Prognostic Markers in Pediatric T-cell Lymphoblastic Leukemia/Lymphoma

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Abstract: Background: Historically, the diagnosis of T-cell acute lymphoblastic leukemia (T-ALL) or T-cell lymphoblastic lymphoma (T-LBL) predicted a higher risk of induction failure, early relapse, and worse event-free survival (EFS) compared with B-precursor childhood leukemia or lymphoma. Treatment intensification has dramatically improved the general prognosis of childhood T-cell precursor acute lymphoblastic leukaemia (T-ALL). Nevertheless, approximately20–25% of cases still relapse early. Various prognostic parameters have been sought for better risk stratification and treatment adjustments for patients with markers of poor prognosis. A task that has been considerably difficult, with conflicting results from different studies. In this study we aimed at highlighting prognostic parameters that would help in better risk stratification and treatment planning of pediatric T cell ALL and LBL patients. **Material and methods**: **105** pediatric T-cell leukemia/lymphoma patients were enrolled in the study. T-ALL were 72 cases and 33 cases were T lymphoblastic Leukemia (LBL), stage III. Patients were diagnosed by thorough examination and extensive lab workup. Patients were further evaluated for response to therapy. The T ALL cases at days 8, 14 and again at day 43, end of induction, together with the LBL cases. Patients were followed up for a median of 20 months and survival was correlated to parameters that could carry prognostic significance. **Results:** The patients' mean age was 9.75 years and male : female ratio was 3.2:1. The 3 years overall survival (OS) was, for the whole 105 patients 77.4 ±5.1%, for the leukemia group 81.6±5%, and for the lymphoma group 77.6±8.1%. The disease free survival (DFS) was 60.42±5.6%, 93.3±6.4%, 47.1±8.6% and event free survival (EFS) was 50.71 ±8.3%, 48.5±6.2% and 59.2 ±9.2% respectively. Age significantly correlated with OS of patients (p= 0.04). For T ALL, male gender, TLC <50 x10^9/L and good prednisone responders correlated with DFS (p = 0.01), (p < 0.001) and (p =0.02) respectively. Also, male gender and good prednisone response correlated with EFS (p = 0.05) and (p < 0.001) respectively. **Conclusion:** further studies are needed to establish concrete prognostic markers that would be used for better risk stratification of pediatric T-cell L/L and hence improve the management of pediatric T-cell ALL and LBL and achieve better outcome for the patients.

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1. Introduction

ALL being the most common cancer diagnosed in children, represents 23% of cancer diagnoses among children younger than 15 years, with T-cell ALL, accounting for about 10-15% of the pediatric ALL cases (1, 2).

Through the Cancer registration at the NCI Egypt 2002-2003, ALL was recorded to constitute 19.6% of all childhood malignancies. TALL accounted for about 26% of the pediatric ALL (3, 4).

T-cell lymphoid malignancies are associated with distinctive biologic, cytogenetic, and clinical features which set them apart from non-T lymphoid malignancies. (5-9)

T-cell ALL and advanced stage lymphoblastic lymphomas (LBL) are aggressive malignancies once associated with a very poor prognosis (10)

The distinction between T-cell ALL and T-cell lymphoblastic lymphoma is also ill-defined. There is some evidence that these disorders arise from different stages of T-cell differentiation and consequently have immunophenotypes reflecting different stages of T-cell maturation. Because this distinction does not occur in every case, it does not provide a reliable basis for delineating between the two diseases. In the absence of more refined biologic criteria, the percentage of blasts in the bone marrow is conventionally used to differentiate between T-cell ALL and T-cell non-Hodgkin's lymphoma according to the WHO2008.

The Children's Oncology Group adopted a strategy of lineage-specific treatment for T-ALL and T-LBL using protocols different from those used to treat patients with B lineage disease. (11, 12).

Despite the improvements of the past 3 decades, outcomes for patients with T-ALL and T- LBL are not optimal (10).

Risk-based treatment assignment has been utilized for children with ALL so that those children who have a very good outcome with modest therapy can be spared more intensive and toxic treatment, while a more aggressive, thus more toxic, therapeutic

approach can be provided for patients who have a lower probability of long-term survival (13).

Identifying reliable prognostic factors for T-ALL treatment planning has been considerably more difficult than for B-precursor ALL (14). Nevertheless, age, WBC at diagnosis, and gender held prognostic significance (5-10, 15-18). Also assessment of day 8 peripheral blood blast count, day 7 and/or day 14 marrow blast percentage, and minimal residual disease determinations in bone marrow and/or peripheral blood during or at the end of induction have been shown to be of strong prognostic significance (10, 15, 19, 20).

It is noteworthy that T-ALL is infrequently characterized by cytogenetic or molecular markers such as hyperdiploidy or chromosomal translocations that are considered parameters of good prognosis (21). In addition our Egyptian pediatric T ALL cases showed low frequency of the most recurring abnormalities that has a possible impact on prognosis. SIL/TAL occurs in about 4% and HOX11L2 ectopic expression in about 12% (22), compared to about 25% of either molecular event in patients in developed countries (23, 24). The specificity of immunoglobulin and TCR gene rearrangements is useful basically as marker of clonality and for MRD detection (25).

In this study we aimed at highlighting prognostic parameters that would help in better risk stratification and treatment planning of pediatric T cell ALL and LBL patients.

2. Patient and Methods:

Patients:

Cases enrolled in this study **were 105** newly diagnosed T-cell ALL and stage III T lymphoblastic Leukemia (LBL) cases. They presented to the Pediatric Oncology Department at the National Cancer Institute, during the time period from beginning of November 2004 till the end of July 2007. The age range was 2-18 years. Patient characteristics are shown in Table 1. Patients were diagnosed by thorough examination including history, physical examination with complete nodal assessment, cytologic assessment of CSF, pleural, peritoneal fluids and radiography. The lab workup included chemistry profile, complete blood count, Bone marrow (BM) aspiration/biopsy, immunophenotyping and molecular testing for T cell receptor (TCR) γ, δ, and immunoglobulin heavy chain (IgH) gene rearrangements. Written informed consent was obtained from the patients' parents and the protocol was approved by the Institutional Review Board (IRB)

Immunophenotyping: on peripheral blood (PB) or bone marrow for T ALL cases and pleural effusion for T LBL, included the monoclonal antibodies panel (obtained from Becman coulter and Dako), CD1, CD2, CD3, CD4,CD5, CD7, CD8, CD10, CD19, CD22, anti κ, anti λ, CD13, CD33, anti class II MHC (26), cytoplasmic μ and TdT (27) analyzed on Coulter XL. T- Cell ALL cases were diagnosed by the expression of T-cell antigens and the absence of B-cell and myeloid antigens.

Molecular testing for antigen receptor gene rearrangement by Polymerase Chain Reaction (PCR):

Antigen receptor gene rearrangements, T cell receptor (TCR) γ and δ and immunoglobulin heavy chain (IgH) gene were done on peripheral blood (PB) or bone marrow for T-cell ALL cases and on bone marrow for NHL cases, as previously described, (28-30). Molecular testing was done on confirmation of the immunophenotyping and immunohistochemical studies. In brief: DNA was isolated from peripheral blood and/or B.M at diagnosis (**31).**

TCR-γ gene rearrangement analysis:

The demonstration of TCR-γ gene rearrangements was performed by using two rounds of multiplex PCR with a set of seven primers **(**Table 2**).** These primers were used in two mixes: Mix 1 contained VγIcons in Jγ2S2, JP and JP1/2 primers, and Mix 2 contained V γ 9.2, Vγ10, and Vγ11, Jγ2S2, JP and JP1/2 primers. For each sample, two PCR amplifications were performed, using first Mix 1 and then Mix 2, respectively.

The amplification reaction was performed in 25 μL reaction mix, containing 0.5 μg of genomic DNA, 0.3 μmol of each primer, 100 μmol of each dNTPs, 1.5 mmol $MgCl_2$, 10 mmol Tris-HCl (pH 8.3), 50 mmol KCl, and 2U NativeTaq DNA polymerase (Finnzymes). After initial denaturation for 6 minutes at 94°C, the PCR was performed for 45 cycles of 1 minute at 94°C, 1 minute at 55°C, and 1 minute at 72°C. The last elongation step was extended to 20 minutes. Positive control sample was included in each amplification series. PCR products were separated on ethidium bromide-stained 3% agarose gels, at 100 volt for 30 min. PCR products for TcR-γ chain gene rearrangements were approximately of 230 and 125 bp for Mix 1 and Mix 2 primer pairs, respectively (**29**).

TCRδ and IgH gene rearrangement analysis:

The reaction mixtures contained 1μg DNA, 0.5 μmol of each oligonucleotide primer (table 2), 100 μmol dNTPs, 10 mmol Tris-HCl (pH 8.3), 1.5 mmol $MgCl_2$, 50 mmol KCl, and, 2U NativeTaq DNA polymerase (Finnzymes). The PCR cyclic conditions consisted of an initial denaturation at 94°C for 6 min, followed by 35 cycles at 94°C, 55°C, and 72°C, each for 30 sec, and a final extension at 72°C for7 min. The PCR product was analyzed by electrophoresis on a 3 % agarose gel, at 100 volt for 30 min.

Table 1: Characteristics of 105 pediatric T cell leukemia/lymphoma Patients

	No of patients	Percentage (%)
Total	105	100
T-ALL	72	(68.6%)
T-Lymphoblastic Lymphoma (lll)	33	(31.4%)
Age (in years) Range	 (2-18)	
Gender Male Female	 80 25	 (76.2%), (23.8%),
Clinical features		
Fever	41	39%
Pallor	48	45.7%
Respiratory distress	42	40.0%
Bleeding tendency	21	20.0%
Bony pains	32	30.5%
Liver Not Palpable Palpable < 5cm MCL ≥ 5cm MCL	 51 54 36 15	 48.6% 51.4% 70.5% 29.5%
Spleen Not palpable Palpable <5cmMCL (below costal margin) ≥5cmMCL (below costal margin)	 46 59 43 16	 43.8% 56.2% 72.8% 27.2%
Lymph Nodes Not enlarged Enlarged Mediastinal/Hilar LNS	 29 76 54	 27.7% 72.3% 51.4%
Effusion Pleural Pericardial Both	 25 3 5	 23.8% 2.85% 4.76%
Ascites	11	10.5%
CNS involvement	11	10.5%
Testicular involvement	2	1.9%

Positive control sample was systematically included in each PCR run. Controls were Pre-B acute lymphobalstic leukemia cases for IgH gene rearrangement and T ALL cases with 100% blasts showing rearrangement for either TCR-γ or TCR δ.

Amplification of a fragment of the housekeeping gene β-globin was used as a positive control for successful amplification of the extracted DNA (Table 2).

The LBL cases were diagnosed by lymph node biopsy. Bone marrow biopsy and immunohistochemical staining were done to confirm that the cases were stage III. Molecular testing was done at diagnosis for 17/33 suspicious cases to confirm lack of BM involvement and for 41/72(56.9%) cases of T-cell ALL. Minimal residual disease (MRD) was assessed at day 14 and at end of induction (day 43) by cytomorphology for all T-cell ALL cases. MRD by Antigen receptor gene rearrangement testing was done for 13/41 T-ALL at end of induction.

Treatment Plan:

Patients were treated according to protocol adopted from POG 9404 for T-cell ALL

Evaluation criteria:

T-ALL Complete remission M-1: marrow status with restoration of normal hematopoiesis and normal performance status. Partial remission: M-2 marrow or >50% but<100% decrease in the extramedullary

leukemia. LBL: Complete remission was defined as disappearance of all evidence of disease from all sites, Partial remission: >50% decrease in the sum of the products of the maximum perpendicular diameter of the lesions, no new lesion

Any of the above findings must be present for at least one month.

No response: Failure to qualify for a PR. Progressive disease: Worsening of disease or appearance of new disease. Relapse: ≥25% leukemic blasts in marrow or histophathologically documented disease in the CNS, testes or else where after initial remission.

Follow up period:

The follow-up period was 6-36 months, with a median of 20 months.

Overall Survival (OS):

Calculated from the date of first admission till the date of last follow up for patients who ended the study in continuous complete remission (CCR), or to the date of death.

Disease Free Survival (DFS):

Included time to relapse or development of a second malignancy, measured from the end of induction for patients who achieved CR (induction deaths and non responders were excluded).

Event Free Survival (EFS):

Measured from the time of CR till the time of occurrence of any events leading to remission failure (early death, non responders) or the end of a first remission period (relapse, second malignancy or death of any causes).

Statistical analysis

SPSS for Windows (32) was used for data management. Data was described in terms of mean, standard deviation, median, range, frequencies (number of cases) and relative frequencies (percentages). For comparing categorical data, Chi square test and Fisher exact tests were performed. A probability value ($p \leq 0.05$) is considered significant. Survival was estimated using Kaplan Meier method and log-rank test for comparing curves. Cox regression analysis was done to detect effect of independent prognostic factors on different outcome including overall survival, disease free survival and event free survival.

Table 2: The oligonucleotide primer sequences used for amplification of the candidate genes

Primer	Primer sequence	Gene Specificity	Reference
VγI cons	5ó-CTGGTACCTACACCAGGAGGGGAA-3ó	TCR-γ	28, 29
Vγ9.2	5ó-GAAAGGAATCTGGCATTCCG-3ó		
Vγ10	5ó-GCAGCATGGGTAAGACAAGC-3ó		
V γ 11	5ó-GATTGCTCAGGTGGGAAGAC-3ó		
Jγ2S2	5ó-CCTGTGACAACAAGTGTTGT-3ó		
JP	5ó-TTGTTCCGGGACCAAATACC-3ó		
JP1/2	5ó-CCAGGTGAAGTTACTATGAG-3ó		
Vδ2	5ó-CAAGGTGACATTGATA TTGC-3ó	TCR-δ	30
Dδ2	5ó-CGGGTGGTGATGGCAAAGTGCC-3ó		
Dδ3	5ó-GAAATGGCACTTTTGCCCCTGCAG-3ó		
VU	5ó-ACGGCCGTGTATTACTG-3ó	IgH	30)
JU	5ó-CTGAGGAGACGGTGACC-3ó		
HBG7-F	5ó-GAAGAGCCAAGGACAGGTAC-3ó	β-globin	30
HBG7-R	5ó-CAACTTCATCCACGTTCACC-3ó		

3.Results

This study included 105 eligible patients with newly diagnosed T-cell ALL and advanced stage lymphoblastic lymphoma (stage III) who were presented to the Pediatric Oncology Department at the National Cancer Institute, Cairo University during the time period from November 2004 till July 2007. T-cell ALL represented 72 cases (68.6%), while stage III lymphoblastic lymphoma represented 33 cases (31.4%). Originally, the T- ALL were 66 patients and the advanced LBL 39 but with bone marrow aspirate examination and immunophenotyping, 6 patients turned out to be in leukemic phase (BM blasts >20%) and hence were added to the leukemia group.

The age of the patients ranged between 2 and 18 years. The mean age was 9.75 years and the median was 10 years, the peak incidence was recorded in the (> 10 -15 years age group) constituting around 34.3% of patients.

In this study, 80 patients were males (76.2%) and 25 were females (23.8%). The male: female ratio was 3.2:1. Initial laboratory findings are shown it Table 3.

Table 3: Initial laboratory findings for T-ALL and stage III T lymphoblastic lymphoma pediatric cases

parameter	No of patients	No of patients (%)
TLC x10^9/L T-ALL	72	(100)
≥ 100.		38 (52.8)
50-100		14 (19.4)
< 50		20 (27.8)
IPT T-ALL	72	(100)
T- Early		27(37.5)
T- Intermediate		30*(41.6)
T- Late		10 (13.8)
Unclassified		5 (7.1)
Immunophenotyping on pleural effusion of LBL patients:	9	(100)
T- Early		4 (44.4)
T-Intermediate		4 (44.4)
T- Late		1 (11.2)
CSF analysis	105	(100)
CNS free		94 (89.5)
Contaminated CSF		4 (3.8)
CNS positive:	7	7/105 (6.7)
CSF Positive		6 (85.7)
Meningeal infiltration		1 (14.3)

*co-expression with CD 33 was found in 2 patients

Initial antigen receptors Gene rearrangement status:

LBL group:

The BM of the suspicious17/33 lymphoma cases (51.5%) tested for TCR and IgH showed no detectable rearrangements, confirming they were stage III.

T-ALL group:

Molecular testing for T cell receptor (TCR) γ, δ, and immunoglobulin heavy chain (IgH) gene rearrangements was done for 41/72(56.9%) cases of T-cell ALL.

Molecular testing showed the following: TCR-γ gene rearrangement had the highest percentage, 43.80% (18/41 cases). TCR δ gene rearrangement was detected in 12.5% (5 cases). Both TCR-γ and TCR δ gene rearrangement were detected in 17% (7 cases). Immunoglobulin heavy (IgH) chain gene rearrangement was detected in 2(4.8%) cases. No detectable rearrangement in 21.9% (9 cases).

Assessment of response to therapy:

Assessment of early response in T ALL included peripheral blood response to steroids at day 8, day 14 marrow blast percentage. Minimal residual disease was determined in bone marrow and/or peripheral blood at the end of induction, day 43, for T ALL as well as response to induction in lymphoma cases.

T ALL response to steroids (Prednisone Response) at day 8: was assessed in 67 patients that had blasts in their PB. The good responders were 37 (55.2%) cases that showed drop of blasts to less than 1000/ul.

T ALL Day 14:

At day 14, one patient had died and 2 were resistant to therapy. The response in the remaining 69 patients was assessed, 55 cases (79.8%) had M1 marrow, while M2 and M3 were found in 7 cases (10.1%) for each of them.

Minimal residual disease (MRD*)* at Day 43:

Evaluation at post induction therapy showed that 94 cases (89.5%) achieved complete remission.

For T-cell ALL BM cytomorphology showed that 65/72 patients were in CR, antigen gene rearrangements was tested in 13 cases of the 41that showed clonal rearrangement at diagnosis and there was none detectable.

For the LBL cases, at end of induction 29 were in CR, 2 failed induction, 1 died and 1 lost follow up.

Follow up period (6-36 months, median 20 months):

At the conclusion of the follow-up period, 58/105 cases (55.2%) maintained complete remission (CR) while 22/105 (20.9%) cases relapsed.

Survival analysis:

For the whole patients the 3 years overall survival (OS) was 77.4 ±5.1%, disease free survival (DFS) 60.42±5.6% and event free survival (EFS) 50.71 ±8.3%. For the leukemia group, 3 years OS was 81.6±5%, DFS 47.1±8.6% and EFS 48.5±6.2%. For the lymphoma group, the 3 years OS was 77.6±8.1%, DFS 93.3±6.4% and EFS 59.2 ±9.2%.

Impact of prognostic parameters on OS, DFS, and/or EFS (Table 4)

Age:

OS of patients in the age group >5-10 years was 96.5%,±3.8 as compared to OS of patients ≤ 5 years, which was 68± 12.1%, > 10-15 years was 70.9±5.3%, and that for patients >15 years was 58.9±3.2%. Difference was statistically significant (p= 0.04).

Gender:

For males the DFS was 62% and EFS was 52.4±4.1% as compared to females with DFS 51% and EFS 41.6±3.1%, (*P*= 0.01) and (*P* = 0.05) respectively. OS Difference was statistically not significant (*P* = 0.64).

Initial TLC for T ALL:

DFS was 93.65% for patients with TLC <50 x10^9/L, difference was statistically highly significant (*P* < 0.001).

OS and EFS: Difference was statistically insignificant (*P* = 0.62 and 0.16 respectively).

T ALL Prednisone Response day 8:

In good prednisone responders DFS was 84.7±3.2% and EFS was 80.2±7.2% while, for patients with poor prednisone responders were 25.9±7.2% and 27.5±11.2% (*P* =0.02) and (*P* < 0.001) respectively.

OS Difference was statistically not significant (*P* = 0.08).

OS, DFS and EFS had no statistically significant correlation to: stage of maturation by immunophenotyping, antigen receptor rearrangement, CSF status or BM status at Day 14 (M1, M2, M3).

Table (4): survival in relation to prognostic parameters

Parameter	OS (%±SE)		DFS (%±SE)		EFS (%±SE)	
	3 Years	*p*-	3 Years	*p* -	3 Years	*p* -
Age:						
≤5years	68 ± 12.1%		59.3±12%		52.4±6.3%	
>5-10years	96.5±3.8%	0.04*	77.6±3.8%	0.33	76.5±3.8%	0.14
>10years	70.9±5.3%		82.6±5.3%		60. ± 5.3%	
>15 years	58.9± 3.2%		80 ±3.4%		46.8±10.1%	
SEX:						
Male	77.1 ± 4.1%		62 ± 4.1%		52.4 ± 4.1%	
Female	75.1 ± 4.1%	0.64	51 ± 3.1%	0.01*	41.6 ± 3.1%	0.05*
TLC:						
<50x10^9/L	75.2+4.5%		79.6±4.5%		71.9±4.5%	
50-100 x10^9/L	81.2±4.8%	0.62	91.4±4.8%	0.0001*	59 ± 4.8%	0.16
>100 x10^9/L	79.4±5.8%		72.9±5.8%		55.4±5.8%	
CXR:						
Free	77.8±3.2%	0.99	66.3±3.2%	0.07	59.4 ±3.2%	0.44
Positive	76.9±4.4%		76.6±8.4%		67.6±8.4%	
IPT:						
T-early	63±6.1%		53.8±6.1%		60.3±6.1%	
T-intermediate	69±4.8%	0.45	74.4±5.4%	0.98	71.1±8.8%	0.63
T-late	65±5.4%		78±3.8%		64.1±5.4%	
CSF:						
Free	78.4±3.2%		60.8±3.2%		51.2±3.2%	
Contaminated	50.4±9.3%	0.21	100%	0.42	66.6±9.3%	0.81
Positive	80 ±7 %		66.6±11%		66.6±11%	
BM morphology						
L1	79.8±10.4%	0.51	68.9± 5.2%	0.92	61.6 ±6.8%	0.85
L2	87.5 ± 3.2%		72.7± 6.4%		70 ± 4.8%	
BM day 14					61.9±3.3%	
M1 (<5% blasts)	82.7±3.3%	0.61	70.6±3.3%	0.27	41.6 ±11%	0.59
M2 (>5-25% blasts)	80 ± 4.1%		55.5±7.1%		71.4±4.7%	
M3(>25% blasts)	49 ±21.7%		100%			
Steroids response						
Good responders	88.9 ±3.8%	0.08	84.7 ± 3.2%	0.02*	80.2±7.2%	0.0001*
Poor responders	68.3±10.2%		25.9 ±7.2%		27.5±11.2%	

*= Statistically significant

4.Discussion

Historically, the diagnosis of T-cell acute lymphoblastic leukemia (T-ALL) or T-cell lymphoblastic lymphoma (T-LBL) predicted a higher risk of induction failure, early relapse, and worse event-free survival (EFS) compared with B-precursor childhood leukemia or lymphoma (33-38). With increasingly intensive regimens of multi-agent chemotherapy, survival rates have improved to more than 70%. (10-12). Although these regimens all demonstrate some degree of efficacy in T-cell disease, true lineage-specific, highly efficacious therapy has not been identified (10). Treatment intensification has dramatically improved the general prognosis of childhood T-cell precursor acute lymphoblastic leukaemia (T-ALL). Nevertheless, approximately20–25% of cases still relapse early, which significantly diminishes the prognostic outlook of this phenotypic ALL subtype (24, 39-41)

Various prognostic markers have been sought for better risk stratification and treatment adjustments for those patients with parameters of poor prognosis. A task that has been considerably difficult, with conflicting results from different studies (10, 15-18).

In view of this study, for the whole 105 patients, the OS was (77.4%), the estimated 3 years DFS and EFS were (60.4%) and (50.71%) respectively. These figures were mostly lower than reported (16, 17, 39, 42, 44).

When comparing groups, the leukemia group had better OS 81.6±5%, than the lymphoma group (77.6±8.1%).

However, the lymphoma group had better DFS and EFS (93.3±6.4%, 59.2 ±9.2%) compared to the DFS and EFS (47.1±8.6% and 48.5±6.2%) of the leukemia group. In an overview, this was in agreement to a recent report in which patients received the same therapy protocol as our patients. However, the report gave a higher 5 years EFS for T-ALL patients (73.4% ± 2.6%) and T-NHL (84.6% ± 3.2%) (10). Only one report had comparable OS (78%) for T-cell ALL. (45).

When analyzing the prognostic parameters, the following were of statistical significant; the age groups of the 105 patients, gender, initial total leucocytic count, and initial steroids response to therapy for the T ALL patients.

As regards age, it had impact on OS which was higher in the age group >5-10 years (96.5%)(p= 0.04), but was low for the other age groups which was in agreement with various studies (16-18). However age had no statistical significance on EFS contrary to report by others (10).

More controversy occurred with the statistical significance of gender on survival of T ALL cases. We reported a statistically significant DFS and EFS (62% and 52.4±4.1%) for males as compared to DFS and EFS (51% 41.6±3.1%) for females (p = 0.01) and (p = 0.05). Some clinical trials showed that male gender was no longer an adverse risk factor (44) and most reports showed that gender has no statistical significance to survival (16, 17, 42, 45). One report showed that the gender could be of statistical significance on the EFS depending on the treatment protocol applied (10).

Initial TLC for our T ALL was statistically insignificant on OS and EFS (p = 0.62 and 0.16 respectively). Patients with TLC <50 x10^9/L, had a statistically highly significant DFS, 93.65% (p < 0.001). Different studies reported contradicting results, some reported that TLC had no effect on survival (16, 17, 42, 45, 46), while others reported initial TLC a significant prognostic factor on EFS. Patients with TLC <50 x10^9/L, had EFS, 81.5% ±3.6%, vs EFS 67.6% ±3.6% for patients with TLC higher than 50 000 x10^9/L) (p < 0.009) (10).

The rapidity with which leukemia cells are eliminated following onset of treatment (cytomorphological) is also associated with outcome. For many years, cytomorphological response has been the leading criterion for stratifying patients into risk groups within the ALL trials. Poor cytomorphological response at a response evaluation point qualified a patient for high-risk treatment. (15). Day 8, 14 and 43 are the important response evaluation points. In this study, assessment of the Prednisone response day 8 for T ALL, good prednisone responders had significantly better DFS and EFS (84.7±3.2% and 80.2±7.2%) (p =0.02) and (p < 0.001), however, had no effect on the OS. This was in agreement to other reports (15, 46)

Other factors showed statistically insignificant impact on OS, DFS, and EFS of our patients, although many studies reported their prognostic significance as mediastinal masses (47), initial CSF analysis (17, 42, 48), immunophenotyping (37, 49, 43) bone marrow day 14, Minimal Residual Disease (MRD) at the end of induction (day 43) (15, 16, 50). On the other hand some reports gave findings similar to our study. (10, 45)

In this study, univariate analysis indicated that age groups, gender, initial Total leucocytic count, and initial steroids response to therapy were significant prognostic factors. However, on multivariate analysis, poor response to steroids therapy and TLC counts >100x10^9/L were found to be independent prognostic factors for DFS (p = 0.03 and 0.05) respectively. Similarly, poor response to steroids therapy was found to be an adverse prognostic factor for EFS. Poor responders to steroid therapy developed an earlier event than good responders.

In conclusion, management of pediatric T-cell ALL and LBL still needs modifications to achieve better outcome for patients. Further multicentric studies are needed to provide accumulating evidences of which prognostic parameters are the most valuable in risk stratification of the patients and hence a consensus treatment protocol can be applied. Also, true lineage-specific, highly efficacious therapy seems to be the hope to true cure.

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