

Analysis of the presence of *FLT3* gene mutation and association with prognostic factors in adult and pediatric acute leukemia patients

Graciele Burnatt¹, Marley Aparecida Licínio¹, Pâmela Cristina Gaspar¹, Arthur Schweitzer Ferreira², Manoela Lira Reis¹, Ana Carolina Rabello de Moraes^{1,2}, Thaís Cristine Marques Sincero^{1,2}, Maria Cláudia Santos-Silva^{1,2*}

¹Postgraduate Program in Pharmacy, Department of Clinical Analyses, Health Sciences Centre, Federal University of Santa Catarina, Florianópolis, SC, Brazil, ²Department of Clinical Analyses, Health Sciences Centre, Federal University of Santa Catarina, Florianópolis, SC, Brazil

When the *FLT3* gene is mutated, it originates a modified receptor with structural changes, which give survival advantage and malignant hematopoietic cell proliferation. Thus, the presence of mutations in this gene is considered an unfavorable prognostic factor. A total of 85 consecutive samples of newly diagnosed untreated patients with AL were included in the study after they provided their informed consent. *FLT3* gene mutations were detected by PCR. For the pediatric group, a positive correlation was observed between WBC count and the presence of *FLT3-ITD* in patients with AML and ALL. Furthermore, children with AML who had the *FLT3-ITD* mutation showed a tendency to express CD34 in blast cells. In the adult group, the AML patients with *FLT3-ITD* who expressed CD34 in blast cells had a tendency to worse progression. The present data indicate no association between the prognostic factors evaluated and *FLT3* gene mutations in adult with AL. Yet, the presence of *FLT3-ITD* mutation was significantly related with WBC count in the pediatric group. These findings demonstrate that *FLT3* gene mutations can be considered as independent poor prognostic factors.

Uniterms: FLT3-ITD/mutation. Acute leucemia/prognostic factors. Acute leucemia/adults patients. Acute leucemia/pediatric patients. AML. ALL. *FLT3-D835*.

INTRODUCTION

Acute leukemia (AL) is a group of heterogeneous diseases characterized by fast and uncontrolled proliferation of hematopoietic progenitor cells in bone marrow, which eventually reach the peripheral circulation and can infiltrate other organic systems (McCulloch, 1982). In recent years, progress in the understanding of genetics and cell-signaling pathways has provided useful tools to stratify acute leukemia patients into different prognostic subgroups based on cytogenetic and molecular characteristics (Liersch *et al.*, 2014). Prognostic factors in malignant diseases are positively or negatively associated with one or more

outcome parameters. Thus, they prove to be useful in deciding upon appropriate therapy (Liersch *et al.*, 2014). According to current diagnostic criteria of the World Health Organization (WHO) (Swerdlow *et al.*, 2008), immunophenotyping, cytogenetics, and molecular genetic characteristics, among other prognostic correlations such as white-blood-cell (WBC) count, patient age, and percentage of blasts are related to prognosis and contribute to the stratification of AL (Swerdlow *et al.*, 2008). The risk stratification into favorable, intermediate, and adverse is based upon cytogenetic risks and molecular genetic alterations such as *FLT3*, *KIT*, *NPM1*, and *CEBPBA*. Among these, mutations on the gene that transcribes the FMS-like tyrosine kinase 3 (*FLT3*) receptor are very important because they are found in approximately 30% of patients with acute myeloblastic leukemia (AML) and correlated with a poor prognosis (Koh *et al.*, 2009).

*Correspondence: M.C. Santos-Silva. Departamento de Análises Clínicas. Centro de Ciências da Saúde. Universidade Federal de Santa Catarina. Campus Trindade - 88040-900 - Florianópolis - SC, Brasil. E-mail: maria.claudia.silva@ufsc.br

The *FLT3* gene is located in chromosome 13q12 and encodes a transmembrane protein that plays a crucial role in normal hematopoiesis (Baldus *et al.*, 2007). The two main types of mutation in this gene are an internal tandem duplication (*FLT3-ITD*) in exons 14 and 15, and a missense point mutation in codon 835 in exon 20 (*FLT3-D835*). Both mutations produce a constitutively active tyrosine-kinase receptor, despite the absence of its ligand, leading to disturbance in the normal hematopoiesis (Baldus *et al.*, 2007; Small, 2006; Gale, Green, Allen, 2008).

In this context, the objective of the present study is to evaluate the association between the presence of *FLT3* gene mutations in adults and children with AL and other prognostic factors or clinical progression.

PATIENTS AND METHODS

A total of 85 consecutive samples of bone marrow and/or peripheral blood were collected from patients

with AL. The subjects comprised adult patients from University Hospital of Federal University of Santa Catarina, Florianopolis, Brazil, and pediatric patients from Children Hospital Joana de Gusmão of Santa Catarina, Florianopolis, Brazil. The samples were collected and evaluated for the presence of *FLT3* mutations in the period of January 2012 to September 2014. All the patients were included in the study after they provided their informed consent.

Due to differences in outcome between children and adults with AL, patients were divided into pediatric and adult groups according to age. Patients below 16 years old were considered as belonging to the pediatric group, while patients 16 years old or more were included in the adult group. A total of 45 patients (22 females and 23 males; median age of 41 years, ranging from 16 to 87 years old) were included in the adult group, while 40 children (17 female and 22 male, median age of 4.5 years, ranging from one month to 15 years old) were included in the pediatric group (Table I).

TABLE I - Clinical and laboratory characteristics of patients diagnosed with LA at the time of diagnosis

Parameters	ADULT GROUP		PEDIATRIC GROUP	
	N (45)	%	N (40)	%
Gender				
Female	22	35.7	17	42.5
Male	23	64.3	23	57.5
Subtipo de leucemia				
AML	27	60.0	12	30.0
LLA	18	30.0	28	70.0
Age (years)				
Mediana	41.0		4.5	
Interval	16 – 87		0.1 - 15	
CD34				
Positive	28	62.2	35	87.5
Negative	17	37.8	05	12.5
WBC (x10³/mm³)				
Median	6.7		24.5	
Interval	0.4 – 288.7		2.0 - 450.0	
LDH (U/L)				
Median	327.5		967.0	
Interval	61 - 6.500		322 - 14.559	
Blasts (%)				
Median	38,0		84,5	
Interval	0,0 - 97,0		21.0 – 98.0	
FLT3 Mutation				
Positive	07		08	20.0
Negative	38		32	80.0

WBC - White-blood-cell; LDH – Lactate dehydrogenase; AL - Acute leukemia.

Diagnosis and subclassification of AL were based on morphological evaluation, immunophenotyping and molecular genetic studies [t(15;17), t(8;21), t(16;16) and t(9;22)] according to the criteria of the World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissue (Swerdlow *et al.*, 2008). Because many patients did not have karyotype request or because patient's samples did not have cell growth, it was not possible to access all karyotypes results from all patients included in this study. Thus, only the results of genetic alterations investigated by molecular biology were considered for AL classification. A total of 39 patients were diagnosed with AML (27 adults and 12 children) and 46 patients with acute lymphoblastic leukemia (ALL - 18 adults and 28 children). The distribution was as follows: AML (5 cases; 5.9%), AML without maturation (5 cases; 5.9%), AML with maturation (6 cases; 7.0%), AML with t(8;21) (1 case; 1.2%), acute promyelocytic leukemia with t(15;17) (14 cases; 16.5%), acute myelomonocytic leukemia (1 case; 1.2%), acute monoblastic leukemia (5 cases; 5.9%), acute monocytic leukemia (1 case; 1.2%), acute megakaryoblastic leukemia (1 case; 1.2%), B-derived ALL (35 cases; 41.2%), B-derived ALL with t(9;22) (2 cases; 2.3%) and T-derived ALL (9 cases; 10.5%).

For the analysis of correlation between the presence of *FLT3* mutations (ITD or D835) and age or WBC count, the adult and pediatric groups were divided according to prognosis. For the adult group, age above 50 years for AML and over 60 years for ALL were considered poor prognosis. Regarding WBC count, AML and ALL were considered of poor prognosis when counts were greater than $20.0 \times 10^3/\text{mm}^3$ and $10.0 \times 10^3/\text{mm}^3$, respectively. For the pediatric group, age above nine years and WBC count greater than $50.0 \times 10^3/\text{mm}^3$ were included in the poor prognosis group. Moreover, the association between the presence of *FLT3* gene mutations and the progression of patients after six months of diagnosis was also evaluated; the patients were divided into four groups: remission, relapse, death in remission, and death without remission.

Genomic DNA extraction

The peripheral blood was used only in the cases that WBC was above $20.0 \times 10^3/\text{mm}^3$ and perceptual of blast cells were about 20% at diagnosis. Due the fact that the method of detecting the *FLT3* mutation with polymerase chain reaction (PCR) has a limit of detection of approximately 1 in 100 cells (Murphy *et al.*, 2003), when the peripheral blood samples did not match this criterion, the bone marrow sample was used. DNA from peripheral

blood or bone marrow was obtained using the commercial QIAamp DNA kit (QIAGEN, USA). Briefly, 200 μL of the sample was mixed with 20 μL QIAGEN protease in a 1.5 mL microtube. Cells were lysed using 200 μL of AL buffer in the sample, homogenized for 15 seconds and incubated at 56 °C for 10 minutes. Absoluted ethanol (96-100%) was added and again the samples were homogenized for 15 seconds. The obtained solution was transferred to the QIAamp column and centrifuged at 6,000 x g for 1 minute. The filtrate was discarded and 500 μL AW1 wash solution was added and the column was centrifuged at 6,000 x g for 1 minute. The column was washed by adding 500 μL of a second wash buffer (AW2) and centrifuged at 20-20,000 x g for 3 minutes. The purified DNA was eluted from the spin columns with 100 μL of buffer AE or 100 μL of distilled water. Subsequently, the column was incubated for 5 minutes at room temperature and centrifuged at 6,000 x g for 1 minute. The obtained DNA was stored at -20 °C to be used in the next PCR reaction.

Detection of *FLT3*-ITD mutation

The Polymerase Chain Reaction (PCR) technique was employed to detect *FLT3*-ITD mutation. The primers 5'-GCAATTTAGGTATGAAAGCCAGC-3' and 5'-CTTTCAGCATTTTGACGGCAACC-3' used to amplify exons 14 and 15 of the *FLT3* gene were previously described by Meshinchi *et al.* (2001) (Meshinchi *et al.*, 2006). The PCR products were visualized in 12% polyacrylamide denaturing gel with ethidium bromide staining under 320 nm UV light transilluminator. The presence of ITD mutation in the *FLT3* gene was determined by visualization of a single band larger than 329 bp, wild-type gene fragment (homozygous mutation), or by the visualization of 329 bp wild-type gene fragment and one or more fragments larger than 329 bp (heterozygous mutation) as demonstrated in Figure 1. All PCR reagents were purchased from Invitrogen (Carlsbad, CA, USA).

Detection of *FLT3*-D835 mutation

The PCR technique followed by the restriction fragment length polymorphism (RFLP) technique described by Liang *et al.* (2003) were used to detect the *FLT3*-D835 mutation (Liang *et al.*, 2003). First, the region of exon 20 of the *FLT3* gene was amplified using specific primers (5'-CCGCCAGGAACGTGCTTG-3' and 5'-GCAGCCTCACATTGCC-3'). Next, the PCR products underwent enzymatic digestion with the enzyme *ECO*-RV. The digestion products were visualized in a 12% polyacrylamide gel with ethidium bromide staining under

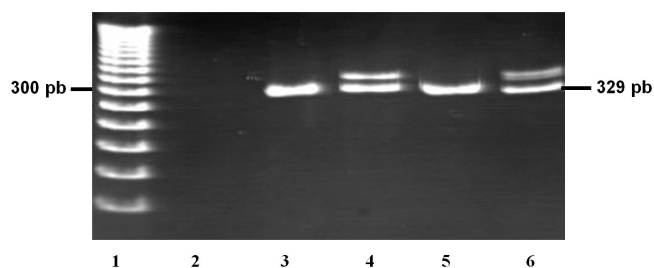


FIGURE 1 - PCR products of FLT3-ITD, analyzed by gel electrophoresis. Reverse view of ethidium-bromide-stained 12% polyacrylamide gel FLT3-ITD was detected as extra-bands in addition to normal band of 329 bp. Line 1- Molecular weight marker (50 bp); Line 2-Water control; Line 3-Patient sample wild type; Line 4- Patient sample with FLT3-ITD mutation; Line 5-wild type control; Line 6-) FLT3-ITD mutation control.

320 nm UV light transilluminator. The samples without mutation underwent complete enzymatic digestion, which resulted in the presence of two bands (68 bp/46 bp). Heterozygous samples for *FLT3-D835* mutation showed three bands (114 bp/68 bp/46 bp) after digestion, while homozygous samples showed a completely undigested band of 114 bp (Figure 2). All PCR reagents were purchased from Invitrogen (Carlsbad, CA, USA).

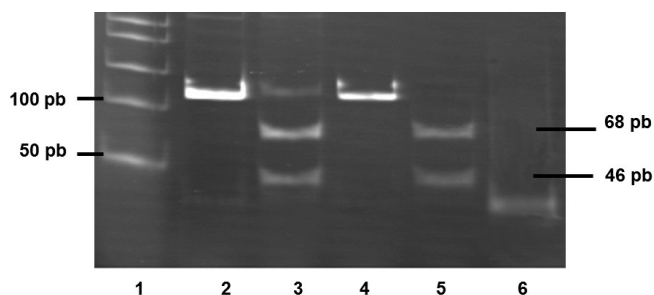


FIGURE 2 - PCR products of FLT3-D835, analyzed by gel electrophoresis. Reverse view of ethidium-bromide-stained 12% polyacrylamide gel FLT3-D835 was detected as extra-band after enzymatic digestion in addition to normal bands of 68 bp and 46 bp. Line 1- Molecular weight marker (50 bp); Line 2-PCR product of a patient sample with FLT3-D835 mutation before enzymatic digestion; Line 3-PCR product of a patient sample with FLT3-D835 mutation after enzymatic digestion ; Line 4-PCR product of a patient sample with wild type before enzymatic digestion; Line 5-PCR product of a patient sample with wild type after enzymatic digestion; Line 6-Water control.

Statistical analysis

The association between the presence of *FLT3* mutations and nominal variables such as gender, CD34 expression, WBC count at diagnosis, and patient outcome after six months of diagnosis were analyzed

using the Chi-square test. Numeric variables such as lactate dehydrogenase activity (LDH) and percentage of blasts were associated with the group of interest using Mann-Whitney U test. A p-value ≤ 0.05 was considered statistically significant for all analyses. Statistical analyses were done with the software SPSS (version 17.0).

RESULTS

During the period of this study, 85 samples of bone marrow and/or peripheral blood from patients with AL were evaluated for the presence of *FLT3* mutations.

As displayed in Table II, the *FLT3-ITD* mutation was observed in three patients in the adult group (6.7%) and *FLT3-D835* mutation in five patients (11.1%). In the pediatric group, *FLT3-IDT* mutation was observed in seven patients (17.5%) and *FLT3-D835* mutation in just one patient (2.5%).

According to the subtype in the adult group, it was observed that, of all cases of AML, 7.4% (two cases) and 14.8% (four cases) had *FLT3-ITD* and *FLT3-D835*, respectively. In adult patients with ALL, 5.6% (one case) had either *FLT3-ITD* or *FLT3-D835*. These results show that there was no correlation between AL subtype and presence of ITD or D835 mutations in adult patients.

The same was observed with the pediatric group, where 33.3% (four cases) of the patients with AML had the *FLT3-ITD* mutation, while 10.7% (three cases) of the patients with ALL showed the same mutation. Although the *FLT3-ITD* mutation was not associated with the AL subtypes (AML or ALL; $P=0.168$), it was observed that this mutation was more prevalent in children with AML. Mutation *FLT3-D835* was detected only in one child (8.3%) with AML.

Regarding gender, *FLT3* mutations were present in 20.8% (five cases) of males and 13.6% (three cases) of females in the adult group. In the pediatric group, 17.4% (four cases) and 23.5% (four cases) of males and females, respectively, showed *FLT3* mutations. No correlation was found between gender and the presence of *FLT3* mutations in either group.

As presented in the Table II, there was no correlation between the presence of *FLT3-ITD* or *FLT3-D835* and prognostic factors such as age, WBC count, percentage of blasts, CD34 expression, and LDH activity in the adult group. However, it is important to note that the patients who had *FLT3-ITD* mutation showed WBC count (median $29.4 \times 10^3/\text{mm}^3$) and LDH activity (median 875.0 U/L) above the upper reference values ($11.0 \times 10^3/\text{mm}^3$ and 190.0 U/L, respectively).

As demonstrated in Table III, for the pediatric group, a

TABLE II - Values of age, percentage of blasts, WBC count, LDH activity, CD34 expression, and progression of patients with AL according to the absence or presence of *FLT3* mutation and group

ADULT GROUP	Presence of <i>FLT3</i> mutation (n=7)		Absence of <i>FLT3</i> mutation (n=38)	
	Median	Limits	Median	Limits
Age (years)	41.0	25.0 - 73.0	41.0	15.0 - 87.0
Blasts (%)	50.0	27.0 - 96.0	46.0	0.0 - 98.0
WBC (x10 ³ /mm ³)	12.9	1.6 - 288.7	5.9	0.4 - 177.2
LDH (U/L)	875.0	259.0 - 5,189.0	300.0	61.0 - 6,500.0
	N	%	N	%
CD34 Expression	4	57.2	24	63.1
Death	3	42.8	18	47.4
Remission	4	57.2	20	52.6
PEDIATRIC GROUP	Presence of <i>FLT3</i> mutation (n=8)		Absence of <i>FLT3</i> mutation (n=32)	
	Median	Limits	Median	Limits
Age (years)	6.5	0.10 - 14.0	4.0	0.3 - 14.0
Blasts (%)	83.0	80.0 - 96.0	83.0	21.0 - 98.0
WBC (x10 ³ /mm ³)	51.3	27.5 - 220.0	14.8	2.0 - 450.0
LDH (U/L)	1,227.0	786.0 - 13,087.0	955.0	322.0 - 14,559.0
	N	%	N	%
CD34 Expression	7	87.5	28	87.5
Death	5	62.5	8	25.0
Remission	3	37.5	24	75.0

WBC - White-blood-cell; LDH – Lactate dehydrogenase; AL - Acute leukemia.

positive correlation was observed between WBC count and the presence of *FLT3-ITD* in patients with AML ($p=0.007$) and ALL ($p=0.005$). Furthermore, children with AML who had the *FLT3-ITD* mutation showed a tendency to express CD34 ($p=0.078$). Similar to the adult group, children who had *FLT3-ITD* mutation showed LDH activity (median 1,292.0 U/L) above the maximum reference value. There was no correlation between *FLT3-D835* mutation and the prognostic factors for the pediatric group. The association between the presence of *FLT3* gene mutations and the patient progression after six months of diagnosis was also evaluated. In the adult group, the AML patients with *FLT3-ITD* who expressed CD34 tended to worse progression ($p=0.067$). Regarding the pediatric group, there were no significant correlations between patient progression and presence of *FLT3* gene mutations.

DISCUSSION

Treatment of patients with *FLT3* mutation represents a great clinical challenge. Patients with these mutations carry a particularly poor prognosis, with greater relapse rate, and inferior overall survival when compared to

patients with AL without this mutation (Taketani *et al.*, 2004; Fedders *et al.*, 2015; Wellmann *et al.*, 2015). Given the poor prognosis of patients with AL and *FLT3* mutation, mainly *FLT3-ITD*, it has become a common practice to do allogeneic bone marrow transplantation during first remission in patients with these mutations. However, there remains a significant relapse rate following transplant for *FLT3-ITD*, and it is possible that maintenance therapy with *FLT3* inhibitors will reduce the recurrence rates (Taketani *et al.*, 2004; Bornhauser *et al.*, 2007; Schlenk *et al.*, 2008; Dezern *et al.*, 2011; Hassanein *et al.*, 2016). Recently, *FLT3* inhibitors have emerged as a new option of treatment for these patients. Several *FLT3* inhibitors have been tried as monotherapy or combined with other chemotherapeutic agents (Hassanein *et al.*, 2016).

Nevertheless, it is still unclear how and when these agents should be used during AML treatment (Hassanein *et al.*, 2016). Because of the impact of *FLT3* mutation on prognosis and treatment protocols, the guidelines from various major organizations, such as National Comprehensive Cancer Network and European LeukemiaNet, recommend *FLT3* mutation analysis on diagnosis.

TABLE III - Association between prognostic factors and presence of *FLT3-ITD* and *FLT3-D835* mutations in adults with acute leukemia

	LMA (n=27)		LLA (n=18)	
	<i>FLT3-ITD</i> (P values)	<i>FLT3-D835</i> (P values)	<i>FLT3-ITD</i> (P values)	<i>FLT3-D835</i> (P values)
Age ^a	0.430	0.608	0.167	0.500
WBC count at diagnosis ^a	0.156	1.000	0.444	0.444
% Blasts	1.000	0.612	0.111	0.667
CD34 expression	0.091	0.609	0.111	1.000
LDH	0.271	0.157	0.556	0.222

WBC - White-blood-cell; LDH – Lactate dehydrogenase; ^a For this analysis the variables age and WBC count at diagnosis were divided into two groups according to prognosis, as follows: Age - poor prognosis is ≥ 50 years old for AML and ≥ 60 years old for ALL, good prognosis is < 50 years old for AML and < 60 years old for ALL; WBC - poor prognosis is $\geq 20,000/\text{mm}^3$ for AML and $\geq 10,000/\text{mm}^3$ for ALL, good prognosis is $< 20,000/\text{mm}^3$ for AML and $< 10,000/\text{mm}^3$ for ALL. * $P \leq 0.05$ is considered to be statistically significant.

TABLE IV - Association between prognostic factors and presence of *FLT3-ITD* and *FLT3-D835* mutations in children with acute leukemia

	LMA (n=12)		LLA (n=28)	
	<i>FLT3-ITD</i> (P values)	<i>FLT3-D835</i> (P values)	<i>FLT3-ITD</i> (P values)	<i>FLT3-D835</i> (P values)
Age ^a	0.295	0.417	0.904	-
WBC count at diagnosis ^a	0.007*	1.000	0.005*	-
% Blasts	0.093	0.167	0.856	-
CD34 expression	0.078	1.000	0.124	-
LDH	0.111	0.667	0.514	-

WBC - White-blood-cell; LDH – Lactate dehydrogenase. ^a For this analysis, the variables age and WBC count at diagnosis were divided into two groups according to prognosis, as follows: Age - poor prognosis is ≥ 9 years old, good prognosis is < 9 years old; WBC: poor prognosis is $> 50,000/\text{mm}^3$ for AML and ALL; LDH – lactate dehydrogenase. * $P \leq 0.05$ is considered to be statistically significant.

Worldwide, the reported incidence of *FLT3-ITD* in adults with AML is 15-35% (Nakao *et al.*, 1996; Abu-Duhier *et al.*, 2000; Kottaridis *et al.*, 2001; Xu *et al.*, 2005; Amir, Yi-Bin, 2011). Additionally, the *FLT3-D835* mutation incidence is 8-12% in AML cases (Small, 2006). Lucena-Araujo *et al.* demonstrated that the incidence of *FLT3-ITD* in Brazilian patients with AML is similar to the other countries, but the *FLT3-TKD* is lower. This reinforces the idea that there are geographic differences in *FLT3* mutation frequency. Considering that Brazil has continental proportions and multiethnic population, it is expected that the incidence of these mutations are different among Brazilian regions. The incidence and clinical relevance of *FLT3* mutations in AL patients from Santa Catarina are unknown, and it is important to create regional data to establish public health policies that

improve AL with *FLT3* mutation treatments in this region (Lucena-Araujo *et al.*, 2010).

The results of this study showed a lower frequency of *FLT3-ITD* mutation (7.4%). This discrepancy can be explained by one of the two facts: i) the small number of patients included in the study, or ii) the intrinsic characteristics of the population studied. Furthermore, it should be noted that, as mentioned by Bao *et al.* (2006), the incidences reported in other studies might be overestimated because the mutation analysis was usually performed on archived samples from patients who usually have leukocytosis, which is generally present in AML carrying *FLT3* mutations (Bao *et al.*, 2006). On the other hand, the incidence of the *FLT3-ITD* mutation in adults with ALL described in other studies (5 - 10%) is similar to that found in the present study (5.6%) (Kottaridis *et*

al., 2001; Amir, Yi-Bin, 2011). However, because only one case was identified in this study, it was difficult to assess properly the relation between this mutation and the prognostic factors.

The incidence of *FLT3-ITD* in children with AML is lower than in adults (5-16%) (Gale, Green, Allen, 2008). Regarding this aspect, the results obtained in this study showed higher percentages (33.3%). To the best of our knowledge, Al-Tonbary *et al.* (2009) conducted the only available study on the presence of *FLT3-ITD* in children with ALL. Unlike the present study, which found an incidence of 10.7%, Al-Tonbary *et al.* reported that children with ALL did not show the *FLT3-ITD* mutation. As in the case of the adult group, this difference can be explained by the small number of patients included in this study and by the intrinsic characteristics of the population studied.

In this study, *FLT3-D835* incidence rate was 14.8% in AML cases, which is higher than the rates reported in other works (Small, 2006; Lucena-Araujo *et al.*, 2010). In ALL cases, our results showed *FLT3-D835* frequency of 5.6%, with contrast with the literature results, that demonstrated a frequency of 1-3% (Abu-Duhier *et al.*, 2001; Yamamoto *et al.*, 2001; Thiede *et al.*, 2002).

Concerning the *FLT3-D835* mutations in the pediatric group, there was only one case of AML (8.3%) and no ALL case positive for the mutation. The frequency of this mutation in AML children is in agreement with that mentioned in other pieces of research, which report it as rare (7 - 10%) (Meshinchi *et al.*, 2006; Markova *et al.*, 2009). However, the reported incidence of *FLT3-D835* in ALL children is 7.5% (Wang *et al.*, 2010), which is higher than that found in the present study. Based on the incidences described for *FLT3-ITD* and *FLT3-D835* mutations, a variation was observed among studies, which indicates that there are differences in the frequency of these mutations according to geographical areas.

In relation to the distribution of *FLT3* mutations according to gender, the present study is consistent with other findings where there is no significant correlation between these two variables (Al-Tonbary *et al.*, 2009; Karabacak *et al.*, 2010).

The prevalence of *FLT3* mutations increases with age, but the presence of this mutation seems to have less impact as a prognostic factor in patients above 60 years old than other adverse prognostic factors (Foran, 2010). In this study, no relation was observed between patient age and the presence of *FLT3* mutations in either group, which is in agreement with other studies, which also found no association between these variables (Parcell *et al.*, 2006; Wang *et al.*, 2010).

WBC count, LDH activity, and cytogenetic abnormalities at diagnosis are important prognostic factors (Stone, 2009), as are the mutations in the *FLT3* gene (Betz, Hess, 2010). The findings related to whether there is a correlation between the mutations in *FLT3* gene and WBC count are controversial. While some groups reported an association between these two variables in AML patients (Kottaridis *et al.*, 2001; Thiede *et al.*, 2002; Emerenciano *et al.*, 2008; Peng *et al.*, 2008; Wang *et al.*, 2010), others found no such relationship (Schlenk *et al.*, 2008; Karabacak *et al.*, 2010). According to the classification of genetic abnormalities, mutations in the *FLT3* gene are considered class I mutations, which confer a survival advantage and increase cell proliferation (Betz, Hess, 2010). This may explain why so many studies found an association between mutations in the *FLT3* gene and WBC count. In the present study, this relationship was not statistically significant in the adult group, but it was observed that the total WBC count was higher for patients who had the *FLT3-ITD* mutation. In the pediatric group, a significant association was observed between high WBC and presence of *FLT3-ITD* mutation.

In patients with AL, an increase in LDH activity is considered a poor prognostic factor (Varma, Varma, 2008). A study with adult AL patients showed an association between the presence of *FLT3* gene mutations and increase in LDH activity (Peng *et al.*, 2008). However, this same observation was not found in another study (Karabacak *et al.*, 2010), as well as in the present one. Nevertheless, in both the adult and the pediatric groups, patients with *FLT3* gene mutations showed higher LDH activity (875.0 U/L and 1,227.0 U/L, respectively) than the highest reference limit (190.0 U/L).

Although the patients with *FLT3* gene mutations included in this study had high percentages of blast cells in both groups, as reported by Liu *et al.* (2007), the presence of *FLT3* gene mutations and percentage of blasts were not statistically related.

Another relevant prognostic factor is the CD34 expression in blast cells. A study by Zhu *et al.* (2013) showed that CD34 expression is an *FLT3-ITD*-independent predictor of poor prognosis. In contrast, other studies reported an association between *FLT3-ITD* mutation and the presence of the CD34 marker in AML patients (Barragán *et al.*, 2011; Zhu *et al.*, 2013). In the present study, no correlation between *FLT3* gene mutations and CD34 expression were found. However, four of the five positive patients for *FLT3-D835* expressed CD34.

Several studies demonstrated that the presence of *FLT3-ITD* in AML patients might indicate unfavorable disease course including higher death rate and shorter

disease-free survival clearly associated with leukocytosis. These findings classify *FLT3-ITD* as a poor-prognostic mutation (Zhu *et al.*, 2013). Mathews *et al.* (1991) suggest that there is an inverse relationship between the rate of molecular remission and patients with the presence of *FLT3-ITD* mutation. In the present study, no association was found between the presence of *FLT3-ITD* mutation and a poor outcome (death or relapse) in adult patients. Nevertheless, the AML pediatric group showed a tendency of association between *FLT3-ITD* and death, which characterizes it as an indicator of poor prognosis. Studies have not shown a significant relation between *FLT3-D835* mutation and complete remission, disease-free survival, or overall survival (Mathews *et al.*, 1991; Wang *et al.*, 2010). These findings are consistent with the results presented in this study.

CONCLUSIONS

In conclusion, the present analysis showed that there is no association between the evaluated prognostic factors such as age, LDH activity, and CD34 expression and *FLT3* gene mutations in adult and pediatric patients with AL. Nevertheless, the presence of *FLT3-ITD* mutation was significantly related to WBC count in the pediatric group. These results suggest that the *FLT3* gene mutations are independent poor prognostic factors.

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