Original Article

# Immunophenotyping of Leukemia in Children, Gorgan, Iran

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## Abstract

### Background

Leukemia is one of the most common tumors in children and it is divided up into two main groups; acute and chronic leukemia. The acute leukemia is more prevalent than chronic in children. Generally acute type is included acute lymphoid leukemia (ALL) and acute myeloid leukemia (AML). In this study, patients with leukemia who were admitted in Talghani hospital of Gorgan were examined for immune markers.

### Materials and Methods

Forty one patients (34 persons with ALL and 7 persons with AML) were examined. Bone marrow aspiration samples were obtained in tubes containing EDTA and were sent to pathology center of Baghiatallah hospital, Tehran. Immunophenotyping was conducted by Flow cytometry and results were recorded in profiles of patients.

### Results

The mean age of ALL and AML patients was 5.64±3.43 and 7.45±5.68 years respectively. It was determined that ALL risk in males is 1.086 times more than females. Mann-Whitney test did not show significant difference between mean age of AML and ALL groups (p=0.5). Highest markers in ALL were CD19 (90.2%), CD10 (84.36%), I3 (HLA-DR) (70.58%), and in AML CD45 (81.8%), I3 (HLA-DR) (63.64%) and CD34 (54.5%).

### Conclusion

The prevalence of markers in ALL and AML patients is different, and some of them are common. These results could be used for differentiation of ALM from ALL. Further study was recommended on bigger sample-size to achieve a definite conclusion.

## Keywords

Leukemia, Child, Immunophenotyping

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## Introduction

Of all cancers in childhood, leukemias are one of the most important that are prevalent in children with the incidence of 25-30% of all childhood cancers (1-3).

Generally, leukemia is divided into two categories, acute and chronic leukemia. Acute leukemia is a heterogeneous group of neoplastic diseases and is categorized into two main subgroups: acute lymphoid leukemia (ALL) and acute myeloid leukemia (AML) (4). ALL is a heterogeneous disease with abnormal proliferation and accumulation of immature lymphoid cells within the bone marrow, peripheral blood and lymphoid tissues. ALL patients are subdivided into three morphological subsets including L1, L2 and L3 (5, 6). AML is an aggressive malignancy same as ALL and it is characterized by accumulation of immature myeloid progenitors in the bone marrow (7).

Immunophenotyping is important not only in the classification and diagnosis of leukemia, but also in the prediction and prognosis of these disorders (8). It makes complete morphological, cytochemical, cytogenetic and molecular studies for defining diagnosis and prognosis. Then various investigations have been conducted in this field.

Immunophenotyping in patients with ALL in China beside clinical and cytogenetic features reveal the importance of Immunophenotyping in diagnosis and determining ALL type (9).

Immunophenotyping CD antigens were studied in patients with acute leukemia in Iran (10).

Immunophenotyping in children is so important, which present study for the first time was done among leukemic patients in Golestan.

## Materials and Methods

*Cases*

This was a descriptive and analytical study and 62 patients with leukemia were examined during 2004-2009.

*Sample preparing and immunophenotype detection*

Patients suspected to leukemia that were admitted in Talghani hospital of Gorgan were checked by CBC test and then if their test were suspicious bone marrow aspiration would be done. After confirming for leukemia, results of hematological, preclinical and clinical studies and morphological tests were recorded in patients' profiles. Bone marrow aspiration samples were collected in tubes containing EDTA and sent to pathology center of Baghiatallah hospital of Tehran. The steps of Immunophenotyping were included: 1) preparing 4 glass slides of bone marrow and 1 glass slide of peripheral blood; 2) staining one glass slide of each sample by Wright method. Then stained glass slides were checked by a pathologist. According to flow cytometry instruction, it is determined which markers should be characterized for each patient; 3) diluting samples according to required marker (50µl sample+ 5 µl monoclonal antibody); 4) incubation all of samples for 20 minutes in refrigerator; 5) putting the samples in Q-Prep. In this device 3 solutions are added to samples during 35 seconds. Solutions include A (lyses buffer), B (buffer), C (fixative buffer); 6) finally all of sample tubes were transferred to flow cytometry device and then the results were recorded.

## Statistical analysis

Statistical analysis was performed by SPSS 18 and Mann-Whitney test, Leven test, relative risk and Shopiro-Wilk test were used to compare the groups.

## Results

Analyzed data of 62 patients showed that 51 cases were ALL and the remaining 7 with AML. There were 51 ALL patients (22 female and 29 male) and 11 AML patients (6 female and 5male) (table 1). The ratio of male to female in all of patients was 1.21 to 1. This ratio in ALL patients was 1.32 to 1 and in AML patients was 0.83 to 1. In all patients, the mean age was 5.96± 3.92 years and the age range was 0.5-15. The mean age and the age range in ALL -group were 5.64± 3.43 and 0.5-13 respectively and about AML group these parameters were 7.45± 5.68 and 0.75-15 respectively.

Relative risk (RR) was used for verifying effect of gender on leukemia type and was determined that ALL risk in males is 1.086 times more than females.

R.R= 1.086 CI 95% (0.0.855-1.378)

*Table 1: Frequency distribution of leukemia type according to gender*

| | | | ALL | AML | Total |
|---|---|---|---|---|---|
| **Gender** | male | count | 29 | 5 | 34 |
| | | % within gender | 85.3% | 14.7% | 100% |
| | female | count | 22 | 6 | 28 |
| | | % within gender | 78.6% | 21.4% | 100% |
| **Total** | | count | 51 | 11 | 62 |
| | | % within gender | 82.3% | 17.7% | 100% |

Mann-Whitney test was used for comparing mean age in ALL and AML groups. Using Shapiro-Wilk test, age distribution in ALL group was not normal (P=0.007), but in AML group was normal (P=0.068). Also Leven test demonstrated non homogeneity of variances. Then nonparametric Mann-Whitney test was applied. It showed that there was no significant difference between mean age of AML and ALL groups (p=0.5).

The frequencies of AML and ALL morphological cell types were shown in table 2. L1 and L2 were the most common types in ALL and L3 was not found among patients. Also there were 2 AML patients with unknown leukemia morphological type.

*Table 2: Frequency distribution of leukemia morphological types*

| Leukemia type | AML | | | | | ALL | |
|---|---|---|---|---|---|---|---|
| **Type** | M2 | M3 | M4 | M7 | unknown | L1 | L2 |
| **Number** | 2 | 3 | 2 | 2 | 2 | 25 | 26 |
| **Percent %** | 18.2 | 29 | 18.2 | 18.2 | 18.2 | 49 | 51 |

Immunophenotyping results are listed in Table 3. The most prevalent line in flow cytometry was pre-B-cell, 29 patients (46.77%).

*Table 3: Frequency distribution of the most common markers according to leukemia type and morphological type*

| Marker | | Leukemia morphological type | | | | | | | Leukemia type | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | L1 | L2 | M2 | M3 | M4 | M7 | Unknown AML | ALL | AML | |
| **CD19** | **number** | 22 | 24 | 1 | 1 | 0 | 0 | 0 | 46 | 2 | 48 |
| | **% in type** | 88 | 92.3 | 50 | 33.3 | 0 | 0 | 0 | 90.2 | 18.2 | 80 |
| **CD10** | **number** | 20 | 23 | 0 | 0 | 0 | 0 | 0 | 43 | 0 | 43 |
| | **% in type** | 80 | 88.5 | 0 | 0 | 0 | 0 | 0 | 84.36 | 0 | 71.7 |
| **HLA-** | **number** | 17 | 19 | 2 | 2 | 1 | 0 | 2 | 36 | 7 | 43 |
| **DR** | **% in type** | 68 | 73.1 | 100 | 66.7 | 50 | 0 | 100 | 70.58 | 63.64 | 26.66 |
| **CD34** | **number** | 8 | 7 | 1 | 2 | 1 | 1 | 1 | 15 | 6 | 21 |
| | **% in type** | 32 | 26.9 | 50 | 66.7 | 50 | 50 | 50 | 29.4 | 54.5 | 33.87 |
| **CD45** | **number** | 3 | 3 | 2 | 2 | 2 | 1 | 2 | 6 | 9 | 15 |
| | **% in type** | 12 | 11.5 | 100 | 66.7 | 100 | 50 | 100 | 11.8 | 81.8 | 24.2 |

The positive predictive value of CD19 for ALL is 95.8 % and negative p-value of it is 35.7%. Also positive p-value of CD10 for ALL is 100% and negative p-value of it is 42.1%. About HLA-DR, positive and negative p-value is 88% and 78.4 %, respectively.

In this study, in addition to immunophenotyping, some factors such as hemoglobin value and count of WBC were considered.

Hemoglobin value was in 27 (43.5%) patients less than 7.5 mg/dl, in 25 (40.3%) between 7.5-10 mg/dl, and in 10 (16.1%) more than 10 mg/dl. In ALL patients, frequency was 20 (39.2%), 25 (49%) and 6 (11.8%), respectively. In AML patients,in seven (63.6%) patients less than 7.5 mg/dl and in 4 more than 10 mg/dl.Difference between AML and ALL was significant (P=0.007).

The count of WBC in ALL was in 29 (58%) less than 2000, in 11 (22%) between 2000 to 50000, and10 (20%) more than 50000. One patient has (2%) not report. The results in AML patients were 4 (36.4%), 3 (27.3%) and 4 (36.4%), respectively.Difference between AML and ALL was not significant (P=0.379).

The count of neutrophil was in 7 (14.3%) patients less than 500, in 8 (16.3%) between 500 and 1000, in 7 (14.3%) between 1000 and 1500, in 27(55.1%) more than 1500, and 2 patients (3.92%) not reported. This count in AML patients, 2 (22.2%) was less than 500, 2 between 1000 and 1500, 5 (55.6%) more than 1500, and 2 (18.18%) had not reported. Difference between AML and ALL was not significant (P=0.555).

## Discussion

In the present discussion, HLA-DRwas 70.58 % in ALL patients and CD19 was the most common marker in these patients. Philip Lanzkowsky has demonstrated that the most common marker is HLA-DR, and CD19 is the second most important. The prevalence of L1 is almost same as L2 in present study, but in Lanzkowsky book, L1 was 84% and L2 was 15% in ALL (11).

According to other studies, it is possible to be a relationship between higher prevalence of HLA-DR and L1 and then connection between markers and morphology was considered here and its results are presented as following.

In our investigation the prevalence of markers in L1, which was found in 25 patients and involved 49% of all of ALL patients, was CD19, CD10 and I3 (HLA-DR) and also in L2, which involved 51% remaining of ALL patients, was as same as L1. Therefore the incidence

of markers in ALL patients was CD19 (90.2%), CD10 (84.36 %) and HLA-DR (70.58%) in general. It is noteworthy that in this investigation the majority of considered population was ALL patients. Based on frequency of HLA-DR and morphology type in our results, it may be concluded that ALL prognosis is poor.

Reviewing other studies express that there is a few differences from ours in some studies and some of them confirm our results (9, 12 and 13).

Asvadi Kermani in northwestern, Tabriz of Iran (2002) showed the most frequent markers in ALL patients were CD7 (11-28%), CD2 (5-21%) and CD19 (3-14%). CD10 (1-5%) and CD20 (9%) were in the next positions (10). In this study there was not especial age range. Similarly, Haxia Tong et al (2010) studied on 113 patients with ALL in China. In this study, the most prevalent markers of B-cells included CD19, CD10, CD22 and CD20. Frequency of these markers was 99%, 82.5%, 74.8% and 37.5% respectively (9).

In general, CD19 was found to be common in this and other studies. Wenxiu et al, (2005) achieved those results in B-cell line. They studied on 81 ALL patients including 43 children and 38 adults. In this investigation CD5 and CD7 were the most common markers in T-cell line (12). It should be noted that there are variety in these antigens in ALL patients of different regions. In research of Shen and colleges (2003) on 222 Chinese patients with leukemia, different results were obtained. In this population, 124 patients were ALL including 94 patients with B- cell line and 30 people in T-cell line. In this study CD13 was the most common antigen and after it, the most frequent markers were CD15 (11.3%), CD11b (6.5%) and CD33 (4.3%) (13).

The frequency of the different antigens was various in various lines of ALL. Study of Ramiar et al (2007) showed in Pre-B1, Pre-B2 and Pre-B of B-cell ALL, frequency of CD20 increases and CD10 decreases (14).

In present study, the most common markers in AML patients were CD45, I3 (HLA-DR) and CD19 in M2, CD45, I3 (HLA-DR) and CD34 in M3, CD45, CD33, I3, CD34 AND CD64 in M4 and CD34, CD45, CD41, CD61 and CD7 in M7. CD45 (81.8%), I3 (HLA-DR) (63.64%) and CD34 (54.5%) were the most common antigens.

The most markers in Asvadi Kermani research were CD13 (71%) and CD33 (74%) in M1, CD33 in M2 and CD13 in M3 (10).

Tong HX et al, (2009) investigated the immunophenotypic subtype profiles of 192 patients with AML. The results showed the CD33, CD13, myeloproxidase (MPO) and CD117 were the most commonly expressed antigens in AML. CD117 expressed in 84.6% of AML-M3 cases (15). Also Shen et al observed that the most common antigens in AML were CD7 (12.8%), CD19 (6.4%) and CD2 (5.1%) (12). Sovariety of markers is observed in different AML subgroups. Nevertheless, the presence of markers like CD34 is remarkable in this study such that frequency of CD34 is 65.1% and in our study is 54.5%.

In our study, prevalence of some markers in ALL and AML patients were different from other studies and some of them were the same. These results could be used for differential diagnosis of AML from ALL. Present investigation was done on a small population of children, and further studies with bigger sample size will be needed to achieve a clear conclusion.

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## References


1. Pui CH. Childhood Ieukemias. N EngI J Med .1995; 332,1618-1630.
2. Parkin DM, Stiller CA, Draper GJ, Bieber CA. The international incidence of childhood cancer. Int J Cancer. 1988 Oct 15;42(4):511-20.
3. Pui CH, Schrappe M, Ribeiro RC, Niemeyer CM. Childhood and adolescent lymphoid and myeloid leukemia. Hematology Am Soc Hematol Educ Program. 2004:118-45.
4. Kinney M, Lukens JN. Classification and differentiation of the acute leukemias. In: Richard Lee GR, Bithell TC, Foerster J, Athens JW, Lukens J, et al, eds. Wintrobe's Clinical Hematology. Vol 2, 10th ed. Phil: Lippincott Williams & Wilkins Co, 1998: 2209-2240.
5. Bene MC. Immunophenotyping of acute leukemias. Immunol Lett 2005; 98, 9-21.
6. Chan NP, Ma ES, Wan TS, Chan LC. The spectrum of acute lymphoblastic leukemia with mature B-cell phenotype. Leuk Res. 2003 Mar;27(3):231-4.
7. Löwenberg B, Downing JR, Burnett A. Acute myeloid leukemia. N Engl J Med 1999; 341,1051–1062.
8. Jennings CD, Foon KA. Recent advances in flow cytometry: application to thediagnosis of hematologic malignancy. Blood. 1997 Oct 15;90(8):2863-92.
9. Tong H, Zhang J, Lu C, Liu Z, Zheng Y. Immunophenotypic, cytogenetic andclinical features of 113 acute lymphoblastic leukaemia patients in China. Ann Acad Med Singapore. 2010 Jan ;39(1):49-53.
10. Asvadi Kermani I. Immunophenotyping of Acute Leukemia in Northwestern Iran. IJMS. 2002; 27(3):136-138.
11. Philip Lanzkowsky. Manual of pediatric hematology and oncology.Fifth edition.Elsevier. 2011
12. Wenxiu.S and Yan. Ch. The characteristics of immunophenotype in acute lymphoblastic leukemia and its clinical significance. 2005; 4(6), 334-337.
13. Shen HQ, Tang YM, Yang SL, Qian BQ, Song H, Shi SW, et al. [Immunophenotyping of 222 children with acute leukemia by multi-color flow cytometry]. Zhonghua ErKe Za Zhi. 2003 May;41(5):334-7.
14. Ramyar A, Shafiei M, Rezaei N, Asgarian-Omran H, Esfahani SA, Moazzami K, et al. Cytologic phenotypes of B-cell acute lymphoblastic leukemia-a single center study. Iran J Allergy Asthma Immunol. 2009Jun;8(2):99-106.
15. Tong HX, Wang HH, Zhang JH, Liu ZG, Zheng YC, Wang YX. [Immunophenotypes,cytogenetics and clinical features of 192 patients with acute myeloid leukemia]. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2009 Oct;17(5):1174-8.