RESEARCH PAPER

Absolute Lymphocyte Count at the End of Induction as a Surrogate Marker for Minimal Residual Disease in T-cell Acute Lymphoblastic Leukemia

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Objective: The relation of absolute lymphocyte count (ALC) with minimal residual disease (MRD) in T cell – acute lymphoblastic leukemia (T-ALL) is not known. The objective of the study was to correlate ALC with MRD, steroid-response and complete remission (CR). **Methods:** *De-novo* T- ALL patients (age 1-18 y) recruited prospectively; 52 enrolled, 9 excluded, and 43 analyzed. 39 achieved CR and MRD was available for 28 patients; 23 were MRD negative. **Results**: ALC did not correlate with steroid response and CR. Median (range) ALC at the end of induction was significantly higher in patients who were MRD negative compared to MRD positive [1.24 (0.12, 6.69) *vs* 0.62 (0.15, 0.87); *P*=0.03], respectively. Patients having ALC ≥700 $\times 10^9$ /L were significantly more likely to be MRD negative than those with lower values *(P*= 0.028) ***Conclusion*****:** Our study suggests that ALC is a favorable factor, and may act as surrogate marker for MRD.

Keywords: *Complete remission, Outcome, Prognosis, Steroid response*.

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Absolute lymphocyte count (ALC) has been correlated with overall survival and event free survival in acute lymphoblasti leukemia (ALL). In T-cell Acute lymphoblastic leukemia (T-ALL), studies have reported the data in combination with B-ALL [1-7]; separate analysis of T-ALL is lacking. Minimal residual disease (MRD) has emerged as the most important predictor of outcome [8]. Current protocols use MRD for risk stratification and treatment [9]. The drawback of MRD is that it is not available at all centers. ALC is the most readily available prognostic factor. Hence, we studied if ALC at baseline, day 8, day 15 and at the end of induction (EOI) correlates with MRD, steroid response (SR), complete response (CR), and if it can be used as a surrogate marker for MRD in T-ALL.

METHODS

We conducted a prospective study from July 2015 to June 2017. The study was approved by the institutional ethics committee of our institute, and informed consent was obtained from parents. Assent was obtained from those patients who were above 7 years of age. Newly diagnosed T-ALL cases with age ≥1 to 18 years were included. Patients partially treated outside the institution and those with relapsed ALL, were excluded.

Patients were treated on Indian Childhood Collaborative Leukemia Group protocol (Unpublished-CTRI/2015/12/006434). All patients were treated with steroid prophase for seven days. Good SR was defined as day 8 blasts in peripheral smear less than 1×10^9 /l. All patients irrespective of SR and baseline features received four doses of vincristine and eight doses of L-asparaginase. MRD and bone marrow morphology was assessed on day 35 at the EOI.

Total leukocyte count was done in MS95s [Melet Schloesing Laboratories, Osny, France] at baseline, day 8, day 15 and day 35. Simultaneously, peripheral smear was made and manual differential count was done to determine the percentage of lymphocytes. ALC was calculated as percentage of lymphocytes multiplied by the total leukocyte count.

MRD assessment was done by 10-color flow cytometry using stain-lyse-wash method [10]. Briefly, bone marrow aspirate, was incubated for 15-20 minutes in the dark at room temperature with antibody panel: anti-CD20-FITC, CD123-PE, CD34-ECD, CD10-PC5, CD 19-PC7, CD58-APC, CD38-AF700, CD33-APC-AF 750, (Beckmann Coulter/Immunotech, Miami, FL, USA), CD13-BV421, CD45-BV510 (Biolegend, San Diego, USA). Samples were lysed using ammonium

chloridelysis buffer, then washed twice in phosphate-buffered saline, re-suspended in 1 ml of phosphate-buffered saline and analyzed by flow cytometry. All samples were processed within 24 hours after collection. For flow cytometry analysis, at least 10,00,000 cells were acquired in all cases, and data were stored in list mode files. Data analysis was done using Kaluza software (version 1.3). The cutoff levels used for MRD positivity were ≥0.01%. MRD was said to be not evaluable when two consecutive bone marrow samples were not analyzable for MRD.

The association between continuous and categorical data was examined by Mann-Whitney U-test. For analyzing cut-off of ALC with MRD Fisher's Exact test was used. Statistical analyses were carried out using Stata ver. 14 software. The criterion for significance in all analyses was $P<0.05$.

RESULTS

A total of 52 patients presented during the study period. Nine patients were excluded (5 deaths during induction and 4 opted for no treatment); and remaining 43 were analyzed. Baseline characteristics and induction outcome are shown in ***Table* I**. Out of 43 patients, 39 (90.7%) achieved CR. MRD data were available for 28 patients. Correlation of ALC with a day 8 SR, CR and MRD is shown in ***Table* II**. At the EOI ALC was significantly higher in patients who were MRD negative, so cutoffs were analyzed. Patients having ALC ≥0.7×10^9/l (*n*=16, MRD negative 15) were significantly more likely to be MRD negative than patients with ALC<0.7×10^9/l (*n*=8, MRD positive 4), $P= 0.028$.

TABLE I BASELINE CHARACTERISTICS AND INDUCTION OUTCOMES IN CHILDREN WITH T-ALL (*N*=43)

Characteristics	*Values*
*Age (y)	5 (1-18)
Male sex (%)	37 (86)
*Hemoglobin (g/L)	90 (40-147)
*Total leucocyte count ($\times10^9$ cells/L)	69 (1.3-500)
*Platelet ($\times10^9$ cells /L)	41 (5-505)
*ALC- day 0 (*n*=41)	5.01 (0-80.64)
*ALC- day 8 (*n*=43)	1.22 (0.117-15.142)
*ALC- day 15 (*n*=37)	1.1 (0.248-12.384)
*ALC- day 35 (*n*=39)	1.09 (0.117-6.691)
Steroid response	
Good	16 (37.2)
Poor	27 (62.8)
Complete remission	39 (91)
Minimal residual disease (n=28)	
Negative	23 (82)

*ALC: Absolute lymphocyte count in $X10^9$ cells/L; Values in n (%) except *median (range).*

TABLE II CORRELATION OF ABSOLUTE LYMPHOCYTE COUNT WITH VARIOUS OUTCOME MEASURES IN CHILDREN WITH T-ALL (*N*=43)

	ALC day 0	*ALC day 8*	*ALC day 15*	*ALC day 35*
**Steroid response*				
Poor	25	27	21	24
Median (range)	4.92 (0-60)	1.15 (0.12-4.90)	0.94 (0.24-12.38)	1.24 (0.12-6.50)
Good	16	16	16	15
Median (range)	6.35 (0.69-806.4)	1.72 (0.13-15.14)	1.19 (0.30-2.5)	0.87 (0.15-6.69)
**Complete remission*				
Yes	37	39	33	35
Median (range)	5.01 (0-80.64)	1.22 (0.12-15.14)	1.06 (0.25-12.38)	1.09 (0.12-6.69)
No	4	4	4	4
Median (range)	6.20 (0.69-25.65)	1.69 (0.78-2.99)	1.29 (0.93-1.80)	1.06 (0.53-1.94)
#MRD				
Negative	23	23	19	19
Median (range)	5.01 (0-80.64)	1.26 (0.13-15.14)	0.94 (0.27-12.38)	1.24 (0.12-6.69)
Positive	5	5	3	5
Median (range)	2.88 (0.78-6.99)	0.8 (0.12-6.99)	0.45 (0.36-1.73)	0.62 (0.15-0.87)

*ALC: Absolute lymphocyte count; MRD: Minimal residual disease. The numbers may not add up to 43 due to missing data; *P>0.5; #P=0.03 for Minimal residual disease with ALC at day 35.*

DISCUSSION

We found that ALC at any point during induction does not correlate with SR and CR. We propose following explanations for our negative results. Mutations in interleukin 7 receptor signalling pathways form the basis of steroid resistance in T ALL [11]. So, lymphocytes may have a limited role in the attainment of SR. In the achievement of CR, instead of ALC, specific lymphocyte subsets could be responsible in eradicating leukemic cells. Since lymphocyte subsets were not analyzed and correlated with CR, it might explain the lack of relationship between CR and ALC. Another possibility is that the number of patients, who failed to achieve CR, was too small to yield statistically significant results.

MRD at the EOI is a favorable factor in T-ALL [12]. We interpret that ALC $\geq 0.7\times10^9$/l is a favorable factor and ALC may act as a surrogate marker of MRD at the EOI. Rolf, *et al*. [13] have shown that ALC at the EOI in B-ALL has a very high correlation with $CD3^+$T cells and dendritic cells, which are known to mediate potent antileukemic activity. Probably, lymphocytes have a role in eradicating residual leukemic cells in T-ALL at the EOI, as higher ALC correlated with MRD negative status.

The limitations of this study are that sample size is small and follow-up duration is short for event free survival, relapse and overall survival. Current study provides evidence that in T-ALL, lymphocytes may have an important role to play in attaining MRD negative status at the EOI. As patients who are MRD negative at the end of consolidation have more favorable outcomes even if they had been MRD positive at the EOI, we suggest further studies of on correlation of ALC with MRD at the end of consolidation.

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