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IKAROS Gene Deleted B-Cell Acute Lymphoblastic Leukemia in Mexican Mestizos: Observations in Seven Patients and a Short Review of the Literature

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ABSTRACT

Background: In B-cell acute lymphoblastic leukemia, one of the most frequent cytogenetic alterations is the presence of the Philadelphia chromosome. Recently, newly identified genetic alterations have been studied, among them the *IKZF1* deletion. *IKZF1* encodes IKAROS, a zinc finger protein that plays an important role in hematopoiesis involving the regulation process of adhesion, cellular migration, and as a tumor suppressor. **Objective:** We aimed to study the impact of *IKAROS* deletion in the evolution and prognosis of B-cell acute lymphoblastic leukemia. **Materials and Methods:** At a single center we prospectively studied patients diagnosed with B-cell acute lymphoblastic leukemia and screened for *IKZF1* deletion using the multiplex ligation-dependent probe amplification method. We did a descriptive analysis of patients positive for the *IKZF1* deletion to determine its impact on the evolution of the disease and survival rate. **Results:** Between 2010 and 2015, 16 Mexican mestizo patients with B-cell acute lymphoblastic leukemia were prospectively screened for *IKZF1* deletion; seven (43%) were positive and were included for further analysis. The age range of patients was 13-60 years; six were males and one female. All cases had type B acute lymphoblastic leukemia. Of the seven patients, two died, three were lost to follow-up, and two continue in complete remission with treatment. Results are worse than those in a group of patients with non-mutated *IKAROS* B-cell acute lymphoblastic leukemia previously studied in our center. **Conclusions:** Although this is a small sample, the presence of *IKAROS* deletion in acute lymphoblastic leukemia patients could represent a poor-prognosis marker and was probably related to therapy failure. It is also possible that this variant of leukemia may be more prevalent in Mexico. More studies are needed to define the role of *IKZF1* deletion in acute lymphoblastic leukemia and the real prevalence of the disease in different populations. (REV INVES CLIN. 2015;68:210-4)

Key words: B-cell acute lymphoblastic leukemia. IKAROS. IKZF1.

INTRODUCTION

B-cell acute lymphoblastic leukemia (ALL) is a hematological malignant disorder characterized by clonal proliferation and tissue infiltration with lymphoid progenitor cells that can be presented in both children and adults[1,2]. The incidence of ALL decreases with age and represents 30% of all childhood cancers and 6% of all cancers in teenagers[3,4]. In almost all patients with B-cell ALL, structural chromosomal changes, such as translocations,

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inversions, and deletions, can be found[2]. One of the most common cytogenetic abnormalities in adults with ALL is the Philadelphia chromosome, representing 20-30% of all cases[5]. The fusion of *ABL1* gene in chromosome 9 to the *BCR* gene on region q11 in chromosome 22 results in the BCR-ABL tyrosine kinase activation and acts as an oncogene for ALL[6].

There is also a rare deletion in the *IKAROS* (*IKZF1*) gene involved in ALL that may increase the risk of relapse in this type of disease[6]. This gene regulates lymphocyte differentiation, is restricted to the fetal and adult hemo-lymphopoietic system, and is localized on 7p12.2[7]. *IKAROS* is involved in the process of multipotential hematopoietic stem cell differentiation into the three major hematopoietic lineages: erythroid, myeloid, and lymphoid[7]. The *IKAROS* dysfunction is linked to hematologic malignancies; loss of *IKAROS* activity would decrease the numbers of differentiating erythroid cells[8]. The loss of activity in *IKZF1* is determined by molecular mechanisms: deletions, intragenic deletions, or loss of the entire *IKZF1* gene[9].

Although *IKAROS* may function as a tumor suppressor gene, the exact mechanism for this activity remains unknown. However, this characteristic is related to target genes that improve different mechanisms such as a positive regulation in the B-cell and T-cell differentiation, downregulation of the Notch signaling pathway, negative regulation of cellular proliferation (that is given by c-Myc oncogene repression by *IKAROS* activation of p27 and downregulation of cyclin D3), and regulation of apoptosis (*IKAROS* may regulate Bcl-xL expression)[10-14]. The function of *IKAROS* may be regulated by ubiquitination, sumoylation, and phosphorylation[15].

The aim of this study was to conduct a descriptive analysis of patients with *IKZF1* deletion and to correlate this cytogenetic abnormality with the evolution of the disease and survival rate.

MATERIALS AND METHODS

We conducted a prospective study of patients diagnosed with B-cell ALL at a single center and screened for *IKZF1* deletion using multiplex ligation-dependent probe amplification (MLPA) method. Other translocations such as t(12;21), t(9;22)BCR-ABL, and t(1;19) were also screened for. MLPA is a multiplex PCR assay that identifies variations in the copy number of human genes using up to 40 probes specific for a different DNA sequence and is employed for the molecular diagnosis of genetic diseases. MLPA reaction involves five steps: (i) DNA denaturation and probes hybridization, (ii) ligation reaction, (iii) PCR amplification, (iv) separation of amplification products by electrophoresis, and (v) data analysis[16].

In the patients positive for *IKZF1*, different variables were analyzed: white blood cell (WBC) count at diagnosis, type of treatment, survival, and relapse. The variables were analyzed to make a descriptive study and correlate them with the impact of *IKAROS* on the evolution of the disease and the survival rate of the patients studied.

RESULTS

A total of 16 consecutive patients with ALL studied after 2010 were prospectively screened for the *IKZF1* deletions and seven (43%) were found to be positive. The descriptive analysis was focused on the latter patients. The age range of patients was 13-60 years; six were male and one female. The ALL type was B in all cases. Regarding the presence of other translocations, all patients were negative for t(12;21) and t(1;19), and only one was positive for t(9;22)BCR-ABL. In one patient, del(1)(q32), t(5;15), (q31;q22) was also found. Table 1 depicts patient characteristics and translocations. Analysis of the WBC count at diagnosis showed that five patients had leukocytosis and two, leukopenia. The treatment used was a modification of St. Jude Total Therapy XI[17], which consists of a combination of systemic chemotherapy and central nervous system-directed therapy. Currently, only two patients continue with the treatment; two patients achieved remission, two had relapse, three were lost to follow-up, and two have died. One patient was given dasatinib 50 mg/day in addition to the chemotherapy. Figure 1 shows the overall survival of the *IKAROS*-positive (n = 7) versus *IKAROS*-negative (n = 9) patients with B-cell ALL studied.

DISCUSSION

B-cell ALL is linked to dysfunctional IKAROS proteins; *IKAROS* is a key regulator to the homeostasis, development, and proliferation of normal lymphoid cells[18,19].

Table 1. Salient features of patients with deleted *IKZF1*

Patient	Age at diagnosis (years)	Diagnosis	*IKZF1*	White blood cell count at diagnosis	Other genetic alterations	Treatment	Relapse during treatment	Survival status
1	23	B-ALL	+	1.2	Chromosome 7 aneuploidy t(9;22) BCR-ABL	Chemotherapy	-	Lost at 334 days
2	18	B-ALL	+	21.3	del (1)(q32), t(5;15),(q31;q22), dup(6)(p12p25) add(17)(q22), t(7;15)(p15;q11.2)	Chemotherapy	+	Lost at 236 days
3	52	B-ALL	+	151.6	None	Chemotherapy	-	Lost at 55 days
4	23	B-ALL	+	1.9	None	HSCT	+ 11 months after treatment	Dead after 365 days
5	13	B-ALL	+	67.0	MLL gene (4 cell +)	Chemotherapy + dasatinib 50 mg/day	-	In treatment 243 days
6	58	B-ALL	+	94.4	MLL gene (7 cell +)	Allogeneic HSCT	-	Dead after 171 days
7	59	B-ALL	+	164.0	None	Chemotherapy	-	In treatment 38 days

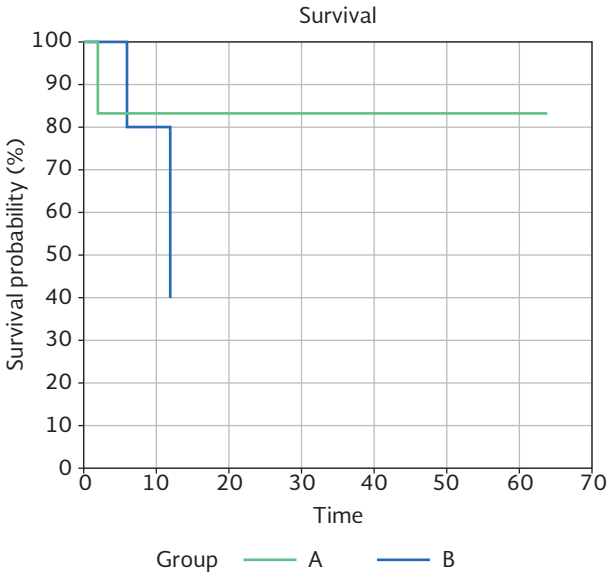
B-ALL: B-cell acute lymphoblastic leukemia; HSCT: hematopoietic stem cell transplantation.

In hematopoietic stem cells (HSC) some transcription factors mediate gene expression[20]. The *IKAROS* gene encodes a novel zinc finger with N-terminal DNA-binding protein and dimerization domains in C-terminal; it confers the *IKAROS* gene the ability to play an important role in the regulation of the lymphoid lineages development[7,20], particularly in hematopoiesis (stem cells, T and B lymphocytes, dendritic cells, natural killer cells)[21,22]. Both regions, N-terminal and C-terminal, have sub-regions that compound zinc fingers: the N-terminal has four amino terminal zinc fingers that regulate their binding to DNA; C-terminal has two zinc fingers that mediate the interaction with other type of IKAROS proteins (Fig. 2); an alternative splicing after transcription is involved in the creation of isoforms. These isoforms conserve in their structure the exons one and two, although in the end the total number of exons varies[7,21,23]. There are 11 isoforms as a result of the alternative splicing from which only IK-1, IK-2, IK-3, and IKX are functional, in fact, for their capacity to bind to DNA. IK-1 and IK-2 are localized in the nucleus, regulating lymphocyte differentiation, being the most important transcriptional factors in this process. In thymocytes, the presence of IK-4 is abundant, and IK-3, IK-5, and IK-6 are in smaller amounts. IK-5, IK-6, IK-7, and IK-8 have a poor activity to bind DNA, and are considered as dominant-negative[21-24].

IKAROS protein zinc finger is involved in the regulation of the process of adhesion and cellular migration; this process begins with the activation of genes and chemokine receptors controlling the adhesion and migration of pro-B-cells[25]. IKAROS plays an important role in hematopoiesis, as stated previously, mainly through the relationship between IKAROS and the nucleosome remodeling and deacetylase (NuRD) complex, which controls chromatin organization. IKAROS, CDK9, and the NuRD subunit Mi2 are factors that perform an important function during transcription elongation; studies demonstrate that the NuRD complex helps polymerase II progression during transcription elongation[26].

An alteration in *IKZF1* may change the proliferation process in lymphoid cells, and is linked to a poor

Figure 1. Overall survival of acute lymphoblastic leukemia patients with the *IKAROS* deletion (Group B) versus *IKAROS* non-deleted (Group A).



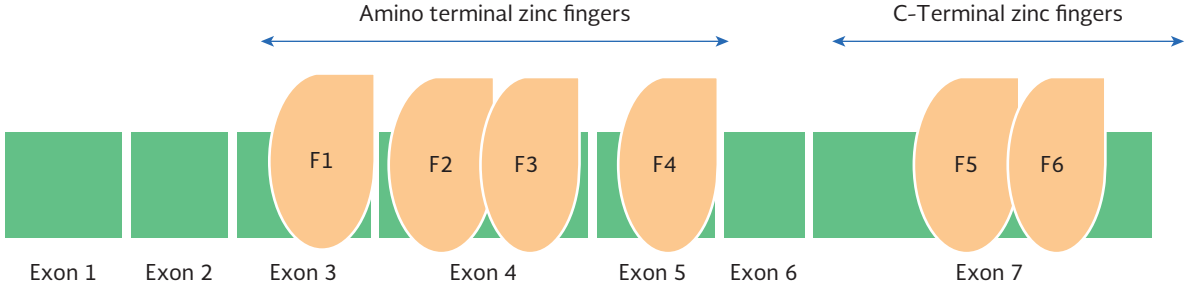
relapse-free survival in adult ALL. These changes in *IKZF1* expression are key to understanding and stratifying a therapeutic plan[27]. Other abnormalities that may modify the outcome and the therapy are the cytogenetic alterations, with the Philadelphia chromosome (Ph) being the worst prognostic factor[28]. The Ph chromosomal abnormality was the first one described in association with chronic myeloid leukemia (CML) and linked with malignant disease. It is the most characteristic structural chromosome alteration in CML, observed in 95% of these patients and in 15-30% of adults with ALL. Ph-positive ALL is related to a poor outcome in adults and in children, with a shorter remission and shorter survival. According to the literature, patients with Ph-positive ALL are older and can achieve complete remission with induction chemotherapy[29]. In this study, in only one patient we found a *IKZF1* deletion with Ph-positive with aneuploidy, but this patient was lost to follow-up. Chromosome gains or losses are not rare in ALL, although their incidence is higher in myeloid leukemias, the most observed abnormalities being the trisomy or monosomy[1].

In a group of 221 patients, Mullighan, et al.[30] found the *IKZF1* gene factor affected in 15% of B-cell ALL patients, and mutations of *IKZF1* were found in 70-80% of Ph-positive B-cell ALL, which the authors related with a poor outcome[15,30]. These data contrast with what we have observed in our study, in which 7/16 patients with B-cell ALL had a mutated form of *IKAROS*. Despite the low number of patients in our study, we can speculate that *IKAROS*-positive ALL may be more frequent in Mexico than in other populations.

The induction of complete remission and the event-free survival may be predicted by the leukocyte count: hyperleukocytosis (> 100 x 10^9/l) is associated with an increased risk of therapy failure[31]. Only two of our patients had hyperleukocytosis; one was lost to follow-up and the other patient is currently under treatment with chemotherapy. Although one patient had leukopenia, he relapsed and died.

The chemotherapy regimen used in all of our patients was a modification of the St. Jude Total Therapy XI. Although this therapy was originally employed for children, we have used it in adults with a favorable response[32]. Recent information suggests that patients with the *IKAROS* deletion and BCR-ABL-positive

Figure 2. Exon composition of *IKAROS*. The first four zinc fingers, which conform the N-terminal DNA binding domain and C-terminal, conformed by two zinc fingers that are in charge of the interactions with other types of *IKAROS*.



ALL may improve their prognosis by using tyrosine kinase inhibitors (TKI) during the maintenance therapy. We have employed dasatinib in one of the patients who is the one with the longest survival, both overall and relapse-free. It may be possible that ALL patients with the *IKAROS* deletion could benefit from the treatment with TKIs[6].

According to the literature, hematopoietic stem cell transplantation (HSCT) may cure some patients, even if the transplant is performed during the first remission or after relapse, showing a similar response and disease-free survival two years after the procedure[33]. In our group of patients, two were given an allogeneic HSCT; one of them relapsed and was treated with further chemotherapy for one year before his death, whereas the other patient was treated for six months and subsequently relapsed and died.

Although this is a study from a small sample, it could suggest that *IKAROS* gene deletion is an aggressive marker similar to BCR-ABL and that this deletion may be more frequent in Mexican mestizos. Larger studies are needed to define the role of the *IKZF1* deletion in B-cell ALL and its prevalence in different populations.

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