of distinct varieties has been imposed simply by virtue of their cytomorphological diversity [8]. This finding led to the meeting of a working group composed of French, American and British hematologists in 1974. Their work led to the publication in 1976 of the Franco-American-British classification "FAB" based on the Examination of stained slides and the use of cytochemical reactions, which made it possible to dissociate myeloid leukemia from non-myeloid leukemia "lymphoblastic" [9].In 2001, WHO proposed a classification by adding genetic and clinical data on the FAB classification criteria, as well as an update of the data in 2008[10], and a revision dating from 2016[11].

Biological work on acute leukemia, especially in molecular genetics, has made important progress in understanding leukemogenesis in recent years[12,13].Diagnosis and prognosis are based on morphological examination of blasts, blood cells and bone marrow, as well as immunophenotyping and cytogenetic and molecular studies. The place of the biologist is essential especially for the diagnosis, the monitoring during the treatment then the follow-up after the remission [14].

Aims of the study:

The description of the epidemiological, clinical and cytological characteristics of acute leukemia cases collected at the Hematology Laboratory of Avicenna Military Hospital over a period of 6 years.

Patients and methods:

1-Study design and Setting:

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This is a descriptive and analytical retrospective study of cases of patients collected at the Hematology Laboratory of the Avicenna Military Hospital (AMH) of Marrakech, between June 2010 and June 2016.

2-Study participants:

All patients in whom acute leukemia was diagnosed, confirmed with myelogram, The diagnosis was made after an analysis of the clinical data mentioned on the sending forms sent with the samples in the laboratory, a morphological examination of the patients' blood films, as well as a study of the May-Grunwald-Giemsa colored myelogram (MGG) and myeloperoxidase (MPO). The total number of patients in this study was 60.

3-Data collection:

The data was collected by clinical-biological information sheets sent at the same time as myelograms and blood counts at the AMH Hematology Laboratory in Marrakesh and the laboratory records.These sheets served as a basis for the exploitation of their data in a pre-established form which made it possible to collect and analyze the main epidemiological, clinical and biological characteristics indicated. This sheet contains the following items: Epidemiological data, Clinical data, and Biological data.

The hemogram was determined on "Sysmex XT 4000". The blood and marrow smears were stained with MGG (May¬Grünwald-Giemsa) by a manual method. For each patient, at least two independent readings of blood and marrow smears were performed and validated by cytologists.

The diagnosis of AL was made as soon as there were more than 20% of blasts in the bone marrow. The blood test established the blood leukocyte formula and contributed to the classification of LAs according to the FAB group. The separation between LA subgroups was based on the assessment of the percentage of blasts in the marrow, the type of blasts and the absolute count of blood monocytes [15].The morphological sub classification of acute leukemia was based on a scoring system using the following cellular characteristics: the nucleocytoplasmic ratio, the presence of nucleoli, granulations and Auer's Body, the irregularity of the nuclear profile and the presence of large cells [16].

The cytochemical study completes the cytological interpretation, so its purpose is to confirm the lineage of blast membership [17]. The cytochemical study is usually performed on blood smears or medulla. Myeloperoxidase characterizes granulocyte myeloid cells and, to a lesser degree, monocytes cells. It is absent in lymphocytes and red blood cells. In ALL, leukemic cells are negative for the MPO reaction (The positivity of the MPO reaction cannot exceed 3% of the blasts) [18]. AML is when at least 3% of blasts are MPO positive. The immunophenotyping was requested when the AL was cytological unclassifiable.

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4-Statistical analysis:

The data listed were then computerized on the statistical processing software (SPSS.16). Descriptive statistics (averages, frequencies) were used to summarize the data.

Results:

The mean age of study population was 38, 2 years (SD 4, 67), with minimum age of 9 months and maximum age being 83 years. The most common age group in children was between 1 and 5 years old (50%) (Figure 1), and 57% of adults were between 21 and 59 years old (figure 2). There was male preponderance in the patients studied, with 60% males and 40% females, with Male- Female ratio is 1,5.

For clinical signs: Fever was the most common general and functional symptom seen in 70%(n=42), followed by fatigue in 35%(n=21), weight lost in 16,7%(n=10), bone pain in 6,7%(n=4), jaundice in 4%(n=2).Pallor was the most common physical sign seen in 75%(n=45), and bleeding in 46,7%(n=28), followed by lymphadenopathy in 41,7%(n=25), splenomegaly in26,7%(n=16), hepatomegaly in 15% (n= 9), and gum hypertrophy in 1,7% (n=1) (Table I).

The study of the hemogram of these 60 cases with acute leukemia concluded with the various anomalies illustrated by figure 3: Anemia was the most common hematological abnormality 90%(n=54),mean corpuscular volume ranged from 57 to 110 fl, the average corpuscular size in hemoglobin ranged from 14 to 37pg, reticulocytes were between 5 G/L and 32 G/L. Based on the WHO classification of anemia, 38 % of the patients had severe anemia, 60% had moderate, and 2% had no anemia ; followed by thrombocytopenia in 86, 7%(n=52).45% (n=31) of the patients had leukocytosis at presentation, 23, 3% (n=14) hadleucopenia. The average of circulating blasts was 46%, ranged between 7% and 98% (Table I).

The morphological examination of the medullar smear and the reaction to myeloperoxidase allowed us to classify the acute leukemia of our series in AML in 61.6% (37 cases) of the cases and LAL in 26.6% (16 cases), and in 11, 6% of cases (7cas) it was impossible to classify the blasts of smears examined; the acute leukemia was considered in these cases as acute myeloperoxidase-negative leukemia with the demand for immunophenotyping. In children, the blast rate ranged from 30% to 96% with an average of 75%. In adults, the blast rate ranged from 25% to 90% with an average of 63%. The blast rate for ALL was 82% compared to 61% for ALM. ALL1was the most common subtype, 50% in children, and AML2 was the most common type in adults, accounting for 23% of all ALM cases (Table II).

Only misclassified cases that had benefited from immunophenotyping were 7 cases (Figure 3).

Discussion:

The mean age of patients in our study was 38, 2 years (SD 11.56) which is similar to Rego et al [19] who notedit to 34,9 years, and Ratnamalaet al[20]demonstrated 41 years(SD 17,26).

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We know that age is an important prognostic factor. It is associated with poor evolution when it is less than a year or more than 10 years [21,22,23].
In our series, 28% of children had an age of poor prognosis. The most affected children are between 1 year and 1 0 years old (72% of children). This is the same as in the literature, which states that L.A. (especially LAL) affects children younger than 10 years more frequently [24,25].
AML are rather pathologies of the elderly with an average age of 63 years and a sharp increase in incidence from age 60 [26].Age> 60 is a poor prognosis. In our series, 29% of adults were older than 60 years [27].

Of the 60 patients in our study, 60% were males and 40% were females which was similar to studies by Nwaandi et al[28], Shahab et al[29]. Male preponderance in acute leukemia can be attributed to exposure to environmental and occupational carcinogens.

In most of the studies including ours, fever is the most common symptom. Fever (70%) is followed by bleeding (46,7%), fatigue(35%), weight loss (16,7%), bone pain (6,7%), jaundice (3,4%), and gum hypertrophy (1,7%). Nwannadi et al showed that fatigue (82.2%) was the most common symptom, followed by fever (78.5%), weight loss (54.6%), lymph node enlargement (53.4%), bone pain (31.9%), and bleeding (10.4%)[28].

Pallor is the most common sign similar to previous studies. It was followed by lymphadenopathy (41,7%), splenomegaly (26,7%), hepatomegaly (15%). Nwannadi et al showed that pallor was the most common physical sign (71.2%), followed by splenomegaly (66.3%) and hepatomegaly (14.8%)[28].

In our study hepatomegaly and splenomegaly were seen in 14, 8% and 28% respectively. In studies conducted by Shome et al[30], hepatomegaly was seen in 73% and splenomegaly in 52% while Mathur et al[31], noted it to be in 76% and 73% respectively. Sharma et al[32], found that 7.59% presented with hepatomegaly and 39.24% patients presented with splenomegaly.

Incidence of lymphadenopathy in our study is 42% which correlates with the studies of Shome et al[30], and Mathur et al[31] but, in a study by Advani et al[33], lymphadenopathy was seen in only 4%.

In our study anemia is the most common haematological abnormality 90% followed by thrombocytopenia 87, 7% which has also been illustrated by Rathee et al[34]. Study done by Preeti et al[35], showed thrombocytopenia to be the most common haematological abnormality, followed by anemia and leukocytosis. In a study by Manisha B et al[36], anemia was the most common (50%), followed by leukocytosis (60%) and thrombocytopenia (75%). Study by Burn CP et al[37], showed that 50% had leukopenia, 25% had normal leukocyte count and 25% had leukocytosis, while in our study 23,3% had leukopenia, 31,7% had normal leucocyte count and 45% had leukocytosis.

Median peripheral smear blast percentage in our study is 46%(7-98%). In bone marrow it is 26,6% in ALL and 61,6% in AML. In a study by Rathee et al[34], median blast percentage was

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45% in AML and 38% in ALL. Ghosh et al[38], demonstrated mean values and range for peripheral blood blasts in AML as 41.4% (5-77%) and bone marrow blasts as 57.6% (34-96%). Preethi et al[35], noted a mean blast percentage of 62% in AML correlating with the study conducted by Mathur et al[32].

26,6% of ALL and 61,6% of AML in our study show peripheral blood blast >20%. It was recommended by Cheson et al that the diagnosis of AML can be made when the percentage of blasts in peripheral blood samples is 20% or more, even if the blast count in the BM is less than 20%[39,40].

Bone marrow was done in all patients, after which, 61, 6% were confirmed to have AML, 26,6% ALL and 7 remained unclassified. It was almost similar to a study done by Kusum et al where 25.4% had ALL while 37.7% had AML[41]. Prad han et al in his study noted that 35.95% were ALL and 21.9% were AML, thus ALL being more common than AML[42].

Among 7 patients who still remained as unclassified acute leukemia after bone marrow biopsy, but only 3 patients(5%) could be grouped into either AML or ALL by flow cytometry. Andoljsek et al, showed that immunophenotyping is of great use to distinguish between AML and lymphoid leukemia, as well as when defining hybrid and biphenotypic leukemia where leukemic cells are atypical[43].

Sazawal etal showed that CD13, CD33 were the most useful markers in diagnosis of AML similar to our study. CD14 and CD36 were most often seen in monocyticand myelomonocytic leukemias[44].Both bone marrow study and flow cytometry not only help to classify acute leukemias but also subtype both myeloid and lymphoid leukemias, which are essential for prognosis and treatment.

Conclusion:

A complete blood count with a careful reading of blood and marrow smears supplemented by cytochemical reactions still allows the classification of most AL. However, the study of other cytogenetic, immunological and molecular markers has become necessary to confirm the diagnosis of ALL and to identify atypical LA. The newly proposed WHO classification uses a combination of all these approaches, taking into account their ability to define biological entities that, together with age and abnormalities of the hemogram, help define the treatment regimen and represent the elements useful to the prognosis [45,46].

Conflict of Interest:

The authors declare no potential conflicts of interest, financial or otherwise.

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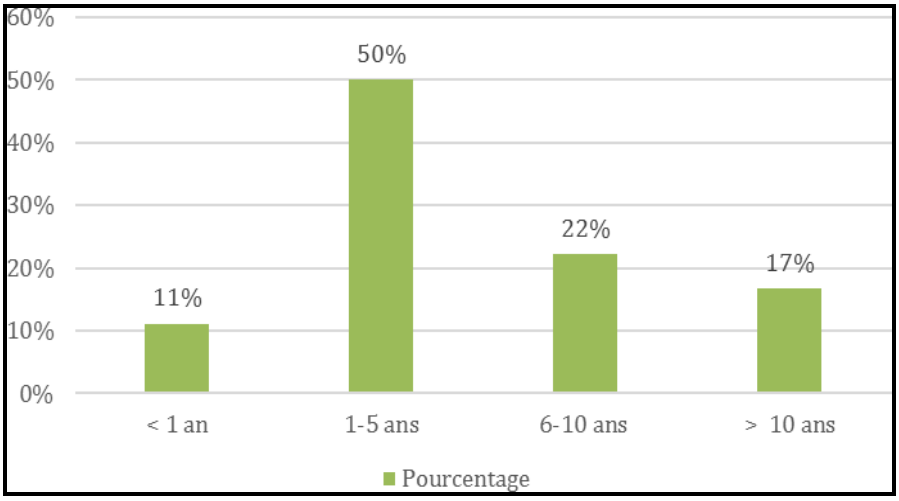

Figure 1 : Age distribution of the child by age group

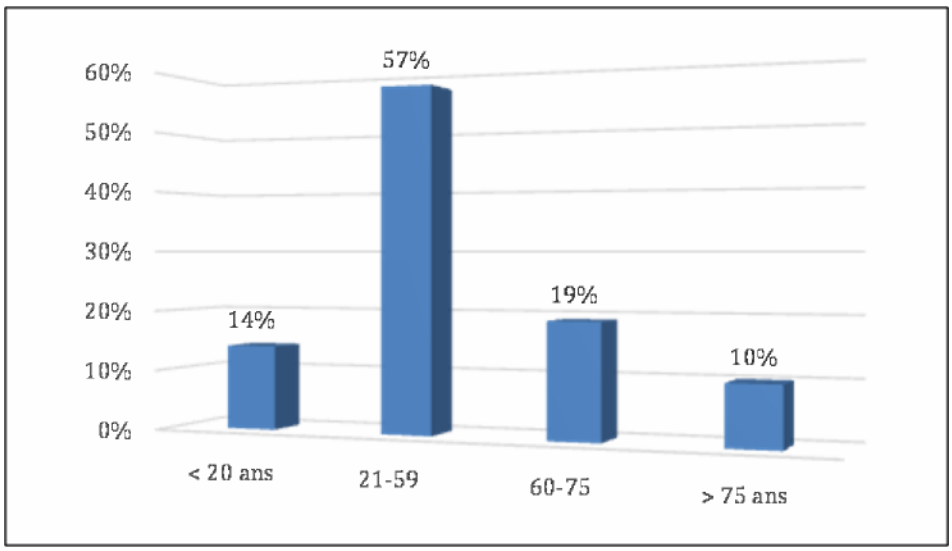

Figure 2 : Brekdown of age groups in adults

items	**N**	**%**
Functional signs	-	-
fever	42	70
fatigue	21	35

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Weight lost	10	16,7
Bone pain	4	6,7
jaundice	2	3,4
Physical signs	-	-
Pallor	45	75
Bleeding	28	46,7
Lymphoadenopathy	25	41,7
Splenomegaly	16	26,7
Hepatomegaly	9	15
Gum hypertrophy	1	1 ,7
Abnormalities of hemogram	-	-
Anemia	54	90
Leucopenia	14	23 ,3
leukocytosis	31	45
Thrombocytopenia	52	86,7
Circulants Blasts	60	Mean=46% Range=7-98%

Table I : Clinical and biological aspects in our studied population

Subtypes	Children		Adults		Total	
	Effectifs	%	Effectifs	%	Effectifs	%
LAL 1	9	50 %	-	-	9	15 %
LAL 2	3	16,6 %	2	4,8 %	5	8,6 %
LAL 3	1	5,6 %	1	2,4 %	2	3,3 %

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LAM 1	-	-	4	9,6 %	4	6,6 %
LAM 2	1	5,6 %	10	23,8 %	11	18,3 %
LAM 3	1	5,6 %	5	12 %	6	10 %
LAM 4	1	5,6 %	5	12 %	6	10 %
LAM 5	-	-	3	7 %	3	5 %
LAM 6	-	-	6	14 %	6	10 %
LAM 7	-	-	1	2,4 %	1	1,6 %
MPO (-)	2	11 %	5	12 %	7	11,6 %

Table II : Distribution of workforce according to classes and subclasses of acute leukemia

Subtype of acute leukemia	**Positifs markers**
LALB	CD19 ,CD22, CD79
LAM1	CD117,CD33,CD 13,MPO
LAM4	CD13,CD3,CD14,MPO

Table III : Immunophenotyping results