Annals of Medical Research

DOI: 10.5455/annalsmedres.2019.10.610 2019;26(12):2928-36

Original Article

Single center 10 years' experience of acute lymphoblastic leukemia in childhood: Retrospective cohort study

Selma Erol Aytekin[1], Gokhan Aytekin[2], Huseyin Tokgoz[3], Umran Caliskan[4]

[1]University of Health Sciences, Konya Education and Research Hospital, Department of Pediatrics, Konya, Turkey
[2]Necmettin Erbakan University, Meram Faculty of Medicine, Department of Internal Medicine, Division of Clinical Immunology and Allergy, Konya, Turkey
[3]Necmettin Erbakan University, Meram Faculty of Medicine, Department of Pediatrics, Division of Pediatric Hematology, Konya, Turkey
[4]KTO Karatay University, Karatay Faculty of Medicine, Department of Pediatrics, Division of Pediatric Hematology, Konya, Turkey

Abstract

Aim: We reviewed the files of 188 patients diagnosed with acute lymphoblastic leukemia at Pediatric Hematology Department, Necmettin Erbakan University Faculty of Medicine, Konya, between April 2006 and April 2016 retrospectively. 167 patients, who had sufficient records and accepting to participate in the study, were included. Patients were classified 3 groups according to their treatment protocols. These protocols were Saint Jude Total Therapy (St. Jude) protocol, Berlin-Frankfurt-Munster 2000 (BFM 2000) protocol and BFM 2009 protocol. Acute lymphoblastic leukemia (ALL) is a malignant disorder characterized with clonal enlargement of lymphoid progenitor cells. It is most common malignancy in childhood. Recent developments in immunologic and genetic methods have significantly altered the diagnostic and classification approaches. Nowadays advanced studies such as immunologic and cytogenetic studies have become more important in prognosis and treatment response. In this study, we aimed to present the clinical and laboratory features of patient with ALL who were followed in our Pediatric Hematology Clinic (Necmettin Erbakan University Faculty of Medicine, Konya) and determine the factors affecting the mortality and morbidity in patients with ALL.

Results: When the results of the study were evaluated, we found that uric acid levels, blast ratio on the 15th day bone marrow evaluation, presence of relapse and relapse type were effective on overall survival. Also, we found that blast ratio on 15th day bone marrow evaluation, high levels of uric acid, and lactate dehydrogenase (LDH) were effective for event-free survival.

Conclusion: In conclusion, our overall survival and disease-free survival rates are similar to those performed by St. Jude Total therapy XIIIB, BFM 2000 and BFM 2009 protocol.

Keywords: Acute lymphoblastic leukemia; BFM 2000; BFM 2009; St. Jude

INTRODUCTION

Acute lymphoblastic leukemia is a malignant disorder characterized with clonal enlargement of lymphoid progenitor cells (1). Clonal enlargement of lymphoid progenitor cells which causes anemia, thrombocytopenia and granulocytopenia, inhibits normal hematopoiesis. It is seen in patients suffering pallor, fatigue, bleeding, fever and severe infections.

ALL is the most common malignancy in childhood (2). Also, ALL is the most important malignant disease of this age group in terms of the faculty of achievement achieved in treatment. In recent years, risk-oriented treatment protocols have been used in the treatment of leukemia, thereby aiming to increase the rate of uneventful survival in patients and to reduce the toxic effects of the treatments given.

Recent developments in immunologic and genetic methods have significantly altered the diagnostic and classification approaches. Nowadays advanced studies such as immunologic and cytogenetic studies have become more important in prognosis and treatment response. In this study, we aimed to present the clinical and laboratory features of patient with ALL who were followed in our Pediatric Hematology Clinic (Necmettin Erbakan

Received: 14.10.2019 **Accepted:** 17.12.2019 **Available online:** 09.01.2020
Corresponding Author: Gokhan Aytekin, Necmettin Erbakan University, Meram Faculty of Medicine, Department of Internal Medicine, Division of Clinical Immunology and Allergy, Konya, Turkey **E-mail:** ayteking@gmail.com

University Faculty of Medicine, Konya) and determine the factors affecting the mortality and morbidity in patients with ALL.

MATERIAL and METHODS

Study Group

We reviewed the files of 188 patients diagnosed with acute lymphoblastic leukemia at Pediatric Hematology Department, Necmettin Erbakan University Faculty of Medicine, Konya, between April 2006 and April 2016 retrospectively. One hundred sixty-seven patients, who had sufficient records and accepting to participate in the study, were included. The study protocol was approved by the Ethics committee of the Necmettin Erbakan University, Faculty of Medicine. Informed consent was obtained from study participants. Patients were classified 3 groups according to their treatment protocols. These protocols were Saint Jude Total Therapy (St. Jude) protocol, Berlin-Frankfurt-Munster 2000 (BFM 2000) protocol and BFM 2009 protocol.

Statistical Method

Statistical analysis was performed using the SPSS version 16.0 package program. In the evaluation of the numerical variables, mean values were determined as the mean of the central tendency, the mean values for the distribution range of the values as well as the standard error values. In cases where the distribution range is too wide, the median value is given as the central tendency criterion. Life curves were obtained using the "Kaplan Meier" method. Prognostic factors were assessed using the "log rank" test with the univariate model. In all cases, values below 0.05 were interpreted significantly.

RESULTS

Of the 167 patients included in the study, 106 were male (63.5%) and 61 were female (36.5%). The ratio of males to females was M/F: 1.7/1. The ages of the patients ranged from 9 months to 204 months (median 80 months). In terms of gender there was no significant difference between the treatment protocols of St. Jude, BFM 2000 and BFM 2009 (p: 0.89).

It was seen that the patients had very different complaints and fever was the most common (30%). Other common causes were fatigue (20%) and neck swelling (10.8%). Among the physical examination findings were frequently hepatomegaly (51.5%), splenomegaly (51.5%) and lymphadenopathy (40.7%). Mediastinal involvement was detected in five patients. There were no patients with the central nervous system and testicular involvement at the time of diagnosis. All patients with mediastinal involvement were T-cell ALL. The clinical and laboratory values of the patients are summarized in Table 1.

The mean follow-up period was 47.9 months (1-113 months, median 37 months). In patients receiving the St. Jude protocol the mean follow-up period was 66.9 months (1-113 months; median 100 months), in patients receiving the BFM 2000 protocol the mean follow-up time was 24.8 months (1-41 months; median 25 months) and in patients receiving the BFM 2009 protocol the mean follow-up period was 9.4 months (1-17 months; median 9 months). The OAS rate was 88%. There was no difference between treatment protocols in terms of mortality (p: 0.442). The 5-year survival of the patients was also 88%.

When the univariate analysis was performed

•Gender,

•The number of age groups in patients receiving St. Jude and BFM 2009 protocol,

•The leukocyte count is less or more than to 50000/mm³ in patients receiving the St. Jude protocol,

•The leucocyte count is less or more than to 20000/mm³ in patients receiving the BFM 2009 and BFM 2000 protocol,

•The grouped form of hemoglobin and platelet values,

•The presence of high or low lactate dehydrogenase value or greater/lower than 1000 IU/L,

•Morphological FAB classification,

•Presence of negative cytogenetic changes [t (9,22), t (4,11), t (1,19)],

•The risk groups of the treatment protocols

•Immune phenotyping,

•On the 8th day, the presence of blast above or below 1000 / mm³ of peripheral smear,

•On the 33rd day, bone marrow examination

•Treatment protocols revealed that did not have a statistically significant effect on prognosis (Table 2).

When the univariate analysis was performed, it was found that

•Age group of patients receiving BFM 2000 protocol,

•High or low uric acid values,

•Presence of blast at the 15th day of bone marrow assessment,

•Presence of relapse and relapse pattern had a statistically significant effect on prognosis (Table 2).

The most common complication during treatment was febrile neutropenia. All (100%) patients developed febrile neutropenia. Systemic inflammatory response syndrome developed in 24 patients (14.4%), osteoporosis developed in 21 patients (12.6%), vincristine neuropathy developed in 12 patients (7.2%), invasive bronchopulmonary aspergillosis developed in 9 patients (5.4%), gastrointestinal hemorrhage developed in 2 patients (1.2%), methotrexate encephalopathy developed in 2 patients (1.2%).

Twenty of 167 patients who were diagnosed with acute lymphoblastic leukemia died during our follow-up (12.0%). The cause of death was sepsis in 16 of 20 patients (80%). Other causes of death were pulmonary thromboembolism, arterial thrombosis and intracranial hemorrhage (Table 1).

Table 1. Clinical and Laboratory Characteristics of ALL Patients

		St. Jude (n: 91)	BFM 2000 (n: 46)	BFM 2009 (n: 30)	Total	p
Gender	Boy (n %) Girl (n %)	59 (64.8) 32 (35.2)	29 (63.0) 17(39.9)	18 (60%) 12 (40%)	106 (66.5%) 61 (33.5%)	0.89
Age (month)	(n %)	0-24 month : 11 (12.1) 24-120 month : 61 (67.0) ≥120 month: 19 (20.9)	0-12 month : 0 (0) 12-72 month : 25 (54.3) ≥72 month : 21 (45.7)	0-12 month : 0 (0) 12-72 month : 20 (66.6) ≥72 month : 10 (33.3)	9-204 month (median 80 month)	---
Lymphocyte (/mm³)		≤ 50000 mm³ : 66 (72.6) > 50000 mm³ : 25 (27.4)	≤ 20000 mm³ : 34 (73.9) > 20000 mm³ : 12 (26.1)	≤ 20000 mm³ : 24 > 20000 mm³ : 6		----
Hemoglobin (g/L)	≤ 7 g/L (n %) 7-11 g/L (n %) ≥11 g/L (n %)	38 (41.8) 34 (37.4) 19 (20.9)	16 (34.8) 17 (37.0) 13 (28.3)	13 (43.3) 16 (53.3) 1 (3.3)	67 (40.1) 67 (40.1) 33 (19.8)	0.162
Platelet (/mm³) (n %)	<20000 /mm³ 20000-100000 /mm³ >100000 /mm³	20 (22.0) 50 (55.0) 21 (23.0)	9 (19.6) 19 (41.3) 18 (39.1)	8 (26.7) 13 (43.3) 9 (30)	37 (22.2) 82 (49.1) 48 (28.8)	0.352
LDH (Lactate dehydrogenase)	<1000 IU/L >1000 IU/L	20 (22.0) 71 (78.0)	11 (24.0) 35 (76.0)	6 (20) 24 (80)	37 (22.2) 130 (77.8)	0.921
Uric acid	Normal (n %) High (n %)	49 (53.9) 42 (46.1)	7 (15.2) 39 (84.8)	19 (63.3) 11 (36.6)	75 (45.0) 92 (55.0)	0.646
Immunphenotype	L1 (n %) L2 (n %) L3 (n %)	67 (73.6) 23 (25.3) 1 (1.1)	33 (71.8) 13 (28.2) 0	25 (83.3) 5 (16.1) 0	125 (74.9) 41 (24.6) 1 (0.5)	0.749
Flow cytometyric classification	Pre B cell (n %) Pre pre B cell (n %) B cell (n %) T cell (n %) Bi-fenotype cell(n%)	25 (27.5) 49 (53.9) 1 (1.1) 13 (14.3) 3 (3.3)	18 (39.1) 17 (37.0) 4 (8.7) 7 (15.2) 0	12 (40) 16 (53.3) 1 (3.3) 1 (3.3) 0	55 (33.0) 82 (49.1) 6 (3.6) 21 (12.6) 3 (1.8)	0.910
Cytogenetic evaluation (n %)	t (9.22) t (12.21) t (4.11) t (8.14) t (1.19)	2 (2.2) 0 3 (3.3) 1 (1.1) 1 (1.1)	2 (4.4) 8 (17.6) 0 0 1 (2.2)	0 4 (13.3) 1 (3.3) 0 4 (13.3)	4 (2.4) 12 (7.2) 4 (2.4) 1 (0.6) 6 (3.6)	0.472 0 0.459 0.657 **0.006**
Cytogenetic evaluation (n %)	No mutation Positive cytogenetic Negatif cytogenetic	83 (91.2) 6 (3.6) 2 (1.2)	33 (71.8) 3 (6.5) 10 (21.7)	21 (70) 5 (16.7) 4 (13.3)	137 (82.0) 14 (8.4) 16 (9.6)	**0.001**
8. Day peripheral smear evaluation	Blast <1000 /mm³ Blast ≥1000 /mm³	-- --	38 (82.6) 8 (17.4)	24 (80) 6 (20)	62 (81.6) 14 (18.4)	0.471
15. Day bone marrow evaluation (n %)	Blast < %0-5 Blast %5-20 Blast ≥ 20%	88 (96.7) 1 (1.1) 2 (2.2)	46 (100) 0 0	28 (93.3) 1 (3.3) 1 (3.3)	162 (97.0) 2 (1.2) 3 (1.8)	0.553

33. Day bone marrow evaluation	Blast ≤ 5 %	--	46	29	75	0,392
	Blast 5-20 %	--	1	1	2	
Risk groups (n %)	Standart risk group	40 (44.0)	19 (41.3)	16 (53.3)	72 (43.1)	
	Moderate risk group	---	24 (52.2)	12 (40.0)	36 (21.6)	--
	High risk group	51 (56.0)	3 (6.5)	2 (6.7)	56 (33.5)	
	None	81 (89.0)	45 (97.8)	29 (100)	155 (93.3)	
		Early Late				
Relapse (n %)	CNS	2 → 2 0	0	0	2 (1.2)	0.847
	Bone Marrow	5 → 3 2	1 (very early relaps)	0	6 (3.6)	
	Testicular	1 → 0 1	0	0	1 (0.6)	
	BM+CNS	1 → 1 0	0	0	1 (0.6)	
Mortality (n %)	Alive	78 (85.7)	43 (93.5)	26 (86.7)	147 (88.0)	0.442
	Death	13 (14.3)	3 (6.5)	4 (13.3)	20 (12.0)	
Cause of mortality (n %)	Sepsis	9 (69.2)	4 (100)	3 (100)	16	
	Pulmonary embolism	1 (7.7)	0	0	1	
	Arterial thrombosis	2 (15.4)	0	0	2	
	Intracranial bleeding	1 (7.7)	0	0	1	
	Total	13	4	3	20	

Table 2. Factors Affecting Overall Survival and Event-Free survival survival in ALL Patients

	Overall survival (%)	Event-Free Survival (%)		Overall Survival (%)	Event-Free Survival (%)
Age (St. Jude)			**Risk groups- BFM 2000**		
- 0-24 month	72.7	72.7	- Low	94.7	94.7
- 24-120 mont	90.2	83.6	- Moderate	91.7	91.7
- >120 month	78.9	73.7	- High	100	100
Log-rank	0.171	0.378	**Log rank**	0.840	0.840
Age (BFM 2000)			**Risk groups- BFM 2009**		
- <12 month	0	0	**- Low**	87.5	87.5
- 12-72 month	85.7	85.7	**- Moderate**	83.3	83.3
- >72 month	100	100	**- High**	100	100
Log rank	**0.049**	**0.049**	**Log rank**	0.777	0.777
Age (BFM 2009)			**Immunophenotype**		
- <12 month	0	0	- B cell	89.5	86.7
- 12-72 month	80	90	- T cell	81	76.2
- >72 month	90	80	- Biphenotypic	66.7	66.7
Log rank	0.446	0,446	**Log rank**	0.276	0.385
WBC count-St. Jude			**8. Day Peripheral Smear**		
- <50000/mm³		80.3	- Blast <1000/mm³	90.3	90.3
- >50000/mm³		80	- Blast >1000/mm³	92.9	92.9
Log rank		0.929	**Log rank**	0.742	0.742

WBC count-BFM 2000		
- <20000/mm³	86.4	94.1
- >20000//mm³	84	91.7
Log rank	0.720	0.78
Morphology		
- L1	86.4	84.8
- L2	90.2	85.4
- L3	100	100
Log rank	0.737	0.919
Cytogenetics		
- Favorable	100	
- Unfavorable	100	
- Undetected	85,4	
Log rank	0.123	
Uric acid		
- Normal	91.9	89.9
- High	80.9	83.1
Log rank	**0.038**	**0.019**
Hemoglobin		
- ≤ 7 gr/dl	92.5	89.6
- 7-11 gr/dl	85.1	82.7
- ≥11 gr/dl	81.8	80 %
Log rank	0.235	0.419
Relapse		
- **None**	90.4	
- **Present**	50.0	
Log rank	0.001	
Treatment Protocols		
- St. Jude	85.7	
- BFM 2000	93.5	
- BFM 2009	86.7	
Log rank	0.249	

WBC Count-BFM 2009		
- <20000/mm³	83.3	83.3
- >20000/mm³	100	100
Log rank	0.305	0.305
15. Day Bone Marrow Evaluation		
- %0-5 Blast	89.4	86.9
- %5-20 Blast	100	50
- >%20 Blast	33.3	33.3
Log rank	**0.001**	**0.001**
33. Day Bone Marrow Evaluation		
- %0-5 Blast	91.8	91.8
- %5-20 blast	100	100
Log rank	0.784	0.784
LDH		
- Normal	96	96.0
- High	85.9	83.1
Log rank	0.154	**0.035**
Platelet Count		
- <20000/mm³	81.3	81.1
- 20000-100000/mm³	89.0	87.8
- ≥100000/mm³	89.6	83.3 %
Log rank	0.378	0.666
Relapse Type		
- None	90.4	
- CNS	50	
- Bone marrow	50	
- Testicle	100	
- CNS +Bone marro	0	
Log rank		
Immunophenotype		
- **Prepre B**	89	
- **Pre B**	89.1	
- **B cell**	100	
- **T cell**	81	
- **Biphenotypic**	66.7	
Log rank	0.525	

DISCUSSION

Leukemias constitute 30-35% of childhood cancers. Acute lymphoblastic leukemia constitutes 75-80% of leukemias (2,3). With the help of randomized controlled trials, rapid development in the fields of cytogenetic and immunophenotyping, CNS prophylaxis and multiple chemotherapies in the treatment of ALL, most patients can be cured and serious improvements in OAS and disease-free survival are achieved. While OAS rates were 20% in the early 1960s, it has now reached almost 90% (4).

In a study by Pui, 5-year EFS in boys was 79.1%, in girls 83.3%; In the Schrappe study, the 6-year EFS rate in boys

was 75%, in girls 82%; In Stary, 5-year EFS rate was 72% in boys and 76% in girls (6-8). In current study, the OAS of boys was 86.8% and the OAS of girls was 88.5%, and the effect of gender on the prognosis was not found (p: 0.446). Similarly, EFS rates are 84% in males and 86% in females. Gender has no effect on EFS (p: 0.446). Reduction of testicular relapse as a result of intensive chemotherapy protocols today may reduce mortality and morbidity in male patients.

Although different age groups were defined in different treatment protocols, age was considered as an important prognostic factor in all treatment protocols. The EFS rates of our under 2 years old patients receiving St. Jude protocol were 72.7%; 83.6% from 2 to 10 years of age and it was 73.7% over the age of 10 years. Although the EFS of 2-10-year-old patients was higher than other age groups, this rate was not statistically significant (p: 0.378).

In the ALL BFM study in Turkey, 5-year EFS were 73% in patients aged 1 to 6 years, and 58% in patients over 6 years of age (12). In our study, we found that age for the BFM 2000 protocol has adverse prognostic significance with EFS. In terms of event free survival, the 1-6 year age group was better than the patients above the age of 6 years, but it was not statistically significant (p: 0.446). The reason why we could not find a relationship between age groups and prognosis when we evaluate the protocols separately can be explained by the low number of patients treated with the BFM 2000 and 2009 protocols.

High leukocyte counts at the time of diagnosis are still a poor prognostic factor. In a study of Pui, 44.9% of patients had leukocyte count of less than 10000/mm³, and 5-year EFS of these patients was 82.7%, 28% of patients had leukocyte count of 10000-49000/mm³ and the 5-year EFS of these patients was 88.6%, the leukocyte count of 11.3% is between 50000-99000/mm³ and the 5-year event free survival of these patients is 78.6%, the leukocyte count of 15.4% of the patients is above 100000/mm³ and the 5-year EFS of these patients is 63% (6). In our study, 27.5% of the patients treated with the St. Jude protocol had a leukocyte count of over 50.000 / mm³. The OAS rate of patients with leukocyte count <50000 / mm³ from patients receiving the St. Jude protocol was 86.4%, while the OAS rate of patients with leukocyte count> 50000 / mm³ was 84%. However, this difference was not statistically significant (p: 0.898). The EFS of these patients was 80.3% and 80%, respectively. In our study, we could not show the relationship between the number of leukocytes and prognosis, it might be because of the number of patients is relatively low and more intensive chemotherapies applied to patient with the leukocyte count > 50000/mm³.

Pui stated that, values above 1000 IU / L for LDH as high-risk, while values below 1000 IU / L are low-risk (14). In 85% of our patients, LDH levels were above our hospitals laboratory limits (LDH 0-220 IU / L). There was no significant difference between the treatment protocols in terms of LDH values (p: 0.352). Although the OAS rate of our patients with normal LDH level (96%) was higher than the patients with high LDH levels (85.9%), this difference was not significant (p: 0.154). The EFS rate of patients with normal LDH levels was statistically significant (96.1%) compared to patients with high LDH levels (83.1%) (p: 0.035). In our study, when we classified LDH levels as, the OAS rate in patients with LDH <1000 IU / L was 89.2% and in patients with LDH> 1000 IU / L was 83.8% and there was no statistically significant difference between the groups in terms of prognosis. The different results of Pui and our study may be due to different devices in different centers, hemolysis during blood collection, and additional diseases such as liver diseases which may be associated with it.

Uric acid level is important in terms of increased tumor load, increased white blood cell, stage of disease and renal function. During induction therapy, many patients are lost due to electrolyte and renal dysfunction. Uric acid is also important because it is part of the tumor lysis syndrome. Bassan reported that patients with creatinine> 1.6 mg / dl and uric acid> 8 mg / dl were at risk for renal failure (15). Crews showed that low uric acid levels in ALL patients were associated with less dialysis and less nephrotoxicity (16). In 40.7% of our patients, uric acid levels were above the laboratory limits of our hospital (uric acid 0-5.5 mg / dl). There was no significant difference in term of uric acid levels between treatment protocols (p: 0.646). The OAS rate of patients with normal uric acid levels was 91.9%, which was higher than patients with high uric acid levels (80.9%) and this difference was statistically significant (p: 0.038). Similarly, the EFS rates of patients with normal uric acid level (89.9%) were statistically significantly higher than the EFS rates of patients with high uric acid levels (77.9%) (p: 0.019).

One of the best indicators of prognosis in leukemia is response to chemotherapy. The BFM protocol accepted the steroid response on the 8th day as a favorable prognostic factor (17). In a study of Stary with 5060 patients who underwent the ALL IC-BFM 2002 treatment protocol, the steroid response rate was 90.2% and their 5-year EFS was 75%. In the same study, the ratio of patients who responded poorly to steroids was 9.2%, and the 5-year EFS rate was 59% (7). In the study performed by Hazar, the rate of patients who did not respond well to the steroid was 12.7%. In our study, there was no statistically significant difference between the OAS rates of patients with blast > 1000 / mm³ (92.9%) and those with blast <1000 / mm³ (88.7%). Similarly, patients with blast > 1000 / mm³ had a 5-year EFS rate of 90.3% and patients with blast <1000 / mm³ had 5-year EFS rates of 92.9%. In our study, the ratio of patients who did not respond to steroids was high compared to both studies.

In a study of 5060 patients, Stary evaluated the bone marrow examination of 66.5% of the patients on the 15th day as M1 bone marrow and the 5-year disease-free survival of these patients was found to be 78%. Twenty

four-percent of the patients were evaluated as bone marrow M2 and their survival rate was 72%. The bone marrow of 9.6% of the patients was evaluated as M3 and the 5-year EFS of these patients was 50% (7). Wei, in the study of 74 patients diagnosed with T-cell ALL, was able to perform bone marrow aspiration in 65 patients on the 15th day. Of these patients, 55.4% were M1, 29.2% were M2, 15.4% were M3 bone marrow and 5 years of EFS were 61.2%, 73.7% and 50%, respectively. Although patients with M3 bone marrow had lower incidence of EFS, bone marrow assessment on the 15th day was not found to be statistically significant (p: 0.129)(22). In our study, bone marrow aspiration was performed in 165 patients on the 15th day, M1 bone marrow in 160 patients (97%), M2 bone marrow in 2 patients (1.2%) and M3 bone marrow in 3 patients (1.8%). No significant difference was found between treatment protocols on the 15th day in terms of bone marrow evaluation (p: 0.553). On the 15th day of bone marrow evaluation, the OAS rate of patients with M3 bone marrow was found to be statistically lower than that of patients with M1 and M2 bone marrow with a rate of 33.3% (89.4%; 100%, respectively) (p: 0.001). EFS rates in patients with M3 bone marrow were also significantly lower (33.3%) than in other groups (p: 0.01). Our study also confirmed that bone marrow assessment at 15th day had a prognostic significance.

Stary performed ALL IC BFM 2002 protocol on 5060 patients, 96.9% of patients had remission at bone marrow evaluation on the 33rd day, with a 5-year eventual survival rate of 76%. In 3.1% of patients, no remission was observed and 5-year EFS was 39% (7). On the 33rd day, Moricke reported a survival rate of 80.6% in patients with remission in bone marrow assessment, whereas the survival rate of patients not in remission was significantly lower (36.3%) (23). Of the patients treated with the BFM protocol (total 76 patients) were underwent bone marrow biopsy on the 33rd day and no blast was detected in bone marrow in 75 patient (98.7%), in 1 patient (1.3%) M2 bone marrow was detected. No statistically significant difference was found between the BFM 2000 and 2009 protocols on the 33rd day for patients who were evaluated for bone marrow (p: 0.392). There was no significant difference between M1 and M2 bone marrow in terms of OAS rates. The presence of single patient with M2 bone marrow may have been effective in this result. Similarly, there was no significant difference in terms of EFS (p = 0.784).

In a study of Pui's 247 patients who underwent St. Jude protocol, 47.4% of patients were accepted as low risk, 52.6% were at high risk group and EFS was 99.1% and 96.9%, respectively (6). In the Hazar's study, 38% of the patients evaluated standard risk, 43.7% moderate risk, 18.3% in high risk group (12). In the literature, standard risk is reported as 28-36%, moderate risk is 50-61% and high risk group is 10-14% (18,23). Atay concluded that there is no difference in survival between risk groups in his study (13). Ninety-one (54.5%) of our patients were treated with the St.Jude protocol, 46 (27.5%) with BFM 2000 and 30 (18%) with the BFM 2009 protocol. Forty four percent of the patients treated with the St. Jude protocol were at the standard risk and 56.1% were at the high-risk group. 41.3% of the patients treated with the BFM 2000 protocol were at standard risk, 52.2% at moderate risk and 6.5% at high risk group. Fifty-three percent of those treated with the BFM 2009 protocol were at the standard risk, 40% at moderate risk and 6.6% at the high risk group. Among patients treated with the St. Jude protocol, the OAS rate was 85% in the standard risk group and 86.3% in the high-risk group. The EFS of these patients was 77.5% and 82.4%, respectively. Patients treated with the BFM 2000 protocol, the 5-year OAS in the standard risk group was 94.7%, in the middle-risk group was 91.7% and high-risk group was 100%. The 5-year EFS of these patients were 94.7%, 91.7% and 100%, respectively. Patients treated with the BFM 2009 protocol, the OAS in the standard risk group was 87.5%, in the middle-risk group was 83.3% and in the high-risk group was 100%. The EFS of these patients was 87.5%, 83.3% and 100%, respectively. Both our and Atay's study (13) it was found that there was no effect of risk groups on the survival of the patients and it may be, because of low number of patients and relatively short follow-up periods.

In a study of 5050 patients who receivied Stary's ALL IC-BFM 2002 protocol, 19% of patients developed relapse. Of these relapses, 12.5% were isolated bone marrow, 1.9% was CNS, and 1.3% was testicular relapse. Combined CNS and bone marrow relapse developed in 1.6% of patients (7). Moricke reported a 16.2% rate of relapse in her study. In the same study, 1.8% of the isolated CNS, 10% of isolated bone marrow, 2.2% of CNS and bone marrow relapse were reported, and 0.5% of isolated testicular relapse (23). The rate of relapse in patients receiving BFM 95 protocol by Bajel was 30.4% (24). This rate is 20.4% in the study of Hazar (12). In our study, relapse occurred in 10 patients (6.1%). The most common type of relapse was bone marrow relapse with 3.6%. 1.2% of the patients developed CNS relapse and 0.6% of the patients developed testicular relapse. In 1 patient, both bone marrow and CNS combined relapse developed (0.6%). In 9 of our patients treated with St. Jude protocol, relapse occurred. Six of these relapses were early relapse and 3 were late relapses. Of the early relapses, 3 were bone marrow, 2 were CNS, 1 were combined relapse of both BM and CNS. Two of the late relapse cases were BM and 1 were testicular relapse. 1 of the patients treated with the BFM 2000 protocol developed relapse. This relapse is very early relapse of bone marrow. The OAS rate of patients without relapse was 90.4%, while the OAS rate of patients with relapse was 50% and this difference was statistically significant (p: 0.001). When we classified the relapses according to the types of relapses, the OAS rate of the patient with CNS + bone marrow relapse was 0% while this rate was 50% in patients with CNS or BM relapse. The difference was statistically significant (p: 0.002). The relapse rates in our

study were lower than in the literature. This may be due to the fact that the follow-up period of patients who received the BFM 2009 and BFM 2000 protocol was shorter than the patients who received the St. Jude protocol. Nine of 10 patients who had relapse had St. Jude protocol, 1 patient who had relapse had BFM 2000 protocol and no relapse developed in patients who received BFM 2009 protocol and this supports our hypothesis. In addition, the number of patients who received the BFM 2000 and 2009 protocol in our study was relatively low. Although our relapse frequency is lower than in the literature, our relapse types are similar to other studies in the literature.

In Stary's study, 5-year EFS was 74% and OAS was 82% (7). In this study, 109 patients died without complete remission, and the causes of death were infection / sepsis in 60 patients, cerebral hemorrhage in 20 patients, and multi-organ failure in 5 patients, progressive ALL in 5 patients, and no cause was found in 19 patients. Two hundred fifty-five patients died after remission, 158 of them died of infection / sepsis, 19 of them hemorrhage, 16 of them had multiple organ insufficiency and 41 were lost due to other / unknown reasons. During our follow-up, 20 of our patients (12%) died. There was no significant difference in terms of mortality between treatment protocols (p: 0.424). The most common cause of mortality is sepsis with 80%. The 5-year OAS rate is 88%. The EFS rate is 85%. The OAS rate was 82.3% in the Hazar's study and the most common cause of death is infection with rate of 33% (12). This ratio is similar to our study. In our study, OAS or EFS was relatively high due to the fact that our median follow-up period was relatively short like 37 months and the number of our patients was low, especially in the groups receiving the BFM 2000 and BFM 2009 protocol.

CONCLUSION

When the results of the study were evaluated, uric acid elevation, the presence or absence of remission on the 15th day of bone marrow biopsy, the presence of relapse and the type of relapse, have a prognostic significance in terms of OAS rates; LDH, uric acid elevation and remission in bone marrow on the 15th day have prognostic significance on EFS. It was concluded that age had prognostic significance in terms of EFS for who receive BFM 2000 protocol. OAS and EFS rates of Jude Total therapy XIIIB, BFM 2000 and BFM 2009 protocols are similar to the centers applied, it is pleasant.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: The study protocol was approved by the Ethics committee of the Necmettin Erbakan University, Faculty of Medicine. Informed consent was obtained from study participants.

Selma Erol Aytekin ORCID: 0000-0001-6256-5988
Gokhan Aytekin ORCID: 0000-0002-9089-5914
Huseyin Tokgoz ORCID: 0000-0002-3064-4646
Umran Caliskan ORCID: 0000-0003-4695-7046

REFERENCES

1. Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. Lancet 2008;37:1030-43.
2. Ward E, DeSantis C, Robbins A, et al. Childhood and adolescent cancer statistics, 2014. CA: A cancer journal for clinicians 2014;64:83-103.
3. Bhojwani D, Yang JJ, Pui CH. Biology of childhood acute lymphoblastic leukemia. Pediatric clinics of North America 2015;62:47-60.
4. Svendsen AL, Feychting M, Klaeboe L, et al. Time trends in the incidence of acute lymphoblastic leukemia among children 1976-2002: a population-based Nordic study. The J Pediatrics 2007;151:548-50.
5. Dores GM, Devesa SS, Curtis RE, et al. Acute leukemia incidence and patient survival among children and adults in the United States, 2001-2007. Blood 2012;119:34-43.
6. Pui CH, Sandlund JT, Pei D et al. Improved outcome for children with acute lymphoblastic leukemia: results of Total Therapy Study XIIIB at St Jude Children's Research Hospital. Blood 2004;104:2690-6.
7. Stary J, Zimmermann M, Campbell M et al. Intensive Chemotherapy for Childhood Acute Lymphoblastic Leukemia: Results of the Randomized Intercontinental Trial ALL IC-BFM 2002, 2013.
8. Schrappe M, Reiter A, Ludwig WD et al. Improved outcome in childhood acute lymphoblastic leukemia despite reduced use of anthracyclines and cranial radiotherapy: results of trial ALL-BFM 90. German-Austrian-Swiss ALL-BFM Study Group. Blood 2000;95:3310-22.
9. Gatta G, Capocaccia R, Stiller C et al. Childhood cancer survival trends in Europe: a EUROCARE Working Group study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2005;23:3742-51.
10. Siegel R, Ward E, Brawley O, et al. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. CA: a cancer journal for clinicians 2011;61:212-36.
11. Pui CH, Campana D, Pei D, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. The New England J Med 2009;360:2730-41.
12. Hazar V, Karasu GT, Uygun V, et al. Childhood acute lymphoblastic leukemia in Turkey: factors influencing treatment and outcome: a single center experience. Journal of pediatric hematology/oncology 2010;32:317-22.
13. Atay A, Kürekci E, Kesik V et al. Akut lenfoblastik lösemili olgularimizin retrospektif analizi. Gülhane Tip Dergisi 2005;47:183-6.
14. Pui CH, Williams DL, Kalwinsky DK et al. Cytogenetic features and serum lactic dehydrogenase level predict a poor treatment outcome for children with pre-B-cell leukemia. Blood 1986;67:1688-92.
15. Bassan R, Gatta G, Tondini C, et al. Adult acute lymphoblastic leukaemia. Critical reviews in oncology/hematology 2004;50:223-61.

16. Crews KR, Zhou Y, Pauley JL et al. Effect of allopurinol versus urate oxidase on methotrexate pharmacokinetics in children with newly diagnosed acute lymphoblastic leukemia. Cancer 2010;116:227-32.
17. Margolin J, Rabin K, Steuber C, et al. Acute lymphoblastic leukemia. In: Pizzo P, Poplack D, editors. Acute lymphoblastic leukemia. Philadelphia: ippincott Williams & Wilkins 2011:518-65.
18. PL. Leukemias. In: P L, editor Manual of Pediatric Hematology Oncology. 6th ed: Elseiver Academic Pres 2005:415-50.
19. Aziz Z, Zahid M, Mahmood R, et al. Modified BFM protocol for childhood acute lymphoblastic leukemia: a retrospective analysis. Medical and Pediatric Oncology 1997;28:48-53.
20. Hoelzer D, Bassan R, Dombret H et al. Acute lymphoblastic leukaemia in adult patients: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of oncology : official journal of the European Society for Medical Oncology 2016;27:69-82.
21. Rubnitz JE, Pui CH. Childhood Acute Lymphoblastic Leukemia. The oncologist 1997;2:374-80.
22. Wei W, Chen X, Zou Y et al. Prediction of outcomes by early treatment responses in childhood T-cell acute lymphoblastic leukemia: a retrospective study in China. BMC Pediatrics 2015;15:80.
23. Moricke A, Reiter A, Zimmermann M et al. Risk-adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95. Blood 2008;111:4477-89.
24. Bajel A, George B, Mathews V et al. Treatment of children with acute lymphoblastic leukemia in India using a BFM protocol. Pediatric blood & cancer 2008;51:621-5.