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Cytogenetics and Molecular Biology in Acute Lymphoblastic Leukemia (ALL): A Study on 141 Patient in the Hematology and Pediatric Oncology Department at the August 20 Hospital - Morocco

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Abstract

Introduction: Acute lymphoblastic leukemia is a group of heterogeneous diseases. Actually, 20% of acute leukemia cases are reported for adults against 80% for children. Several clinical, biological and therapeutic factors are essential for defining optimal treatment modalities. In children, acute lymphoblastic leukemia is known by the presence of recurrent genetic abnormalities. These abnormalities are described as specific markers which represent an important clinical aspect in the identification of significant risks. Cytogenetic analysis (karyotype along with, if necessary, adequate FISH analyzes) is an essential examination when diagnosing Acute Lymphoblastic Leukemia (ALL).

Aim: Our focus in this study is to define the cytogenetic abnormalities considering some Moroccan patients and their frequency. A comparison of the cytogenetic profiles of cancer cells with other prognostic factors is also demonstrated with the evolution of ALL.

Patient and Methods: We established a descriptive study covering a period from 2014 to 2018 with an established diagnosis of ALL children and patients less than 20 years in the Hematology and Pediatric Oncology Department at the August 20 Hospital. The data concerning cytogenetic profile were collected from patients' charts and we classified cytogenetic abnormalities according to French cytogenetic guidelines. The three identified groups are favorable, intermediate and unfavorable. In the karyotype the sample containing the blasts is cultured and treated to obtain a sufficient number of mitotic cells which will be analyzed in conventional cytogenetics. The material used in this study is the bone marrow or the peripheral blood, when it contains blast cells. The Fish technique is used as a complementary test to confirm the prognosis of the ALL patients.

Results: The 141 patients were collected for this study and analyzed in 2020. The karyotype was performed on 105 patients. We analyzed 75 patients with B ALL. It was normal in 33 cases (37%). A hyperdiploidy between 51 and 65 chromosomes was found in 17 cases (17%). Ten cases showed karyotype failure. 25% of karyotypes were complex. The use of molecular biology allowed the detection of MLL + gene in 4 patients, and BCR-ABL gene in 5 patients during this study.

Conclusion: The management of pediatric ALL has progressed enormously in these recent years, resulting in improved patient survival. In our study we identified several cytogenetic abnormalities where the prognosis is unknown, as well as intermediate prognostic abnormalities, which encouraged us to set up a collaboration between hemato-biologists, geneticists and hematologists.

Keywords: Acute lymphoblastic leukemia; Cytogenetic; Karyotype; Hematology; Oncology

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Introduction

Acute Lymphoblastic Leukemia (ALL) is the most common pediatric cancer, accounting for about 30% of all pediatric tumors [1]. In Morocco, the number of cases of lymphoid leukemia recorded between 2013 and 2017 was 217, representing 0.9% of the total number of cancer cases

recorded and 38.5% of the total number of leukemia cases [2]. It is also about 5 times more prevalent than AML [3]. The diagnosis of ALL is mainly based on cytological and immunophenotypic criteria of blasts [4]. The role of immunophenotyping, cytogenetics and molecular biology is very important to describe subtypes in detail and to establish the diagnosis and prognostic factors allowing the treatment to be adapted to the severity of the disease [4]. In children, acute lymphoblastic leukemia is known by the presence of recurrent genetic abnormalities. These abnormalities are described as specific markers which represent an important clinical aspect in the identification of significant risks [5]. The immunological phenotype established using monoclonal antibodies labeling cellular antigens makes it possible to affirm the lymphoid nature of proliferation in order to characterize ALL B (85% of cases) or T [6]. Cytogenetic analysis (karyotype along with, if necessary, an adequate FISH analyzes) is an essential examination when diagnosing Acute Lymphoblastic Leukemia (ALL) [7]. Fluorescence *in situ* Hybridization (FISH) studies complement the diagnostic karyotype by providing a higher resolution of analysis with clarification of rearrangements observed by G-banding and identification of cryptic abnormalities not observed by karyotyping [8]. Although many chromosomal abnormalities have been described in ALL, approximately 30% of pediatric patients with ALL do not have cytogenetic abnormalities of clinical significance [9].

Patients and Methods

Population

We reviewed all cases from January 2014 to December 2018 with a confirmed diagnosis of childhood ALL. The diagnosis in all cases was established based on morphology, immunophenotypic, and genetic studies. This study included patients between 1 and 20 years of age. Patients were excluded if they were under 1 year of age or had insufficient data or because cytogenetic studies were not performed in some cases. The medical records were reviewed for epidemiologic, demographic, and laboratory data including immunophenotype, cytogenetic, and molecular analysis in addition to patient outcome variables such as remission status or relapsed disease, chemotherapeutic responses, follow-up, and morbidity. The patients with ALL were treated according to MARALL protocol. Patients were classified into 3 risk groups based on prognostic factors such as age, sex, white blood cell count at diagnosis, immunophenotype, and cytogenetic results.

Immunophenotypic study

It is performed by flow cytometry on blood or medullary samples taken on EDTA tubes. The panel of antibodies used is that recommended by the European Group for Immunological Leukemia (EGIL): for line B: CD19, CD22, CD20, CD10, anti-heavy chains mu, gamma and light chains kappa, lambda, for the T line: CD2, CD3, CD5, CD7 [10]. Characterization of blasts is performed in two consecutive steps. Reading is done by flow cytometer (Becton-Dickinson) in three colors. An antigen is considered positive when at least 20% of the cells express [11].

Cytogenetic analysis

In the karyotype the sample containing the blasts is cultured and treated to obtain a sufficient number of mitotic cells which will be analyzed in conventional cytogenetics [12]. The material used in this study is the bone marrow or the peripheral blood, when it contains blast cells. Sampling must be carried out sterilely on a heparinized tube. After counting the sample, the cells are cultured in a synthetic medium without the addition of mitogen and then in an oven (37°C.) for a period of 24 h to 48 h. The technical steps then include stopping the culture with colchicine followed by hypotonic shock and several fixations (methanol-acetic acid). The cell suspension obtained is spread on slides, which are then denatured by heat and stained to obtain bands R: RHG allowing the identification of chromosomes (the classic chromosome banding). Molecular cytogenetics techniques such as FISH will be used secondarily to specify complex reshuffle, identify a cryptic abnormally or assess the residual disease.

Results

The patient characteristics included in the study are summarized in Table 1. Of the 141 patients, 76 were B-lineage ALL (58%), and 55 were T-cell ALL (41%). The range of age was 1 to 20 years with a median of 9 years, with a Sex-Ratio H/F of 1.6. In total, 87 of the patients were male and 54 were female. Most of the patients were within a good risk age category (<10 years). The most common symptom that revealed the disease is anemic syndrome (65%).

The white cell rate at diagnosis varied from 560 to 756 930/mm^3 with a median of 21,600/mm^3. Leukocytosis greater than 10,000 was found in 90 patients (70%) of examined patients. In Table 2 a correlation between phenotype and leukocytosis.

The bone marrow aspiration performed in all patients made it possible to make the diagnosis of ALL in 97% of patients, with an average of 80% blasts, 70% of them were ALL2.

The immunophenotyping was performed on 131 patients. The phenotypes observed were B in 76 cases (58%) and T in 55 cases (41%), the phenotype B was associated with a younger age with a median of

Table 1: Patient characteristics and outcome.

Parameters	ALL (N= 141)
Male: Female	87:54
Median age	9
Median WBC count, mm^3	21,600/mm^3
Median BM Blasts, %	80
Immunophenotype	
B-ALL, %	58
T-ALL, %	41
Karyotype results	
Favorable, %	29.7
Intermediate, %	42.9
Unfavorable, %	24.2
Outcome	
Complete Remission, %	72
Died, %	2.4
Relapsed, %	25

Table 2: Correlation between phenotype and leukocytosis.

	B-ALL (N=76)	%	T-ALL (N=55)	%	Total	%
<4000	10	7	8	6	18	14
4000<WBC<10,000	12	9	10	7	22	17
10,000<WBC<50,000	32	24	14	11	46	35
>50,000	9	5	3	2	12	9
>100,000	13	10	19	14	32	25
					131	100

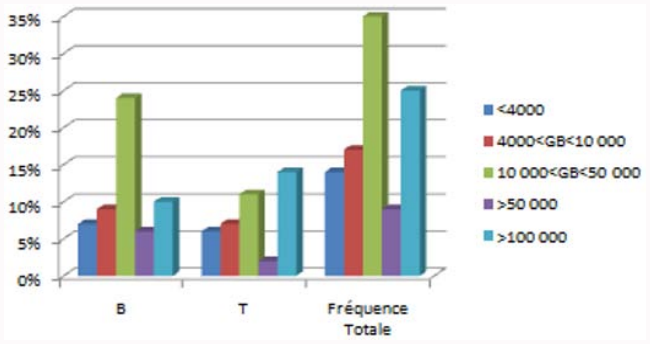


Figure 1: Distribution of white blood cell counts by immunophenotyping.

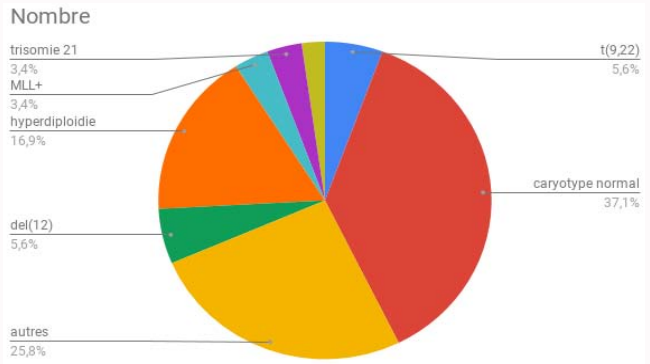


Figure 2: Distribution of cytogenetic abnormalities.

Table 3: Comparison of therapeutic results with cytogenetic abnormalities.

	Hyper diploidy >50 chromosomes	Normal Karyotype	T (9, 22)
CR	82%	70%	33%
Relapse	17%	30%	66%
N	17	33	6

N: Number of patients; CR: Complete Remission

8 years, *vs.* 14 years for the T phenotype. Distribution of white blood cell counts by immunophenotyping demonstrated on Figure 1.

Mediastinal widening and neurological involvements were often associated with T-ALL.

The karyotype was performed on 105 patients. It was normal in 33 cases (37%). A hyperdiploidy between 51 and 65 chromosomes was found in 17 cases (17%). The 9 patients were aged between 1 and 10 years. The different cytogenetic abnormalities are summarized in Figure 2.

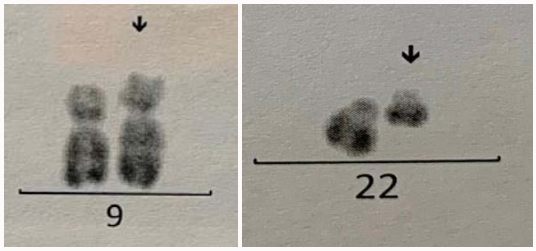
Cytogenetic abnormalities were classified according to French cytogenetic guidelines. The three groups that emerged were Favorable, Intermediate, and Unfavorable.

Some cytogenetic abnormalities of patients in this study were collected from the laboratory, Figure 3.

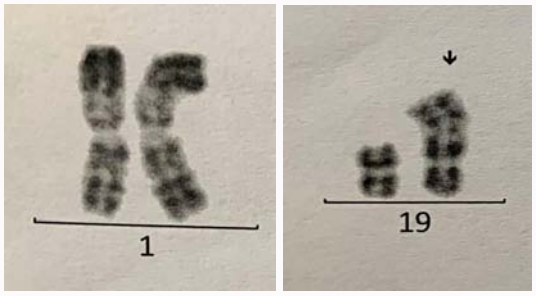
The use of molecular biology allowed the detection of MLL + gene in 4 patients, and BCR-ABL gene in 5 patients during this study.

Based on the analysis of the collected data, it was demonstrated that 88% of B-ALL have a favorable prognosis. There was a significant association between phenotype and prognosis (p=0.006).

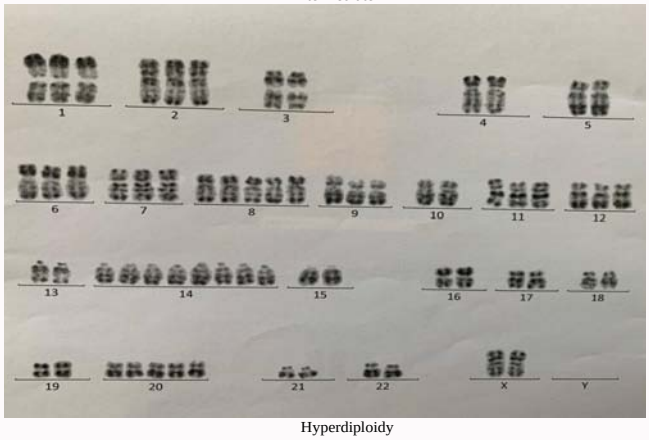
At the end of the induction, 72% of the patients were in complete



46, XY, t (9;22) (q34;q11)
Unfavorable.



46, XY, t(1;19)(q23,p13)
Intermediate



Hyperdiploidy
Favorable

Figure 3: Cytogenetic abnormalities of ALL patients.

medullary remission, 2.4% have died, 25% relapsed, 70% of them were medullary. The frequency of relapse was associated with T-ALL.

The karyotype is an important prognostic factor, hyperdiploidies are associated with the highest rate of complete remission and the lowest relapse as for t (9;22) that is related to an unfavorable prognostic, the relapse rate higher than the complete remission rate, Table 3 represents a comparison of therapeutic results with cytogenetic abnormalities.

Discussion

The progress made in the treatment of the child's ALL could be obtained thanks to a better definition of the initial prognostic factors, making it possible to adapt the therapeutic strategy. In Morocco, several published studies have described the immunophenotypic profile of the child's ALL and correlated it with other clinical and biological prognostic factors. Few studies, however, have described the cytogenetic abnormalities. This cohort study describes these cytogenetic aspects of leukemic cells and their impact on the evolution of ALL.

In the present work, the T-ALL phenotype is high. Actually, it

Table 4: Frequency of chromosome abnormalities in the literature.

Reference	Country	Year	Patients (N)	Normal (%)	Chromosomal abnormalities (%)
[17]	Sweden	1997	381	36	53
[18]	India	2003	47	68	32
[16]	Oman	2006	47	46.8	53.2
Present Study		2014-2018	141	37	55

is comparable to other Moroccan studies with values from 37% to 38.8% [13,14]. Nonetheless, the ALL T phenotype is high compared to western studies that report a rate of only 20% [15]. This frequency is also higher than 26.6% T-ALL of an Egyptian series [16]. The majority of childhood ALL has been reported to occur before the age of 10 years [17], which is comparable to our study. The interpretation of phenotypic characteristics must be correlated with epidemiological, clinical, biological and cytogenetic data.

The karyotype performed in 72% of patients, was normal in 37% of cases which is similar to some series made in Oman [18], Sweden [19], and India [20].

According to the literature, with a frequency of 30%, hyperdiploidy is the most common abnormality in children at B-ALL [20]. In our study hyperdiploidy is estimated only at 17%, but remains the most common abnormality among the other 105 cytogenetic abnormalities.

According to the literature [21] hyperdiploidies >50 chromosomes are of favorable prognosis.

The translocation t (9;22) was found in 5% of cases tested in the present work. This result is similar to that of the Omani series and other related series [18]. This anomaly is associated with an adverse diagnosis according to the Francophone Cytogenetics Group hematologic [7]. In our study, this anomaly; t (9;22) was associated with a rate of relapse of 66% which demonstrates its poor prognosis. The t (12;21) good prognosis was detected in one patient. His diagnosis requires FISH or RT-PCR techniques. The cytogenetic characteristics in this cohort were similar to those reported in other countries (Table 4), but with varying frequencies of some abnormalities. This could be due to geographic heterogeneity. The results of this study provide a better knowledge of the biological profile of ALL in Morocco, which allows the development of currently applicable chemotherapy protocols.

Conclusion

The management of pediatric ALL has progressed enormously in these recent years, resulting in improved patient survival.

In our study, we identified several cytogenetic abnormalities with unknown prognoses, as well as intermediate prognostic abnormalities. This encourages us to establish a collaboration between hemato-biologists, geneticists, and hematologists.

Although the cytogenetic characteristics in this cohort were similar to those reported in other countries (Table 4), the frequencies of some anomalies vary. This could be due to ethnic differences and geographical heterogeneity. However, a large number of patients is required for future studies in molecular cytogenetics in order to provide better detection of cryptic anomalies and stratify more precisely the prognosis factors and adapt the therapeutic regimens.

Cytogenetic analysis is crucial not only for initial prognosis stratification but also used for evaluation of the residual disease.

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