RESEARCH COMMUNICATION

Myeloid Antigen Positivity in Turkish Children With Acute Lymphoblastic Leukemia Lacks Influence on Prognosis

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Abstract

Introduction: Several studies have suggested that the presence of myeloid antigens is a poor prognostic factor in patients with acute lymphoid leukemia (ALL). Objective: We aimed to assess this possibility in Turkish patients. Materials and Methods: Seventy-three children with a diagnosis of ALL-L1 and 38 with ALL-L2 were included. Flow cytometry and fluorescein-isothiocynate conjugated antibodies were used to determined surface antigens on blasts. Results: Myeloid antigens were positive in 48.4% with ALL-L1 and 60.5% with ALL-L2, the difference not being significant. Overall survival rates of myeloid antigen positive patients at 36, 60, and 72 months were 76%, 58%, and 48%, respectively, comparable to the corresponding 70%, 56%, and 46% in myeloid antigen negative patients (p >0.05). Conclusion: We did not find any association between myeloid antigen positivity and clinical and laboratory features of ALL.

Keywords: Acute lymphoblastic leukemia - myeloid antigen - childhood

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Introduction

Leukemic blasts in acute lymphoid leukemia (ALL) may have immunological features of both the myeloid and lymphoid lineages, which is called "acute mixed lineage", "hybrid", "chimeric" or "biphenotypic leukemia" (Margolin et al., 2002; Campana and Behm, 2000; Ben-Bassat and Gale, 1986). Another immunological definition for such cases is "myeloid antigen positive ALL" (My+ALL). Myeloid antigens including CD13, CD14, CD15 or CD33 may be detected in 5-10% of childhood ALL cases and 10-20% of adult ALL cases (Drexler, 1991). The most frequently encountered antigens are CD13 (6-16%) and CD33 (3-10%). Although some studies suggest that the presence of myeloid antigens is a poor prognostic factor in patients with ALL, others suggest no independent prognostic significance (Lauria et al., 1994; Campana and Behm, 2000). However presence of these antigens is associated with poor prognosis in adult ALL (Nakase et al., 1996). In this study, we aimed to investigate the frequency and prognostic significance of the positiveness of myeloid antigen in childhood ALL cases according to the French-American-British (FAB) classification in Cukurova region of our country.

Materials and Methods

The study was performed in Cukurova University, Department of Pediatric Oncology between March 1996 and February 2003. Seventy three cases with the diagnosis of ALL-L1 and 38 cases with ALL-L2 were included.

Physical examination findings including hepatomegaly, splenomegaly, lymphadenopathy and signs of hemorrhage were recorded for all the patients. Blood counts and peripheral blood smears were examined. Hepatic and renal function tests, coagulation tests and other tests were performed as needed with routine methods. Bone marrow aspirates of all patients were evaluated morphologically according to the FAB classification. Flow cytometric analysis of bone marrow aspirate was also performed. Myeloid antigens were specified as CD13, CD14 and CD33. More than 20% expression of these antigens was considered positive.

After morphological and immunological diagnosis was made, modified ALL-BMF-95 (Berlin-Frankfurt-Münster) protocol designed in our country was started. The patients were divided into 3 groups based on the response to 2 mg/kg prednisolone therapy on the 8th day of treatment: standard risk group (SRG), medium risk group (MRG) and high risk group (HRG).

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

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) 14.0. The Chi-square test, Mann-Whitney U tests and Kaplan-Meier survival analysis were employed for data analyses. A p value of <0.05 was considered statistically significant in all cases.

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Results

The patients with ALL-L1 (n=73) consisted of 37 females (50.7%) and 36 males (49.3%). Mean age of the patients with ALL-L1 was 64 ± 42.5 months (ranges 9 months to 14 years). Patients with ALL-L2 consisted of 17 females (44.7%) and 21 males (55.3%). Mean age of the patients with ALL-L2 was 66.8 ± 42.5 months (ranges 5 months to 13 years). Age and gender distribution of the two groups were comparable (p >0.05).

The frequency of organomegaly, hematocrit and hemoglobin values, leukocyte and platelet counts, serum blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase and uric acid levels were also similar between the two groups.

Myeloid antigens were positive in 36 of 73 patients (48.4%) with ALL-L1 and in 23 of 38 patients (60.5%) with ALL-L2. The positive rates of the myeloid antigen were similar between the groups. The positive rates of CD7, CD20, CD14, CD25 and CD10 were higher in the ALL-L1 group when compared to those in the ALL-L2 group, but the difference did not reach statistical significance (p >0.05). Similarly, the positive rates of CD2, CD19, CD13, CD41, CD56 and I2 were higher in ALL-L2 group when compared to those in the ALL-L1 group, but the difference did not reach statistical significance (p >0.05). Monoclonal antibody levels of the patients are demonstrated in Table 1.

During the study period, 4 cases quit the study to receive treatment in another center, 15 cases quit the study on their own will, 20 cases were lost to follow-up and 17 cases died. The remaining 55 cases were eligible for analysis. Fifteen of the remaining 34 cases (44.1%) with ALL-L1 had one myeloid antigen positive and 5 cases (14.7%) in this group had two myeloid antigens positive. Eight of the remaining 21 cases (38%) with ALL-L2 had one myeloid antigen positive and 2 cases (9.5%) in this group had two myeloid antigens positive. When all the cases were evaluated (73 cases with ALL-L1 and 38 with ALL-L2), 28 cases (38.4%) with ALL-L1 had one myeloid antigen positive and 20 cases (52.6%) with ALL-L2 had one myeloid antigen positive.

No association was observed between relapse rates and positive rates of myeloid antigens (p >0.05). Among the cases who died, more patients in ALL-L2 group (57.1%) had one myeloid antigen positive compared to

Table 1. Monoclonal Antibody Levels of the Patients

	ALL-L1	ALL-L2	Total	P
CD2	31.4±28.3 (1.2-98.9)	38.9±31.5 (0.7-99.5)	33.9±29.4 (0.7-99.5)	(p>0.05)
High	38 (%54.3)	22 (%62.9)	60 (%57.1)	(p>0.05)
Low	32 (%45.7)	13 (%37.1)	45 (%42.9)	
CD7	35.4±31.8 (0-98)	29.9±27.5 (0.2-82.9)	33.4±30.3 (0-98.5)	(p>0.05)
High	34 (%54.0)	18 (%51.4)	52 (%53.1)	(p>0.05)
Low	29 (%46.0)	17 (%48.6)	46 (%46.9)	
CD19	37.2±31.5 (0.1-97.6)	40.2±33 (0.9-98)	38.2±31.9 (0.1-98)	(p>0.05)
High	43 (%59.7)	25 (%65.8)	68 (%61.8)	(p>0.05)
Low	29 (%40.3)	13 (%34.2)	42 (%38.2)	
CD20	16.7±17.1 (0.1-69)	11.7±12.8 (0.1-49.7)	14.8±15.7 (0.1-69)	(p>0.05)
High	15 (%24.6)	6 (%16.7)	21 (%21.6)	(p>0.05)
Low	46 (%75.4)	30 (%83.3)	76 (%78.4)	
CD13	22.8±23.9 (0.1-86)	21.3±18.9 (0.1-71.5)	22.2± 22.1 (0.1-86)	(p>0.05)
High	25 (%34.2)	19 (%50.0)	44 (%43.1)	(p>0.05)
Low	39 (%60.9)	19 (%50.0)	58 (%56.9)	
CD14	6.2±15 (0-67)	2.90.1±9.7 (0-56.2)	5±13.3 (0-67.2)	(p>0.05)
High	8 (%12.5)	2 (%5.3)	10 (%9.8)	(p>0.05)
Low	56 (%87.5)	36 (%94.7)	92 (%90.2)	
CD33	5.6±13.8 (0-74.3)	7.2±20.9 (0-90)	6.2±16.7 (0-90)	(p>0.05)
High	5 (%8.2)	3 (%8.3)	8 (%8.2)	(p>0.05)
Low	56 (%91.8)	33 (%91.7)	89 (%91.8)	
CD41	42.2±31.2 (0-96.6)	49.4±32.2 (0-97.3)	45.2±31.6 (0-97.3)	(p>0.05)
High	27 (%77.1)	19 (%79.2)	46 (%78.0)	(p>0.05)
Low	8 (%22.9)	5 (%20.8)	13 (%22.0)	
CD56	2.4±3.3 (0-12.7)	4.6±13.3 (0-68.2)	3.2±8.5 (0-68.2)	(p>0.05)
High	(-)	1 (%3.8)	1 (%1.4)	(p>0.05)
Low	44 (%100.0)	25 (%96.2)	69 (%98.6)	
CD25	7.2±21.1 (0-98)	2.3±3.4 (0-10.6)	5.5±17.2 (0-98)	(p>0.05)
High	1 (%4.8)	(-)	1 (%3.1)	(p>0.05)
Low	20 (%95.2)	22 (%100.0)	31 (%96.9)	
CD10	38.9±35.5 (0.1-98)	38.1±37.6 (0.7-97.9)	38.6±35.9 (0.1-98)	(p>0.05)
High	23 (%51.1)	10 (%45.5)	33 (%49.3)	(p>0.05)
Low	22 (%48.9)	12 (%54.5)	34 (%50.7)	
I2	50±32.9 (0.3-96)	56.8±28.8 (0.7-97.7)	52.6±31.3 (0.3-97.7)	(p>0.05)
High	28 (%75.7)	19 (%82.6)	47 (%78.3)	(p>0.05)
Low	9 (%24.3)	4 (%17.4)	13 (%21.7)	

CD levels of 20% and over were considered high and CD levels of less than 20% were considered low. For statistical analyses, Chi-square test and Mann-Whitney U test were used

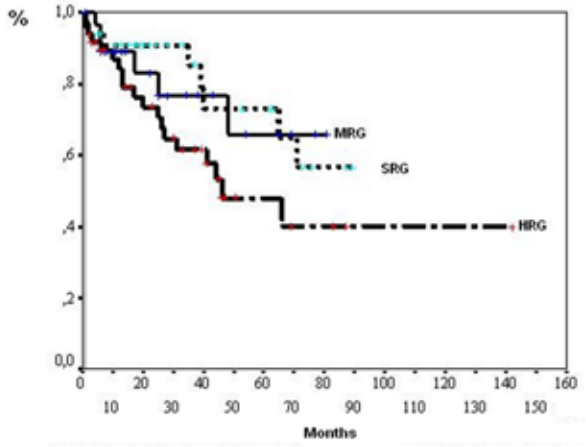

Figure 1. Overall Survival According to Risk Groups (SRG, MRG and HRG), Kaplan Meier Test

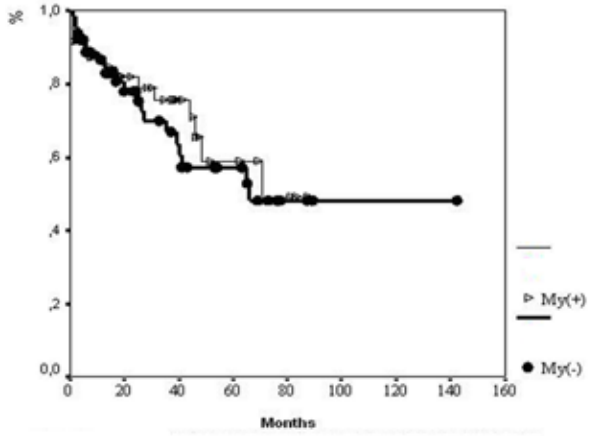

Figure 2. Overall Survival According to Myeloid Antigen Positivity, Kaplan Meier Test

those in ALL-L1 group (20%), but the difference was not statistically significant (p >0.05).

After the first induction therapy, complete remission was achieved in 101 cases (91%) while 10 cases (9%) had no remission. On follow-up, 15 patients relapsed (13.5%), and 17 patients (15.3%) died. The overall survival (OS), event-free survival (EFS), and disease free survival (DFS) rates at 36 months were 72%, 72%, and 74%, respectively. These rates at 60 months were 58%, 64%, and 62%, respectively. However, at the end of 120 months, OS, EFS, and DFS rates were found as 48%, 52%, and 56%, respectively.

Forty eight patients in our study (43.3%) were classified as HRG, 33 (29.7%) cases as SRG, and 30 patients (27%) as MRG. Survival analysis according to these risk groups of ALL-BFM revealed that OS rates at 36, 60, and 72 months were 62%, 48%, and 40% for HRG, 78%, 66%, and 66% for MRG, and 92%, 74%, and 58% for SRG, respectively (Figure 1).

Overall survival rates of myeloid antigen positive patients at 36, 60, and 72 months were 76%, 58%, and 48%, respectively, and were comparable to the corresponding OS rates of 70%, 56%, and 46% in the myeloid antigen negative patients (p >0.05) (Figure 2).

Discussion

The prognosis of ALL is closely associated with immunophenotype. Drexler et al., (1991), investigated the presence of myeloid antigens CD13, CD14, CD15, CD33, and CDw65 in patients with ALL and found the rate of My+ALL to be 10-20% in adults and 5-10% in children. They observed that the positiveness of myeloid antigen did not have any prognostic importance in childhood, but the rate of myeloid antigen positiveness was higher in adults at higher risk. Similarly, Lauria et al. (1994), reported a higher rate of CD13 and CD33 positiveness in adult ALL cases (54%). Sobol et al. (1987), claimed that positiveness of myeloid antigen was associated with lower rates of complete remission and short survival in adult ALL cases. Hanson et al., (1993), determined the rate of positiveness of myeloid antigen to be 6.9% in ALL cases, including both children and adults. Czuczman et al., (1999), investigated the immunophenotypical features of ALL in 259 adults and found that at least one-third of B-cell ALL cases (79%) and one-fourth of T-cell ALL cases (17%) expressed myeloid antigens. They reported that positiveness of myeloid antigen had no association with response to treatment, duration of remission, and survival.

Pui et al., (1990), found that myeloid antigens were positive in 61 of 372 children (16.4%) with ALL and that two or more myeloid antigens were positive in 18 children (4.8%). They also observed that out of 372 children, 8.9%, 6.5%, 3.2%, 1.9%, 1.6%, 1.3%, and 1.1% had positive antigens of CD11b, CD13, CD33, CD36, CD15, CD14, and CDw12, respectively. However, consistent with the results of our study, they also found no significant association between the positiveness of myeloid antigen and prognostic factors, such as high WBC count, race, and age.

Buccheri et al., (1993), reported the positiveness of myeloid antigen in 25% of 91 cases with ALL. Kurec et al., (1991), found the positive rate of CD13, CD14, CD15, and CD33 to be 16% in 51 children with ALL, but reported no significant association between the positiveness of myeloid antigen and gender, age, leukocyte count, FAB morphological classification or the presence of organomegaly or lymphadenopathy, showing consistency with our results. They did not find any difference in the duration of remission and survival between patients with and without myeloid antigen expression.

Rajalekshmy et al., (1994), noted that the rate of the positiveness of myeloid antigen was higher in children with null-ALL, and suggested using more aggressive treatment protocols for this type of leukemia. They reported the rate of positiveness to be 38% for CD13, 1.8% for CD14, 2.8% for CD33, and 4.3% for CD11b and CD15. Killick et al., (1999), found that 25 of 693 adults and children (3.6%) with acute leukemia had biphenotypic features. Of all cases with biphenotypic leukemia, 15 were B-lymphoid/myeloid, 8 were T-lymphoid/myeloid, 1 was T-/B-lymphoid, and 1 had trilineage differentiation. The rate of early death was high (25%) and the rate of 2-year survival was 39.4%.

In this study, we explored the presence of myeloid antigens CD13, CD14, and CD33, which have been most-frequently investigated in the literature. The CD13, CD14,

and CD33 were positive in 34.2%, 12.5%, and 8.2% of ALL-L1 cases, and in 50.0%, 5.3%, and 8.3% of ALL-L2 cases, respectively, which are consistent with the pertinent literature. Moreover, there was no significant difference between the two groups of patients.

Pui et al., (1991), found that 6.1% of 410 children with ALL had myeloid antigens, with ALL-L2 cases showing a higher rate than ALL-L1 cases (36% and 19%, respectively). However, they did not detect a significant association between the positiveness of myeloid antigen, gender, age, presence of a mediastinal mass or hepatosplenomegaly, hemoglobin and leukocyte counts, except for high platelet count and central nervous system involvement, which is consistent with our observation as well. In this study, although ALL-L2 cases had more frequent myeloid antigen expression, particularly CD13, the difference between ALL-L1 and ALL-L2 cases was not significant given the small sample-size of the study.

Owing to the improvements in treatment in the last 30 years, the disease free survival rate has reached 80% in ALL (Margolin et al., 2002). However, OS, EFS, and DFS rates at 36 months in our patients were 72%, 72%, and 74%, respectively. Unfortunately these rates dropped to 58%, 64%, and 62% at the end of 60 months, and to 48%, 52%, and 56% at the end of 120 months, mostly because of disease relapses.

There have been studies with conflicting results on the incidence of ALL-L2 morphology and the positiveness of myeloid antigen in biphenotypic, mixed-lineage, and hybrid leukemias (Sobol et al., 1987; Buccheri et al., 1993). Thus, we can conclude that the results of this study supported the relevant literature. We did not find any association between the positiveness of myeloid antigen and the clinical and laboratory features of ALL can be elucidated by further studies with larger sample sizes.

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