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**ARAŞTIRMA / RESEARCH**

# Flow cytometric evaluation and outcomes of pediatric acute leukemia patients

## Çocukluk çağında akut lösemi tanısı alan hastaların sonuçları ve akım sitometri ile değerlendirilmesi

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**Abstract**

**Purpose:** In this study, the outcomes and flow cytometry results of our pediatric acute leukemia patients and their relationship with the prognosis and other clinical conditions were investigated.

**Materials and Methods:** A total of 93 patients, 69 acute lymphoblastic leukemia (ALL) and 24 acute myeloid leukemia (AML) diagnosed at our clinic between January 2008 and November 2013, were included.

**Results:** 5-year overall survival (OS) was 66% in ALL patients and 80% in AML patients. When leukemia patients were classified flow cytometrically according to their cell surface antigens (CD); 5-year OS was 58% in CD2(+) patients and 77% in CD2(-) patients. Five-year OS was 82% in patients with CD10(+) and 61% in patients with CD10(-). ALL patients were divided into two groups as ALL with positive T cell markers and ALL with positive B cell markers in the foreground and evaluated accordingly, and the OS of ALL patients with positive T cell markers in the foreground was lower than ALL patients with positive B cell markers in the 5 year (59% and 75%, respectively).

**Conclusion:** In the flow cytometric evaluation of our patients with leukemia, patients with CD10(+) had a better 5-year OS and patients with CD2(+) had a lower 5-year OS. The results of patients with positive B cell markers were better in ALL patients, and the results of patients with positive lymphoid markers were better in AML patients.

**Keywords:** Acute leukemia, flow cytometry, prognosis

**Öz**

**Amaç:** Bu çalışmada; çocukluk çağı akut lösemi hastalarının sonuçlarının ve akım sitometri sonuçlarının değerlendirilmesi, prognoz ve diğer klinik durumlar ile ilişkisinin araştırılması planlandı.

**Gereç ve Yöntem:** Bu çalışmaya, Ocak 2008 ile Kasım 2013 arasında hastanemizde tanı alan 69 akut lenfoblastik lösemi (ALL) ve 24 akut miyeloid lösemi (AML) hastası olmak üzere toplam 93 hasta dahil edildi.

**Bulgular:** Çalışmamızda, ALL hastalarında 5 yıllık genel sağkalım (OS) % 66, AML hastalarında ise % 80 saptandı. Lösemi hastaları, akım sitometrik olarak yüzey antijenlerine (CD) göre sınıflandırıldığında; CD2(+) hastalarda 5 yıllık OS % 58, CD2(-) hastalarda ise % 77 idi. CD10(+) hastalarda, 5 yıllık OS % 82, CD10(-) olan hastalarda ise % 61 idi. ALL hastaları, T ve B hücre belirteçleri pozitif ALL olarak iki gruba ayrılarak değerlendirildi; T hücre belirteçleri pozitif ALL hastalarının 5 yıllık OS'leri, B hücre belirteçleri pozitif ALL hastalarına göre daha düşük saptandı (sırasıyla % 59 ve % 75) (p=0.518)

**Sonuç:** Lösemi hastalarımızın akım sitometri ile değerlendirilmesinde, CD10(+) olan hastaların 5 yıllık OS'lerinin daha iyi olduğu, CD2(+) olan hastalarda ise daha düşük olduğu saptandı. ALL hastalarında B hücre belirteçleri pozitif hastaların, AML hastalarında ise lenfoid belirteçleri pozitif hastaların sonuçları daha iyiydi.

**Anahtar kelimeler**: Akut lösemi, akım sitometri, prognoz.

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## INTRODUCTION

Acute lymphoblastic leukemia (ALL), described as the abnormal proliferation of immature lymphoid cells or lymphoblasts, is the most common malignancy seen in children[1]. ALL accounts for 1/4 of the childhood cancers and 72% of the childhood leukemias[2]. Acute myeloid leukemia (AML) is characterized by abnormal proliferation and differentiation of myeloid precursors and accounts for about 20% of childhood leukemias[3]. Survival rates for leukemias have improved dramatically since the 1980s. This improvement in survival is due to treatment of a large number of children on sequential standardized research protocols[1]. Classification of acute leukemia was first attempted in 1976 when a panel of experts from France, the USA and the UK developed the so-called FAB (French-American-British) criteria[4]. FAB classification is based on criteria derived from the morphologic and cytochemical examination of the bone marrow specimens[5]. This criteria was followed by the Europen Group for Immunophenotyping of Leukaemias (EGIL) criteria in 1995 (which included flow cytometric assessment) and has culminated in the 2008 WHO Classification, which further incorporates results from cytogenetic and molecular testing[6]. Immunophenotyping with flow cytometry has an invaluable place in diagnosis, classification, determining prognosis and monitoring treatment in leukemia. Prognostic significance of single antigens remains controversial but it is important to recognize different prognostic factors on contemporary treatment regimens, since the specific of each treatment regimen affects outcomes[7]. In this study, we aimed to evaluate the overall results and flow cytometric results of our acute leukemia patients and the relationship of these results with the prognosis.

## MATERIALS AND METHODS

### Patients

Patients who were diagnosed with acute leukemia at the Pediatric Oncology Clinic of Çukurova University Hospital between January 2008 and November 2013 were included in the study. The number of patients diagnosed with acute leukemia within this period was 117, and 24 of these patients continued their treatments so patients who were treated in another hospital, and those who discontinued the therapy were excluded. Therefore, 93 patients with acute leukemia were included, 69 of which had ALL and 24 had AML. We analyzed hospital files of the remaining 93 patients retrospectively and their clinical and epidemiological characteristics, laboratory parameters, flow cytometric results, latest conditions of patients after treatment and survival rates were noted in data form.

The ethics committee approval with the decision number 28/15 dated 14.02.2014 was obtained from Çukurova University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee. Verbal and written informed consent were received from the legal guardians of the patients and controls.

### Procedure

Morphological, cytochemical, and immunological identifications of the patients were made and morphological subgroups were created according to FAB classification. Immunophenotyping was performed using flow cytometry. Flow cytometry was studied in the central laboratory of our hospital by specialist doctors in the immunology department. All blood and bone marrow samples were taken at the time of diagnosis.

Monoclonal antibodies for lymphocyte surface antigens and surface markers were analyzed. For this procedure, bone marrow aspiration samples of 2 ml were taken from the patients into tubes containing ethylenediaminetetraacetic acid (EDTA), and the samples were processed in the flow cytometry (BD fluorescence-activated cell sorting (FACS) Calibur) device, and their percentages within the blastic cell population were determined. The terminology of "Cluster of Differentiation, CD" is used to identify cell surface antigens. Immunophenotyping of blasts with CD2, CD3, CD7, CD10, CD13, CD19, CD20, CD22, CD33, CD34, HLA DR, intracytoplasmic MPO, CD117, and Anti TdT was performed in our center. If the cells in the cell population taken express 20% or more antigens, they were considered positive[8].

Patients diagnosed with ALL and AML were divided into subgroups according to FAB classification and immunophenotyping. ALLs were immunophenotypically divided into the subgroups of B-ALL and T-ALL. CD2, CD3, and CD7 positivity was regarded as T-cell marker positivity, CD19, CD20, and CD22 positivity was regarded as B cell marker positivity, and CD13, CD33, and MPO positivity was regarded as myeloid cell marker

positivity[9]. ALL patients were treated with ALL-BFM 2000 chemotherapy protocol and AML patients were treated with idarubicin + ARA-C based chemotherapy protocol[10,11].

### Statistical analysis

All statistical analyses were performed with SPSS v22 (statistical package for social sciences) software package. Mean, median and percentage were used for demographic characteristics. Pearson correlation test was conducted for correlation between laboratory results and flow cytometry results. Kaplan-Meier test was used for survival analysis in the evaluation of the patients' data. In addition, ANOVA test (one-way variance analysis) and Mann-Whitney U test were also used in the comparison of the groups. $P<0.05$ was considered statistically significant.

## RESULTS

This study was performed with 93 childhood acute leukemia patients, including 69 ALL and 24 AML patients diagnosed in our clinic. Thirty-four (49.3%) of ALL patients had ALL L1 and 35 (50.7%) had ALL L2. Eight of the AML patients had AML M4 (33.3%) and the rest had other AML types. In ALL patients, 24 were classified as the standard risk group (SRG) (34.8%), 33 as the intermediate risk group (MRG) (47.8%), and 12 were classified as the high-risk group (HRG) (17.4%) according to the TRALL BFM. Demographic characteristics of the patients are given in Table 1.

The CD markers at the time of diagnosis did not affect the survival of the patients ($p>0.05$) (Table 2). However, considering 5-year OS, it was observed that the presence of CD2 (+) led to a poor prognosis in patients and the presence of CD10 (+) led to a good prognosis. Flow cytometric parameters were evaluated in patients with relapse and central nervous system (CNS) involvement. However, no statistically significant results were found.

After starting treatment in ALL patients, remission was achieved in 67 (97%) of 69 patients undergoing the bone marrow examination performed on day 33. However, remission was not achieved in two patients (3%). In patients with AML, remission was achieved in 21 (87.5%) of 24 patients after induction, and remission could not be achieved in 3 (12.5%) of them. Treatment, relapse, and final conditions of patients with acute leukemia are provided in Table 3.

When overall survival (OS) was evaluated in ALL patients, it was found to be 69% in the 36th month and 66% in the 60th month. OS of ALL patients is given in Figure 1. When OS of AML patients was evaluated, it was found to be 80% in the 36th and 60th months, and the OS of AML patients is given in Figure 2.

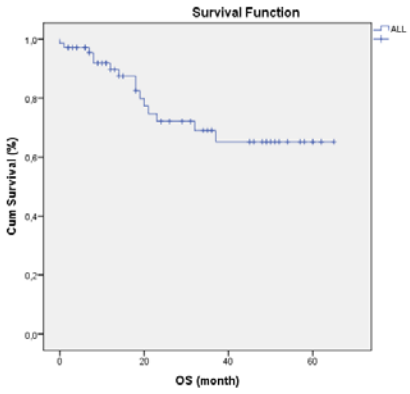


**Figure 1. Overall survival in ALL patients.**

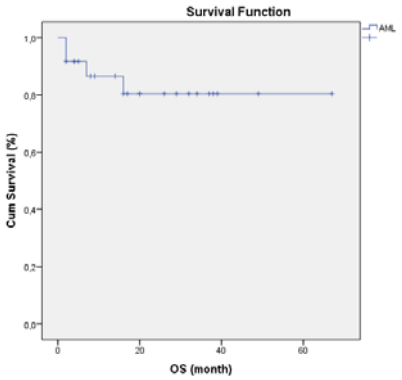


**Figure 2. Overall survival in AML patients**

**Table 1. Demographic characteristics of the patients**

| Characteristics | ALL (n=69) | AML (n=24) |
|---|---|---|
| Age (months) (mean±SD) | 83.8±56.6 | 99.9±55.2 |
| (min-max) | 7-239 | 12-193 |
| Median | 67 | 119 |
| Gender, n (%) | | |
| Male | 44 (% 64) | 14 (% 58) |
| Female | 25 (% 36) | 10 (% 42) |
| Male/female ratio | 1.76 | 1.4 |
| Presenting signs and symptoms (+) n (%) | | |
| Hepatomegaly | 37 (% 53.6) | 11 (% 45.8) |
| Splenomegaly | 32 (% 46.4) | 9 (% 37.5) |
| Lymphadenopathy | 28 (% 40.6) | 8 (% 33.3) |
| Fever | 21 (% 30.4) | 3 (% 12.5) |
| Bruising | 11 (% 15.9) | 1 (% 4.2) |
| Pallor | 7 (% 10.1) | 2 (% 8.3) |
| Laboratory results at the time of presentation | | |
| WBC (/mm$^3$) (mean± SD) (min-max) | 39806±77005 (1420-454000) | 38388±72089 (949-278000) |
| Hemoglobin (gr/dL) (mean±SD) (min-max) | 8.385±14.22 (3.4-13.1) | 8.12±1.69 (4.8-11.2) |
| Platelet (/mm$^3$) (mean±SD) (min-max) | 87610±96264 (3700-394000) | 100387±103338 (8000-355000) |
| LDH (U/L) (mean±SD) (min-max) | 1731.79±3292.42 (150-22960) | 692.66±471.20 (185-1845) |
| Uric Acid (mg/dL)(mean±SD) (min-max) | 4.84±4.88 (0.3-39.3) | 3.81±1.53 (2-7,8) |
| AST (U/L) (mean±SD) (min-max) | 46.05±38.2 (10-178) | 22.37±8.10 (12-45) |
| ALT (U/L) (mean±SD) (min-max) | 35.2±42.14 (9-304) | 18.83±15.03 (0-79) |
| BUN (mg/dL) (mean±SD) (min-max) | 12.69±11.89 (1-93) | 10.97±3.32 (5.6-21) |
| Creatinine (mg/dL) (mean±SD) (min-max) | 0.56±0.52 (0.17-3.4) | 0.37±0.15 (0.10-0.67) |

WBC: white blood cell, LDH: lactic dehydrogenase acid, AST: aspartate aminotransferase, ALT: alanine aminotransferase, BUN: blood urea nitrogen

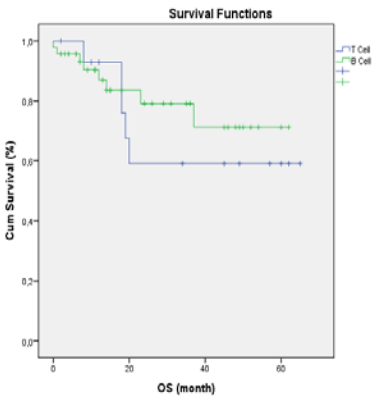


**Figure 3. Overall survival in T cell and B cell ALL**

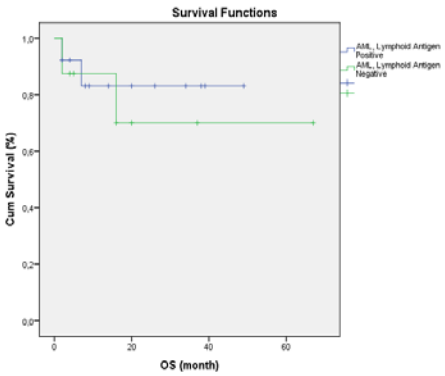


**Figure 4. Overall survival in AML**

**Table 2. OS assessment in patients with leukemia according to CDs**

| | Number of cases | Mean Follow-Up Period | 3rd year OS (%) | 5th year OS (%) | p** |
|---|---|---|---|---|---|
| **CD2 (-)** | 57 | 55.60±3.67 | 83 | 77 | 0.115 |
| **CD2 (+)** | 21 | 42.86±6.41 | 58 | 58 | |
| **CD3 (-)** | 61 | 50.93±4.01 | 75 | 69 | 0.913 |
| **CD3 (+)** | 13 | 47.43±7.00 | 69 | 69 | |
| **CD7 (-)** | 59 | 48.59±3.60 | 78 | 71 | 0.978 |
| **CD7 (+)** | 20 | 50.72±6.02 | 68 | 68 | |
| **CD10 (-)** | 37 | 47.78±4.89 | 68 | 61 | 0.209 |
| **CD10 (+)** | 38 | 53.04±3.68 | 82 | 82 | |
| **CD19 (-)** | 24 | 48.79±6.16 | 68 | 68 | 0.452 |
| **CD19 (+)** | 55 | 50.20±3.50 | 80 | 73 | |
| **CD22 (-)** | 26 | 51.79±5.25 | 75 | 69 | 0.690 |
| **CD22 (+)** | 42 | 45.17±3.26 | 79 | 79* | |
| **CD13 (-)** | 53 | 47.90±3.99 | 68 | 68 | 0.382 |
| **CD13 (+)** | 27 | 53.85±5.77 | 82 | 70 | |
| **CD33 (-)** | 64 | 49.36±3.70 | 72 | 68 | 0.961 |
| **CD33 (+)** | 14 | 52.58±7.35 | 78 | 78 | |
| **CD34 (-)** | 27 | 45.32±4.58 | 73 | 73 | 0.703 |
| **CD34 (+)** | 48 | 49.82±4.38 | 72 | 68 | |
| **CD117(-)** | 55 | 51.50±4.02 | 79 | 70 | 0.548 |
| **CD117(+)** | 15 | 48.29±7.65 | 65 | 65 | |
| **TdT (-)** | 25 | 49.00±6.16 | 68 | 68 | 0.389 |
| **TdT (+)** | 47 | 53.14±4.03 | 78 | 78 | |
| **MPO (-)** | 56 | 50.71±4.22 | 70 | 70 | 0.430 |
| **MPO (+)** | 16 | 39.59±3.54 | 85 | X | |

*4-year follow-up period is available,**log-rank test, x: no 4-year follow-up period available.

**Table 3. Latest conditions of patients with acute leukemia after treatment**

| | ALL | | | AML | | |
|---|---|---|---|---|---|---|
| | **Relapse (+)** | **Relapse (-)** | **Total (n/%)** | **Relapse (+)** | **Relapse (-)** | **Total (n/%)** |
| Exitus | 9 (% 13) | 6 (% 8.7) | 15 (% 21.7) | 1 (% 4.2) | 3 (% 12,5) | 4 (% 16.7) |
| Not Followed Up | 2 (% 2.9) | 0 (% 0) | 2 (% 2.9) | 1 (% 4.2) | 0 (% 0) | 1 (% 4.2) |
| Survived | 6 (% 8.7) | 46 (% 66.7) | 52 (% 75.4) | 1 (% 4.2) | 18 (% 75) | 19 (% 79.1) |
| Total | 17 (% 24.6) | 52 (% 75.4) | 69 (% 100) | 3 (% 12.5) | 21 (% 87,5) | 24 (% 100) |

At the time of diagnosis, flow cytometric analysis could be performed in 61 of 69 ALL patients and 21 of 24 AML patients. Eight ALL patients from whom bone marrow samples could not be taken for flow cytometry were considered B-ALL, and their treatment was planned accordingly. ALL patients were divided into two groups as ALL with positive T cell markers and ALL with positive B cell markers in the foreground. On the other hand, AML patients were divided into two groups as AML with positive lymphoid markers and AML with negative lymphoid markers. The OSs of the groups were evaluated among themselves. Fifteen (24.6%) ALL patients with positive T cell markers in the foreground had an OS of 59% in the 36th and 60th months, while 46 (75.4%) ALL patients with positive B cell markers had an OS of 79% in the 36th month and 71% in the 60th month (p=0.518) (Figure 3). Thirteen (61.9%) AML patients with positive lymphoid markers had an OS of 83% in the 36th and 60th months, and 8 (38.1%) AML patients with negative lymphoid markers had an OS of 70% in the 36th and 60th months (p=0.638) (Figure 4).

When the effects of some clinical and hematological conditions on OS were evaluated in ALL patients, it was found that OS of patients with only relapse and with CNS involvement was statistically significantly lower (Table 4). Due to the lack of a sufficient number of cases in AML patients, only the

parameters in Table 5 were evaluated. According to the age groups in the table, since there are 3 cases under the age of 2, the statistics are not statistically significant. There was no statistically significant finding in the parameters analyzed.

Flow cytometric results of both ALL and AML patients were compared with some of the laboratory results (Table 6). There was a positive correlation between CD2, CD7, and CD117 and urea, creatinine, and uric acid in ALL. A negative correlation was found between CD10 and CD19 and urea and creatinine.

**Table 4. The effect of some clinical and hematological conditions on OS in ALL patients**

| | Number of cases | Mean Follow-up Periods | 3rd year OS (%) | 5th year OS (%) | p** |
|---|---|---|---|---|---|
| Gender | | | | | |
| Female | 25 | 48.79±6.22 | 79 | 64 | 0.98 |
| Male | 44 | 46.24±4.07 | 64 | 64 | |
| Age | | | | | |
| Under 2 years | 8 | 19.38±17.80 | 100 | 100* | 0.017 |
| 2-6 years | 30 | 30.40±22.20 | 86 | 79 | |
| Over 6 years old | 31 | 19.60±16.20 | 45 | 45 | |
| WBC count | | | | | |
| Below 50000/mm$^3$ | 56 | 48.31±3.95 | 68 | 62 | 0.94 |
| Above 50000/mm$^3$ | 13 | 47.14±7.35 | 71 | 71 | |
| WBC count | | | | | |
| Below 100000/mm$^3$ | 63 | 50.39±3.56 | 72 | 68 | 0.089 |
| Above 100000/mm$^3$ | 6 | 33.11±11.50 | 45 | 45 | |
| ALL risk group | | | | | |
| SRG | 22 | 44.41±5.14 | 62 | 62 | 0.90 |
| MRG | 35 | 48.83±5.39 | 71 | 62 | |
| HRG | 12 | 46.12±7.89 | 73 | 73 | |
| Relapse | | | | | |
| Yes | 17 | 35.01±4.01 | 52 | 43 | 0.047 |
| No | 52 | 55.72±3.57 | 83 | 83 | |
| CNS involvement | | | | | |
| Yes | 14 | 34.16±4.11 | 48 | 36* | 0.041 |
| No | 55 | 55.08±3.49 | 82 | 82 | |

*4-year follow-up period is available,**log-rank test. OS: overall survival, WBC: white blood cell, SRG: standard risk group, MRI: intermediate risk group, HRG: high-risk group, CNS: central nervous system.

**Table 5. The effect of some clinical and hematological conditions on OS in AML patients**

| | **Number of cases** | **Mean Follow-Up Period** | **3rd year OS (%)** | **5th year OS (%)** | **P**** |
|---|---|---|---|---|---|
| Gender | | | | | |
| Female | 10 | 38.90±6.33 | 78 | 78* | 0.66 |
| Male | 14 | 57.09±6.42 | 82 | 82 | |
| Age | | | | | |
| Under 2 years | 3 | 38.70±25.00 | 100 | 100 | 0.517 |
| 2-6 years | 7 | 15.70±16.10 | 64 | 64* | |
| Over 6 years old | 14 | 19.41±14.54 | 83 | x | |
| WBC count | | | | | |
| Below 20000/mm$^3$ | 15 | 16.52±13.12 | 69 | 69* | 0.104 |
| Above 20000/mm$^3$ | 9 | 27.74±21.12 | 100 | 100 | |

*4-year follow-up period is available,**log-rank test, x: No 4-year follow-up period available.; OS: overall survival, WBC: white blood cell.

**Table 6. Correlation of flow cytometry results with other parameters in ALL and AML patients**

| | ALL | | AML | |
|---|---|---|---|---|
| | **Positive** | **Negative** | **Positive** | **Negative** |
| WBC | CD2 (p:0.048; r:0.261)<br>CD3 (p:0.026; r:0.303) | CD22 (p:0.018; r :-0.333) | CD7 (p:0.017;r:0.529) | |
| Urea | CD2 (p:0.001;r:0.409)<br>CD7 (p:0.001;r:0.445)<br>CD117 (p:0.001;r:0.650) | CD10 (p:0.037; r :-0.285)<br>CD19 (p:0.010;r:-0.335)<br>CD22 (p:0.013; r :-0.349)<br>TdT (p:0.041; r :-0.285) | | |
| Cr | CD2 (p:0.023; r:0.297)<br>CD7 (p:0.003; r:0.378)<br>CD117(p:0.001r:0.436) | CD10 (p:0.05; r :-0.269)<br>CD19 (p:0.007; r :-0.352)<br>CD22 (p:0.028;r:-0.311) | CD3 (p:0.048;r:0.448) | |
| AST | CD2 (p:0.01; r:0.425)<br>CD13 (p:0.017; r:0.312) | CD10 (p:0.008;r:-0.357)<br>CD22 (p:0,001;r:-0.437)<br>CD117 (p:0.012; r :-0.348) | CD2 (p:0.027;r:0.494)<br>CD13 (p:0.019;r.0.547) | |
| Uric acid | CD2 (p:0.031;r:0.284)<br>CD7 (p:0.05; r:0.361)<br>CD117 (p:0.001;r:0.549) | CD19 (p:0.002; r :-0.391)<br>CD22 (p:0.014; r :-0.347) | CD3 (p:0.006; r:0.596) | CD13 (p:0.019;r:-0.505) |
| LDH | CD2 (p:0.001;r:0.425) | | | |

WBC: white blood count, AST: aspartate aminotransferase, LDH: lactic dehydrogenase acid.

## DISCUSSION

The course and outcomes of leukemia, previously known as a fatal disease, have changed due to better understanding of the biology of the disease, newly developed treatment strategies, allogeneic stem cell transplantation and supportive therapies[2]. Identifying the prognostic factors well, can also contribute further to achieving better outcomes.

The prevalence of childhood cancers is greater in male patients, and this difference is pronounced in ALL patients[12]. In a study conducted by Ward et al. in North America between 2006 and 2010, the male/female ratio was found to be 1.27[13]. In the present study, this difference was more pronounced and the male/female ratio was 1.76 in ALL patients. A study by Ishii et al. showed that male patients receiving the same treatment had a worse prognosis and that additional treatment methods should be developed for male patients[14]. In the present study, when OS was evaluated according to gender, OS was 79% in the 3rd year and 64% in the 5th year in male patients, while it was 64% in the 3rd year and 5th year in female patients, and no statistically significant difference was observed ($p>0.05$).

Hastings et al. have shown that a leukocyte count higher than 50000/mm$^3$ at the time of diagnosis is a cause of poor prognosis in ALL patients[15]. In the present study, there were no statistically significant results between leukocyte count and survival. According to Stiller et al., the age at diagnosis of ALL peaks between 2 and 4 years of age[16]. Ward et al. also investigated the age at diagnosis for the Spanish race, Caucasian race, and black race, and found that it peaked between the ages of 2 and 5[13]. In a study by Ratei et al., the age at diagnosis peaked between 1 and 5 years of age[17]. In addition, age is another important parameter in the prognosis for ALL patients. The age at the time of diagnosis being over 10 and older age at diagnosis are associated with a poor prognosis[18]. In the present study, the number of patients aged 2-6 years was 30 cases (43%) and the median age was 5.5 years in ALL patients. There was no relationship between the age at diagnosis and the prognosis in ALL patients.

Considering the findings and symptoms of the ALL patients in the present study at the time of presentation, hepatomegaly, splenomegaly, lymphadenopathy, and fever were the most common symptoms, respectively. In a meta-analysis, in which

33 studies were examined and which included more than 3000 children with acute leukemia, it was reported that the most common manifestations were hepatomegaly (64%), splenomegaly (61%), pallor (54%), fever (53%), and bruising (52%)[19]. Our results are similar to that meta-analysis. In the previous studies, leukemic cell load and the degree of involvement of extramedullary disease were associated; and hepatosplenomegaly and lymphadenopathy were mentioned as prognostic factors in ALL[20,21]. However, in the present study and in the latest literature, it has been observed that hepatosplenomegaly and lymphadenopathy no longer affect the prognosis[2].

According to the BFM protocol, ALL patients are divided into risk groups according to the initial white blood cell count, blast count on the 8th day, and bone marrow analyzed 1 month after diagnosis. Accordingly, ALL patients are divided into 3 groups: SRG, MRG, and HRG, and according to the BFM study group, their incidence rates are 33%, 48%, and 19%, respectively[22]. In a study by Ratei et al., values close to these were found[17]. In the present study, 22 of the patients presenting to our clinic were SRG (31.9%), 35 were MRG (50.7%), and 12 were HRG (17.4%), which were consistent with the previous studies. In a study conducted by the BFM group, relapse was most common in HRG (48%) group, and less common in SRG (14%) and MRG (38%) groups[22]. However, in the present study, relapse was most common in patients in SRG with 59% (10 patients), and least common in patients in HRG with 6% (1 patient). This difference may be associated with geographical and racial factors. There was no statistically significant difference when OS was evaluated according to the risk groups in our patients.

The timing of bone marrow relapse is the most important prognostic factor in ALL with a relapse. In a study by Raetz et al., 5-year event-free survival (EFS) was about 11 ± 7% in relapses occurring before the 18th month, while this rate was about 40 ± 7% in relapses occurring after the 36$^{th}$ month[23]. The time of bone marrow relapse was not evaluated in the present study, but it was found that the OSs of patients with relapse and patients with central nervous system (CNS) involvement were significantly lower (p=0.047 and 0.041, respectively).

In the studies conducted, survival has been shown to be significantly better in B-ALL compared with T-ALL[18]. In general, other specific immunophenotypic features are no longer commonly used to assess prognosis, as they tend to be associated with genetic alterations, which are more stable prognostic markers. Specific immunophenotypic markers do continue to retain importance as targets for specific therapeutic agents; for example, the monoclonal antibodies epratuzumab (anti-CD22) and rituximab (anti-CD20)[2].

The five-year EFS for ALL has currently approached 90% in developed countries. Five-year and estimated 10-year OS rates were reported to be 90%[24,25]. In the present study, 3-year survival was 69% and 5-year survival was 66% in ALL patients.

Prognostic factors vary depending on the patient, characteristics of the disease and the response to treatment in AML patients. Age, race, and the presence of concomitant diseases are the characteristics of the patient that determine the prognosis. The characteristics of the disease vary according to the patient's remission status and existing translocations, and other factors such as chromosome anomaly and FLT3 gene mutations are also among the prognostic factors[3]. There was no difference in incidence by gender in AML patients[13]. In a study conducted by Rubnitz et al. between 1987 and 2002 in 191 patients, the female/male ratio was found to be 1.17[26]. In our patients, there were more male patients and the female/male ratio was 1/1.4. In the study by Rubnitz et al., 61% of the patients were <10 years and 39% of the patients were >10 years. There was a statistically significant effect of the age at diagnosis on event-free survival[26]. In the present study, the patients were divided into three groups according to the age at diagnosis, and 13% (3 patients) were under 2 years, 29% (7 patients) were aged 2-6 years, 58% (14 patients) were older than 6 years of age at diagnosis. No significant difference was found in the OS of these patients according to the age groups. However, since there were 3 patients under the age of 2, no statistical evaluation could be made.

The most common signs and symptoms of AML are hepatosplenomegaly, lymphadenopathy, fever, weakness, and hemorrhage[19]. Our AML patients presented with the findings and symptoms indicated in the literature.

In a study conducted by Liang et al. in AML patients, the patients were divided into three groups as those with a white blood cell count under 20000/mm$^3$, those with a white blood cell count of 20000-100000/mm$^3$ and those with a white blood cell count

above 100000/mm$^3$ at the time of diagnosis, and OS was found to be statistically significant between these groups[27]. We grouped our patients into those with an initial WBC count of below (n=15) and above (n=9) 20000/mm$^3$ because it was a small group but no statistically significant difference was found between the groups (p>0.05).

Full remission can be achieved in 80-90% of the patients with modern treatment modalities in AML patients. However, relapse is observed in 30-40% of these patients, and long-term survival is observed in only 50% of them[26]. The incidence of CNS relapse is observed to be around 2-8.8%. In a study conducted by Johnston et al. in AML patients, this rate was observed to be around 4.8%[28]. In a study by Rubnitz et al., relapses were observed at a rate of 37.5%; 4.3% of which were reported as CNS relapse[26]. In the present study, relapses were observed in only 3 (12.5%) of 24 patients, and only one (4%) of all AML patients had CNS relapse. In AML patients, 3-year and 5-year OS was 80%.

Jemal et al. showed that the 5-year survival, which was 14% in the 1975, has increased to 54% for AML patients[29]. In a study involving 38 patients diagnosed with AML between 1992 and 1999 in our clinic, 1-year survival was 40% and 3-year survival was 23%[30]. In the present study, 3-year and 5-year survival was 80% for AML patients. An increase was observed in survival rates compared with our previous results. We believe that our efforts on improvement of patient care and reduction in drug toxicity had a role in this increase[11].

Immunophenotypic evaluation of leukemia is essential to confirm the diagnosis and perform further subclassification. Detection of specific antigens may have prognostic or therapeutic implications even within a single acute leukemia subtype. After initial diagnosis, a leukemia's immunophenotypic evaluation provides a useful reference to monitor response to therapy, minimal residual disease, prognosis and recurrence. In leukemia patients, CD10 expression was associated with good prognostic outcomes and CD2 expression was found to be a poor prognostic indicator[31].

In our study, in the flow cytometric evaluation performed at the time of diagnosis, CD2 (n=21), CD3 (n=13), CD7 (n=20), CD10 (n=38), CD19 (n=55), CD22 (n=42), CD33 (n=14), CD34 (n=48), CD117 (n=15), TdT (n=47), and MPO (n=16) were positive. These parameters analyzed were not found to be statistically significant in OS. However, considering 5-year OS, 5-year survival in CD2(-) patients was 77% and 5-year survival in CD2(+) patients was 58%, and accordingly, CD2 is an indicator of poor prognosis. The 5-year survival of patients with CD10 (-) was 61% and the 5-year survival of patients with CD10(+) was 82%, and we can say that CD10 causes a good prognosis for patients with leukemia.

ALL is five times more common than acute AML in children[13,32]. The distribution of ALL categories is B lineage (85 %), T lineage (10 to 15 %), and NK lineage (<1%)[27]. In the present study, 75.4% of ALL patients were B-ALL and 24.6% of them were T-ALL consistent with the literature.

ALL patients with positive T cell markers had a lower 5 year OS compared to ALL patients with positive B cell markers (59% and 75%, respectively) (p=0.518). Studies of Children Oncology Group (COG) have shown that between 2000 and 2005, the 5-year OS in T-ALL and B-ALL was 81% and 91.6%, respectively with a significant difference of 10% between them[18]. In our study, OSs were lower than in COG studies but there was no statistically significant difference, which is likely due to the low number of cases.

When AML patients were divided into two groups as AML with positive lymphoid markers and AML with negative lymphoid markers, 5-year OSs of AML patients with positive lymphoid markers were higher than AML patients with negative lymphoid markers (83% and 70%, respectively) (p=0.638). These results were not statistically significant. In a study by Ding et al., OS of patients with positive lymphoid markers and patients with negative lymphoid markers was 24% and 32% in the 5th year, respectively[33]. In the present study, the OSs of AML patients were found to be better.

In ALL patients, there was a positive correlation of elevated WBC count with CD2 and CD3 levels, while a negative correlation was found with CD22. It was observed that there was a positive correlation of elevated urea, creatinine, and uric acid with CD2, CD7, and CD117, while there was a negative correlation with positive B cell markers (CD19, CD10, and CD22). In other words, it may be suggested that T-cell marker positivity (elevated CD2 and CD7 markers) increases and B cell marker positivity (CD19, CD10, CD22) decreases tumor lysis.

In AML patients, there was a positive correlation between elevated WBC and elevated CD7 marker, as well as between elevated creatinine and uric acid levels and elevated CD3 marker. We may suggest that T cell marker poses a risk in terms of tumor lysis in AML patients, although there is less risk compared with ALL patients. While there was a positive correlation between elevated CD3 and uric acid level, there was a negative correlation between elevated CD13 and uric acid level in AML patients. Interestingly, there was a positive correlation between AST elevation and high CD2 and CD13 markers in both ALL patients and AML patients. It can be considered that this result may be associated with liver infiltration.

There were some limitations in our study. At the time of diagnosis, flow cytometric analysis could be performed in 61 of 69 ALL patients and 21 of 24 AML patients. As it is a retrospective study, data of all patients could not be reached. The number of AML patients was not sufficient for optimal statistical analysis. Since the parameters studied in flow cytometry were studied on different dates, standard parameters could not be studied in all patients.

As a result, 3 and 5 year OS were significantly lower in ALL patients with CNS involvement and relapse. While the results of the ALL patients were lower than in the literature, the results of the AML patients were much better compared to the results indicated in the literature. In ALL patients, OSs of patients with positive T-cell markers were lower than those of patients with positive B cell markers. We believe that because we had more T-ALL patients (24.6%) than in the literature, the results of our ALL patients were found to be lower. On the other hand, the OS of AML patients was found to be better than the literature.

In the flow cytometric evaluation, there was generally a positive correlation of WBC, urea, uric acid, LDH and AST with T-cell markers, and negative correlation with B cell markers. It can be suggested that while T cell marker positivity increases tumor lysis; B cell marker positivity decreases it. T cell marker poses a risk in terms of tumor lysis in AML patients although they have less risk compared to ALL patients. Though the results were not statistically significant, patients with CD10 (+) had a better 5-year survival and patients with CD2 (+) had a lower survival. We believe that morphological examination of peripheral blood and bone marrow aspiration smears is fundamental following a good medical history and physical examination in the evaluation, diagnosis and treatment planning of the leukemia patients, and recently flow cytometry has become an indispensible tool for diagnosing and monitoring of leukemia.

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