RESEARCH ARTICLE

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Outcome of the Modified St. Jude Total XV Protocol in Turkish Children with Newly Diagnosed Acute Lymphoblastic Leukemia: A Single-Center Retrospective Analysis

Yeni Tanı Almış Akut Lenfoblastik Lösemili Türk Çocuklarında Modifiye St. Jude Total XV Protokolünün Sonuçları: Tek Merkezli Retrospektif Bir Analiz

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Abstract

Objective: The prognostic factors and outcomes of Turkish children with newly diagnosed acute lymphoblastic leukemia (ALL), treated with the Modified St. Jude Total XV Protocol, which was adjusted by adding high-dose methylprednisolone (HDMP) before induction in the original protocol, were assessed in this study.

Materials and Methods: The Modified St. Jude Total XV Protocol was administered to 183 newly diagnosed ALL patients, aged 1-18 years, between 1 January 2008 and 30 January 2016. HDMP was applied at doses of either 10 mg/kg/day (Group A) or 20 mg/kg/day (Group B) for 7 days before induction and then tapered over the next 7 days to 5 or 10 mg/kg/day, and continued at 2 mg/kg/day for 2 weeks during the induction phase. Absolute blast count (ABC) in peripheral blood and minimal residual disease (MRD) in bone marrow were assessed at the end of the initial 7-day HDMP treatment. MRD in the bone marrow was evaluated on day 15 and at the end of the induction period. The follow-up for these patients ended on 15 July 2019.

Results: The 5-year event-free (EFS) and overall survival (OS) rates for all patients were 85.6±2.6% and 89.2±2.3%, respectively. The rate of good response to steroids (defined as ABC in peripheral blood of less than 1000/mm^3 on day 7) was 88% and 97% of children achieved complete remission after induction. The survival rate and infection frequency did not show statistically significant differences between Group A and B. EFS and OS correlated with initial leukocyte count, age of 10-18 years at diagnosis, CD20 positivity at diagnosis, and gram-negative bacterial infection during remission induction.

Conclusion: The remarkable response rates on days 7 and 15, along with the promising EFS and OS results in childhood ALL patients treated with the Modified St. Jude Total XV Protocol, highlight the early and substantial response effect of HDMP. At the onset of induction, short-term HDMP can be initiated, preferably at 10 mg/kg/day for the first 7 days, to minimize potential side effects.

Keywords: Acute lymphoblastic leukemia, Child, Survival, Prognostic factors

Öz

Amaç: Bu çalışmada, akut lenfoblastik lösemi (ALL) tanısı almış ve orijinal protokole indüksiyon fazının öncesine yüksek doz metilprednizolon (YDMP) tedavisi eklenerek modifiye edilmiş Modifiye St, Jude Total XV protokolü uygulanan çocuk hastalarda sağkalım ve prognostik faktörleri incelemektedir.

Gereç ve Yöntemler: 1 Ocak 2008'den 30 Ocak 2016'ya kadar tanı almış 183 ALL hastasına (1-18 yaş), Modifiye St. Jude Total XV tedavisi uygulandı. YDMP tedavisi, indüksiyon öncesinde 7 gün, 10 veya 20 mg/kg/gün dozlarında randomize olarak verildi. Takip eden 7 gün, 5 ve 10 mg/kg/gün ve ardından 2 hafta boyunca 2 mg/kg/gün olarak uygulandı. Periferik kanda absolü blast sayısı (ABS) ve kemik iliğinde minimal kalıntı hastalık (MKH), YDMP tedavisinin sonunda (7. gün) değerlendirilirken, indüksiyonun 15. gün ve sonunda kemik iliğinde MKH incelemesi yapıldı. Hastaların takibi 15 Temmuz 2019'da sonlandırıldı.

Bulgular: Tüm hastalar için 5 yıllık olaysız sağkalım (OSK) oranı %85,6±2,6 ve genel sağkalım (GSK) oranı %89,2±2,3'tü. Steroide iyi yanıt veren (YDMP tedavisi 7. günde periferik kanda ABS <1000/mm^3) hastaların oranı %88 iken indüksiyon sonunda tam remisyon %97 hastada kaydedildi. İki grup karşılaştırıldığında, sağkalım oranları ve enfeksiyon sıklığında anlamlı farklılık gözlemlenmedi. OSK ve GSK, tanı anındaki lökosit sayısı, tanı yaşının 10-18 olması, tanıda CD20 pozitifliği ve indüksiyon döneminde gram-negatif bakteriyel enfeksiyon geçirme durumu ile ilişkili bulundu.

Sonuç: Modifiye St. Jude Total XV tedavisi uygulanan çocukluk çağı ALL hastalarında, indüksiyon tedavisinin 7. ve 15. gününde gözlemlenen dikkate değer yanıt oranları ve teşvik edici sağkalım sonuçları, YDMP'nin erken ve yüksek tedavi yanıtına katkı sağlayabileceğini vurgulamaktadır. İndüksiyon tedavisinin başlangıcında, kısa süreli, olası yan etkileri de en aza indirmek adına 10 mg/kg/gün dozunda YDMP tedavisi uygulanabilir.

Anahtar Sözcükler: Akut lenfoblastik lösemi, Çocuk, Sağkalım, Prognostik faktörler

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Introduction

Childhood acute lymphoblastic leukemia (ALL) is the most common malignancy in children, with 5-year survival rates reaching 85%-90% owing to advancements in risk-stratified treatment protocols and supportive care [1]. Advances in clinical trials and a deeper understanding of ALL pathobiology have enhanced precise and effective risk classifications, directing intensive treatment to high-risk groups and protecting others from drug-induced toxicities [2,3,4,5,6,7].

The primary aim in the induction phase is the maximal eradication of lymphoblasts to ensure remission while preventing the development of drug resistance. Glucocorticoids have been the backbone agent in the induction treatment of ALL, used at conventional doses (40-60 mg/m^2/day prednisolone) varying in bioequivalence across different clinical studies [8,9,10,11]. In our center, high-dose methylprednisolone (HDMP) therapy has been used for patients with ALL before and during remission induction since 1990, following encouraging results in various clinical trials, including trials with patients with both acute myeloid leukemia and ALL. Corticosteroid-dependent reduction of lymphoblasts in peripheral blood has been shown as a good prognostic marker for survival [12,13,14,15].

In this study, we investigate the impact of different HDMP doses in induction on early treatment response and survival in childhood ALL. Our aim is to contribute to a new therapeutic approach for enhancing survival rates and bolster research on childhood ALL survival in Türkiye. To address the efficacy of HDMP and the limited experience with diverse treatment protocols, we conducted a retrospective analysis of patients who were treated with the Modified St. Jude Total XV Protocol at our hospital, one of the leading cancer centers in Türkiye.

Materials and Methods

Study Population

The medical records of 207 patients newly diagnosed with ALL at Hacettepe University's İhsan Doğramacı Children's Hospital between 1 January 2008 and 30 January 2016 were reviewed. Exclusion criteria were age under 1 year (n=8), treatment at another hospital before admission to our center (n=1), protocol changes (n=6), and post-diagnosis transfer to another hospital (n=1). Data on the sociodemographic, clinical, laboratory, imaging, and treatment characteristics of the 183 included patients were extracted from electronic health records and patient files.

Treatment Protocol

The St. Jude Total XV Protocol was modified to include a 7-day HDMP therapy before induction and the omission of upfront high-dose methotrexate (HDMTX) and intrathecal cytarabine. Consequently, days 19 and 26 of induction corresponded to days 15 and 22, respectively. The second modification was administration of the first dose of HDMTX at 2.5 g/m^2 to all risk groups in consolidation treatment due to unknown methotrexate (MTX) pharmacokinetics. Notably, the original protocol established the safety of excluding cranial radiotherapy (RT) from childhood ALL treatment, and no patients in our study received cranial RT. Additionally, as a minor change, the DNA index was not used in risk classification as it was not assessed at our hospital. Aside from these adjustments, our protocol and treatment response assessment were identical to those of the original protocol [16]. The details of remission induction and the criteria for risk classification are summarized in the Supplementary Information.

Patients were consecutively randomized into Group A (n=65, 36%) or Group B (n=90, 49%), receiving HDMP at 10 or 20 mg/kg/day, respectively (maximum: 1000 mg/day) for 7 days before induction. HDMP doses were tapered to 5 or 10 mg/kg/day, respectively, the following week. Both groups then received 2 mg/kg/day MP for the subsequent 2 weeks. A third group, Group C, included patients with high tumor burden (hyperleukocytosis [>100x10^9/L] and significant organomegaly) who received lower MP doses (<10 mg/kg/day) for the first 7 days to forestall acute tumor lysis syndrome (TLS). Upon completion of the HDMP treatment, on day 7, the peripheral absolute blast count (ABC) was recorded to assess early treatment response, with <1x10^9/L in peripheral blood accepted as a good steroid response. Bone marrow (BM) minimal residual disease (MRD) was assessed on day 15 and at the end of induction for all patients and on day 22 for those with MRD of 1% or more on day 15. Poor response was defined as BM MRD of >1%, 1%, and 0.01% on days 15 and 22 and at the end of induction, respectively.

Morphological assessment, cytogenetic analysis (including classical karyotyping, fluorescence in situ hybridization, and/or polymerase chain reaction), and immunophenotyping were performed at diagnosis for all patients. Cerebrospinal fluid (CSF) analysis involved cytocentrifugation and cytological methods to determine central nervous system (CNS) involvement, categorized as CNS-1 (no detectable blast cells), CNS-2 (<5 leukocytes/mm^3 with blasts and <10 erythrocytes/mm^3), CNS-3 (≥5 leukocytes/mm^3 with blasts and <10 erythrocytes/mm^3), or traumatic status (>10 erythrocytes/µL CSF with blasts) as per the original protocol. Even if blasts were not observed, we classified patients with erythrocytes in their CSF as traumatic to adhere to the protocol and administered additional triple intrathecal treatment.

Immunophenotyping and MRD assessment were performed by flow cytometry (FCM), defining CD positivity as more than 20% expression in leukemia blasts within BM samples. Patients were categorized into being of low, standard, or high risk after

remission induction treatment according to the criteria of the original protocol.

This study was approved by the Ethics Committee of Hacettepe University Faculty of Medicine (decision no: 2019/24-18, date: 15.10.2019).

Statistical Analysis

Chi-square and Fisher exact tests were used to compare differences in the distribution of parameters among patient subgroups. Survival analysis included event-free survival (EFS), measured from diagnosis to first outcome event (treatment failure, death, relapse, secondary malignancy, or treatment discontinuation), and overall survival (OS), measured from diagnosis to death from any cause or loss of contact. The statistical impact of demographic, clinical, and laboratory characteristics of patients on survival was evaluated via Kaplan-Meier analysis with the log-rank test. Variables with $p<0.10$ in univariate analysis were included in the Cox proportional hazard regression model using the enter method to identify the most impactful factors. IBM SPSS Statistics 22.0 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses, with $p<0.05$ considered statistically significant.

Results

Initial Characteristics of Patients

Table 1 details the clinical and laboratory features of the patients. The risk group distribution was 49% low, 41% standard, and 10% high. CNS involvement (i.e., CNS-3) was found at diagnosis in 5% of patients. Among these, a significantly higher proportion had an initial leukocyte count of $\geq 50x10^9$/L and T-cell leukemia compared to those without CNS involvement ($p=0.014$ and $p<0.001$, respectively). Additionally, significantly more patients in the group with leukocyte counts of $\geq 50x10^9$/L at diagnosis had T-cell ALL and CNS-3 status compared to the group with initial leukocyte counts of $<50x10^9$/L ($p<0.001$ and $p=0.012$, respectively). Furthermore, hyperdiploidy and t(12;21) (*ETV6-RUNX1*) were not detected in the patients with initial leukocyte counts of $\geq 50x10^9$/L.

Treatment Outcomes

The treatment outcomes of all patients according to clinical and biological characteristics are displayed in Table 2. On day 7 of HDMP, peripheral ABC was assessed for 156 of 183 patients, 137 (88%) of whom had a good response to steroids. The rate of good response to steroids was significantly higher (92%) in those receiving HDMP (Groups A and B) compared to Group C (60%), which had higher rates of T-cell ALL and high-risk patients ($p<0.01$). The MRD-negative rate was 96% on day 15 and 92% at the end of induction, with 97% of patients achieving complete morphological remission. On day 15, MRD of >1% was detected in 7 patients, all of whom had MRD values above 0.01% at the end of induction. Additionally, 6 patients who showed steroid response on day 7 and had MRD of <1% on day 15 had MRD values above 0.01% at the end of induction and were treated as being of high risk. Among the 13 patients

Table 1. Clinical and biological characteristics of patients.

Characteristics	Number and percentage of patients	Mean ± SD	Median (range)
Age at diagnosis (years)		6.7±4.4	5.3 (1-18)
1-10	140 (76)		
>10	43 (24)		
Sex			
Male	108 (59)		
Female	75 (41)		
Complete blood count at diagnosis			
Hemoglobin (g/dL)		8.6±2.2	8.6 (2.7-17.8)
Leukocytes ($x10^9$/L)		45.6±91.3	9.9 (1.3-574.6)
Platelets ($x10^9$/L)		114.4±117.6	74.5 (5-703)
Involvement of systems			
Hepatomegaly	158 (86)		
Lymphadenopathy	85 (46)		
Splenomegaly	78 (43)		
Skeletal system involvement	20 (25)		
Skin involvement	2 (1)		
Testicular involvement	1 (0.5)		
CNS status			
CNS-1	118 (65)		
CNS-2	8 (4)		
CNS-3	9 (5)		
Traumatic with blasts	47 (26)		
Cytology			
Hypodiploidy	13/68 (19)		
t(12;21)	12/84 (14)		
Hyperdiploidy	7/68 (10)		
t(9;22)	7/138 (5)		
KMT2A rearrangements	6/48 (12)		
t(1;19)	4/65 (6)		
Trisomy 21	3/163 (2)		
Others*	7/163 (4)		
Immunophenotype			
B-cell precursor	167 (91)		
CALLA +	152 (91)		
CALLA -	15 (9)		
T-cell	16 (9)		

*: t(8;21), t(10;14), t(7;9) del(9), del(9p), del(7q)del(12p), del(6q)del(12p)der(14), and der(9)der(11)der(15)der(19). SD: Standard deviation; CNS: central nervous system; CALLA: common acute lymphoblastic leukemia antigen.

Table 2. Treatment outcomes by clinical and biological characteristics.

Characteristics	Number of patients	Five-year event-free survival, % (95% CI)	p	Five-year overall survival, % (95% CI)	p
Age at diagnosis (years)					
1-10	140	89.9	0.003	92.6	**0.003**
>10	42	70.8		77.6	
Sex					
Male	107	86.9	0.683	88.7	0.871
Female	75	83.8		90.0	
Leukocyte count (x10^9/L)					
≥50	34	67.6	<0.001	78.9	**0.011**
<50	148	89.7		91.5	
Immunophenotype					
B-lineage	167	85.5	0.643	89.5	0.351
T-lineage	15	86.7		86.7	
CD20 positivity at diagnosis					
Present	68	79.1	0.080	81.9	**0.021**
Absent	110	90.0		93.5	
Genetic abnormalities					
Hypodiploidy					
Present	13	83.3	0.748	83.3	0.959
Absent	55	81.8		86.8	
Hyperdiploidy					
Present	7	85.7	0.730	85.7	0.895
Absent	61	81.7		86.2	
BCR-ABL1					
Present	7	100.0	0.283	100.0	0.372
Absent	131	85.3		89.8	
***KMT2A* rearrangement**					
Present	6	83.3	0.862	100.0	0.408
Absent	42	83.3		90.3	
t(1;19) *(TCF3-PBX1)*					
Present	4	50.0	**0.041**	50.0	**0.006**
Absent	61	86.9		91.4	
t(12;21) *(ETV6-RUNX1)*					
Present	12	91.7	0.462	91.7	0.758
Absent	72	84.5		89.8	
CNS involvement					
CNS-1	118	86.3	0.373	90.2	0.233
CNS-2	8	87.5		87.5	
CNS-3	9	100.0		100.0	
Traumatic with/without blasts	47	80.8		84.9	
Risk group					
Low[a]	88	94.2	**0.015**	93.9	0.082
Standard	72	80.3		86.8	
High	18	83.3		94.4	

Table 2. Continued.

Characteristics	Number of patients	Five-year event-free survival, % (95% CI)	p	Five-year overall survival, % (95% CI)	p
HDMP dose (mg/kg/day)					
10 (Group A)	90	87.6	**0.043**	89.4	0.435
20 (Group B)	65	89.2		92.2	
<10 (Group C)[b]	27	70.4		80.7	
Peripheral ABC on day 7 (×10^9/L)					
<1	137	86.0	0.683	88.7	0.377
≥1	19	94.7		100.0	
MRD on day 15 (%)					
≥1	7	85.7	0.939	100.0	0.351
<1	170	86.3		89.6	
MRD on remission date (%)					
≥0.01	13	76.9	0.188	92.3	0.779
<0.01	149	89.2		91.6	
Infection during induction					
Present	148	86.9	0.366	90.8	0.104
Absent	34	80.0		82.9	
Infection with gram-positive bacteria during induction					
Present	51	82.4	0.266	89.9	0.940
Absent	131	86.9		88.9	
Infection with gram-negative bacteria during induction					
Present	24	70.8	**0.005**	75.0	**0.003**
Absent	158	87.9		91.5	

Event-free survival differences: [a]: Low-risk and standard-risk groups differed significantly (p=0.004); [b]: Groups A and C differed significantly (p=0.025). CI: Confidence interval; CNS: central nervous system; HDMP: high-dose methylprednisolone; ABC: absolute blast count; MRD: minimal residual disease.

with MRD values above 0.01% at the end of induction, 12 were considered to be in morphological remission, while one case was evaluated as refractory ALL. Induction failures occurred due to TLS-related death (n=1), refractory leukemia (n=1), and fatal infections including acute respiratory distress syndrome related to pneumonia (n=2), *Escherichia coli* sepsis (n=1), and *Clostridium* sepsis (n=1).

During follow-up, 6 patients classified as low risk and one patient classified as standard risk transitioned to the high-risk protocol in the 7th week of maintenance therapy due to the detection of MRD of >0.1% in the BM.

BM transplantation was performed for a total of 9 patients, including 7 high-risk patients and 2 standard-risk patients with BM relapse and recurrent isolated CNS relapse. Among these patients, 3 died. Of the 7 patients with t(9;22), 4 underwent BM transplantation, and all survived until the end of the study.

Comparison of Groups A and B

No significant differences were observed between Groups A and B in terms of age, sex, initial leukocyte count, immunophenotype, CNS status at diagnosis, or risk group classification (p>0.05). Additionally, the proportions of patients with a good response to steroids on day 7 and BM FCM-MRD-negative patients on day 15 and at the end of induction were similar between the two groups (p>0.05). More details are provided in the Supplementary Information.

Survival Rates

Excluding one patient who died early in induction due to TLS, the 182 eligible patients (median follow-up: 6.6 years) achieved 5-year EFS of 85.6% (95% confidence interval [CI]: 83.0%-88.2%) and OS of 89.2% (95% CI: 86.9%-91.5%) (Figure 1). Groups A and B demonstrated similar survival rates (Group A: EFS 87.6%, OS 89.4%; Group B: EFS 89.2%, OS 92.2%), while Group C exhibited significantly lower EFS compared to Groups A and B (p=0.043), though the OS rate was similar (p=0.435).

Prognostic Factors

Age of 10-18 years, initial leukocyte count of ≥50x10^9/L, t(1;19) (*E2A-PBX1*) positivity at diagnosis, and gram-negative bacterial infections during induction were significantly linked

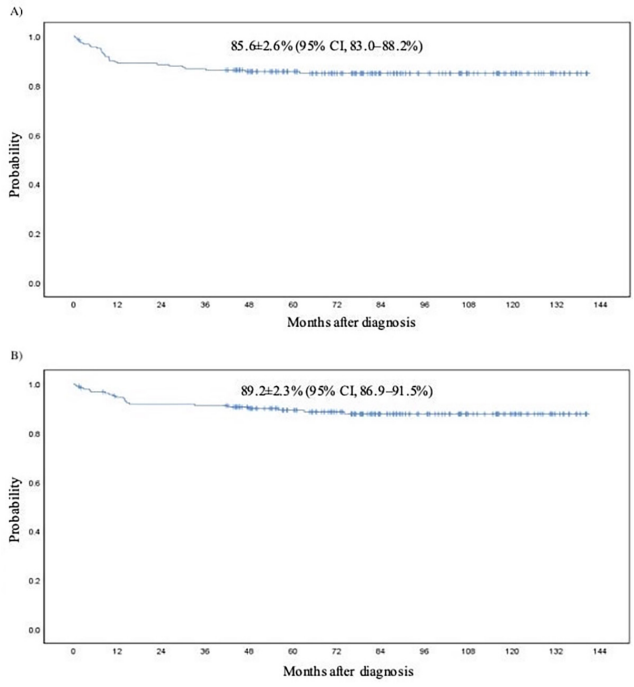

Figure 1. Excluding one patient who died during induction, patients achieved 5-year event-free survival of 85.6% (A) and overall survival of 89.2% (B).

CI: Confidence interval.

to poorer EFS and OS in univariate analysis. Standard-risk and CD20-positive patients had significantly lower EFS and OS, respectively, compared to low-risk and CD20-negative patients. Trends toward increased survival for patients with a good response to steroids on day 7 and FCM-MRD-negative patients on day 15 and at the end of induction were observed but were not significant (p>0.1).

Cox regression multivariate analysis was conducted on factors with a p-value of <0.1 in univariate analysis, yielding age of 10-18 years, leukocyte count of ≥50x10^9/L, and CD20 positivity at diagnosis as independent adverse predictors of both EFS and OS. Gram-negative bacterial infection during induction was also an independent predictor of poorer OS (Table 3).

Infections in Remission Induction

Infections and microbiological causative agents were evaluated during induction. At least one infection episode was experienced during induction by 148 patients (81%), with 255 episodes in total. The predominant infections were febrile neutropenia (n=115, 45%), pneumonia (n=38, 15%), and central venous catheter/port infections (n=255, 9%). Investigation into the relationship between HDMP dosage and vulnerability to infection revealed no significant variation between Groups A and B (p>0.05).

Table 3. Independent risk factors for major adverse events and mortality.

Major adverse events	Number of patients	Hazard ratio (95% CI)	p
Age at diagnosis (years)			
1-10	140	1.0	**0.007**
>10	42	5.012 (1.541-16.302)	
Leukocyte count (x10^9/L)			
≥50	34	5.980 (1.484-24.101)	**0.012**
<50	148	1.0	
CD20 positivity at diagnosis			
Present	68	2.531 (0.997-6.427)	**0.050**
Absent	110	1.0	
Death			
Age at diagnosis (years)			
1-10	140	1.0	**0.018**
>10	42	6.735 (1.395-32.524)	
Leukocyte count (x10^9/L)			
≥50	34	5.934 (1.397-25.204)	**0.016**
<50	148	1.0	
CD20 positivity at diagnosis			
Present	68	3.786 (1.304-10.993)	**0.014**
Absent	110	1.0	
Infection with gram-negative bacteria during induction			
Present	24	4.449 (1.205-16.428)	**0.025**
Absent	158	1.0	

CI: Confidence interval.

Discussion

The five-year EFS of 85.6% and OS of 89.2% achieved in this study are comparable to those of the St. Jude Total Therapy XV Study (EFS: 85.6%, OS: 93.5%) and slightly higher than the recent Surveillance, Epidemiology, and End Results Program data for patients under 15 treated between 2010 and 2017 (72%) and other major studies [9,10,17,18,19,20,21]. The hallmark of our study is the use of HDMP treatment in preinduction, likely contributing to our superior survival rates.

Apart from the trials conducted at our institution, the Dana Farber Cancer Institute Protocol 91-01 is the only one in the literature for administering a high steroid dose before induction. Similar to our study's findings, high-dose dexamethasone (18 mg/m^2/day or 150 mg/m^2/day) produced a better BM response, addressing drug insensitivity and reduced glucocorticoid-receptor expression in lymphoblasts compared to prednisolone (40 mg/m^2/day) or standard-dose dexamethasone (6 mg/m^2/day) [22].

In our study, we observed a notable early impact of HDMP, with a higher proportion of MRD-negative patients on day 15 (96%) and at the end of induction (92%) compared to their counterparts from the St. Jude Total XV Protocol (79.9% and 79.6%, respectively), as well as other major collaborative trials [9,10,22,23,24]. Moreover, achieving a morphological remission rate of 99% by the end of induction underscores the significant effect of HDMP in swiftly eradicating lymphoblasts during this phase. However, our rate of good response to steroids on day 7 (88%) was similar to that of protocols using conventional steroid doses [17,25,26,27]. The patients with poor steroid responses had significantly higher rates of T-cell phenotype, leukocyte count of ≥50x10^9/L at diagnosis, CNS-3, and standard or high risk compared to those with good responses to steroids (p=0.001, p<0.001, p=0.005, p=0.015, and p=0.022, respectively). Seventeen of 19 poor responders to steroids had negative MRD on day 15 and at the end of induction. This could suggest that drawing conclusions about the efficacy of HDMP by day 7 might be premature. Furthermore, the possibility of rapid eradication of steroid-resistant lymphoblasts with HDMP and the inability to identify a prognostic impact of MRD on the 15th and 42nd days could be speculated as reflecting the advantageous impact on survival of a higher cumulative dose of HDMP in the first 2 weeks.

In comparisons of patients in Group A (10 mg/kg/day) and Group B (20 mg/kg/day), the differences in early treatment response and survival rates did not achieve statistical significance. While comparisons of the early efficacy, side effects, and survival influence of HDMP with conventional steroid doses were not feasible, the lower EFS of the patients in Group C (irregular dose), who required lower MP doses due to concerns about TLS from hyperleukocytosis, might offer insights into the effectiveness of HDMP treatment.

Expanding our investigation, we explored infections occurring during induction to assess whether HDMP heightened susceptibility. While this study could not directly compare infection frequencies between HDMP and conventional steroid doses, a prior study at our center found no difference [28]. Infection rates during induction vary widely across studies, ranging from 52% in the St. Jude Total XV Protocol to 80% for the protocol of the Pediatric Oncology Group, and the most common infections, acute neutropenia, and isolated bacterial agents were consistent with those reported for the original protocol [29,30]. Our relatively high infection rate (81%) might be attributed to factors such as port placement for all patients (12.5% with port infections), housing leukemia follow-up patients alongside those hospitalized for other conditions due to the absence of a dedicated hematology service at our center during the study, and limited facilities and intermittent construction in our hospital.

This study found that age (10-18 years) and leukocyte count (≥50x10^9/L) at diagnosis, as well-known prognostic factors, significantly impacted EFS and OS. The lower survival rates among adolescents lack a definitive explanation but studies suggest several contributing factors. This age group tends to exhibit fewer favorable prognostic genetic factors, such as (12;21) (*ETV6-RUNX1*) and hyperdiploidy, while showing higher prevalence of unfavorable genetic factors like *BCR::ABL1* and intrachromosomal amplification of chromosome 21. Additionally, postinduction MRD burdens tend to be higher among adolescents, and they experience more treatment-related complications such as pancreatitis, thromboembolism, steroid-induced diabetes mellitus, and osteonecrosis. These disparities may be attributed to age-related changes in drug pharmacokinetics and pharmacodynamics. Alternatively, they could be associated with poorer treatment adherence in adolescent patients compared to younger cohorts [31,32].

In our study, t(1;19) (*E2A-PBX1*) emerged as the sole poor genetic prognostic marker. Two of the four patients with this marker died due to sepsis in the maintenance period and BM relapse. While prior studies linked it to poorer outcomes, more recent research revealed no impact on outcomes, although it might still be associated with CNS relapse [33]. Notably, established indicators like *BCR::ABL1*, *KMT2A* rearrangement, t(12;21) (*ETV6-RUNX1*), hypodiploidy, and hyperdiploidy did not significantly affect EFS or OS in our study, possibly due to the genetic specifics of a small number of patients.

It is intriguing that CD20 positivity emerged as a predictor of adverse events and death in our study. Borowitz et al. [23] found shorter EFS in CD20-positive patients, but later studies suggested that CD20 expression may not affect prognosis [34,35]. Additionally, the meta-analysis conducted by Ou et al. [36] revealed no *KMT2A* rearrangement, associated with

heightened relapse risk, in CD20-positive patients. Surprisingly, *KMT2A* rearrangements were observed in 13% of CD20-positive patients in our study. CD20 expression often indicates poor prognosis in adult ALL, leading to the incorporation of rituximab, a monoclonal antibody targeting CD20, in treatment regimens for adults with B-cell precursor ALL [37]. In future studies, the prognostic impact of CD20 positivity, its correlation with other factors, and potential therapeutic options could be explored in larger patient cohorts.

The St. Jude Total Therapy Study XVI achieved the highest 5-year survival rate in pediatric ALL (EFS: 88.2% [95% CI: 84.9%-91.5%]; OS: 94.1% [95% CI: 91.7%-96.5%]) to date [38]. Despite the reduced rate of CNS relapse and increased EFS in the St. Jude Total Therapy Study XVI, its OS rate matched that of Total Therapy Study XV. In 2002, the ALL IC-Berlin-Frankfurt-Munster (BFM) Study documented EFS and OS of 74±1% and 82±1%, respectively [17]. Koc et al. [39] applied the Modified St. Jude Total XV, using HDMP therapy alongside a multiple chemotherapy regimen during induction, not before, and reported 5-year EFS and OS of 78.3% and 80%, respectively. Hazar et al. [40] reported 8-year EFS and OS rates of 63.2% (95% CI: 54.4%-72.0%) and 72% (95% CI: 63.6%-80.4%), respectively, whereas Kocak et al. [41] reported 12-year EFS and OS of 75.0% and 80.6%, respectively, based on their experiences with the ALL-BFM 95 protocol. Our promising EFS and OS rates achieved with the Modified St. Jude Total XV Protocol in patients with childhood ALL surpass those seen with protocols applied by prominent centers in our country and other major study groups worldwide [10,18,40,41,42].

Conclusion

This study significantly contributes to the literature by revealing favorable survival rates and prognostic factors in a large pediatric ALL population. The high early response in induction using HDMP appears to be pivotal for treatment success. Notably, preinduction steroid treatment is not applied in other major protocols, except for the use of conventional dosages in BFM protocols [43]. In light of our findings, integrating short-term HDMP therapy into protocols as a preinduction phase seems viable. Our study revealed no significant variance in survival rates between HDMP doses of 10 and 20 mg/kg/day. Hence, initiating treatment with the lower dosage during the first week would be more appropriate to reduce potential side effects.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of Hacettepe University Faculty of Medicine (decision no: 2019/24-18, date: 15.10.2019).

Informed Consent: Written informed consent was obtained from the parents or guardians of all pediatric patients included in this study.

Authorship Contributions

Surgical and Medical Practices: S.A., Ş.Ü., B.K., D.Ç., F.G., H.Y.; Concept: S.A., F.G., Ş.Ü., B.K., F.G.; Design:S.A., F.G., H.Y.; Data Collection or Processing: H.Y., S.A.; Analysis or Interpretation: H.Y., S.A., F.G.; Literature Search: H.Y., S.A.; Writing: H.Y., S.A.

Conflict of Interest: No conflict of interest was declared by the authors.

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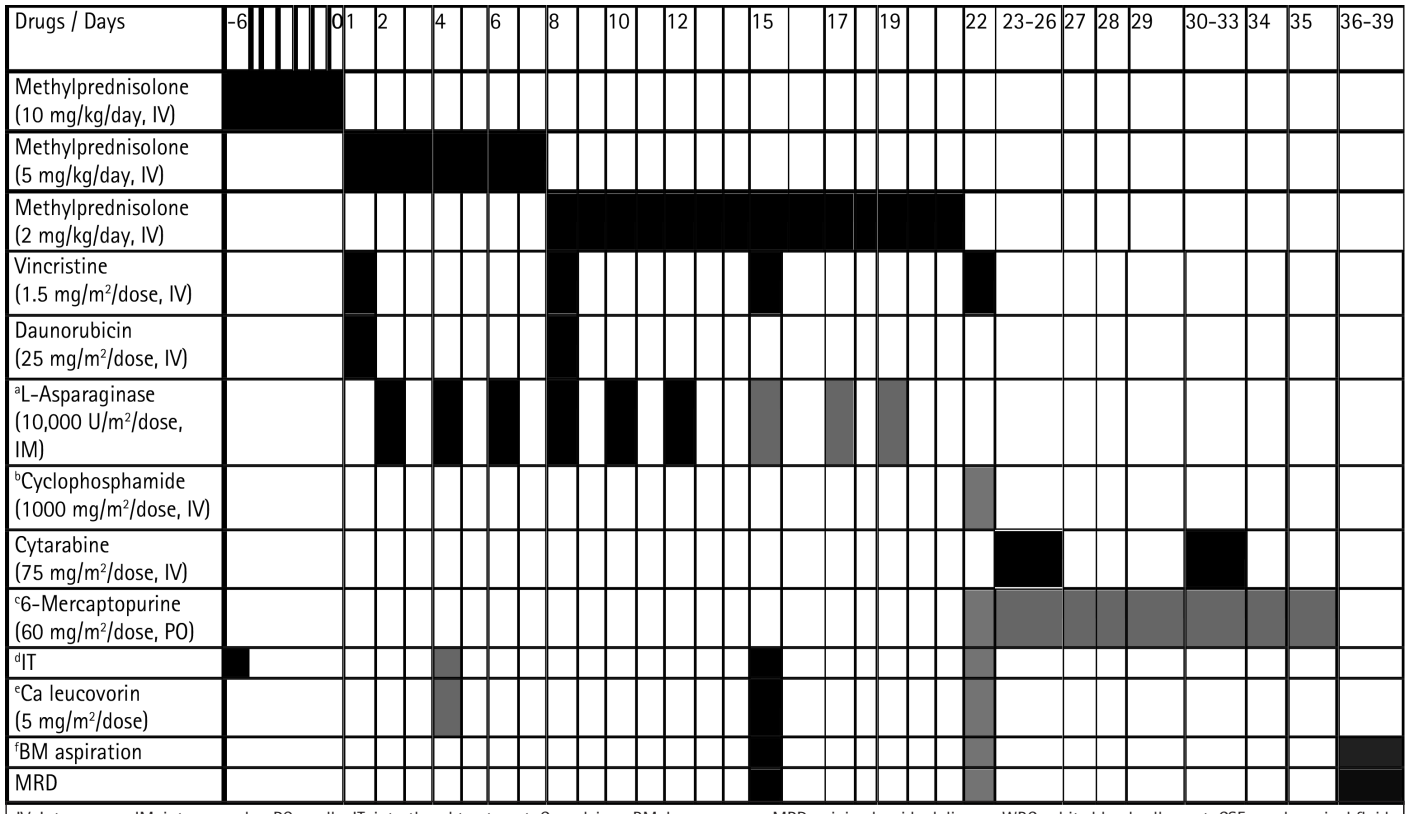
Supplementary Information

1. Modified St. Jude Total XV Protocol (Remission Induction)



IV: Intravenous; IM: intramuscular; PO: orally; IT: intrathecal treatment; Ca: calcium; BM: bone marrow; MRD: minimal residual disease; WBC: white blood cell count; CSF: cerebrospinal fluid; RBC: red blood cell count; HDMTX: high-dose methotrexate.

[a]: The presence of approximately 1% or more leukemic lymphoblasts in the bone marrow identified through morphological examination or MRD analysis is accepted as an indication for three additional doses of L-asparaginase to be applied on days 15, 17, and 19.

[b, c, f]: Bone marrow aspirate on day 22 is performed for patients who received extra doses of asparaginase due to the presence of 1% or more leukemic lymphoblasts in the bone marrow on day 15. Patients with residual leukemia identified on day 22 should receive cyclophosphamide, 6-mercaptopurine, and cytarabine.

[d]: All patients receive triple IT on days -6 and 15. Patients with the following characteristics receive additional triple IT on days 4 and 22:

- CNS-3 status (i.e., ≥5 WBC/μL CSF with blasts or cranial nerve palsy)
- CNS-2 status (<5 WBC/μL CSF with blasts)
- Traumatic status (>10 RBC/μL CSF with blasts)
- T-cell leukemia with WBC of >$50x10^9$/L at presentation
- WBC of >$100x10^9$/L at presentation
- Presence of Philadelphia chromosome, MLL rearrangement, or hypodiploidy (<45)

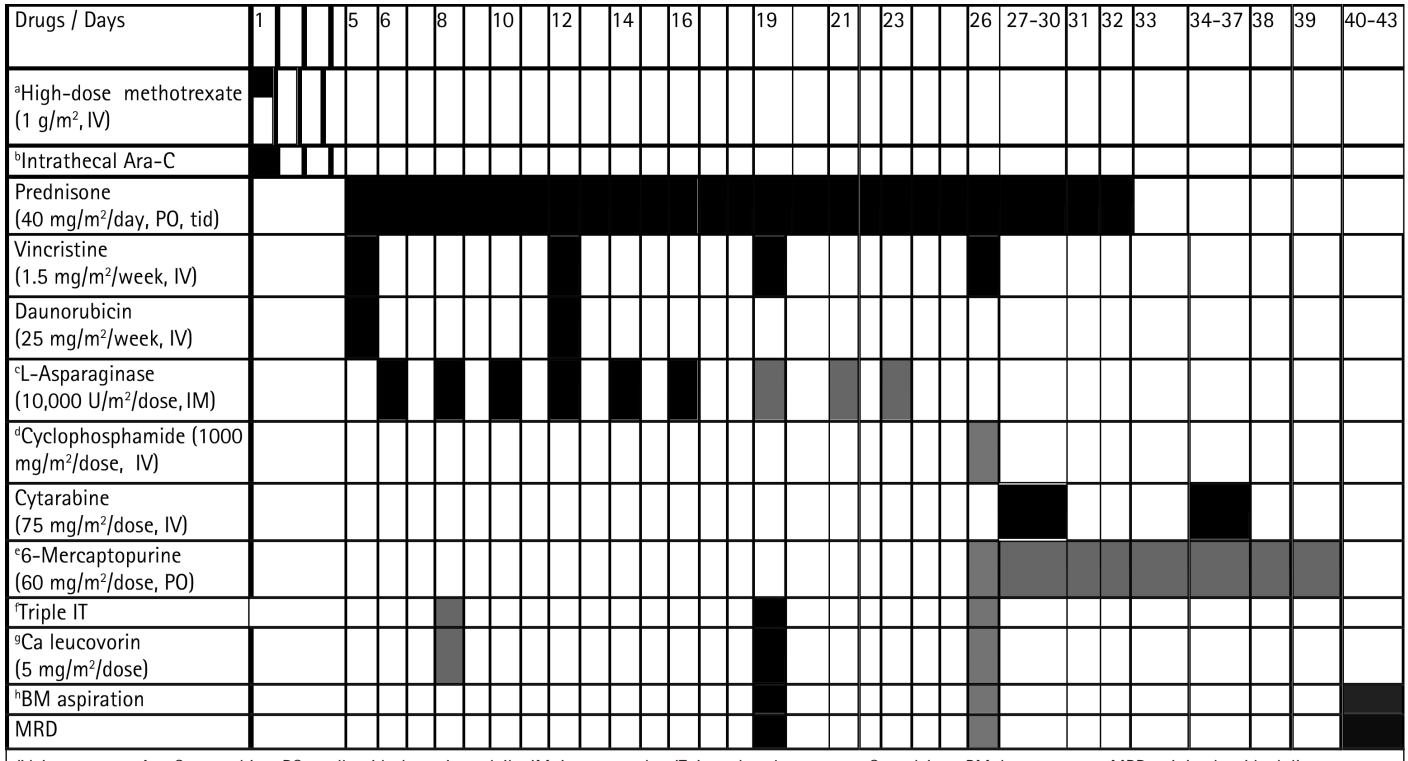
[e]: Leucovorin rescue (5 mg/m²/dose) is given orally at 24 and 30 h after each triple IT during induction and consolidation (IT with HDMTX is rescued by leucovorin for HDMTX only).

Triple Intrathecal Chemotherapy Doses

Age (months)	Methotrexate (mg)	Prednisone (mg)*	Normal saline (mL)	Ara-C (mg)
12-23	8	6	2	24
24-35	10	8	2.5	30
≥36	12	10	3	36

Ara-C: Cytarabine.
*: Prednisone is administered mixed with saline solution. If hydrocortisone is to be used instead of prednisone, the age-dependent intrathecal treatment dose of hydrocortisone would be as follows: 12-23 months, 16 mg; 24-35 months, 20 mg; ≥36 months, 24 mg.

2. St. Jude Total XV Protocol (Remission Induction)



IV: Intravenous; Ara-C: cytarabine; PO: orally; tid: three times daily; IM: intramuscular; IT: intrathecal treatment; Ca: calcium; BM: bone marrow; MRD: minimal residual disease; HDMTX: high-dose methotrexate; CNS: central nervous system; WBC: white blood cell count; RBC: red blood cell count.

[a]: All consenting patients will be stratified by leukemic cell lineage, DNA index, and WBC at presentation and randomized to receive HDMTX (1 g/m²) as a 4-h infusion versus a 24-h infusion. Patients with pre-existing renal failure (i.e., creatinine level of >1.4 mg/dL in children and >2 mg/dL in adolescents aged >13 years or creatinine clearance of <50 mL/min/1.73 m²) or Down syndrome are ineligible for up-front HDMTX treatment.

[b]: Intrathecal Ara-C will be applied immediately following the collection of cerebrospinal fluid for diagnosis with age-dependent doses as follows: 12-23 months, 40 mg IT Ara-C (8 mL); 24-35 months, 50 mg IT Ara-C (10 mL); ≥36 months, 60 mg IT Ara-C (12 mL).

[c]: The presence of approximately 1% or more leukemic lymphoblasts in the bone marrow identified through morphological examination or MRD analysis is accepted as an indication for three additional doses of L-asparaginase to be administered on days 19, 21, and 23.

[d, e, h]: Bone marrow aspirate on day 26 is performed for patients who received extra doses of asparaginase due to the presence of 1% or more leukemic lymphoblasts in the bone marrow on day 19. Patients with residual leukemia identified on day 26 should receive cyclophosphamide, 6-mercaptopurine, and cytarabine.

[f]: All patients receive triple IT on day 19. Patients with the following characteristics receive additional triple IT on days 8 and 26:

- CNS-3 status (i.e., ≥5 WBC/μL CSF with blasts or cranial nerve palsy)
- CNS-2 status (<5 WBC/μL CSF with blasts)
- Traumatic status (>10 RBC/μL CSF with blasts)
- T-cell leukemia with WBC of >50x10⁹/L at presentation
- WBC of >100x10⁹/L at presentation
- Presence of Philadelphia chromosome, MLL rearrangement, or hypodiploidy (<45)

[g]: Leucovorin rescue (5 mg/m²/dose) is given orally at 24 and 30 h after each triple IT during induction and consolidation (IT with HDMTX is rescued by leucovorin for HDMTX only).

Triple Intrathecal Chemotherapy Doses

Age (months)	Methotrexate (mg)	Prednisone (mg)*	Normal saline (mL)	Ara-C (mg)
12-23	8	6	2	24
24-35	10	8	2.5	30
≥36	12	10	3	36

Ara-C: Cytarabine.
*: Prednisone is administered mixed with saline solution. If hydrocortisone is to be used instead of prednisone, the age-dependent intrathecal treatment dose of hydrocortisone would be as follows: 12-23 months, 16 mg; 24-35 months, 20 mg; ≥36 months, 24 mg.

3. Risk Classification

Patients are assigned to one of three risk categories (low, standard, or high) depending on factors including age, leukocyte count, presence or absence of CNS-3 status or testicular leukemia, immunophenotype, cytogenetic and molecular diagnosis, DNA index, and early response to therapy at diagnosis. Final risk classification based on presenting features was established after the completion of remission induction therapy. The criteria for each category, referenced to the St. Jude Total XV Protocol, are provided below.

1. Low-Risk

- B-cell precursor ALL with *TEL-AML1* fusion, DNA index of ≥1.16, or age 1 to 9.9 years and WBC of $<50x10^9$/L at diagnosis.
- **Must not have:** CNS-3 (≥5 WBC/μL CSF with morphologically identifiable blasts or cranial nerve palsy), overt testicular leukemia evidenced by ultrasonogram, adverse genetic features [t(1;19) with *E2A-PBX1* fusion, t(9;22) or *BCR-ABL* fusion, rearranged *MLL*, or hypodiploidy (<45 chromosomes)], or poor early response (≥1% lymphoblasts on day 19 or 26 of induction, ≥0.01% lymphoblasts detected by immunological or molecular methods on the remission date).

2. Standard-Risk

- T-cell ALL.
- B-cell precursor ALL that does not meet the criteria for low-risk or high-risk ALL.

3. High-Risk

- t(9;22) or *BCR-ABL* fusion.
- Induction failure or >1% leukemic lymphoblasts in the bone marrow on the remission date.
- >0.1% leukemic lymphoblasts in the bone marrow in week 7 of continuation of treatment (i.e., before reinduction I).
- Reappearance of leukemic lymphoblasts by MRD (at any level) in patients who were previously MRD-negative.
- Consistently detectable MRD at lower levels.

4. Comparison of Groups Receiving Different Doses of High-Dose Methotrexate

Characteristics	Group A (n=90), n (%)	Group B (n=65), n (%)	p
	6.8±4.4 (1-16.7)	5.7±3.5 (1.8-15.4)	0.197
Age at diagnosis, years			
1-10	67 (74)	57 (88)	0.067
>10	23 (26)	8 (12)	
Sex			
Female	40 (44)	25 (38)	0.456
Male	50 (56)	40 (62)	
Leukocyte count (x10^9/L)			
≥50	9 (10)	2 (3)	0.123
<50	81 (90)	63 (97)	
Immunophenotype			
B-lineage	88 (98)	63 (97)	1.000
T-lineage	2 (2)	2 (3)	
CNS status			
CNS-1	64 (71)	40 (61)	
CNS-2	3 (3)	3 (5)	0.666
CNS-3	2 (2)	2 (3)	
Traumatic with blasts	21 (24)	20 (31)	
Risk group			
Low	45 (51)	41 (64)	0.242
Standard	35 (39)	19 (30)	
High	9 (10)	4 (6)	
Peripheral blast count on day 7 (cells/µL)			
<1000	69 (90)	56 (95)	0.349
≥1000	8 (10)	3 (5)	
MRD on day 15 (%)			
≥1	4 (4)	1 (2)	0.649
<1	86 (96)	61 (98)	
MRD on remission date (%)			
≥0.01	6 (7)	3 (5)	0.736
<0.01	75 (93)	54 (95)	
Infection during induction			
Present	70 (78)	52 (80)	0.893
Absent	20 (22)	13 (20)	
Infection with gram-positive bacteria during induction			
Present	13 (14)	6 (9)	0.329
Absent	77 (86)	59 (91)	

CNS: Central nervous system; MRD: minimal residual disease.