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CYTOGENETIC CHARACTERISATION OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA IN NICARAGUA

THESE

préparée sous la direction du Docteur
Maja Beck-Popovic, Privat-Docent et Maître d'enseignement et de Recherche
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par

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Cytogenetic characterization of childhood acute lymphoblastic leukemia in Nicaragua

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Rapport de synthèse

Introduction:

L'étude proposée s'inscrit dans un projet appelé « La Mascota » qui a débuté en 1986. Il s'agit d'un programme de jumelage entre La Mascota – hôpital pédiatrique de Managua au Nicaragua, - et les hôpitaux de Monza et Milan en Italie et Bellinzona en Suisse. Dans notre étude, nous avons évalué la contribution de la caractérisation cytogénétique de la Leucémie Lymphoblastique Aigue (LLA) comme facteur pronostique comparée aux paramètres cliniques, morphologiques et immunohistochimiques.

Méthode:

Tous les patients avec une LLA, diagnostiquée et traitée dans le seul hôpital d'Oncologie Pédiatrique du Nicaragua en 2006, ont été étudié prospectivement. Le diagnostic de l'immunophénotype a été réalisé sur place, des échantillons de moelle osseuse ou de sang ont été envoyé au laboratoire de cytogénétique de Zürich pour des analyse caryotypique par G-banding et analyses d'hybridation fluorescente in Situ (FISH) à la recherche de marqueurs cytogénétiques de leucémie connus.

Résultats:

Soixante six patients avec une LLA ont été évalué. Leur âge moyen au diagnostique était de 7.3 ans, le 31.8% était ≥10 ans. Trente trois patients (51.5%) présentaient au diagnostic une hyperleucocytose ≥50 10^9/L, 45 (68.2%) une hepatosplenomégalie. L'Immunophénotype a révélé dans 63/66 patients (95%) une LLA de type pré-B, 2 (3%) une LLA de type T et 1 (1.5 %) une LLA de type B-mature.

Les analyses du FISH ont démontré une fusion *TEL/AML1* chez 9/66 patients(14%), la fusion *BCR/ABL* chez 1 patient (1.5%), un réarrangement *MLL* chez 2 patients (3.1%), un *iAMP21* chez 2 patients (3.1%), un réarrangement *MYC* chez 1 patient (1.5%) et une hyperdiploïdie élevée chez 16 patients (24%).

Tous les patients, à l'exception de 2, avec une fusion *TEL/AML1* et une hyperdiploïdie élevée étaient cliniquement et hématologiquement dans le groupe à risque standard. Par contre, ceux qui avaient des facteurs cytogénétiques de mauvais pronostic, avaient des caractéristiques cliniques du groupe à haut risque et étaient traités intensivement.

Conclusions:

Comparé à l'Europe, les enfants atteints de LLA au Nicaragua sont plus âgés, ont une proportion majeure de facteurs de mauvais pronostic clinique et hématologique et reçoivent un traitement plus intensif, tandis que les patients avec la translocation *TEL/AML1* et avec une hyperdiploïdie élevée sont selon la clinique déjà dans le groupe à risque standard. Les résultats cytogénétiques n'ont pas contribué aux décisions thérapeutiques. Du point de vue coût-bénéfice, introduire de routine des analyses cytogénétiques pour la LLA ne semble pas une priorité par rapport à la nécessité de diagnostiquer plus précocement la maladie pour améliorer la compliance au traitement et la survie.

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Cytogenetic Characterization of Childhood Acute Lymphoblastic Leukemia in Nicaragua

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Background. Within the frame of a twinning programme with Nicaragua, The La Mascota project, we evaluated in our study the contribution of cytogenetic characterization of acute lymphoblastic leukemia (ALL) as prognostic factor compared to clinical, morphological, and immunohistochemical parameters. ***Methods.*** All patients with ALL treated at the only cancer pediatric hospital in Nicaragua during 2006 were studied prospectively. Diagnostic immunophenotyping was performed locally and bone marrow or blood samples were sent to the cytogenetic laboratory of Zurich for fluorescence in situ hybridization (FISH) analysis and G-banding. ***Results.*** Sixty-six patients with ALL were evaluated. Their mean age at diagnosis was 7.3 years, 31.8% were ≥10 years. Thirty-four patients (51.5%) presented with hyperleucocytosis $\geq 50 \times 10^9$/L, 45 (68.2%) had hepatosplenomegaly. Immunophenotypically 63/66 patients (95%) had a B-precursor, 2 (3%) a T- and 1 (1.5%) a B-mature ALL. FISH analysis demonstrated a TEL/AML1 fusion in 9/66 (14%), BCR/ABL fusion in 1 (1.5%), MLL rearrangement in 2 (3.1%), iAMP21 in 2 (3.1%), MYC rearrangement in 1 (1.5%), and high-hyperdiploidy in 16 (24%). All patients but two with TEL/AML1 fusion and high-hyperdiploidy were clinically and hematologically in the standard risk group whereas those with poor cytogenetic factors had clinical high-risk features and were treated intensively. ***Conclusions.*** Compared to Europe, the ALL population in Nicaragua is older, has a higher proportion of poor prognostic clinical and hematological features and receives more intensive treatment, while patients with TEL/AML1 translocations and high-hyperdiploidy are clinically in the standard risk group. Cytogenetics did not contribute as an additional prognostic factor in this setting. Pediatr Blood Cancer 2009;53:1238–1241.

Key words: acute lymphoblastic leukemia; cytogenetics; developing countries

INTRODUCTION

Acute Lymphoblastic Leukemia (ALL) is the most frequent type of childhood malignancy, representing one third of all pediatric cancers. It is highly curable with an 80% cure rate in developed countries [1]. Whereas the incidence of childhood cancer in developing countries is comparable [2], outcome results are much lower especially for patients living in low-income countries [3–6]. Cure rate for ALL has been achieved through a better knowledge of risk factors and stratification of treatment according to risk groups [7,8], with reduced treatment intensity for patients with good prognostic factors and treatment intensification for those with adverse prognostic factors [9–13].

There is currently an initiative to reduce the mortality gap between childhood cancer in low-income and developed countries. Such a program exists in Nicaragua, the La Mascota Project. It started in 1986 at the La Mascota pediatric hospital in Managua, as a twinning project between the hospitals of Monza and Milan, Italy, and Bellinzona, Switzerland [14]. In the years following the introduction of the program, the care of ALL patients in Nicaragua improved significantly and is offered now, free of charge, to all Nicaraguan children with cancer [14]. Medical professionals (doctors, nurses, and laboratory technicians) were trained in the 1980s and 1990s in Italy and Switzerland, and the immunophenotyping was introduced in the 1990s permitting more precise diagnosis of acute leukemias [15].

Although cytogenetic and molecular analyses of leukemic cells have significantly contributed to a better comprehension of the pathogenesis of childhood ALL [9,10] and treatment adaptation and are routinely performed in developed countries, they have not yet been introduced in Nicaragua.

The aim of the study was to prospectively perform a cytogenetic characterization of ALL in Nicaragua at diagnosis, to evaluate its contribution as prognostic factor by correlating the results with clinical, morphological and immunohistochemical parameters, and to compare them to Swiss and European data.

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METHODS

From January to December 2006, all newly diagnosed patients with acute ALL in Nicaragua were included into the study. The study was performed in collaboration with the Pediatric Hematology-Oncology Unit of the University Hospital in Lausanne and the cytogenetic laboratory of the University Children's Hospital in Zurich, the Swiss referral centre for cytogenetic analysis in pediatric ALL. A physician trained in Switzerland spent 1 year in Nicaragua and was responsible for the collection of the clinical data at diagnosis, bone marrow aspiration, and preparation of samples for sending them to Switzerland.

The diagnosis was performed locally by bone marrow aspiration, with analysis of morphology, cytochemistry (myeloperoxidase), and immunophenotype. For the latter the alkaline-phosphatase/antialkaline-phosphatase (APAAP) method was used comprising CD10, CD19, CD3, CD13, CD33, and CD45.

Bone marrow aspiration or peripheral blood samples were cultured using a simplified published version of the method employed at the Zurich cytogenetic laboratory [16]. Cells were cultured in RPMI 1640 with Glutamax 1 (Invitrogen, Basel, Switzerland), supplemented with 20% fetal bovine serum (Invitrogen) and 1% penicillin/streptomycin (Invitrogen). Colcemid (Invitrogen) was added prior to harvesting. The cells were fixed in

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methanol 3:1 acetic acid and were stored at −20°C. Once a month a batch of fixed cell preparations was sent by courier to the cytogenetic laboratory of the University Children's Hospital of Zurich where fluorescence in situ hybridization (FISH) analysis and G-banding were performed.

For the FISH analysis the following probes were used: BCR/ABL, TEL/AML1, MLL (Abbott, Baar, Switzerland), and identification of high-hyperdiploidy. For cases with either confirmed or suspected T-ALL, a TLX3 probe was added (Dako, Baar, Switzerland) and for the only case with confirmed B-ALL, a MYC breakapart probe (Abbott) was used.

Due to an intermittent unidentified problem with the quality of the preparations of heparinized bone marrow samples, EDTA (as a recommended substitute of heparin) samples were employed for the last 2 months, and did not influence the outcome of results [16]. The protocol was approved by both the Swiss and local ethics committees, and all parents gave a written consent for cytogenetic analysis.

RESULTS

From January to December 2006, 71 children were diagnosed with ALL, 66 had complete clinical and laboratory data at diagnosis for evaluation summarized in Table I. Three patients were excluded because no BM was done or not enough material was available for cytogenetic analysis. In two other cases, diagnosis was undifferentiated leukemia.

There were 37 females (56%) and 29 males (44%). The mean age at diagnosis was 7.3 (SD ± 4.4) years. The risk distribution according to age comprised 1 child (2%) <1 year, 44 (66%) between 1 and 10 years, and 21 (32%) ≥10 years. Risk distribution according to blood cell counts revealed, a leukocyte count $<50 \times 10^9$/L in 45/66 (68%) patients, and a hyperleukocytosis $\geq 50 \times 10^9$/L in 21 (32%). Ten of the 21 patients had hyperleukocytosis $>100 \times 10^9$/L and 3 presented extreme levels of $>400 \times 10^9$/L (10/21 patients = 47%). Hepatosplenomegaly (HSM) (spleen or liver >2 cm below costal margin assessed by physical examination) was present in 45/66 (68%).

The results of the immunophenotyping in 66 patients showed B-progenitor ALL (CALLA, including Pre-B-ALL) in 54 (81%), and a T-cell immunophenotype in 2/66 (3%). There was only one patient with B-mature ALL (2%). Nine patients showed Pro-B ALL (14%) characteristics, derived from a very immature B-cell precursor.

The cytogenetic results and their distribution by percentage are presented in Figure 1.

Out of 66 patients, 9 (13%) showed a TEL/AML1 fusion. These patients had features of standard risk leukemia with a median age at diagnosis of 4 (2–12) years, with HSM in 6/9 (66%) and a median leukocyte count of 5.2 (3.3–91.3) $\times 10^9$/L. A BCR/ABL fusion product was found in only one case (1.5%) in a patient who presented at diagnosis with hyperleukocytosis of 600×10^9/L concomitantly to a CNS infiltration. His leukemia expressed CD10+, CD19+, and CD13+ and was classified as bi-phenotypic ALL. A MLL (11q23) rearrangement was identified in two patients (3.1%). In the first case, a t(4;11) was seen in an infant with a hyperleukocytosis of 367×10^9/L at diagnosis and a pro-B ALL immunophenotype. The second patient was 6 years old, with a moderate hyperleukocytosis of 61.7×10^9/L at diagnosis and a pro-B ALL immunophenotype. He later showed to be very resistant to treatment. Conventional cytogenetics showed a complex karyotype with an unidentified MLL partner chromosome. Signal pattern consistent with iAMP21 (amplification of AML1) was found in two patients (3.1%), who were ≥6 years old and had a leukocyte count between 10 and 50×10^9/L.

High-hyperdiploidy (51–67 chromosomes) was confirmed by conventional cytogenetics in 11 cases, and inferred in another 5 based on FISH results, for a total of 16 patients (24%). The mean age of the subgroup high-hyperdiploidy was 3.5 (2–8) years and the mean leukocyte count at diagnosis 4.3 (1.1–52.8) $\times 10^9$/L. The only patient with mature B-cell ALL, and morphologic FAB L3 criteria, also had cytogenetic confirmation of a MYC (8q24) rearrangement. The comparison of distribution of age, incidence of TEL/AML and high-hyperdiploidy between ALL patients in Nicaragua and Switzerland is summarized in Table II.

TABLE I. Patient Characteristics

Features	nb	Range	Median age (y)
Total	66		
Gender			
Male	29		
Female	37		
Age (years)			
<1	1		
1 < 10	44	2–9	
≥10	21	10–16	
Hepatosplenomegaly			
Yes	45		
No	21		
WBC (g/L)			
<10	30	0.5–8.4	7 (2–16)
≥10 < 50	15	16.6–48.9	6 (2–16)
≥50	21	52.8–400	6 (1–15)

DISCUSSION

The population of Nicaragua is 5.4 million people with approximately 49% of the population under the age of 18 years. The country has a yearly income per capita of 420 U.S. dollars. Manuel de Jesus Rivera La Mascota Children's Hospital in

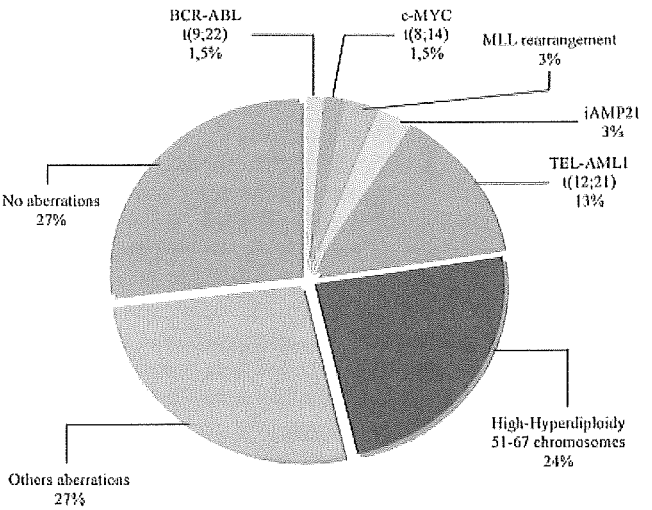

Fig. 1. The distribution by percentage of the cytogenetic results.

TABLE II. Incidence of TEL/AML1 Fusion and H-H According to Age Group in Switzerland and Nicaragua

	Age distribution				TEL/AML1				High-hyperdiploidy			
	Nicaragua		Switzerland		Nicaragua		Switzerland[a]		Nicaragua		Switzerland	
Age (y)	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Infant <1	1	1.5	10	2	0	0	0	0	0	0	0	0
1 to <10	44	66.5	388	76	7	16	86	29	16	36.5	114	29.5
>10	21	32	112	22	2	9.5	7	8.4	0	0	15	13.5
Fisher *t*		$P=0.06$				$P=0.2$				$P=0.3$		

[a]Numbers based on a consecutive series of 376 patients where TEL/AML1 was routinely tested.

Managua, the capital city, is the only national centre for pediatric oncology, to where children are referred, diagnosed, and treated [17].

The yearly incidence of ALL in Nicaragua corresponds to formerly published data (30 cases per million children per year) on countries of Central America [15,18]. Surprisingly we observed a higher percentage of older children (32%) compared to developed countries (20%) [2] whereas the incidence of very young patients <1 year of age was equally small (1.5%). Therefore, only 67% fulfilled criteria of favorable risk factors by age (1–10 years), a phenomenon also observed by others [5,6,15]. There was a female predominance (56%) in contrast to the usually observed male predominance [1].

The incidence of HSM was high with 68% (45/66 patients) in comparison with European data (30–50%) [19] and may be related to delayed diagnosis. Malta et al. [15] made a similar observation in 1996, when they found an incidence of HSM of 70% in Nicaraguan ALL patients. Among the 66 study patients, 21 had a hyperleukocytosis at diagnosis. Other three patients who had more than 300×10^9/L leukocytes had either a T-ALL (one patient) or adverse cytogenetic factors such as MLL rearrangement (one patient) or t(9;22) (one patient).

The incidence of B-progenitor ALL (81%) was very similar to that in Europe [20]. In contrast, T-ALL was found only in two patients (3%), which appears less compared to international results, where approximately 10–15% of children present a T-cell immunophenotype [1]. T-ALL might also be under diagnosed in Nicaragua as only the surface marker CD3 (and not the cytoplasmic CD3) is available to diagnose T-lineage lymphoblasts. Consequently some patients diagnosed as B-progenitor or undifferentiated ALL with clinical characteristics of a T-ALL might have been wrongly diagnosed.

The cytogenetic mutations observed in Swiss patients with ALL are representative of Europe [21]. Therefore, main cytogenetic characteristics were compared to this population. The incidence of the TEL/AML1 fusion (13%) was not statistically different ($P=0.2$), than the 22% reported in Switzerland (Table II). The observed trend, however, might be explained by the smaller patient number studied compared to the Swiss cohort. Another explanation may lay in the different age distribution of ALL patients in Nicaragua. TEL/AML1 patients have a classical prevalence in the <10 years age group. In the studied patient population, 32% of patients were older than 10 years of age, which is a higher proportion than usually observed [7]. These patients also had more often HSM and hyperleukocytosis than patients in developed countries (31/44 patients (70%) <10 years had HSM and 14/21 (66%) >10 years had HSM) [15]. Thus, the low incidence and poor outcome of the TEL/AML1 fusion appears to be real in Nicaragua. It is similar to what has been found in people of Hispanic origin living in the U.S.A., where an incidence of 13% of TEL/AML1 fusion has been reported by Aldrich et al. [22] and might reflect a racial difference of the disease. Among the patients with the TEL/AML1 translocation, only two out of nine patients were older than 10 years and treated with more intensive chemotherapy. A further interesting observation is the under representation of high-hyperdiploidy in ALL. This result is similar as for TEL/AML1 mutation, when considering the different age distribution of ALL, which again showed only a trend without reaching statistically significance ($P=0.3$).

The incidence of adverse prognostic factors such as MLL rearrangement or t(9;22) was very low as observed elsewhere [1]. These patients fulfilled clinical high-risk criteria at presentation with hyperleukocytosis, HSM, age <1 or >10 years, and more intensive therapy.

Only three reports are available in the medical literature regarding cytogenetic analysis in children with ALL in developing countries. The studies of Macedo et al. [23] from Rio de Janeiro, Pérez-Vera et al. [24] from Mexico City, and Chang et al. [25] from Taiwan are all institutional studies; they describe the frequency of the chromosomal abnormalities in ALL patients without making any correlation with clinical risk factors.

In conclusion, cytogenetic analyses of ALL in Nicaragua showed a slightly different distribution than that observed in Western countries with a smaller proportion of favorable risk factors such as TEL/AML1 and high-hyperdiploidy. These results correlated well with a higher proportion of a clinical high-risk population by older age, hyperleukocytosis, and tumor volume by HSM receiving high-risk treatment. On the other hand, all patients with hyperdiploidy and 7/9 patients with TEL/AML1 were already in the favorable treatment-group according to age and were treated as standard risk patients. Thus, the cytogenetic results did not contribute to therapeutic decision. From the cost-benefit point of view, introducing routine cytogenetic analysis for ALL seems not to be a priority in this setting compared to needs of early referral, treatment compliance, and follow-up. There are needs to focus financial support on improvement of basic laboratory facilities and training of non-hospital physicians for timely referral of children for further investigation, on improvement of supportive care and hospital facilities, and on expansion of the existing immunophenotyping for more precise diagnosis of ALL and follow-up. In a second step, introducing a routine FISH analysis in order to allow for better stratification within the HR and LR patient groups in order to change treatment approaches.

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