Oncology
and Radiotherapy ©
17(10) 2023: 472-476 • RESEARCH ARTICLE

Immunological and cytogenetic features, treatment management and prognosis in pediatric relapsed/refractory leukemias

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ABSTRACT

Background: Relapsed Acute Lymphoblastic Leukemia (ALL) has remained challenging to treat in children with survival rates lagging well behind those observed at initial diagnosis. Although there have been some improvements in outcomes over the past few decades, only approximately 50% of children with first relapse of ALL survive long term, and outcome are much worse with second or later relapses.

Material and Methods: In this study, 24 Patients who received leukemia treatment in the pediatric hematology and oncology clinic of our hospital between the years 2020-2023 and were diagnosed as refractory or relapsed were included. Diagnostic and treatment information such as immunophenotype, cytogenetics, treatment protocols, relapse treatment protocols, demographic data, relapse characteristics of the patients were recorded in patient-specific sheets. Considering these data, the survival rates of the patients and the response rates to the treatments were presented.

Results: A total of 24 patients, 7 of them were girls (29%), 17 of them were boys (71%). 19 of the patients were ALL (5 T-ALL, 14 B-ALL) and 5 AML. The mean age at primary diagnosis of the patients was 7.04 years ± 4.51 years, and the mean age at relapse was 8.77 years ± 4.31 years. The relapse times of the patients were 7 very early (30%), 7 early (30), 8 late (32%) relapse, 2 (8%) patients who could not achieve remission. The relapse times of the patients were 7 very early (30%), 7 early (30), 8 late (32%) relapse, 2 (8%) patients who could not achieve remission. At the time of relapse, 7 (30%) of the patients were in the standard risk group and 17 (70%) were in the high-risk group. Isolated bone marrow 16 (66%), Isolated Cerebrospinal Fluid (CSF) 4 (17%), isolated testis 1 (4%), and bone marrow and CSF 3 (13%) were relapsed. In cytogenetic studies, t9.22 gene mutations were found in 4 (17%) patients, complex karyotype in 2 (8%) and FLIT3 gene mutations in 1 (4%).

Conclusion: Until recently, the main treatment options for relapsed ALL were cytotoxic chemotherapy and HSCT. Now there are a variety of treatment options, including highly active immunotherapies for B-ALL, small molecule inhibitors of pathways altered in relapsed T-ALL, and improved HSCT technologies. Risk stratification has been further refined at initial diagnosis and some of these therapies are now also being investigated. Future efforts will sustain the best choices for better porgnostic outcome of Pediatric Leukemias.

Key words: relapsed leukemia, immunology, phenotype, chemotherapy, bone marrow transplant

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Word count: 3547 **Tables:** 04 **Figures:** 01 **References:** 24

Received: 18 September, 2023, Manuscript No.: OAR-23-113819

Editor Assigned: 21 September, 2023, PreQC No.: OAR-23-113819 (PQ)

Reviewed: 07 October, 2023, QC No.: OAR-23-113819 (Q)

Revised: 12 October, 2023, Manuscript OAR-23-113819 (R)

Published: 17 October, 2023, Invoice No. J-23-113819

INTRODUCTION

Relapse of disease, especially in BM, is an ominous event, which is often equated with failure of our endeavour to cure children with ALL. After infection-related toxic deaths, relapse of disease is a major cause of morbidity and mortality in developing nations. Treatment in ALL has evolved over the years to its present day successful risk-adapted approach. Therapy protocols have been modified to overcome problem areas identified by determining a pattern of relapsed disease [1-5].

Recurrences that occur within three years of diagnosis and any T-ALL relapses are particularly difficult to salvage. Acute Myeloid Leukemia (AML) is composed of a heterogeneous group of diseases that can be classified by morphology, lineage, and genetics. Despite the large number of subtypes and the lack of targeted therapy for most subtypes, the treatment outcome has improved markedly for children with AML.

Excellent supportive care, adaptation of therapy on the basis of each patient's response, and the use of intensive chemotherapy or Hematopoietic Stem Cell Transplantation (HSCT) have led to Event-Free Survival (EFS) rates that are greater than 50% and Overall Survival (OS) rates greater than 60% on recent trials. Until recently, treatment options were limited to intensive cytotoxic chemotherapy with or without site-directed, radiotherapy and allogeneic Hematopoietic Stem Cell Transplantation (HSCT). In the past decade, several promising immunotherapeutic have been developed, changing the treatment landscape for children with relapsed ALL and AML [6-9].

MATERIALS AND METHODS

Totally 24 Patients who received leukemia treatment in the pediatric hematology and oncology clinic of our hospital between the years 2020-2023 and were diagnosed as refractory or relapsed were included. Diagnostic and treatment information such as immunophenotype, cytogenetics, treatment protocols, relapse treatment protocols, demographic data, relapse characteristics of the patients were recorded in patient-specific sheets. Considering these data, the survival rates of the patients and the response rates to the treatments were presented.

Statistical analysis

Data were analyzed with IBM SPSS V23. Conformity to the normal distribution was examined using the Shapiro-Wilk Test. Paired Samples T Test was used to compare normally distributed

data in dependent groups, and Wilcoxon Test was used to compare data that were not normally distributed. Cohran's Q Test was used to compare categorical and dependent variables. Analysis results were presented as frequency (percentage) for categorical variables, and as mean ± standard deviation and median (minimum-maximum) for quantitative variables. Significance level was presented as $p<0.050$.

RESULTS

A total of 24 patients; 7 of them were girls (29%), 17 of them were boys (71%). 19 of the patients were ALL (5 T-ALL, 14 B-ALL) and 5 AML. The mean age at primary diagnosis of the patients was 7.04 years ± 4.51 years, and the mean age at relapse was 8.77 years ± 4.31 years. The relapse times of the patients were 7 very early (30%), 7 early (30), 8 late (32%) relapse, 2 (8%) patients who could not achieve remission. The relapse times of the patients were 7 very early (30%), 7 early (30), 8 late (32%) relapse, 2 (8%) patients who could not achieve remission. At the time of relapse, 7 (30%) of the patients were in the standard risk group and 17 (70%) were in the high-risk group Table 1.

Isolated bone marrow 16 (66%), isolated Cerebrospinal Fluid (CSF) 4 (17%), isolated testis 1 (4%), and bone marrow and CSF 3 (13%) were relapsed. In cytogenetic studies, t9.22 gene mutations were found in 4 (17%) patients, complex karyotype in 2 (8%) and FLIT3 gene mutations in 1 (4%). ALL-IC BFM REL2016 protocol was started for ALL patients. 2 patients with AML received idarubicin+clofarabine and 3 patients received Flag+idarubicin chemotherapy. Five of the ALL patients died with neutropenia and other side effects at various stages of salvage chemotherapy, Table 2.

A statistically significant difference was found between the mean age of the patients in primary and relapse ($p=0.013$). While the mean age was 7.04 in primary, it was 8.77 in relapse. A statistically significant difference was found between the median WBC values of patients in primary and relapse ($p=0.008$). While the median WBC value at admission was 22880 in primary, this value was obtained as 2550 in relapse, Tables 3 and 4.

Allogeneic HSCT was performed in 1 AML-M5 patient in remission, 2 allogeneic HSCTs from SMD in 1 T-ALL patient, 2 haploidentic HSCT in 2 B-ALL patients and 2 haploidentic HSCT in 1 B-ALL patient. One of these patients is B-ALL; the patient who underwent haploidentic HSCT is in our follow-up with chronic GVHD. Other patients are in outpatient follow-up as they are in remission. In this study, the patients' OS (mean 2

Tab. 1. Frequency distributions of the variables in Relapse Disease

	Frekans (n)	Yuzde (%)
Translocations		
Flt3	1	5,0
Complex karyotype	2	10,0
t(9:22)	4	20,0
Yok	13	65,0
Sct		
Positive	4	19,0
Negative	17	81,0
Relapse time		
Very Early	7	30,4
Early	7	30,4
Late	8	34,8
No remission	1	4,3
Relapse Risk Group		
Standart	7	30,4
High Risk	16	69,6
Relaps Bone Marrow		
M1	5	20,8
M3	19	79,2
Currently		
Exitus	5	21,7
Live	18	78,3
Relapse findings		
Headache	1	7,1
Bone marrow involvement	1	7,1
Vomiting	1	7,1
Lymphadenopathy	1	7,1
Elaveted LDH	1	7,1
Leukocytosis	1	7,1
Pancytopenia	1	7,1
No remission	1	7,1
Strabismus	1	7,1
Testicular mass	1	7,1
Thrombocytopenia	4	28,6
Relapse moment treatment stage		
Maintanence	13	56,5
Protocol II	1	4,3
Ted Kesimi	10	39

Tab. 2. Comparison of age and WBC values in primary and relapse time

time	Primary		Relapse		Test 1st	p
	Ort. ± ss	Ort. (min-max)	Ort. ± ss	Ort. (min-max)		
Age	7,04 ± 4,51	5,8 (1 - 17)	8,77 ± 4,31	8,5 (2,2 - 19)	-2,685	0,013*
WBC value	76409,09 ± 94268,1	22880 (2250 - 296000)	12544 ± 20873,63	2550 (1000 - 55000)	-2,666b	0,008**

*Paired Sample t Test; **Wilcoxom Test
WBC: White Blood Count

Tab. 3. Comparison of relapse treatments regarding to primary treatment protocols

		Primary treatment protocol /Frequency (percent)			
		ALL IC BFM 2009	AML BFM2013	AML BFM2019	EspHall
Relapse treatment	ALL REL BFM	18 (100)	0 (0)	0 (0)	1 (100)
	flag+klo	0 (0)	0 (0)	3 (75)	0 (0)
	ida+flag	0 (0)	1 (100)	1 (25)	0 (0)

Tab. 4. Involvement sites relapse diseases

		Frequency (percent)
Relapse ınvolvement site	CSF	3 (12,5)
	CSF+Other	1 (4,2)
	BM	16 (66,7)
	BM+CSF	3 (12,5)
	Testiculary	1 (4,2)

years) was 19/24 (80%) after relapse until the end of the relapse chemotherapy protocol.

DISCUSSION AND CONCLUSION

Acute Lymphoblastic Leukemia (ALL) is the most frequently diagnosed cancer in children. Outcomes for children with newly diagnosed ALL are excellent, with an overall survival rate of 90%. 1 Relapse occurs in 15%-20% of children who are diagnosed with ALL, and outcomes after relapse are poor. The treatment for relapsed ALL is intensive chemotherapy followed by allogeneic Hematopoietic Stem Cell Transplant (HSCT) for patients that have an early bone marrow relapse or suboptimal response to reinduction chemotherapy. Standard reinduction chemotherapy includes an anthracycline combined with vincristine, asparaginase, prednisone or dexamethasone, and Centralnervoussystem (CNS) directed therapy [10-15].

In the ALL R3 trial for children with a first relapse of ALL, patients randomized to receive mitoxantrone in reinduction combined with dexamethasone, pegaspargase, vincristine, and intrathecalmethotrexate had a 3-year progression- free survival of 64.6% 3. This was an improvement over historical results and significantly better than the comparison group that received idarubicin who had a lower 3-year progression-free survival of 35.9% [16].

While somatic genetic alterations are not routinely used in risk assignment following relapse, several unfavorable alterations have been identified and could influence treatment decision-making. We would perform gene sequencing technics and RNA-based fusion gene testing. If the Philadelphia chromosome were present, we would add imatinib or dasatinib to reinduction therapy and would strongly consider use of these agents if an ABL class gene fusion was detected. One might also consider addition of ruxolitinib if a JAK2 fusion were detected, but the efficacy and safety of this strategy have not yet been proven, so we would use ruxolitinib only if she were refractory to reinduction or remained MRD positive after 2 cycles of blinatumomab. TP53 mutations can be acquired at relapse and are associated with very poor outcome. If present, it would be reasonable to consider HSCT or CAR T-cells in CR2 regardless of time to relapse or MRD response. If she had a late marrow relapse with a high-risk somatic genetic alteration, such as TCF3-HLF fusion, we would consider HSCT or CAR T-cells in CR2 regardless of MRD response [17-19].

Acute Myeloid Leukemia (AML) is composed of a heterogeneous group of diseases that can be classified by morphology, lineage, and genetics. Despite the large number of subtypes and the lack of targeted therapy for most subtypes, the treatment outcome has improved markedly for children with AML [20, 21].

Excellent supportive care, adaptation of therapy on the basis of each patient's response, and the use of intensive chemotherapy or Hematopoietic Stem cEll Transplantation (HSCT) have led to event-free survival (EFS) rates that are greater than 50% and Overall Survival (OS) rates greater than 60% on recent trials [22-24].

In our clinical trial; a total of 24 patients; 7 of them were girls (29%), 17 of them were boys (71%). 19 (%79) of the patients were relapse ALL (5 T-ALL, 14 B-ALL) and 5 (21%) of them were relapse AML. The relapse times of the patients were 7 very early (30%), 7 early (30%), 8 late (32%) relapse, 2 (8%) patients who could not achieve remission. The relapse times of the patients were 7 very early (30%), 7 early (30), 8 late (32%) relapse, 2 (8%) patients who could not achieve remission. At the time of relapse, 7 (30%) of the patients were in the standard risk group and 17 (70%) were in the high-risk group. Isolated bone marrow 16 (66%), isolated cerebrospinal fluid (CSF) 4 (17%), isolated testis 1 (4%), and bone marrow and CSF 3 (13%) were relapsed.

Initial chemaotheraphy preference for relapse ALL patients was ALLIC-REL BFM protocol 2016, Figure 1.

The risk strafication in relaps ALL patient are goruped standart or high risk chemotheraphy according to egibility for Stem Cell Transplantation (SCT). Any T-cell relaps involving bone marrow, any very early relaps, any early isolated relapse in BM,

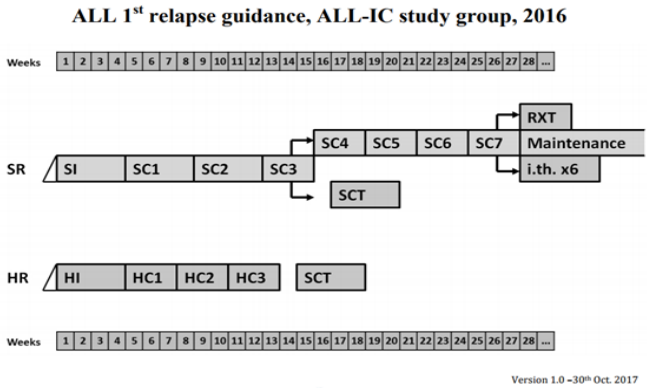

Fig. 1. ALL BFM study group guidance

all relapses after SCT, certain genetic groups stratified as high risk ALL while the rest as standart risk ALL. Any high risk patients, standart risk patients with poor initial treatment response defined as bone marrow MRD>0, 1% and certain genetic groups were stratified as eligible to SCT. The chemotherapy regimen include dexamethasone, 6-mercaptopurine, vincristine, methotrexate, cytarabine, L asparaginase, 6 thiogunaine and cycophosphamide in divided blocs. A statistically significant difference was found between the median WBC values of patients in primary and relapse (p=0.008). While the median WBC value at admission was 22880 in primary, this value was obtained as 2550 in relapse. Although WBC values are found to be higher at the time of initial diagnosis, this does not seem to be present in relapsed disease. However, the most prominent laboratory finding at the time of relapse was found to be thrombocytopenia with a rate of 28.4%.

In cytogenetic studies, t (9.22) gene mutations were found in 4 (20%) patients, complex karyotype in 2 (8%) and FLIT3 gene mutations in 1 (4%). In pediatrci leukemias; t (9.22) gene mutations and and FLIT3 gene mutations are strongly corraleted with worsened outcome and overall survival in these groups. Even usage of imatinib, a tyrosine kinase inhibitor in t (9, 22) positive group suggested better outcome in ALL, in relapse individuals benifits seem unclear and needs morre attention and clinical trials.

Allogeneic HSCT was performed in 1 patient with AML-M5 in remission, 2 allogeneic HSCTs from SMD in 1 patient with T-ALL, 2 haploidentic HSCT in 2 B-ALL patients and 2 haploidentic HSCT in 1 B-ALL patient. One of these patients with B-ALL; The patient who underwent haploidentic HSCT is in our follow-up with chronic GVHD. Other patients are in outpatient follow-up as they are in remission. All of the patients' OS (mean 2 years) was 19/24 (80%) after relapse until the end of the relapse chemotherapy protocol.

In conclusion, relapse of disease was documented in total 24 of cases who are under follow up in 2020 to 2023 in our clinic. The majority of patients relapsed on chemotherapy. We observed a very high incidence of BM (78%) and CNS (16%) relapses. These observations dictate the need for reappraisal of treatment protocols. Very early relapsers necessitate the administration of aggressive chemotherapy upfront. Besides cranial irradiation, high-risk cases probably require administration of intrathecal mtx with and in AML of cytosine arabinoside.More clinical trials involving newly devoloped TARGET treatments will adjust the outcome in pediatric leukemias.

DECLARATIONS

Ethics approval and consent to participate have been taken from Health Sciences University.

Ethical committie

Consent for publication has been taken from the patients' parents. Patient's parents gave informed written consent for their personal or clinical details along with any identifying images to be published in this study. Ethics approval and consent to participate have been taken from Health Sciences University.

ACKNOWLEDGEMENTS

The authors are thankfull to all indivuduals have contributed to this study.

CONFLICTS OF INTEREST

The authors have no conflict of interest to declare.

FUNDING

The authors did not receive support from any organization for the submitted work.

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