RESEARCH COMMUNICATION

Flow Cytometry Results at Diagnosis and Relapse in Childhood Acute Lymphoblastic Leukemia

Ibrahim Bayram[1], Fatih Erbey[1*], Mustafa Kömür[1], Filiz Kibar[2], Atila Tanyeli[1]

Abstract

Introduction: Several studies have focused on the immunophenotype of the leukemic population at the time of relapse compared to that observed at diagnosis. Objectivew: The question of whether differences exist between surface antigens levels on blasts at the time of diagnosis and at relapse in cases of acute lymphoblastic leukemia (ALL) was addressed. Materials and Methods: A total of 25 All patients were included. Flow cytometry and fluorescein-isothiocynate conjugated antibodies were used to determined surface antigens levels. Results: The most frequently detected five antigens were I2 (n=21), CD10 (n=17), CD41 (n=16), CD2 (n=14) and CD7/CD19 (n=13/n=13) at the time of diagnosis and CD41 (n=21), I2 (n=20), CD10 (n=14), CD19 (n=16) and CD2 (n=12) at the time of relapse. There was a significant difference only between CD41 levels at the time of diagnosis and at the time of relapse (p=0.041). Conclusion: We found changes in antigen expressions at the time of relapse in ALL patients. This condition ought to be evaluated with reference to prognosis of leukemia.

Keywords: Acute lymphoblastic leukemia - leukemia - childhood

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Introduction

Surface antigens on leucocytes are defined as cluster of differentiation (CD) antigens and these antigens can be detected by using monoclonal antibodies (MoAb) (Knapp et al., 1989; Campana and Behm, 2000). Acute lymphoblastic leukemia (ALL), depending antigen expressions, is divided into T-cell, B-cell, pre-B-cell and early pre-B-cell ALL (Greaves, 1981). Besides, acute leukemic blasts may occasionally have features that belong to more than one origin (lymphoid and myeloid). These leukemias are called acute mixed lineage, hybrid, chimeric or biphenotypic leukemia (Ben-Bassat and Gale, 1984; Campana and Behm, 2000; Margolin et al., 2002). According to another immunological classification, ALL is classified into myeloid antigen positive ALL (My+ALL) and lymphoid antigen positive AML (Ly+AML). According a classification system developed by Catovsky et al. (1991), CD13, CD14 and CD33 were considered important myeloid antigens. Now, CD3, CD5, CD7 are used for the detection of T-cell leukaemia and CD10, CD19 and CD22 for the detection of early B-cell leukemia (Margolin et al., 2002).

About 20-30% of the children with ALL remitting after initial chemotherapy develops relapses later (Silverman and Sallan, 2003). Original immunotypes and karyotypes may or may not change in cases of relapsing leukemias (Greaves et al., 1980; Lobato-Mendizabal and Ruiz-Arguelles, 1990; Abshire et al., 1992).

Using flow cytometric investigations, we attempted to determine whether there were differences between surface antigen levels on blasts at the time of diagnosis and at relapse in cases of acute lymphoplastic leukemia.

Patients and Methods

This study included 25 patients who were diagnosed with ALL in the Department of Pediatric Oncology, Çukurova University School of Medicine, and who had relapsed later. All patients had the results of flow cytometric investigations. Evaluation of morphologic features was based on the French-American-British (FAB) classification. The patients received the treatment protocol of modified BFM 85-90-95 (Seidemann et al., 2001; Buhrer et al., 1994; Henze et al., 1991; Schrappe et al., 2000; Möricke et al., 2005).

This study was approved by the local ethics committee and all parents provided written informed consent.

Flow Cytometric Analyses

Leukemic blasts were obtained from bone marrow aspirate. Cell surface antigens were detected with flow cytometry (FCM) (Becton Dickinson FAC Scan, Lysis 1 Program) and Fluorescein-isothiocyanate (FITC) conjugated antibodies.

Antigens with a MoAb percentage of over 20% were considered positive. B cell antigens were considered as CD19, CD10, CD20, CD24 and surface (SIg) and

*[1]Division of Pediatric Oncology, Cukurova University Medical Faculty, [2]Central Laboratories of Cukurova University Medical Faculty, Adana, Turkey. *For correspondence : erbeyfa@gmail.com*

cytoplasmic (CyIg) immunoglobulins; T cell antigens as CD2, CD7, CD1a, surface and cytoplasmic CD3 and CD5; myeloid antigens as CD13, CD14, CD33, CD65w, CD11b and CD15; lineage-nonspecific markers as CD34, HLA DR and TdT.

Statistical Analyses

Statistical analyses were made with SPSS 10.01 (statistical package program for social sciences). Data were analysed with Chi-square, Mann-Whitney U test, Kaplan-Meier and Spearman correlation tests. $p<0.05$ was considered significant. EFS rate: At any given time, the proportion of the patients that has survived without relapse, from the time of diagnosis. OS rate: At any given time, the proportion of patients who has survived and has been followed from the time of diagnosis. DFS rate: At any given time, the proportion of patients who showed no signs of relapse after complete remission.

Results

Out of 25 children included into the study, 15 (60%) were boys and ten (40%) girls and 15 (60%) had ALL-L1, eight (32%) ALL-L2 and two (8%) ALL-L3. Twenty-two cases had one relapse and three cases had two relapses. Treatment offered after relapse was rejected by parents in four cases (16%). Out of all cases, 96% died. One was being followed now.

Fourteen patients had CD2, thirteen CD7, thirteen CD19, four CD20, eleven CD13, one CD33, sixteen CD41, two CD25, seventeen CD10 and twentyone I2 positivities at the time of diagnosis. At the time of relapse, twelve cases had CD2, eleven CD7, sixteen CD19, four CD20, ten CD13, one CD14, twentyone CD41, five CD25, fourteen CD10 and twenty I2 positivities. There was a significant difference only between CD41 at the time of diagnosis and relapse ($p=0.041$) (Table 1). A total of 28 antigens were negative at the time of diagnosis, but turned into positive at the time of relapse: CD2 in five cases, CD7 in three cases, CD 19 in five cases, CD20 in two cases, CD13 in four cases, CD14 in one case, CD41 in four cases, CD10 in two cases and I2 in one case. Besides, a total of 38 antigens were positive at the time of diagnosis but later became negative at the time of relapse: CD2 in nine cases, CD7 in seven cases, CD19 in three cases, CD20 in two cases, CD13 in seven cases, CD14 in one case, CD33 in one case, CD41 in one case, CD10 in five cases and I2 in two cases (Table 2).

Flow cytometric investigations revealed that antigen levels determined at the time of diagnosis increased or decreased by 10% at the time of relapse. In fact, CD2 levels increased or decreased in 15 cases, CD7 in 13 cases, CD19 in 12 cases, CD20 in four cases, CD13 in twelve cases, CD14 in one case, CD33 in one case, CD41 in 16 cases, CD56 in two cases, CD10 in 17 cases and I2 in 13 cases (Table 3). Antigens changed at the time of relapse saw in Table 4.

There was no significant relation between CD2, CD7, CD19, CD20, CD13, CD14, CD33, CD25, CD56, CD41, CD10 and I2 positivity and OS, time to relapse and DFS ($p>0.05$). The relation between T-cell antigen positivity (CD2/CD7), B-cell positivity (CD19/CD20), myeloid antigen positivity (CD13/CD14/CD33) and OS, time to relapse and DFS was not significant, either ($p>0.05$ [Log Rank, Breslow, Tarone-Ware test]). However, there was a significant relation between early pre-B antigen positivity (CD10) and time to relapse and DFS (Breslow test [$p=0.0334$, $p=0.0284$ respectively]), though there was no

Table 1. Distribution of Antigen Expression on Flow Cytometry

	At the Time of Diagnoses					At the Time of Relapses					
	n	Minimum	Maximum	Mean	Standard deviation	n	Minimum	Maximum	Mean	Standard deviation	P
CD2	25	,12	92,9	34,8	30,1	28	2,20	97,30	29,9	30,9	,498
CD7	24	,40	99,4	34,2	30,0	28	,39	96,60	27,0	30,2	,279
CD19	25	,50	94,9	36,8	35,4	28	,54	97,70	47,3	39,8	,637
CD20	25	,16	69,0	12,5	16,7	28	,02	72,00	8,8	15,3	,115
CD13	25	,00	62,6	17,5	19,6	28	,07	81,80	22,4	26,7	,556
CD14	25	,00	2,6	,4	,7	28	,00	54,50	3,0	10,5	,591
CD33	25	,00	22,0	1,8	4,7	28	,00	18,80	2,0	4,1	,580
CD41	25	,30	82,4	31,4	21,2	28	,00	90,10	46,0	27,5	,041
CD56	25	,00	16,4	3,3	4,7	26	,00	12,00	1,8	2,6	,130
CD25	21	,04	40,0	10,0	11,4	28	,00	43,70	9,2	13,8	,163
CD10	23	,50	90,0	42,1	28,9	26	1,10	97,60	42,5	37,6	,952
I2	23	,34	99,3	63,2	27,3	24	2,60	98,60	57,7	31,6	,632

Table 2. Distribution of Antigens Positivity at the Time of Diagnosis and Relapse

		CD2	CD7	CD19	CD20	CD13	CD14	CD33	CD41	CD56	CD25	CD10	I2
		n %	n %	n %	n %	n %	n %	n %	n %	n %	n %	n %	n %
At the Time of initial diagnosis	Negative	14 56	13 54,2	13 52	4 16	11 44	- 	1 4	16 72,7	- 	2 10	17 73,9	21 91,3
	Positive	11 44	11 44	12 48	21 84	14 56	25 100	24 96	6 27,3	25 100	18 90	6 26,1	2 8,7
At the Time of Relapse	Negative	12 42,9	11 39,3	16 57,1	4 14,3	10 35,7	1 3,6	- 	21 77,8	- 	5 18,5	14 53,8	20 83,3
	Positive	16 57,1	17 60,7	12 42,9	24 85,7	18 64,3	27 96,4	28 100	6 22,2	26 100	22 81,5	12 46,2	4 16,7

Table 3. Antigenic Changes in Blastic Cells

Differences between antigen levels at the time of diagnoses and at the time of relapses	CD2 n	CD7 n	CD19 n	CD20 n	CD13 n	CD14 n	CD33 n	CD41 n	CD56 n	CD25 n	CD10 n	I2 n
An increase by 10%	4	4	5	1	5	1	0	13	0	0	11	6
A decrease by 10%	11	9	7	3	7	0	1	3	2	0	6	7
Total	15	13	12	4	12	1	1	16	2	0	17	13

Table 4. Antigen Positivity or Negativity at the Time of Diagnosis and Relapse

	CD2 n	CD7 n	CD19 n	CD20 n	CD13 n	CD14 n	CD33 n	CD41 n	CD56 n	CD25 n	CD10 n	I2 n
Antigens negative at the time of diagnosis but became positive at the time of relapse	5	3	5	2	4	1	0	4	0	1	2	1
Antigens positive at the time of diagnosis but became negative at the time of relapse	9	7	3	2	7	1	1	1	0	0	5	2
Total	14	10	8	4	11	2	1	5	0	1	7	3

significant relation between early pre-B antigen positivity and OS (p>0.05 [Log Rank, Breslow, Tarone-Ware test]).

Discussion

There are several explanations of antigenic differences in relapse cases of ALL. First, considering that the lineage at relapse belongs to original biclonal leukemia and chemotherapy has eradicated this clone, the other clone grows and causes relapse. Second, chemotherapy modifies antigenic expressions in the leukemic clone and may cause potential myeloid or lymphoid differentiation to become permanent. Besides, lineage branch differentiates and may cause secondary leukemia (Abshire et al., 1992).

In a study by Tomova and Babusikova (2001) on 90 cases of ALL and 66 cases of AML (156 children and adults), 58 patients developed relapses (45 cases of B-cell ALL) and 32 patients (71%) had the same immunological markers at the times of both diagnoses and relapses. There were changes in markers of 13 cases (29%) and out of 13 cases, 10 had common ALL, one precursor ALL and two pre B-cell ALL. Thirty-one cases had minor phenotypical changes such a loss of markers at the time of initial diagnoses and appearance of the markers at the time of relapses which were absent before and CD34 most frequently disappeared. In fact, 51% of the B-cell ALL cases had CD34 at the time of initial diagnoses, but CD34 significantly decreased at the time of relapses. Only five cases (21.7%) had CD34+ in their original blasts. CD10 disappeared in seven cases, two cases had precursor B-cell ALL antigen and HLA-DR disappeared in two cases. Four cases turned out to have CD19 at the time of relapses, though they did not have it at the time of initial diagnoses. Three out of 20 T-cell ALL cases had relapses and two of them had minor decreases in CD1 and CD2 and the other had B-cell related CD10, myeloid CD13 and CD34, a non-lineage specific antigen.

Guglielmi et al. (1997), found that 39 out of 103 cases of B lineage ALL had a phenotypic change at first relapse (38%), compared to 20/25 of T lineage ALL (80%). The CD10 antigen was acquired at relapse in seven cases and lost in eight. By contrast, TdT, HLA-DR, CD19, SmIg and CD7 rarely changed at relapse. CD34 was positive at diagnosis in 23/33 B lineage ALL and in 5/7 T lineage ALL tested. At relapse, CD34 became negative in four B lineage ALL and in one T lineage cases, while it became positive in three B lineage ALL. CD10 was lost at relapse in 1/4 CD10-positive T-ALL and acquired in 2/21 CD10-negative cases. HLA-DR was acquired in 2/25 HLA-DR-negative cases. All T lineage ALL were TdT and CD3 positive at diagnosis. Two became TdT negative at relapse, but they did not observe any T-ALL lacking cytoplasmic CD3 at relapse. Two of the five cases classified as My^+ALL at diagnosis lost the myeloid antigens (CD13 and/or CD33) at relapse. CD13 and/or CD33 were acquired in 12/99 cases of B lineage ALL and in none of the 24 cases of T lineage ALL.

In current study, the most frequently detected five antigens were I2, CD10, CD41, CD2 and CD7/CD19 at the time of diagnosis and CD41, I2, CD10, CD19 and CD2 at the time of relapse. Flow cytometric investigations revealed that antigen levels determined at the time of diagnosis increased or decreased by 10% at the time of relapse. In fact, CD2 levels increased or decreased in 15 cases, CD7 in 13 cases, CD19 in 12 cases, CD20 in four cases, CD13 in twelve cases, CD14 in one case, CD33 in one case, CD41 in 16 cases, CD56 in two cases, CD10 in 17 cases and I2 in 13 cases.

Tuset et al., (2001), noted that CD52 expression disappeared at the time of relapses in two cases of T-cell leukemia and emphasized that disappearance of CD52 expression played an important role in resistance to treatment. Hashimoto et al., (2002) showed increased CD56 expression and decreased CD13, CD7 and CD3 expressions at the time of relapse in a case of T lymphocytic leukemia. It has been reported from other studies that mature B-cell ALL has poorer prognosis than earlier B-cell subgroups of ALL and that there is no difference between pre-B cell (cIg^+) and early pre-B cell (cIg^-) leukemia in their prognosis, but that ALLs with CALLA and CD34 positivity have better prognosis than B-cell ALLs (Greaves et al., 1981 Crist et al., 1984).

In our study, there was no significant relation between CD2, CD7, CD19, CD20, CD13, CD14, CD33, CD25,

CD56, CD41, CD10 and I2 positivity and OS, time to relapse and DFS. The relation between T-cell antigen positivity (CD2/CD7), B-cell antigen positivity (CD19/CD20), myeloid antigen positivity (CD13/CD14/CD33) and OS, time to relapse and DFS was not significant, either. However, there was a significant relation between early pre-B antigen positivity (CD10) and time to relapse and DFS, though there was no significant relation between early pre-B antigen positivity and OS.

Antigens in ALL cases vary with the predominant immunophenotypical feature at the time of initial diagnoses and/or chemotherapeutic drugs. In fact, we also found changes in antigen expressions at the time of relapse. There have been few studies in the literature on changes in antigen expressions on blasts and their relation with prognosis (Ciudad et al., 1999; Oelschlagel et al., 2000; Tomova and Babusikova, 2001; Tuset et al., 2001; Hashimoto et al., 2002; Cotter et al., 2003). We believe that further studies with larger sample sizes will shed light on the prognosis and treatment of ALL, and this condition ought to be evaluated in prognosis of leukemia.

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