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Research Article

Role of Minimal Residual Disease in the Clinical Course of T cell Acute Lymphoblastic Leukemia in Pediatric Patients

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ABSTRACT

Background: Minimal Residual Disease (MRD) is the most important prognostic parameter in pediatric precursor B cell-Acute Lymphoblastic Leukemia (B-ALL). However, the feasibility of flow cytometry in the detection of Minimal Residual Disease (MRD) in T cell- Acute Lymphoblastic Leukemia (T-ALL) is not well defined

Objectives: We aimed to investigate the prognostic impact of Flow Cytometry-MRD in T-ALL measured at different time points post-induction.

Patients and Methods: In the current study, the impact of MRD was investigated in 58 newly diagnosed pediatric ALL at day 15, 28 and 42 post induction treatment in relation to other clinical and hematological parameters as well as disease-free survival.

Results: At day 15, day 28 and day 42 patients with MRD level ≥ 0.1% had inferior Disease-Free Survival (DFS) compared to patients with lower MRD levels which was significant for day 15, day 28 and 42 (p=0.007, p=0.0148 and p=0.0004 respectively). No association was detected between MRD status and different clinical and laboratory parameters.

Conclusion: Post-induction MRD detection is sensitively reflecting the disease progression in pediatric (T-ALL). This is most evident at day 42 followed by day 15.

Keywords: T-ALL (T-cell Acute Lymphoblastic Leukemia); MRD (Minimal Residual Disease); Flow cytometry

INTRODUCTION

Minimal Residual Disease (MRD) is defined as detection of residual leukemic cells that cannot be recognized by morphologic evaluation. MRD assessment during induction periods in Acute Lymphoblastic Leukemia (ALL) patients is a well-established independent prognostic parameter in precursor B-cell Acute Lymphoblastic Leukemia (B-ALL) [1-13]. However, the role of MRD in T-cell acute lymphoblastic leukemia (T-ALL) is not as clearly defined [9,14]. There are two main methods for MRD detection.

Polymerase Chain Reaction (PCR) of T cell receptor gene rearrangement (TCR) is highly sensitive but it requires pre-characterization of TCR rearrangement. Moreover, it may be limited by the evolution of new clones. The other method, Flow cytometry, is a well standing, available sensitive method for MRD detection [15].

Unlike precursor B-cell Acute Lymphoma Leukemia (B-ALL), only a few controversial studies about the role of MRD in T-cell acute lymphoblastic leukemia (T-ALL) are available in the literature [9,14]. In the current study, we evaluated the influence of MRD detection at different time points by flow cytometry on the clinical course of 58 pediatric *de novo* (T-ALL).

MATERIALS AND METHODS

The study was conducted on 58 *de-novo* pediatric T-ALL presented to pediatric outpatients clinic, NCI, Cairo University, between June 2012 and September 2015. They included 49 males and 8 females with an age range of 1.5-18 and a median of 7.5 years.

The diagnosis was made by standard methods including history and complete clinical examination. Laboratory workup included complete blood picture, bone marrow examination by morphology and cytochemistry as indicated as well as basic chemistry profile.

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Peripheral blood or bone marrow was obtained at diagnosis; samples were analyzed within 24 hours. Comprehensive panel of monoclonal antibodies was used for lineage assignment including CD45, CD19, CD10, CD22, CD34, CD79A, CD1PE, CD7, CD2, CD4, CD8, CD16, CD56, CD99, TDT, CD33, CD13, CD11B, CD15, NG2 and CD117 were purchased from Beckman Coulter, Miami, USA. MPO, CD3, Kappa, Lambda, CD5, cytoplasmic μ, CD20, MHC class II were supplied from Dako, Cytomation, Denmark.

Briefly, 50 μL of a sample with an adjusted cell count of (approximately 1×106 cells/tube) were incubated with monoclonal antibodies according to the manufacturer recommendations in the dark for 30 minutes at room temperature, then lysed and washed with Phosphate Buffer Saline (PBS) to get rid of excess antibodies. Cells were re-suspended in 500 μL of PBS. The analysis was performed on Navious cytometer Beckman Coulter, Miami, USA. Blast population was selected first on forward scatter versus side scatter, and then from CD45 versus side scatter. Cut off the value of surface monoclonal antibodies was considered ≥ 20% while cytoplasmic ≥ 10%. The median number of events quantified in the assay 1.250.000 (750.000-1.600.000). The lower Limit of Detection (LOD)=0.008% 1 in 50,000 cells. The Lower Limit of Quantification (LLOQ)=0.01% 1-10.000.

Treatment protocol

Protocol used was total XV protocol (modified from St. Jude total XV protocol); it consists of three phases, remission induction, consolidation and maintenance [16].

Remission induction/Consolidation

Remission induction therapy began with prednisone, vincristine, daunorubicin, and asparaginase (Table 1). Patients with ≥ 1% MRD on day 15 received three additional doses of asparaginase. Subsequent induction therapy consisted of cyclophosphamide, mercaptopurine and cytarabine. Upon hematopoietic recovery (between days 43 and 46), MRD was assessed, and consolidation therapy began (Table 2).

Table 1: Leukemia Associated Immuno Phenotypes (LAIP) used in 58 pediatric T acute lymphoblastic leukemia cases.

LAIP	N (%)
CD45/TdT/cytoplasmic CD3 /sCD3	41 (70.7)
CD45/CD7/CD13/ CD3	28 (48.3)
CD45TdT/Cytoplasmic CD3/cytoplasmic CD13	15 (25.9)
CD45/CD7/CD34/ CD13	10 (17.2)
CD45/CD2/CD33/ CD5	5 (8.6)
CD45/CD7/CD117/CD34	2 (3.4)
CD45/CD2/CD19/CD3	1 (1.7)

Table 2: Induction-Consolidation therapy for T-ALL.

Induction Therapy		
Agent	**Dosage**	**Schedule**
Prednisone	40 mg/m2/day	Days 1–28
Vincristine	1.5 mg/m2 per week	Days 1, 8, 15, 22
Daunorubicin	25 mg/m2 per week	Days 1, 8
L-asparaginase	10,000 U/m2 per dose IM (thrice weekly)	Days 2, 4, 6, 9, 11, 13, (16, 18, 20)*
Cyclophosphamide	1000 mg/m2 IV	Day 22
Cytarabine	75 mg/m2 per day IV	Days 23-26 , 30- 34
Mercaptopurine	60 mg/m2 per night	Days 22-35
Intrathecal cytarabine	Age-dependent	Day 1
Triple intrathecal	Age-dependent	Day 15 (8, 22)**
Consolidation Therapy		
Agent	Dosage	Schedule
High-dose methotrexate	Targeted to 65μM (standard-/or high-risk)	Days 1, 15, 29 and 43
Mercaptopurine	50 mg/m2 per night	Days 1 to 56
Triple intrathecal	Age-dependent	Day 1, 15, 29 and 43
*additional treatment if MRD more than 1% at d15 ** if CNS 2/CNS 3 Leukemia		

Continuation therapy

Patients received weekly asparaginase and daily mercaptopurine with pulses of doxorubicin plus vincristine plus dexamethasone. They also received two reinduction treatments between weeks 7-9 and weeks 17-20. For the remaining continuation therapy, standard-risk patients received three rotating drug pairs (mercaptopurine plus methotrexate, cyclophosphamide plus cytarabine, and dexamethasone plus vincristine). Dosages of mercaptopurine and methotrexate were adjusted according to the tolerance; total scheduled dosages of anthracyclines and cyclophosphamide were limited to 230 mg/m2 and 4.6 g/m2, for standard-risk patients, respectively. Continuation treatment lasted 120 weeks in girls and 146 weeks in boys.

CNS-directed therapy

Standard-risk patients received triple intrathecal chemotherapy (16 to 25) depending on the presenting features and the CNS status.

Allogeneic hematopoietic stem-cell transplantation

This procedure was an option for patients with high-risk leukemia (poor response to induction treatment MRD>1%).

STATISTICAL ANALYSIS

SPSS 17 was used for statistical analysis. Continuous variables were assessed with independent t-test and categorical variables with Fischer's exact test. Significance was assessed at p value 0.05. Kaplan-Meier Overall survival curves and disease-free survival for groups were derived and compared using log-rank tests.

RESULTS

The study cohort comprised 58 pediatric ALL patients including 49 males and 9 females. Patients' characteristics are presented in Table 3.

Table 3: Clinical and hematological characteristics of 58 pediatric T acute lymphoblastic leukemia.

Parameter	Finding
Age: years *	7.5 (1.5-18)
Gender: male/female	49/9
Lymphadenopathy**	58 (100)
Hepatosplenomegaly**	40 (69)
Splenomegaly **	18 (31)
CNS infiltration **	12 (20.7)

Mediastinal involvement**	21 (36.3)
FAB L2/L1	55/3
BM blasts % *	90 (62- 98)
PB blasts% *	46 (18- 74)
Immunophenotype	
Precursor T-ALL **	5 (8.6)
T early **	16 (27.6)
T intermediate **	22 (37.3)
T late**	8 (13.8)
Unclassified **	7 (12.1)

* Median (range), ** N (%), CNS: Central Nervous System; FAB: French American British;
Hb: Hemoglobin; TLC: Total Leucocytic Count; PLTs: Platelets; BM: Bone Marrow; PB: Peripheral Blood.

At day 15, 48 samples were obtained; 18 (37.5%) patients had MRD ≤ 0.01 and 6 (12.5%) had MRD>0.01-<0.1; all these did not relapse. In contrast the 24 (50%) patients with ≥ 0.1% had 10.3 months median DFS survival (p=0.007, Figure 1).

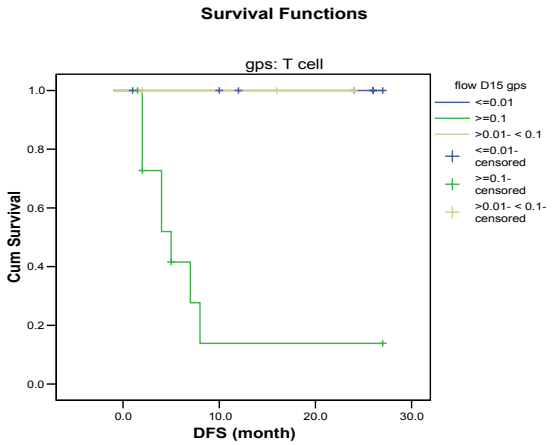

Figure 1: Impact of Minimal Residual Disease (MRD), at day 15 post induction, on disease-free survival of 58 pediatric T-cell acute lymphoblastic leukemia patients (p=0.007).

At day 28, 22 samples were received; all the 14 (63.6%) patients with MRD<0.1% did not relapse. In contrast, 8 (36.4%) patients with MRD level ≥ 0.1% had a median DFS of 6.2 months (p=0.0148, Figure 2).

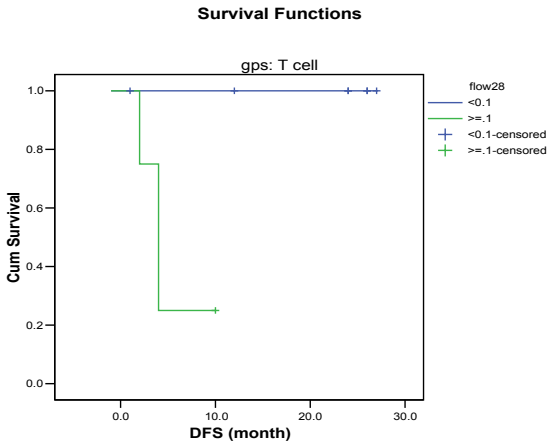

Figure 2: Impact of Minimal Residual Disease (MRD) at day 28 post induction on disease-free survival of pediatric acute lymphoblastic leukemia patients (p=0.0148).

At day 42, 30 samples were evaluated; 18 (60%) patients had MRD <0.01% with a median DFS of 20 months. On the other hand, 12 (40%) patients with MRD level ≥ 0.1% had a median DFS of 4.5 months (p=0.0004, Figure 3). No associations were detected between MRD status and age, gender, lymphadenopathy, hepatosplenomegaly, PB or BM blasts.

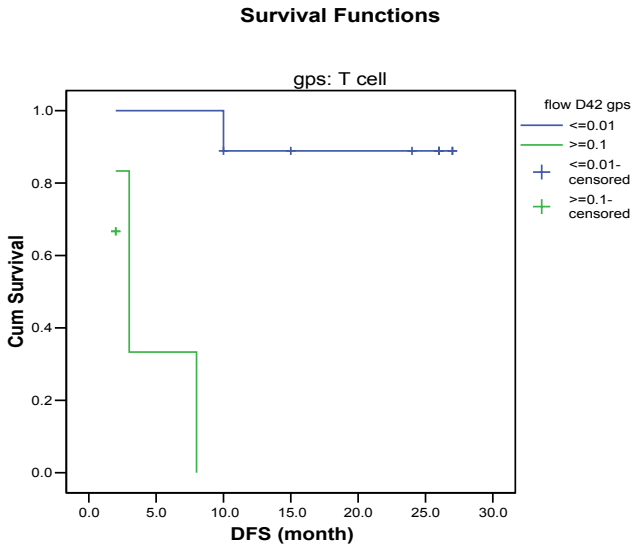

Figure 3: Impact of Minimal Residual Disease (MRD) at day 42 post induction on disease-free survival of pediatric acute lymphoblastic leukemia patients (p=0.0004).

DISCUSSION

The role of MRD is well defined in pediatric precursor B-ALL being a cornerstone in risk stratification and therapy adjustment [1-13]. On the other hand, the role of MRD detection in T-ALL has not been precisely established; few controversial reports have emerged to doubt the significance of MRD detection on treatment outcome [9,14].

In our cohort, 58 pediatric ALL were investigated at day 15, day 28, and day 42 post induction. The persistence of MRD>0.1% raised the possibility of therapy failure and occurrence of relapse. The DFS was shorter in patients with positive MRD at day 15 and day 28 with a median of 10.3 months and 6.2 months respectively. Whereas, patients with MRD>0.1% were still disease free during the follow-up period in both day 15 and day 28 (p=0.007 & p=0.0148 respectively).

In addition, the persistence of MRD at day 42 lowered the disease-free survival to 4.5 months. Patients with negative MRD had free DFS of 20 months as most events occur within two years of diagnosis of T-ALL. Our results had much lower MRD rate than PCR based studies [9,14]. The discordance could be explained not only by the higher sensitivity but also by the detection of non-viable cells by PCR-based assays that are excluded by flow cytometry.

Flow cytometry is a readily sensitive method that discriminates between debris and real events. The cut off value of 0.1% was a discriminating point for risk stratification in the large European study based on PCR detection of MRD. This highlights the impact of MRD on the outcome of childhood (T-ALL) [9].

Parekh et al. detected more than 50% of positive MRD during induction period in 33-childhood T-ALL cohort [14]. This is in line with our study where we identified 50% of MRD<0.1%. In

contrast, Parekh and his team did not encounter any impact of MRD on DFS [14]. This discordance could be attributed to low sample size; besides the majority of their patients were hispanic.

CONCLUSION

In conclusion, our study provides evidence that MRD detection by flow cytometry can predict treatment outcome of pediatric T-ALL. Further monitoring during the consolidation period is recommended with a long follow up period.

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